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- Your phone, and fax numbers, e-mail and mailing addresses
- Dojindo product code and product name
- Quantity of the product
- Date needed

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Requests for custom synthesis are also welcome. For a quotation, please call send e-mail to info@dojindo.com with following information:

- Your phone and fax numbers, e-mail and mailing addresses
- Chemical structure and chemical or product name
- Specification of the chemical
- Synthetic schemes or references, if available

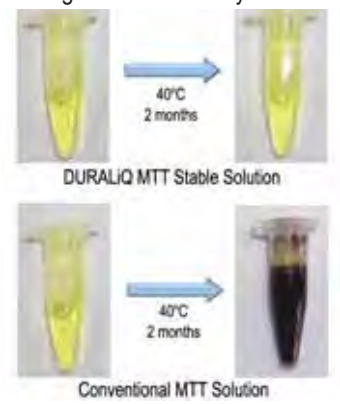
DURALiQ MTT Stable Solution

p. 13

Application: Cell viability and cytotoxicity detection
Features: Long Term Stability
Ready to Use Solution
Economical

Product Description

Cell proliferation/cytotoxicity assays are widely used in the field of drug screening and investigating influences of cytokines, chemicals and growth factors on cell functions. The reduction of tetrazolium salts is one of the most reliable and common methods to determine cell viability and cytotoxicity. MTT(3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) is a well known tetrazolium salt for determining mitochondrial dehydrogenase activity in living cells. However, MTT is really unstable in aqueous solution and, a stock solution of MTT must be prepared as needed Dojindo's DURALiQ MTT Stable Solution is a ready-to-use cell proliferation/cytotoxicity reagent that can be stored in refrigerator for over a year. This MTT solution is an indispensable tool for those who preform frequent assays.



MTT solution was exposed at 40°C for 2 months. The appearance of each solution was compared.

ICG Labeling Kit-NH_2

p. 93

Application: ICG dye labeling of proteins

Features: Suitable wavelength for in vivo imaging
Quick and Easy Labeling to Antibodies : 1.5hr
High Recovery Rate : more than 90%

Product Description

ICG Labeling Kit - NH_2 is used primarily for the preparation of ICG (Iindocyanin green)-labeled antibody for near-infrared fluorescence imaging. ICG offers two remarkable properties: 1) ICG has a strong near-infrared fluorescence even after a few days under physiological conditions. The excitation and emission wavelength of the ICG-labeled proteins are 774 nm and 805 nm, respectively. 2) ICG has been used in clinical fields such as a hepatic deficiency testing. Therefore, ICG and ICG conjugates are materials suitable for in vivo imaging.
This kit contains all required compornents required for labeling, including storage buffer for conjugates. The labeling process is simple:. Add NH_2-reactive ICG to protein solution on a filter membrane, and incubate at 37°C, for 10 minutes. A filtration tube can remove excess ICG molecules.

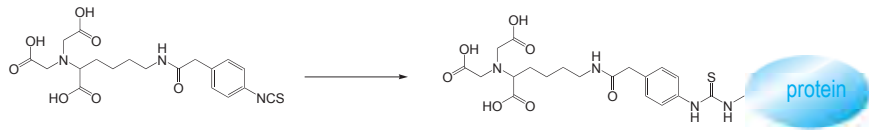
Fluorescent Overlay Imaging

Isothiocyanobenzyl-NTA

p. 108

Application: Chelate labeling of proteins

Chemical Structure



Isothiocyanobenzyl-NTA NTA-conjugated protein

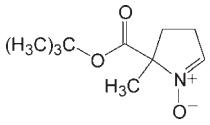
BMPO

p. 146

Application: Spin trap reagent, EPR (ESR) detection
Superoxide anion radical, Hydroxyl radical

Features: Long half-life
High solubility in water
Greater S/N ratio

Chemical Structure



Biotin-SAM Formation Reagent

p. 163

Appearance: White or slightly yellowish solid

Features: Simple preparation of Biotin-SAM on gold surface
Efficient Immobilization of Avidins
Suppress unspecific binding with unknown proteins

Product Description

Dojindo's Biotin-SAM Formation Reagent is utilized in a biosensor that is minimized unspecific binding, and it efficiently immobilizes Avidin such as Streptavidin or NeutrAvidin. In fact, a sensor prepared with Dojindo's Biotin-SAM Formation is presented with less unspecific binding than a conventional product (Fig. 2). Therefore, biotinylated proteins are more efficiently immobilized on the sensor for further analysis. The product can also be utilized with biotinylated DNA or peptides.

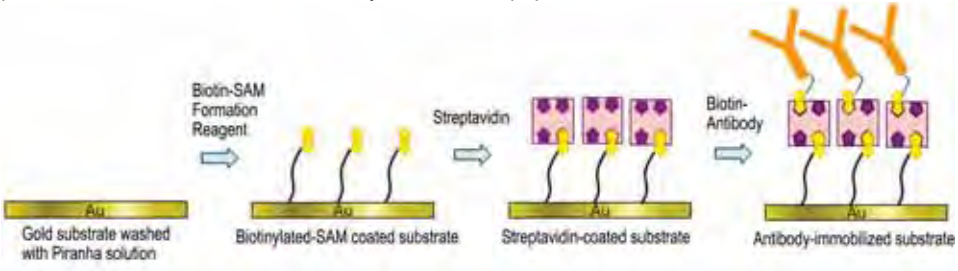


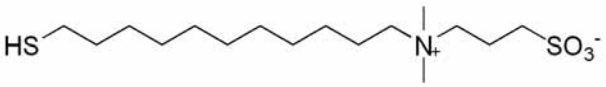
Illustration of biosensor constructed with Biotin-SAM Formation Reagent

Sulfobetaine3-undecanethiol

p. 164

Application: SAM preparation

Chemical Structure

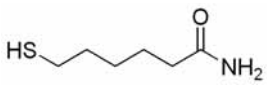


5-Amido-1-pentanethiol

p. 166

Application: SAM preparation, amine group coating

Chemical Structure

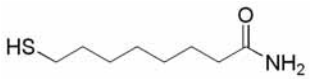


7-Amido-1-heptanethiol

p. 166

Application: SAM preparation, amine group coating

Chemical Structure

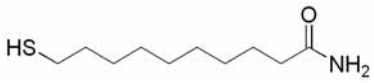


10-Amido-1-decanethiol

p. 167

Application: SAM preparation, amine group coating

Chemical Structure

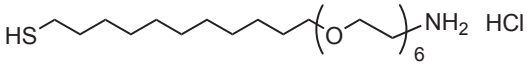


Amino-EG6-hexadecanethiol,hydrochloride

p. 168

Application: SAM preparation, amine group coating

Chemical Structure



Carboxylic acid-SAM Formation Reagent

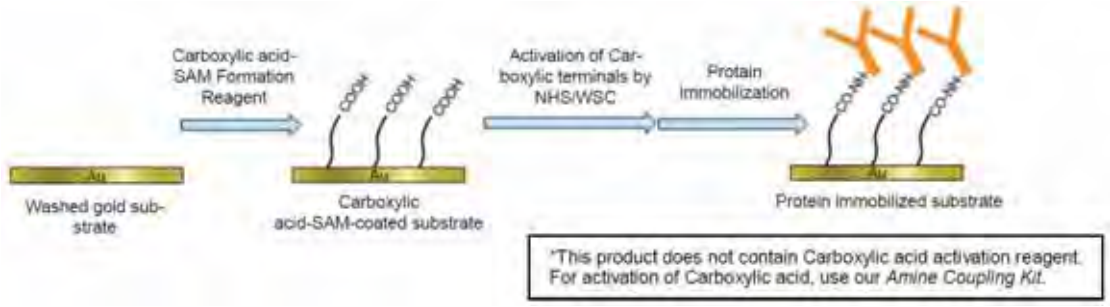
p. 171

Appearance: Colorless or pale yellow liquid

Features: Easily forms carboxy-terminated monolayers on gold
Suppress non-specific binding

Product Description

Carboxylic acid-SAM Formation Reagent is used to prepare carboxylic acid- terminated self-assembled monolayers (SAMs) on gold surfaces as biosensors detected by QCM (Quartz Crystal Microbalance), SPR (Surface Plasmon Resonance), and electrochemical analysis. Coupled with carboxyl group activation method using NHS/WSC, carboxylic acid-SAM can be used as an interface to immobilize proteins or peptides and other ligands on gold surfaces.



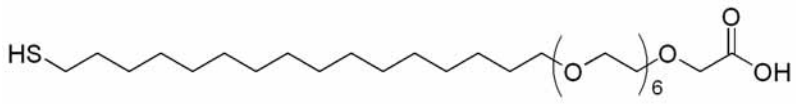
A biosensor construction by Carboxylic acid-SAM Formation Reagent

Carboxy-EG$_6$-hexadecanethiol

p. 174

Application: SAM preparation, carboxylate coating,

Chemical Structure



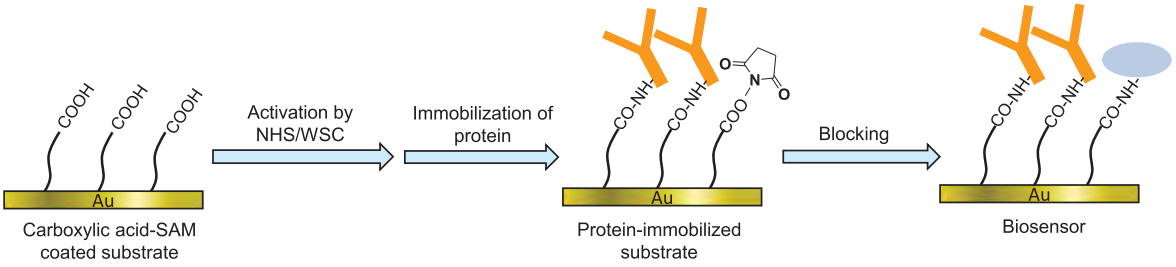
Amine Coupling Kit

p. 177

Appearance: Immobilization of protein and/or peptide to carboxylic acid groups

Features: Sufficient for 40 immobilizations
All reagents necessary for activation, immobilization, and blocking are included
Blocking buffer enables minimization of non-specific absorption by protein

Product Description
Amine and carboxylic acid coupling is one of the most common methods to immobilize a protein or a peptide through a covalent bound onto biosensor surface. Dojindo's Amine Coupling Kit contains all of the reagents and buffers necessary for activation of carboxylic acid, protein immobilization, and blocking. Blocking solution included in the kit minimizes non-specific protein absorption to the surface by capping residual activated esters. Each kit is adequate for approximately 40 immobilizations.



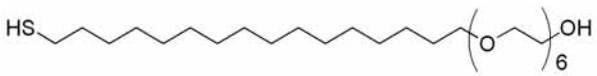
A biosensor prepared by Amine Coupling Kit

Hydroxy-EG$_6$-hexadecanethiol

p. 182

Application: SAM preparation, dilution of functional alkanethiols

Chemical Structure

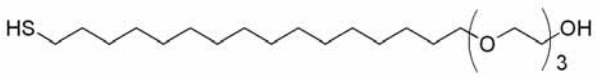


Hydroxy-EG$_3$-hexadecanethiol

p. 183

Application: SAM preparation, dilution of functional alkanethiols

Chemical Structure



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Kits for Life Science Research

Dojindo offers various kits for cell based assay, oxidative stress detection, protein conjugation, and DNA/RNA or IgG purification. All of our kits have simple procedures and yield accurate measurements. Kits are also complete and ready-to-use, no additional reagents are necessary for the experiment.

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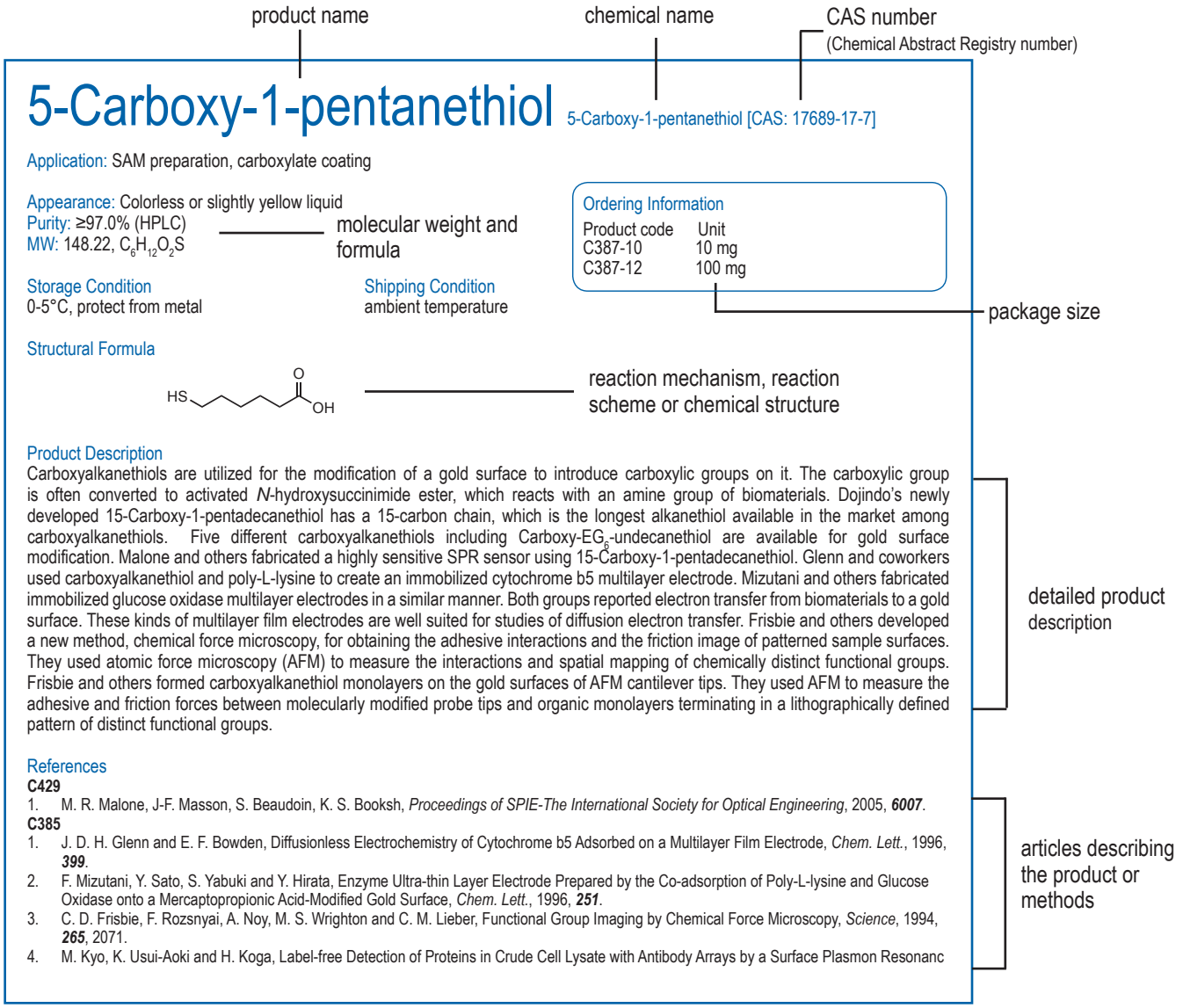


Abbreviations:

Ac: acetyl
ADP:adenosine 5'-diphosphate
AM: acetoxy methyl ester
AMC: aminomethylcoumarin
ALP: alkaline phosphatase
APC: Allophycocyanin
ATP: adenosine 5'-triphosphate
Br: bromide
B-PE: B-phycoerythrin
Cl: chloride
DMF: N,N-dimethylformamide
EIA: enzyme immunoassay
ESR: electron spin resonance
GC: gas chromatography
GSH: glutathione reduced form
GSSG: glutathione oxidized form
HCl: hydrogen chloride
H_2O_2: hydrogen peroxide
HPLC: high performance liquid chromatography
HRP: horseradish peroxidase
Kd: (metal-chelate) dissociation constant
NAD: β-nicotinamide adenine dinucleotide
NADH: β-nicotinamide adenine dinucleotide, reduced form
NADP: β-nicotinamide adenine dinucleotide phosphate
NADPH: β-nicotinamide adenine dinucleotide phosphate, reduced form
NHS: N-hydroxysuccinimidyl ester
NO: nitric oxide
NOS: nitric oxide synthase
OSu: N-hydroxysuccinimidyl ester
PVC: polyvinyl chloride
ROS: reactive oxygen species
R-PE: R-phycoerythrin
SH: sulfhydryl
THF: tetrahydrofuran
TLC: thin layer chromatography
ε: molar absorptivity
λem: emission maximum wavelength
λex: excitation maximum wavelength
λmax: maximum wavelength

Cell Proliferation & Cytotoxicity

Colorimetric Standard Method

Cell Counting Kit-8

- Colorimetric microplate assay
- One solution type (ready-to-use solution)
- No Toxicity to Cells
- No washing required
- No radioisotopes or organic solvents required

For additional information, please see P. 8.

High Sensitive Colorimetric Assay

Cell Counting Kit-SK

- Colorimetric microplate assay
- One solution type (ready-to-use solution)
- Highly sensitive
- No washing required
- No radioisotopes or organic solvents required

For additional information, please see P. 13.

Conventional Colorimetric Assay

DURALiQ
MTT Stable Solution

- Long Term Stability
- One solution type (ready-to-use solution)
- Conventional Method

For additional information, please see P. 13.

High Sensitive Fluorometric Assay

Cell Counting Kit-F

- Fluorometric microplate assay
- Highly sensitive: as low as 50 cells per well
- Quick detection: 10-30 minutes incubation
- No radioisotopes or organic solvents required

For additional information, please see P. 14.

Living & Dead Cell Staining

Fluorescent Staining

-Cellstain- Double Staining Kit

- Fluorometric microscope detection
- Simultaneous determination of viable and dead cells

For additional information, please see P. 31.

Microbial Detection

Bacterial and Yeast Cell Viability Detection

Microbial Viability Assay Kit-WST

- ♦ Colorimetric microplate assay
- ♦ Wide variety of microorganism detection
- ♦ No harvesting or washing required

For additional information, please see P. 16.

Aerobic Bacterial cell Fluorescent Staining

-Bacstain- CTC Rapid Staining Kit

- ♦ Flow cytometry and Microscopy detection
- ♦ Highly sensitive fluorescence detection
- ♦ No washing required

For additional information, please see P. 51.

Transfection Reagents

DNA Transfection Reagent

HilyMax

- ♦ Best reagent for cell signaling research
- ♦ High transfection efficiency in wide variety of cells
- ♦ Optimized protocol for maximizing transfection
- ♦ Great result with insect cell

For additional information, please see P. 58.

Protein Transfection Reagent

CarryMax

- ♦ Simple procedure to optimize delivery condition
- ♦ Low toxicity to cells
- ♦ Antibody and enzyme transfection

For additional information, please see P. 60.

Oxidative Stress Detection Assays

AP Site Detection

DNA Damage Quantification Kit

- ♦Determine the number of abasic sites in genomic DNA
- ♦Colorimetric microplate assay
- ♦Detection range: 1-40 abasic sites per $1x10^5$ base pairs DNA

For additional information, please see P. 129.

SOD or SOD-like Activity Detection

SOD Assay Kit-WST

- ♦ WST-1 based SOD inhibition assay
- ♦ Colorimetric microplate measurement
- ♦ Measures 100% inhibition by SOD
- ♦ pH-independent IC_{50} determination
- ♦ Low background noise measurement

For additional information, please see P. 132.

Total Glutathione Detection

Total Glutathione Quantification Kit

- ♦ Colorimetric microplate measurement
- ♦ Highly sensitive DTNB-based recycling system
- ♦ Wide detection range of 1 µg to 100 µg

For additional information, please see P. 135.

ACE Inhibition Assay

Angiotensin-converting Enzyme Activity Detection

ACE Kit-WST

- ♦Screening of ACE inhibitors
- ♦Colorimetric microplate assay
- ♦Simple protocol
- ♦No organic solvent required
- ♦High reproducibility

For additional information, please see P. 184.

Fluorophore Labeling

Fluorescent Dye (Small Molecule) Labeling Kits

- Only 1 hour to recover conjugates
- All processes in a single filtration tube
- High recovery of conjugates
- Applicable for 50-200 µg IgG

Green Fluorescent Labeling *:* 495 nm (*ex*), 520 nm (*em*)

Fluorescein Labeling Kit-NH_2

For additional information, please see P. 85.

Red Fluorescent Labeling :555 nm (*ex*), 570 nm (*em*)

HiLyte Fluor™ 555 Labeling Kit-NH_2

For additional information, please see P. 87.

Far-Red Fluorescent Labeling : 652 nm (*ex*), 673 nm (*em*)

HiLyte Fluor™ 647 Labeling Kit-NH_2

For additional information, please see P. 89.

Near-Infrared Fluorescent Labeling : 760 nm (*ex*), 780 nm (*em*)

HiLyte Fluor™ 750 Labeling Kit-NH_2

For additional information, please see P. 91.

Near-Infrared Fluorescent Labeling : 774 nm (*ex*), 805 nm (*em*)

ICG Labeling Kit-NH_2

For additional information, please see P. 31.

Fluorescent Protein Labeling Kits

- Only 3 hours to recover conjugates
- All processes in a single filtration tube
- High recovery of conjugates
- Applicable for 50-200 µg IgG

Red Fluorescent Protein Labeling : 490 or 566 nm (*ex*), 578 nm (*em*)

R-Phycoerythrin Labeling Kit-NH_2,-SH

For additional information, please see P. 77 & 79.

Far-Red Fluorescent Protein Labeling : 650 nm (*ex*), 660 nm (*em*)

Allophycocyanin Labeling Kit-NH_2, -SH

For additional information, please see P. 81 & 83.

Enzyme Labeling

HRP Labeling

Peroxidase Labeling Kit-NH_2, -SH

- Only 3 hours to recover conjugates
- All processes in a single filtration tube
- High recovery of conjugates
- Applicable for 50-200 µg or 1 mg IgG

For additional information, please see P. 66 & 69.

AP Labeling

Alkaline Phosphatase Labeling Kit-NH_2, -SH

- Only 3 hours to recover conjugates
- All processes in a single filtration tube
- High recovery of conjugates
- Applicable for 50-200 µg or 1 mg IgG

For additional information, please see P. 72 & 74.

Biotin Labeling

Biotinylation

Biotin Labeling Kit-NH_2, -SH

- Only 1 - 3 hours to recover conjugates
- All processes in a single filtration tube
- High recovery of conjugates
- Applicable for 50-200 µg or 1 mg IgG

For additional information, please see P. 96 & 98.

IgG Purification

Protein A Gel Purification

IgG Purification Kit-A

- All processes take place in a single tube
- IgG recovery is 70-90%
- Purity of the IgG from serum is greater than 80%
- Purified IgG is available in 30 minutes
- High reproducibility and no affinity loss

For additional information, please see P. 113.

Protein G Gel Purification

IgG Purification Kit-G

- All processes take place in a single tube
- IgG recovery is 70-90%
- Purity of the IgG from serum is greater than 80%
- Purified IgG is available in 30 minutes
- High reproducibility and no affinity loss

For additional information, please see P. 113.

DNA, RNA Purification

Genomic DNA Isolation from Cell and Tissue

Get *pure*DNA Kit - Cell, Tissue

- ♦ No phenol or chloroform required
- ♦ Short DNA isolation time
- ♦ No need for spin columns or Filtration tubes
- ♦ DNA recovery from a wide range of sample volumes: up to 6 g tissue or $2x10^9$ cells for 200 samples kit

For additional information, please see P. 186

Genomic DNA Isolation from Agarose Gel

Get *pure*DNA Kit - Agarose

- ♦ No phenol or chloroform required
- ♦ DNA isolation in 30 minutes
- ♦ No need for columns or filtration tubes

For additional information, please see P. 188

Genomic RNA Isolation from Cell, Tissue and Blood

Get *pure*RNA Kit

- ♦ No phenol or chloroform required
- ♦ Short RNA isolation time
- ♦ No need for spin columns or filtration tubes
- ♦ RNA recovery from a wide range of sample volumes: up to $5x10^8$ cells, 1.5 g tissue or 1 ml whole blood

For additional information, please see P. 190

Introduction

Cell viability and cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals. They are based on various cell functions such as enzyme activity, cell membrane permeability, cell adherence, ATP production, co-enzyme production, and nucleotide uptake activity. A widely used assay for staining dead cells is Trypan Blue. In this method, cell viability must be determined by counting the unstained cells with a microscope or other instruments. However, Trypan Blue staining cannot be used to distinguish between healthy cells and cells that are alive but losing cell functions. Because of this, enzyme-based methods have been gaining more attention. Fig. 2-1 indicates various reagents used for detection of cell viability.

Among the enzyme-based assays, the MTT assay is the best known for determining mitochondrial dehydrogenase activities in living cells. MTT is reduced to a purple formazan by NADH. MTT formazan, however, has low solubility in water and it forms purple needle-shaped crystals in cells. Before measuring absorbance, an organic solvent is required to dissolve the crystals. Additionally, the cytotoxicity of MTT formazan makes it difficult to remove cell culture media from the plate wells due to many floating cells with MTT formazan needles, giving significant well-to-well error.

Although the ^{3}[H]thymidine-uptake assay is regarded as a reliable cell viability assay, the use of a radioisotope causes various concerns. The ^{51}Cr method is highly sensitive and is commonly used to determine low levels of cytotoxicity. However, the use of ^{51}Cr also causes problems in handling, storage, and disposal of the material. Cellular enzymes such as lactate dehydrogenase (LDH), adenylate kinase, and glucose-6-phosphate dehydrogenase are also used as cell death markers, and there are several such products available on the market. However, adenylate kinase and glucose-6-phosphate are not stable; only lactate dehydrogenase does not lose its activity during cell death assays. Therefore, cell death assays based on LDH activity are more reliable than other enzyme-based cell death assays.

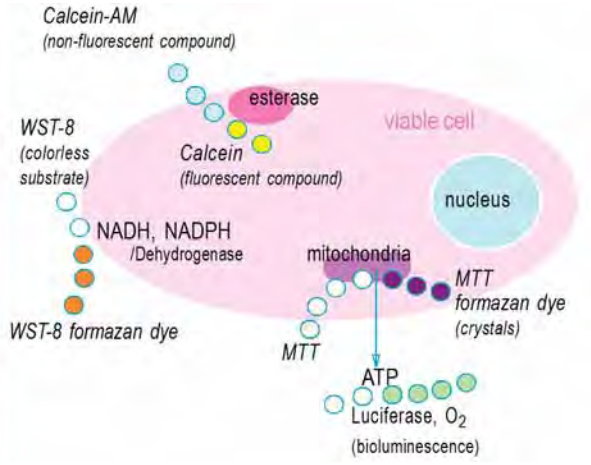


Fig.1 Dehydrogenase Based Assay: Cell Counting Kit-8

Dojindo developed highly water-soluble tetrazolium salts called WSTs. WSTs produce water-soluble formazans and are suitable for assays of cell proliferation and cytotoxicity. WSTs receive two electrons from viable cells to generate a yellow, orange, or purple formazan dye. WST-8, a highly stable WST, is utilized in Cell Counting Kit-8 (CCK-8). The electron mediator used in this kit, 1-Methoxy PMS, is also highly stable. Therefore, CCK-8 is stable for at least 6 months at room temperature and one year at 0-5° C. Since WST-8, WST-8 formazan, and 1-Methoxy PMS have no cytotoxicity in cell culture media, additional experiments may be carried out using the same assay plate. Dehydrogenase-based assays reflect cell conditions with more sensitivity than the other assays because they depend on several elements including dehydrogenases, NAD(H), NADP(H), and mitochondrial activity. The major difference between CCK-8 and the MTT assay, other than MTT's toxicity, is the enzymes involved.

The CCK-8 assay involves most of the dehydrogenase in a cell, while MTT only involves mitochondrial dehydrogenase. Therefore, the MTT assay depends on mitochondrial activity, not the cell itself. Additionally, CCK-8 is far more sensitive than the MTT assay. Since WST-8 formazan is water soluble, it does not form crystals as MTT does. Therefore, after 1-4 hours of incubation with the CCK-8 solution, measurement of O.D. at 450 nm gives the number of viable cells. No extra steps are required.

Esterase Activity Based Assay: Cell Counting Kit-F

Cell Counting Kit-F is another cell viability assay kit based on the esterase activity of viable cells. Fluorescent esterase substrates, such as Calcein-AM, BCECF-AM, CFSE, and FDA, can be passively loaded into viable cells. These electrically neutral molecules, which have acetyl or acetoxymethyl groups capping the phenolic OH or carboxylic groups of Calcein, freely permeate into the cell. Intracellular esterases then convert them into green fluorescent products, which are retained by cells. These esterase substrates, therefore, can serve as viability assay probes for cells. Calcein-AM is used for the kit because it is retained inside of cells better than the other fluorescent esterase substrates.

Dead and Viable Cell Staining: Double Staining Kit

Propidium iodide and Calcein-AM are used to distinguish dead and viable cells simultaneously. Dead cells can be stained by propidium iodide because it passes through deteriorated cell membranes; viable cells can be stained by Calcein-AM because of their esterase activity. Since both calcein and propidium iodide-DNA complex can be excited at 490 nm to emit green and red fluorescence, respectively, dead and viable cells can be observed with a fluorescence microscope. Only dead cells are observed with 545 nm excitation.

Cell Staining Reagents

Fluorescent cell staining reagents are very powerful tools to stain viable cells and dead cells. For more information, see (page 19).

Cell Counting Kit-8

Application: Cell viability and cytotoxicity detection
Features: Colorimetric microplate assay
One solution type (ready-to-use solution)
No toxicity to cells
No washing required
No radioisotopes or organic solvents required

Kit Contents:
Cell Counting Kit-8, 1,000 tests ..... 5 ml bottle x 2
Cell Counting Kit-8, 3,000 tests ..... 5 ml bottle x 6
Cell Counting Kit-8, 10,000 tests ..... 100 ml bottle x 1

Ordering Information

Product code	Unit*
CK04-11	1,000 tests
CK04-13	3,000 tests
CK04-20	10,000 tests

*One test corresponds to one well on a 96-well plate. 500 tests (5 ml) per vial for CK04-11 and CK04-13. 10,000 tests (100 ml) per vial for CK04-20.

Storage Condition
0-5°C, protect from light

Shipping Condition
ambient temperature

Required Equipment and Materials
plate reader with 450 nm filter; 96-well culture plate; 10 µl, 100-200 µl, and multi-channel pipettes; CO_2 incubator

Product Description
Cell Counting Kit-8 (CCK-8) allows convenient assays using Dojindo's tetrazolium salt, WST-8* (2-(2-methoxy-4-nitrophenyl)-3- (4-nitrophenyl)-5-(2,4-disulfophenyl)- 2*H*-tetrazolium, monosodium salt), which produces a water-soluble formazan dye upon bioreduction in the presence of an electron carrier, 1-Methoxy PMS (Fig. 1). CCK-8 solution is added directly to the cells; no pre-mixing of components is required. CCK-8 is a sensitive nonradioactive colorimetric assay for determining the number of viable cells in cell proliferation and cytotoxicity assays. WST-8 is bioreduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium (Fig. 2). The amount of formazan produced is directly proportional to the number of living cells. Typical cytotoxicity assay and cell proliferation assay curves are shown in Fig. 3 and 4. Since adherent cells have a higher metabolism than that of leucocyte cells, the O.D. from adherent cells are much larger than leucocyte cells (Fig. 5). The detection sensitivity of cell proliferation assays using WST-8 is higher than assays using other tetrazolium salts such as MTT, XTT, MTS, or WST-1. Since the CCK-8 solution is very stable and has low cytotoxicity, a longer incubation, such as 24 to 48 hours, is possible. Fig. 6 shows that the toxicity of CCK-8 is much lower than other cell viabilty assay kits. After 24 hours incubation with CCK-8, most of the HeLa cells have survived. Therefore, after the measurement of cell viability, the cells can be used for further experiments. *Patent No. US 6,063,587 EP 0908453 JP 2757348 Canada 2,251,850

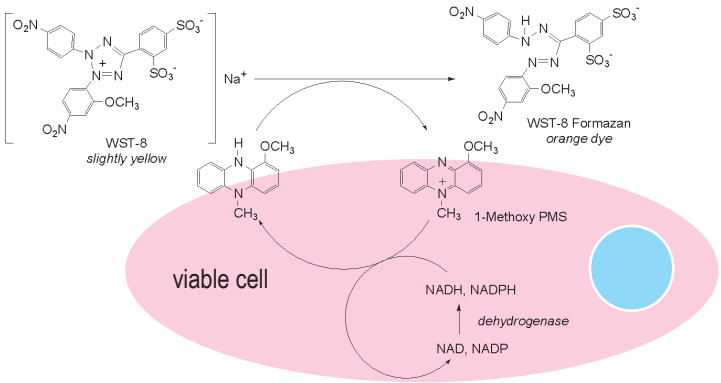


Fig. 1 Cell viability detection mechanism with CCK-8

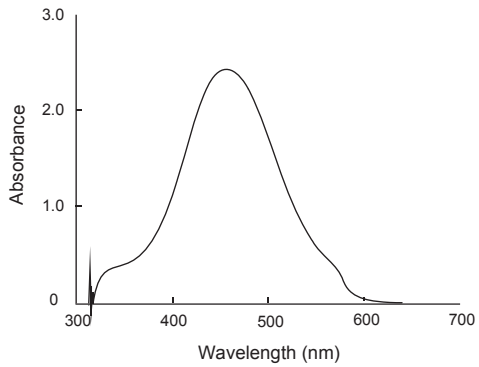
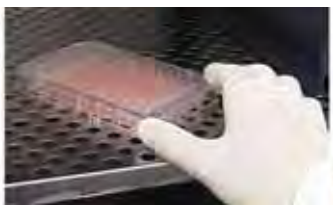


Fig. 2 Absorption spectrum of WST-8 formazan dye

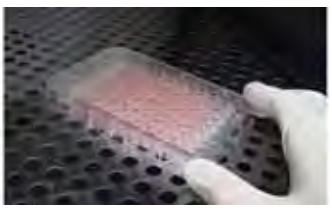
Assay Procedure

Step 1.
Add 100 µl of cell suspension[a] to each well.



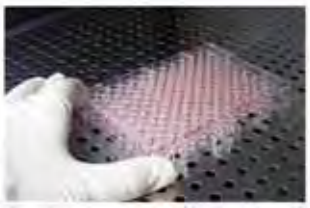
Step 2.
Pre-incubate the plate at 37°C.[b]

Step 3.
Add 10 µl of various concentration solution to be tested.[c]



Step 4.
Incubate the plate at 37°C.

Step 5.
Add 10 µl CCK-8 solution[d] each well.



Step 6.
Incubate the plate at 37°C for 1-4 hours.[e]

Step 7.
Read the O.D. at 450 nm and determine the cell viability.

[a] *Prepare a cell suspension with 5,000 cells per well in a 96 well plate using an appropriate culture medium.*
[b] *Overnight preincubation in a CO_2 incubator is recommended.*
[c] *Use the culture medium or PBS to prepare the solutions.*
[d] *If the solution to be tested has reducing activities, incubate the solution and CCK-8 without cells to determine the background absorbance at 450 nm. If the absorbance is negligibly small, add CCK-8 solution to each well. If the absorbance is high, remove the culture medium and wash cells twice with the medium, then add 100 µl of the culture medium and 10 µl CCK-8 solution.*
[e] *Longer incubation may be necessary for leukocyte cells.*

Calculation

$$\text{cell viability}^* (\%) = \frac{A(\text{substance}) - A(\text{blank})}{A(0\ \text{substance}) - A(\text{blank})} \times 100$$

A(substance) : absorbance of a well with cells, CCK-8 solution and substance solution
A(blank) : absorbance of a well with medium and CCK-8 solution, without cells
A(0 substance) : absorbance of a well with cells and CCK-8 solution, without substance solution

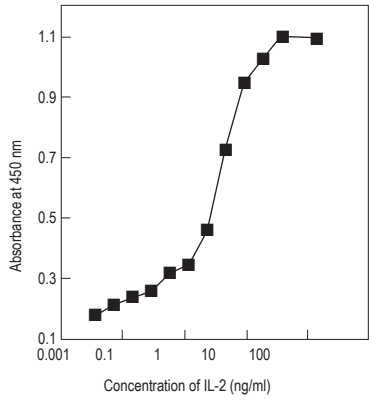


Fig. 3 Cell cytotoxicity test (IL2)
Cell line : CTLL-2
Culture medium : RPMI1640, 10%FBS
Drug :Human Interleukin-2
Exposure : 72hours
Incubation : 37°C, 5% CO_2, 4hours
Detection : 450 nm

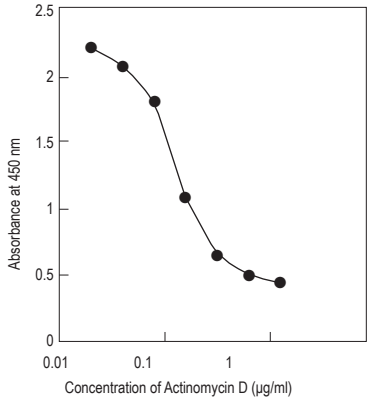


Fig. 4 Cell proliferation test (Actinomycin D)
Cell line : HeLa
Culture medium : DMEM, 10%FBS
Drug : Actinomycin D
Exposure : 24hours
Incubation : 37°C, 5% CO_2, 4hours
Detection : 450 nm

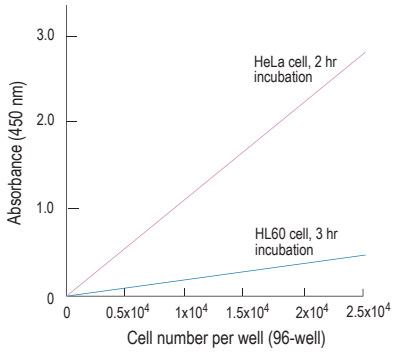
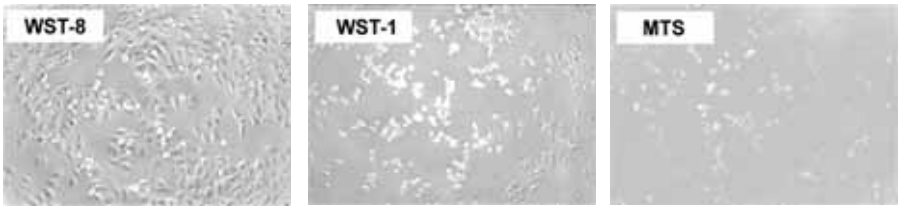


Fig. 5 Comparison of signal strength from adherent cells(HeLa) and leucocyte cell(HL60)



Procedures and Conditions:
Preincubate HeLa cell/ DMEM culture overnight in a CO_2 incubator and add 10 µl of CCK-8 solution to each well. Incubate cells at 37°C for various time periods and observe the cells with a phase-contrast microscope. As seen in the photos, even after 24-hour incubation with CCK-8, cells continue to proliferate. Other cell viability assay kits are toxic to cells, and most of the cells are killed during the first 3 hours of incubation.

Fig. 6 Cytotoxicity of CCK-8 and competitor's cell viability assay kits

FAQ

♦How many cells should there be in a well?

For adhesive cells, at least 1,000 cells are necessary per well (100 µl medium) when using a standard 96-well plate. For leukocytes, at least 2,500 cells are necessary per well (100 µl medium) because of low sensitivity. The recommended maximum number of cells per well for the 96-well plate is 25,000. If 24-well or 6-well plates are used for this assay, please calculate the number of cells per well accordingly, and adjust the volume of the CCK-8 solution in each well to 10% of the total volume.

♦Can this kit be used with a 384-well plate?

Yes, you can use this kit for a 384-well plate. Add CCK-8 solution with 1/10 volume of the culture medium in a well. If the volume of CCK-8 to be added is too small, dilute CCK-8 in half with the medium and add 1/5 of the volume of the culture medium in a well.

♦Can this kit be used with a 24-well plate?

Yes. Add CCK-8 solution with 1/10 volume of the culture medium in a well.

♦Does CCK-8 stain viable cells?

No, CCK-8 does not stain viable cells because a water-soluble tetrazolium salt (WST-8) is used for the CCK-8 solution. The electron mediator, 1-Methoxy PMS, receives electrons from viable cells and transfers the electrons to WST-8 in the culture medium. Since its formazan dye is also highly water soluble, CCK-8 cannot be used for cell staining.

♦Does phenol red affect the assay?

No. The absorption value of phenol red in the culture medium can be removed by subtracting the absorption value of a blank solution from the absorption value of each well. Therefore, a culture medium containing phenol red can be used for the CCK-8 assay.

♦Is there a correlation between CCK-8 and the Thymidine incorporation assay?

Yes. However, please note that since CCK-8 uses a different assay mechanism from that of the thymidine assay, the results may differ. Comparison data are shown in the technical manual, which is available at www.dojindo.com.

♦Is CCK-8 toxic to cells?

CCK-8 solution itself is cytotoxic because of the high concentration of 1-Methoxy PMS. CCK-8 in the culture medium, however, is not cytotoxic because of the ten times dilution with culture medium. Therefore, long incubation, such as overnight and several days, is possible. The same cell culture can be used for other cell proliferation assays, such as the crystal violet assay, neutral red assay, or DNA fluorometric assays after the CCK-8 assay is completed. Since each cell has a different tolerance to CCK-8, incubate cells with CCK-8 solution and check the cell viability if a longer incubation time is necessary.

♦How stable is CCK-8?

CCK-8 is stable over 6 months at room temperature and one year at 0-5°C with protection from light. For longer storage, we recommend storing the solution at -20°C ; CCK-8 is stable over 2 years at -20°C.

♦I do not have a 450 nm filter. What other filters can I use?

You can use filters with an absorbance between 450 nm and 490 nm.

Cell Line	Description	Reference
293T	human kidney carcinoma	H. Fuda, *et al*., *J Lipid Res*. 2007;**48**:1343-1352.
3T3-L1	mouse embryonic fibroblast	J. Lee, *et al*., *J Biol Chem*. 2010;**285**:32647-32656.
A2780	human ovarian cancer cell	J. Song, *et al*., *Clin Cancer Res*. 2007;**13**:6842-6849.
APRE19	human retinal pigment epithelium	E. F. Moreira, *et al*., *Invest Ophthamol Vis Sci*. 2009;**50**:523-532.
AR42J	rat pancreatic acinar cell	R. Szmola, *et al*., *Gut*. 2010;**59**:365-372.
AsPC-1	pancreatic cancer cell	S. Awale, *et al*., *Cancer Res*. 2006;**66**:1751-1757.
BEAS-2B	human bronchial epithelial cell	K. C. Thomas, *et al*., *J Pharmacol Exp Ther*. 2011;**337**:400-410.
BEL7404	human hepatoma cell	X. Liang, *et al*., *Mol Cancer Res*. 2008;**6**:1499-1506.
BMMC	BM(bone marrow)-derived mast cell	S. Arandjelovic, *et al*., *J Leukoc Biol*. 2010;**88**:737-745.
BMSC	bone marrow-derived stem cell	Y. Ikegame, *et al*., *Cytotherapy*. 2011;**13**:675-685.
BT-474	human breast cancer cell	D. Iliopoulos, *et al*., *Clin Cancer Res*. 2007;**13**:268-274.
C2C12	mouse myoblast cell	W. Yoon, *et al*., *J Biol Chem*. 2008;**283**:32751-32761.
C6	rat malignant glioma cell	M. Zhao, *et al*., *Anticancer Res*. 2010;**30**:2217-2224.
Ca9-22	human gingival epithelial cell	N. Ebe, *et al*., *J Dent Res*. 2011;**90**:235-240.
Caki-2	renal carcinoma	T. Morikawa, *et al*., *Clin Cancer Res*. 2007;**13**:5703-5709.
cardiomyocyte	rat	E. E. Hamm, *et al*., *PNAS*. 2006;**103**:14176-14181.
CDC	cardiac-derived stem cell	J. Terrovitis, *et al*., *Circulation*. 2008;**117**:1555-1562.
chondrocyte, primary	from rat knee joint	M. M. Abouheif, *et al*., *Rheumatology*. 2010;**49**:2054-2060.
cortical neurons, primary	from mouse	Z. Zhong, *et al*., *J Neurosci*. 2010;**30**:15521-15534.
COS7	African green monkey kidney cell	H. Bando, *et al*., *Clin Cancer Res*. 2005;**11**:5784-5792.
CV-1	African green monkey kidney cell	J. Peloponese, *et al*., *J Biol Chem*. 2006;**281**:8927-8938.
Daudi	human burkitt lymphoma	J. E. Weldon, *et al*., *Blood*. 2009;**113**:3792-3800.
DDLS	dedifferentiated liposarcoma	G. Ambrosini, *et al*., *Mol Cancer Ther*. 2008;**7**:890-896.
dermal stem cell	from mouse	Y. Hasebe, *et al*., *J Dermatol Sci*. 2011;**62**:98-106.
DPSC	dental pulp stem cell	X. Wang, *et al*., *Arch Oral Biol*. 2011;**56**:837-845.
DU145	human prostate carcinoma	P. Davis-Searles, *et al*., *Cancer Res*. 2005;**65**:4448-4457.
E14TG2a	mouse embryonic stem cell	N. Sasaki, *et al*., *J Biol Chem*. 2008;**283**:3594-3606.
H1299	human lung cancer cell	Y. Su, *et al*., *Cancer Res*. 2007;**67**:3329-3336.
H9c2	embryonic rat heart-derived cell	T. Okazaki, *et al*., *Am J Pathol*. 2007;**171**:1093-1103.
HaCaT	human keratinocyte	H. Lee, *et al*., *J Immunol*. 2011;**186**:1248-1258.
HCT116	human colon carcinoma	A. N, Tse, *et al*., *Mol Pharmacol*. 2009;**75**:124-133.
hematopoietic stem cell	from mouse	S. Kwon, *et al*., *J Mol Cell Cardiol*. 2011;**51**:308-317.
HepG2, spheroid culture	hepatocellular carcinoma	A. Oshikata, *et al*., *J Biosci Bioeng*, 2011;**111**:590-593.
Hippocampal neuron, primary	from Wistar rat embryos	K. Kurata, *et al*., *J Pharmacol Exp Ther*. 2004;**311**:237-245.
HL60	human acute promyelonic leukemia	Y. Li, *et al*., *Toxicol Sci*. 2010;**118**:435-443.
hMSC	human mesenchymal stem cell	J. Kim, *et al*., *Exp Gerontol*. 2011;**46**:500-510.
hPASMC	human pulmonary artery smooth muscle cell	R. Zhang, *et al*., *Am J Physiol Lung Cell Mol Physiol*. 2009;**297**:L631-L640.
HUVEC	human umbilical vein endothelial	M. Narazaki, *et al*., *blood*. 2010;**116**:3099-3107.
ILT-Hod	T-cell	T. Kasai, *et al*., *J Biol Chem*. 2002;**277**:5187-5193.
Jurkat	human T cell	L. Lu, *et al*., *J Biochem*. 2007;**141**:157-172.
Kasumi-1	acute myeloid leukemia cell	G. Zhou, *et al*., *Blood*. 2007;**109**:3441-3450.
KB-3-1	human epidermoid carcinoma cell	M. Okabe, *et al*., *Mol Cancer Ther*. 2008;**7**:3081-3091.
KMBC	cholangiocarcinoma	V. Dudeja, *et al*., *Am J Physiol Gastrointest Liver Physiol*. 2011;**300**:G948-G955.

Cell Line	Description	Reference
KW-807	non-small-cell lung cancer cell	L. Gandhi, *et al*., *Cancer Prev Res*. 2009;**2**:330-337.
L929	mouse fibroblast	X. Yuan, *et al*, *Mol Cancer Ther*. 2009;**8**:1906-1915.
LLC-PK1	porcine renal tubular	T. Yano, *et al*., *Antimicrob Agents Chemother*. 2009;**53**:1420-1426.
LNCaP	human prostate cancer cell	P. S. Koka, *et al*., *Exp Biol Med*. 2010;**235**:751-760.
M28	mesothelioma cell	F. An, *et al*., *Mol Cancer Ther*. 2008;**7**:569-578.
MC38	murine colon adenocarcinoma cell	X. Liang, *et al*., *J Leukoc Biol*. 2009;**86**:599-607.
MDA-MB-231	human breast cancer cell	M. E. Grossmann, *et al*., *Cancer Prev Res*. 2009;**2**:879-886.
MDCK	canine kidney epithelial cell	A. W. Kahsai, *et al*., *J Biol Chem*. 2008;**283**:24534-24545.
mesenchymal stem cell	from mouse lacrimal gland	S. You, *et al*., *Invest Ophthalmol Vis Sci*. 2011;**52**:2087-2094.
MH-S	murine alveolar macrophage	G. Murphy, Jr., *et al*., *Am J Respir Cell Mol Biol*. 2008;**38**:532-540.
MiaPaCa2	pancreatic cancer cell	A. Aghdassi, *et al*., *Cancer Res*. 2007;**67**:616-625.
MKN45	gastric carcinoma	Y. Ye, *et al*., *Cancer Lett*. 2011;**307**:124-131.
MN9	mouse	G. Kweon, *et al*., *J Biol Chem*. 2004;**279**:51783-51792.
mSMC	myometrial-derived smooth muscle cell	G. Suo, *et al*., *Biol Reprod*. 2009;**81**:749-758.
MT4	T-cell	J. Peloponese, Jr., *et al*., *J Virol*. 2009;**83**:3238-3248.
Mycoplasma bovis PG45	mycoplasma	M. K. Soehnlen, *et al*., *J Antimicrob Chemother*. 2011;**66**:574-577.
NCI-H226	human mesothelioma cell	M. Feng, *et al*., *J Cancer*. 2011;**2**:123-131.
NIH3T3	mouse fibroblast	R. Yu, *et al*., *Toxicol Sci*. 2006;**93**:82-95.
NRK-52E	rat renal tubular epithelial cell	S. Nakajima, *et al*., *Mol Cell Biol*. 2011;**31**:1710-1718.
NSC	neural stem cell	Y. Nakatani, *et al*., *Glycobiology*. 2010;**20**:78-86.
P493-6	B-lymphocyte cell	T. Chang, *et al*., *PNAS*. 2009;**106**:3384-3389.
PANC-1	pancreatic cancer cell	P. A. Phillips, *et al*., *Cancer Res*. 2007;**67**:9407-9416.
PBMC	human peripheral blood mononuclear cell	C. Chang, *et al*., *Stem Cells*. 2006;**24**:2466-2477.
PC6	human lung small-cell carcinoma	M. Spencer, *et al*., *J Biol Chem*. 2002;**277**:20160-20168.
primary hepatocyte	from mouse	Y. Jung, *et al*., *Gut*. 2010;**59**:655-665.
RAW 264.7	mouse macrophage	T. S. Devera, *et al*., *Infect Immun*. 2010;**78**:1610-1617.
RP9	B lymphoblastoid cell	R. A. Dalloul, *et al*., *Poult Sci*. 2006;**85**:446-451.
SH-SY5Y	human neuroblastoma	S. A. Vasudevan, *et al*., *Mol Cancer Ther*. 2009;**8**:2478-2489.
SMC-PV	pulmonary vascular smooth muscle cell	W. Zhou, *et al*., *Am J Physiol Heart Circ Physiol*. 2007;**292**:H2773-H2781.
SW620	human colon cancer cell	J. O. Ban, *et al*., *Mol Cancer Ther*. 2009;**8**:1613-1624.
THP-1	human monocytic leukemia cell	D. E. Voth, *et al*., *Infect Immun*. 2009;**77**:205-213.
TK6	human B lymphoblastoid cell	H. Fung, *et al*., *Mol Cell Biol*. 2007;**27**:8834-8847.
U251MG	human glioblastoma	S. Kim, *et al*., *Clin Cancer Res*. 2006;**12**:5550-5556.
U2OS	human osteosarcoma	J. Park, *et al*., *J Biol Chem*. 2010;**285**:3029-35038.
U-87	glioma cell	J. Wei, et al., Clin Cancer Res. 2010;16:461-473.
U87MG	human glioblastoma	T. Arai, et al., Anticancer Res. 2010;30:1057-1064.
UM-UC-14	human bladder urotherial carcinoma	M. Miyake, et al., J Pharmacol Exp Ther. 2010;332:795-802.
V-C8	chinese hamster cell mutant	P. Gottipati, et al., Cancer Res. 2010;70:5389-5398.
Vero	african green monkey kidney cell	K. Sagou, et al., J Virol. 2010;84:2110-2121.
WERI-RB-1	human retinoblastoma	C. L. Dalgard, et al., Invest Ophthalmol Vis Sci. 2009;50:4542-4551.

Visit our website, www.dojindo.com for additional cell line reference information. Simply type "CK04" on the search bar to see the product page.

Cell Counting Kit-SK

Application: Cell viability and cytotoxicity detection
Features: Colorimetric microplate assay
One solution type (ready-to-use solution)
No washing required
No radioisotopes or organic solvents required
Highly sensitive

Ordering Information

Product code	Unit*
CK10-11	1,000 tests
CK10-13	3,000 tests

*One test corresponds to one well on a 96-well plate.

Kit Contents:
Cell Counting Kit-SK, 1,000 tests ..... 5 ml bottle x 2
Cell Counting Kit-SK, 3,000 tests ..... 5 ml bottle x 6

Storage Condition: 0-5°C, protect from light
Shipping Condition: ambient temperature

Required Equipment and Materials
plate reader with 450 nm filter; 96-well culture plate; 10 µl, 100-200 µl, and multi-channel pipettes; CO_2 incubator

Product Description
Cell Counting Kit-SK (CCK-SK) is an alternative kit to CCK-8 for cells or cell lines with low metabolism. The O.D. of the CCK-8 for leukocyte cells is sometimes very low, such as 0.2-0.4 after 2-4 hours incubation because of lower metabolism compared with adherent type cells. CCK-SK enables a shorter incubation time to obtain a higher O.D. increase within 30 minutes to 1 hour. Assay mechanism and assay protocol are the same as CCK-8 (page 8). Fig. 1 shows the sensitivity of various assays.

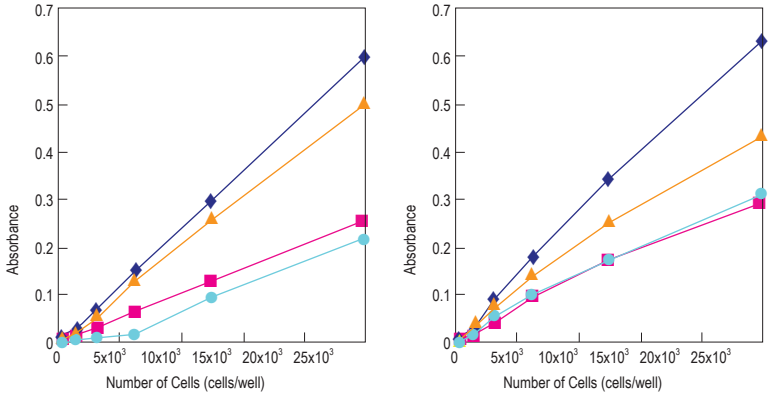


Fig. 1 Cell number determination using CCK-SK and other kits.

Medium: HL60.....RPMI 1640, 10% FBS
Jurkat.....RPMI 1640, 10% FBS
Incubation:37°C, 5% CO_2, 2 hours

Reagent: CCK-SK..... 10 ml/well
CCK-8..... 10 ml/well
Company A..... 10 ml/well
Company B..... 20 ml/well

Detection: CCK-SK(♦)..... 450 nm
CCK-8(■)..... 450 nm
Company A(▲)..... 450 nm
Company B(●)..... 490 nm

DURALiQ MTT Stable Solution

Application: Cell viability and cytotoxicity detection
Features: Long Term Stability
One solution type (ready-to-use solution)
Conventional Method

Ordering Information

Product code	Unit*
CK11-11	1,000 tests
CK11-15	5,000 tests
CK11-20	10,000 tests

*One test corresponds to one well on a 96-well plate.

Kit Contents:
DURALiQ MTT Stable Solution, 1,000 tests ..... 10 ml bottle x 1
DURALiQ MTT Stable Solution, 5,000 tests ..... 10 ml bottle x 5
DURALiQ MTT Stable Solution, 10,000 tests ..... 10 ml bottle x 10

Storage Condition: 0-5°C
Shipping Condition: ambient temperature

Product Description
Cell proliferation/cytotoxicity assays are widely used in the field of drug screening and investigating influences of cytokines, chemicals and growth factors on cell functions. The reduction of tetrazolium salts is one of the most reliable and common methods to determine cell viability and cytotoxicity. MTT(3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide) is a well known tetrazolium salt for determining

mitochondrial dehydrogenase activity in living cells. However, MTT is really unstable in aqueous solution and, a stock solution of MTT must be prepared as needed Dojindo's DURALiQ MTT Stable Solution is a ready-to-use cell proliferation/cytotoxicity reagent that can be stored in refrigerator for over a year. This MTT solution is an indispensable tool for those who preform frequent assays.

Fig. 1 MTT solution was exposed at 40°C for 2 months. The appearance of each solution was compared.

A. No Color change was seen in DURALiQ MTT Stable Solution.
B. Dark-purple color changed was seen in MTT/PBS(-) solution.

Cell Counting Kit-F

Application: Cell viability and cytotoxicity detection
Features: Fluorometric microplate assay
Highly sensitive: as low as 50 cells per well
Quick detection: 10-30 minutes incubation
No radioisotopes or organic solvents required

Ordering Information

Product code	Unit*
CK06-10	500 tests

*One test corresponds to one well on a 96-well plate.

Kit Components
Calcein-AM Solution .....1 tube

Storage Condition	Shipping Condition
-20°C, protect from light	with blue ice or dry ice

Required Equipment and Materials
plate reader with 485 nm excitation and 535 nm emission filters; 96-well culture plate, black or white; 10 µl, 100-200 µl, and multi-channel pipette; CO_2 incubator

Product Description
Cell Counting Kit-F(CCK-F) is utilized for the fluorometric determination of living cell numbers. The amount of the fluorescent dye, calcein, hydrolyzed by esterases in cells is directly proportional to the number of viable cells in culture media (Fig. 1). The 96-well microplate CCK-F assay has a detection range of less than 50 cells to more than 25,000 cells per well (Fig. 2). Since esterases and phenol red in the culture medium interfere with the fluorescence measurement, replacing the cell culture medium with PBS is necessary prior to adding the Calcein-AM assay solution. The excitation and the emission wavelengths of calcein are 485 nm and 535 nm, respectively (Fig. 3). An incubation of 10 to 30 minutes gives sufficient fluorecence intensity for cell viability determination.

Assay Procedure
1. Prepare a cell suspension with 10,000-100,000 cells/ml using an appropriate culture medium.
2. Add 100 µl of the cell suspension to each well.
3. Pre-incubate the plate at 37°C.[a)]
4. Add 10 µl of various concentration solutions[b)] to be tested to each well.
5. Incubate the plate at 37°C for a certain time period such as 24, 48, or 72 hours.
6. Remove the medium from each well and wash cells twice with 100 µl of PBS.[c)] Then, add 100 µl of PBS.
7. Mix 100 µl Calcein-AM solution with 5 ml PBS to prepare an assay solution.
8. Add 10 µl of the assay solution to each well and incubate the plate at 37°C for 10-30 minutes.
9. Measure the fluorescence intensity using a fluorescent microplate reader with 485 nm excitation and 535 nm emission filter.

[a)] Overnight preincubation in a CO_2 incubator is recommended.
[b)] Use the culture medium or PBS to prepare the solutions.
[c)] Warm the PBS solution to 37°C.

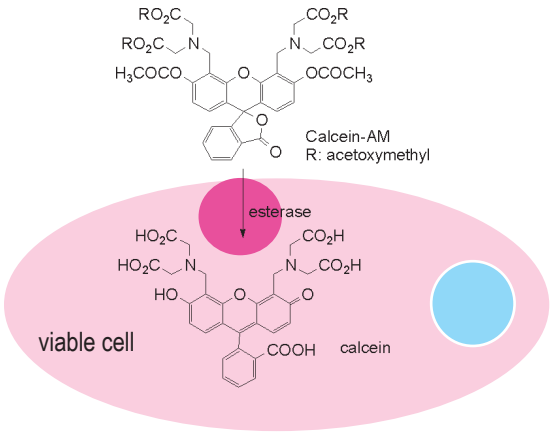


Fig. 1 Cell viability detection mechanism with CCK-F

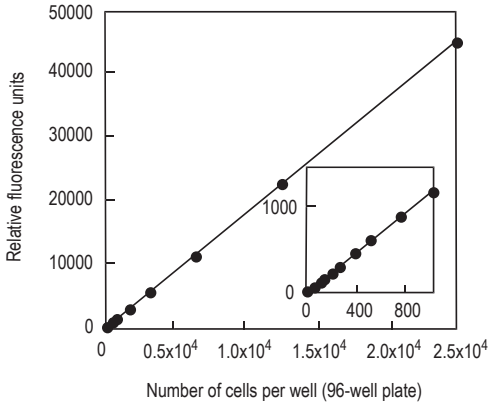


Fig. 2 Typical calibration curve using CCK-F

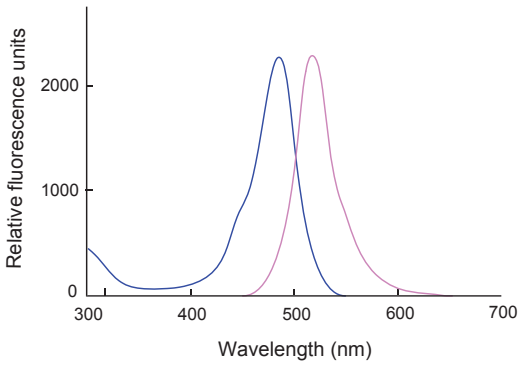


Fig. 3 Fluorescence spectrum of Calcein/PBS solution, pH 7.4

FAQ

♦How many cells should there be in a well?

At least 50 cells are necessary per well (100 µl medium) when using a standard 96-well plate. However, we recommend using at least 1,000 cells per well for more reliable and consistent data.

♦Does calcein stain viable cells?

Yes, calcein stays inside of the cell. The viable cells can be visualized using a fluorescent microscope. If a longer observation period is necessary, please try CFSE (Product Code: C375-10).

♦Can I use this kit for bacterial cell counting?

No. Calcein-AM cannot pass through bacterial cell walls, so they cannot be stained using this kit. Please try Dojindo Bacstain series.

♦Is the CCK-F assay solution stable?

No. Calcein-AM in CCK-F is easily hydrolyzed in PBS. Prepare only the required volume of assay solution for the experiment.

♦Is there a correlation between CCK-F and the thymidine incorporation assay?

Yes. However, please note that since CCK-F uses a different assay mechanism from that of the thymidine assay, results may differ. Comparison data are shown in the technical manual, which is available at www.dojindo.com.

♦Is CCK-F assay solution toxic to cells?

Since the assay solution is prepared with PBS, some cells may be affected by PBS. Also, calcein binds calcium ions in the cell, so the reduction of the free calcium ions causes some damage to cell functions.

♦What other filters can I use?

You can use excitation filters between 460 nm and 490 nm and emission filters between 510 nm and 540 nm.

♦Can I use CCK-F for 384-well plates?

CCK-F can be used for 384-well plates by adding 5 µl (instead of 10 µl) CCK-F assay solution to 50 µl PBS solution per well.

References

1. N. G. Papadopoulos, *et al*., An improved fluorescence assay for the determination of lymphocyte-mediated cytotoxicity using flow cytometry. *J Immunol Methods*. 1994;**177**:101-111.
2. S. Sakamoto, *et al*., Increased Expression of CYR61, an Extracellular Matrix Signaling Protein, in Human Benign Prostatic Hyperplasia and Its Regulation by Lysophosphatidic Acid. *Endocrinology*. 2004;**145**:2929-2940.
3. T. Uruma, *et al*., Chlamydia pneumoniae growth inhibition in human monocytic THP-1 cells and human epithelial HEp-2 cells by a novel phenoxazine derivative. *J Med Microbiol*. 2005;**54**:1143-1149.
4. B. Ashibe, *et al*., Dual Subcellular Localization in the Endoplasmic Reticulum and Peroxisomes and a Vital Role in Protecting against Oxidative Stress of Fatty Aldehyde Dehydrogenase Are Achieved by Alternative Splicing. *J Biol Chem*. 2007;**282**:20763-20773.
5. Z. Bai, *et al*., CXC Chemokine Ligand 12 Promotes CCR7-Dependent Naive T Cell Trafficking to Lymph Nodes and Peyer's Patches. *J Immunol*. 2009;**182**:1287-1295.

Microbial Viability Assay Kit-WST

Application: Bacterial and yeast cell viability detection
Features: Colorimetric microplate assay
Wide variety of microorganism detection
No harvesting or washing required

Ordering Information

Product code	Unit*
M439-10	500 tests

*One test corresponds to one well on a 96-well plate.

Kit Contents:
WST solution ..... 1 ml x 5 tubes
Electron mediator reagent/ DMSO ..... 0.5 ml x 1 tube

Storage Condition	Shipping Condition
0-5°C	ambient temperature

Required Equipment and Materials

plate reader with 450 nm filter; 96-well culture plate; 10 µl, 100-200 µl, and multi-channel pipettes; incubator

Product Description

Viable bacterial cell detections are very important for analyzing bacteria contamination in food or evaluating the cleanliness of facilities in order to protect us from food poisoning and infections. Bacterial cell detections are also used for the screening of sanitizing agents and drug resistance detections. Generally, counting the number of colonies on an agar plate is the standard method for determining the number of viable bacterial cells in samples. However, colony formations require one to several days. Dojindo's Microbial Viability Assay Kit-WST can be used to determine the number of viable bacterial cells in a sample by a colorimetric method and can be applied to 96-well microplate assays. The electron mediator in the kit receives electrons from viable bacterial cells and transfers the electrones to WST, one of the water-soluble tetrazolium salts developed by Dojindo. Bacterial cell viability then can be determined by monitoring the color intensity of WST formazan dye. Since several types of media used for bacterial cell cultures and components do not interfere with the assay(Fig. 5), simply add the assay solution and incubate for one to several hours to determine the initial number of viable bacterial cells in the sample(Fig. 2). This assay kit was co-developed with the Biotechnology and Food Research Institute, Fukuoka Industrial Technology Center.

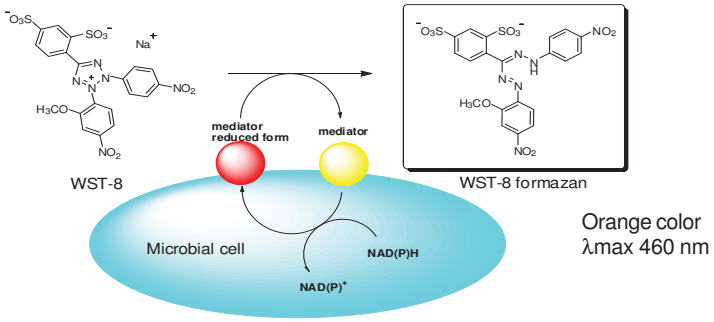


Fig. 1 Bacterial cell viability detection mechanism

General Procedure 1

Determination of the initial number of viable *E. coli* cells

1. Culture *E. coli* with Mueller-Hinton medium.
2. Add Microbial Viability Assay solution with 1/20 volume of the culture medium to the *E. coli* culture.
3. Measure the O.D. every 10 to 15 minutes at 450 nm.
4. Determine the number of *E. coli* from proliferation assay data prepared from a known number of *E. coli* cells (Fig. 2).

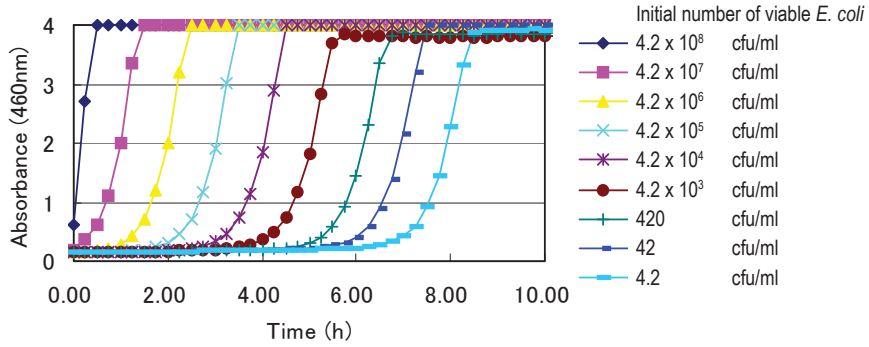


Fig. 2 Correlation between initial number of *E. coli* and time-dependent O. D. increase.
The initial number of viable *E. coli* were determined by a colony counting method.

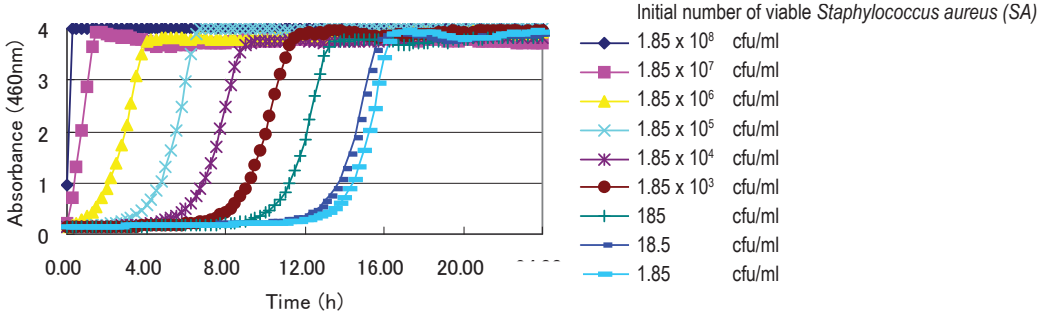


Fig. 3 Correlation between initial number of *SA* and time-dependent O. D. increase.
The initial number of viable *SA* were determined by a colony counting method.

General Procedure 2

Determination of the susceptibility of *Staphylococcus aureus* to oxacillin
Oxacillin: antimicrobial agent: 0-64 μg/ml
Microorganism: *Staphylococcus aureus* (*SA*), Methicillin-resistant *Staphylococcus aureus* (*MRSA*)

1. Culture SA or MRSA with Mueller-Hinton medium containing various concentrations of Oxacillin for 6 hours at 35°C.
2. Add Microbial Viability Assay solution equal to 1/20 the volume of the culture medium.
3. Incubate for 2 hours at 35°C.
4. Measure the O.D. at 450 nm to determine the MIC (Minimum inhibitory concentration).

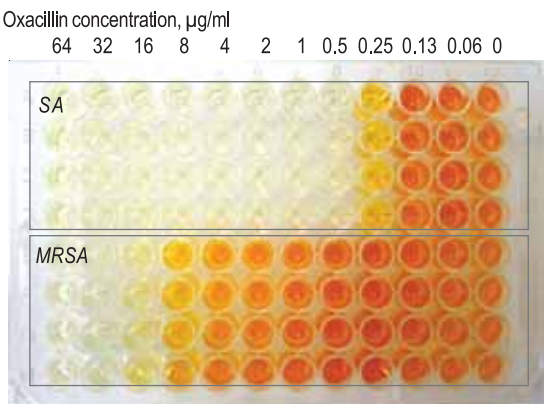


Fig. 4 Susceptibility test of *SA* and *MRSA* against Oxacillin.

The data indicated that MRSA has lower susceptibility than *SA*. The MICs of *MRSA* (32 μg/ml) and *SA* (0.5 μg/ml) are close to the MICs determined by the CLSI (Clinical and Laboratory Standards Institute) method.

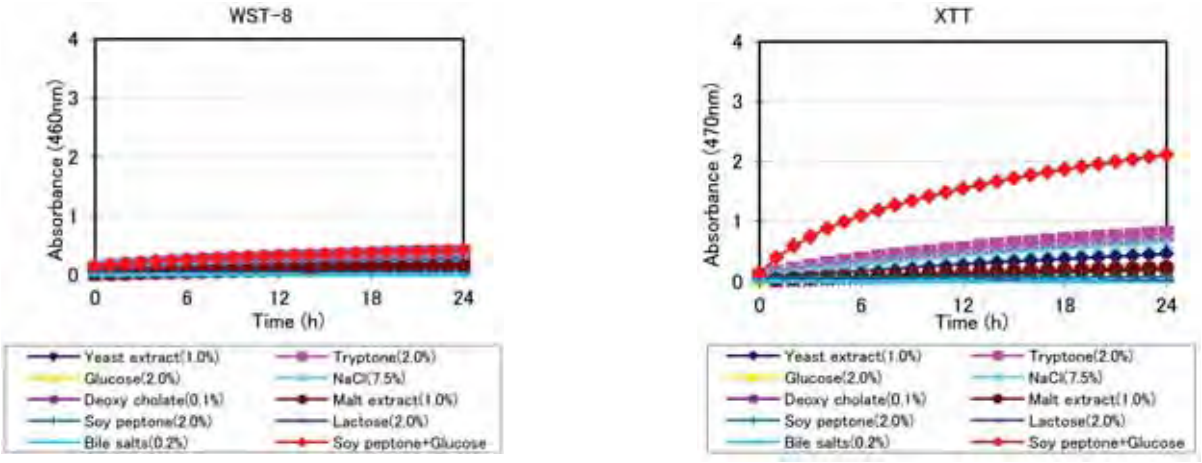


Fig. 5 Influence of culture media or substances used for bacterial cell culture.
The data indicated that WST is less sensitive to various culture media or substances that are used for bacterial cell culture. WST is a better tetrazolium salt than XTT for bacterial cell viability assays.

Table 1 Initial cell number can reach O.D. 0.5 with 1-hour and 4-hours incubation.

Microorganism		Cell Density (CFU/ml)	
		1 hour	4 hours
Yeast	*Candida utilis*	5.53×10^7	6.18×10^6
	Saccharomyces cerevisiae	8.70×10^5	2.65×10^5
	Zygosaccharomyces rouxii	1.65×10^5	2.47×10^4
Gram-positive bacteria	*Bacilus cereus*	6.70×10^5	6.77×10^4
	Bacilus subtilis	2.45×10^6	6.71×10^5
	Corynebacterium glutamicum	1.69×10^6	2.47×10^5
	Enterococcus faecalis	5.18×10^7	1.76×10^6
	Lactobacillus casei	8.40×10^7	2.34×10^6
	Listeria monocytogenes	5.07×10^6	6.46×10^5
	Micrococcus luteus	8.29×10^5	1.29×10^5
	Staphylococcus aureus	2.78×10^6	2.71×10^5
	Staphylococcus epidermidis	5.53×10^6	1.12×10^6
Gram-negative bacteria	*Acetobacter sp.*	2.53×10^7	7.39×10^6
	Escherichia coli	1.31×10^7	2.86×10^5
	Klebsiella pneumoniae	1.76×10^7	5.59×10^5
	Proteus mirabilis	7.42×10^6	1.35×10^6
	Pseudomonas aeruginosa	1.76×10^8	1.78×10^7
	Salmonella enteritidis	2.55×10^7	1.06×10^6
	Salmonella typhimurium	1.73×10^7	2.60×10^6
	Serratia marcescens	7.15×10^7	5.08×10^6
	Vibrio parahaemolyticus	2.90×10^7	1.03×10^7
	Yersinia enterocolitica	1.92×10^7	5.46×10^6

The initial cell number of each microorganism was determined by colony counting. Each microorganism cell culture was diluted with medium and 190 µl of the cell culture was added to each well. Then 10 µl of assay solution was added. The cells were incubated at 30°C or 37°C for 1 hour and 4 hours to determine how many cells are required to reach O.D.=0.5 at 460 nm.

Reference

1. T. Tsukatani, *et al*., Colorimetric cell proliferation assay for microorganisms in microtiter plate using water-soluble tetrazolium salts. *J Microbiol Methods*. 2008;**75**:109-116.
2. T. Tsukatani, *et al*., Colorimetric microbial viability assay based on reduction of water-soluble tetrazolium salts for antimicrobial susceptibility testing and screening of antimicrobial substances. *Anal Biochem*. 2009;**393**:117-125.

Cell Staining

Introduction

Visualization of a cell with fluorescent compounds provides a wide variety of information for the analysis of cell functions. Various activities and structures of a cell can be targeted for staining with fluorescent compounds (Fig. 1). The most commonly stained cell components are cell membranes, proteins, and nucleotides. Small neutral molecules and positively charged molecules can pass through viable cell membranes and remain inside of cells, depending on their reactivity or hydrophilicity. Negatively charged molecules cannot pass through viable cell membranes. Positively charged molecules are usually cell membrane permeable and accumulate in mitochondria.

Ester is a suitable functional group for staining viable cells. Ester can pass through viable cell membranes, where it is hydrolyzed by cellular esterases into a negatively charged molecule under physiological conditions. Several fluorescein analogs with ester groups in their structure are available for staining viable cells. Succinimidyl ester compounds can also be used to improve retention of the fluorescent derivative within the cell. These compounds are neutral molecules that pass through cell membranes and covalently conjugate with cell proteins. Covalently conjugated molecules can stay in the cell for several weeks. Nucleotide staining with fluorescent intercalators is mostly used for dead cell detection.

Cell Cytosol Staining

Fluorogenic esterase substrates that can be passively loaded into viable cells, such as Calcein-AM, BCECF-AM, Carboxyfluorescein succinimidyl ester (CFSE), and Fluorescein diacetate (FDA), are converted by intracellular esterases into fluorescein analogs with green fluorescence. Calcein and BCECF can be converted into electrically neutral molecules by the addition of acetyl or acetoxymethyl groups to their phenolic OH or carboxylic groups, which allows them to freely permeate into the cell. Once converted into fluorescent products by esterase, these compounds are retained by cells because of their negative charges. These esterase substrates, therefore, can serve as assay probes of cell viability.

Fluorescent esterase substrates may also be used in cell viability assays in place of tetrazolium analogs such as MTT or WST. The mechanism for determining cell viability is different: Although both assays determine cell metabolism, esterase substrates detect esterase activity; tetrazolium salts detect dehydrogenase activity of viable cells. CFSE is also an ester compound that passes through viable cell membranes. Since it has an amine-reactive succinimidyl group, fluorescein derived from CFSE can covalently bond to proteins or other amino groups in the cell or on the cell membrane. This covalently attached fluorescein remains stable and allows the cell to be traced over several weeks

Mitochondria Staining

Mitochondria exist in most eukaryotic cells and play a very important role in oxidative metabolism by generating ATP as an energy source. The average number of mitochondria per cell is from 100 to 2,000. Although the typical size is about 0.5-2 mm, the shape, abundance, and location of mitochondria vary by cell type, cell cycle, and cell viability.Therefore, visualization of mitochondra is important. Since mitochondria have electron transport systems, they can be stained with various redox dyes. MitoRed and Rh123 readily pass through cell membranes and accumulate in mitochondria. The fluorescence intensity of Rh123 reflects the amount of ATP generated in mitochondria.

Nucleus Staining

Fluorescent dyes with aromatic amino or guanidine groups, such as propidium iodide (PI), diaminophenylindole (DAPI), acridine orange (AO), and Hoechst dyes, interact with nucleotides to emit fluorescence. PI molecules intercalate inside the DNA double helix. DAPI and Hoechst dye molecules attach at the minor groove of the DNA double helix. On the other hand, AO can form complexes with either double-stranded DNA or single-stranded DNA and RNA. One molecule of AO can intercalate with three base pairs of double-stranded DNA to emit green fluorescence with the maximum wavelength at 526 nm. One molecule of AO can also interact with one phosphate group of single-stranded DNA or RNA to form an aggregated, or stacked, structure that emits red fluorescence with the maximum wavelength at 650 nm. Cell membranes of viable cells are impermeable to these fluorescent dyes,except for the Hoechst dyes, and these dyes can therefore be used as fluorescent indicators of dead cells. Hoechst dyes are positively charged under physiological conditions and can pass through viable cell membranes.

Bacterial Cell Staining

There are several ways to detect bacteria, including agar plate cultivation and bacteria-specific DNA amplification. Fluorescent staining using CTC is another method used to detect viable bacterial cells. The advantage of this method is very quick detection and the possibility of detecting VNC (viable but non-culturable) bacterial cells. CTC is a tetrazolium salt that is converted to formazan dye by bacterial cell activity. The solid state of the formazan dye emits red fluorescence. Therefore, viable bacterial cells can be stained by CTC and are easily detected by fluorescent microscopy.

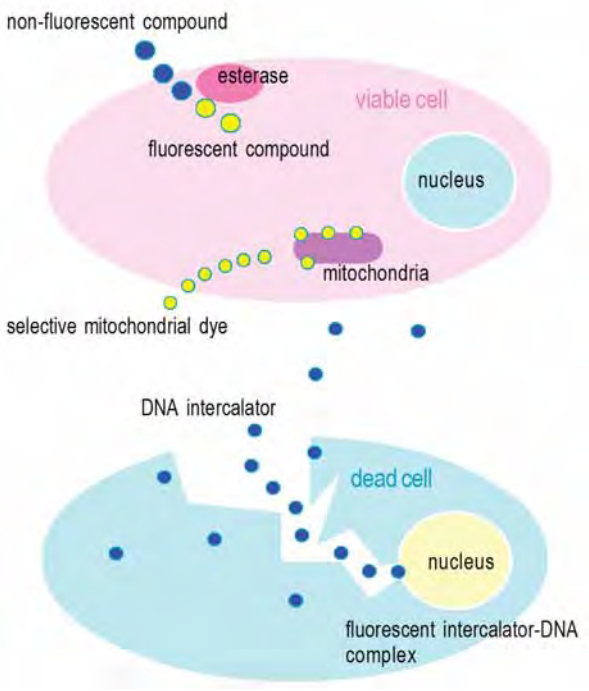


Fig. 1 Cell staining method

BCECF-AM 3'-O-Acetyl-2',7'-bis(carboxyethyl)-4 or 5-carboxyfluorescein, diacetoxymethyl ester [CAS:117464-70-7]

Application: Viable cell staining
Appearance: Orange or orange-brown solid
Purity: ≥90.0% (HPLC)
MW: 688.59, $C_{35}H_{28}O_{15}$

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
B262-10	1 mg

Product Description

BCECF-AM is cell-membrane permeable and is easily hydrolyzed by esterases to BCECF. BCECF is not cell-membrane permeable, and it accumulates inside viable cells (Fig. 1). BCECF emits a strong green fluorescence; therefore, it is easy to visualize viable cells. BCECF-AM is also utilized as an intracellular pH indicator. The excitation and emission wavelengths of BCECF are 500 nm and 530 nm, respectively. Usually, over 80% of loaded BCECF will remain inside the cell for at least 2 hours.

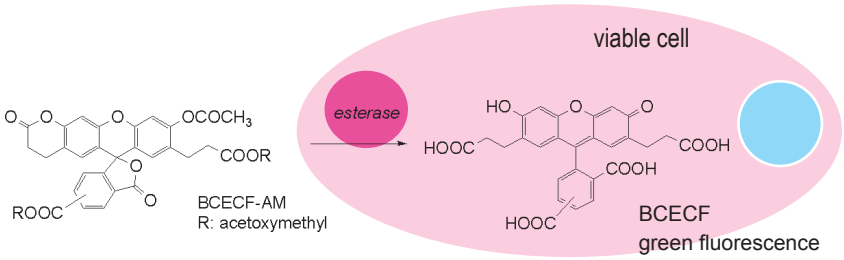


Fig. 1 Cell staining mechanism

General Protocol*

Reagents:
1 mM BCECF-AM/DMSO solution (1 mg BCECF in 1.45 ml DMSO)
HEPES buffer saline (20 mM HEPES, 153 mM NaCl, 5 mM KCl, 5 mM glucose, pH 7.4)

Protocol:
1. Suspend cells in HEPES buffer solution to prepare $4x10^7$ cells per ml.
2. Add 1 mM BCECF-AM/DMSO solution to the cell suspension to prepare 3 µM BCECF-AM (1/300 vol of cell suspension) as the final concentration.
3. Incubate the cell suspension at 37°C for 30 minutes.
4. Wash the cells 3 times with HEPES buffer saline and then prepare $3x10^6$ cells per ml of the cell suspension.
5. Determine the fluorescence intensity using a fluorescence microscope or a confocal laser microscope coupled with an image analyzer.

* Cell staining conditions differ by cell type, so it is necessary to optimize the conditions for each experiment.

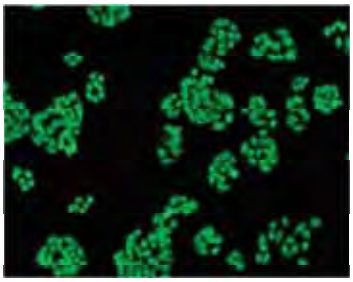


Fig. 2 Cell staining with BCECF-AM
Cell type: HeLa

References

1. R. A. Steinhardt, *et al*., Development of K+-conductance and Membrane Potentials in Unfertilized Sea Urchin Eggs After Exposure to NH_4OH. *Nature*. 1973;**241**:400-401.
2. T. J. Rink, *et al*., Cytoplasmic pH and Free Mg^{2+} in Lymphocytes. *J Cell Biol*. 1982;**95**:189-196.
3. A. M. Paradiso, et al., Na^+ -H^+ Exchange in Gastric Glands as Measured with a Cytoplasmic-trapped, Fluorescent pH Indicator. *PNAS*. 1984;**81**:7436-7440.

4. S. Grinstein, *et al*., Phorbol Ester-induces Changes of Cytoplasmic pH in Neutrophils: Role of Exocytosis in Na^+ - H^+ Exchange. *Am J Physiol*. 1985;**248**:C379-C386.
5. G. B. Zavoico, *et al*., Regulation of Intracellular pH in Human Platelets. *J Biol Chem*. 1986;**261**:13160-13167.
6. G. R. Bright, *et al*., Fluorescence Ratio Imaging Microscopy: Temporal and Spatial Measurements of Cytoplasmic pH. *J Cell Biol*. 1987;**104**:1019-1033.
7. C. Aalkjaer, *et al*., Intracellular pH Regulation in Resting and Contracting Segments of Rat Mesenteric Resistance Vessels. *J Physiol*. 1988;**402**:391-410.
8. K. Tsujimoto, *et al*., Intracellular pH of Halobacteria Can Be Determined by the Fluorescent Dye 2', 7'-bis(carboxyethyl)-5(6)-carboxyfluorescein. *Biochem Biophys Res Commun*. 1988;**155**:123-129.
9. M. A. Kolber, *et al*., Measurament of Cytotoxicity by Target Cell Release and Retention of the Fluorescent Dye Bis-carboxyethyl-carboxyfluorescein(BCECF). *J Immunol Methods*. 1988;**108**:255-264.
10. H. Harada, *et al*., cAMP Activates Cl^-/HCO_3^- Exchange for Regulation of Intracellular pH in Renal Epithelial Cells. *Biochim Biophys Acta*. 1991;**1092**:404-407.
11. C. C. Freudenrich, *et al*., Intracellular pH Modulates Cytosolic Free Magnesium in Cultured Chicken Heart Cells. *Am J Physiol*. 1992;**262**:C1024-C1030.
12. K. Khodakhah *et al*., Functional Heterogeneity of Calcium Release by Inositol Triphosphate in Single Purkinje Neurones, Cultured Cerebellar Astrocytes, and Peripheral Tissues. *PNAS*. 1993;**90**:4976-4980.
13. G. Boyarsky, *et al*., Superiority of in vitro Over in vivo Calibrations of BCECF in Vascular Smooth Muscle Cells. *FASEB J*. 1996;**10**:1205-1212.

-*Cellstain*- Calcein-AM

3',6'-Di(*O*-acetyl)-4',5'-bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein, tetraacetoxymethyl ester [CAS: 148504-34-1]

Application: Viable cell staining
Appearance: White to slightly yellow solid
MW: 994.86, $C_{46}H_{46}N_2O_{23}$

Storage Condition	Shipping Condition
-20°C, protect from light	with blue ice or dry ice

Ordering Information

Product code	Unit
C326-10	1 mg

-*Cellstain*- Calcein-AM solution

3',6'-Di(*O*-acetyl)-4',5'-bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein, tetraacetoxymethyl ester [CAS: 148504-34-1]

Application: Viable cell staining
Product: 1 mM Calcein-AM DMSO solution
(1.0 mg Calcein-AM per 1 ml DMSO)
Appearance: Colorless liquid
MW: 994.86, $C_{46}H_{46}N_2O_{23}$

Storage Condition	Shipping Condition
-20°C, protect from light	with blue ice or dry ice

Ordering Information

Product code	Unit
C396-10	1 ml

Product Description

Calcein-AM readily passes through the cell membrane of viable cells because of its enhanced hydrophobicity compared to Calcein. After Calcein-AM permeates into the cytoplasm, it is hydrolyzed by esterases to Calcein, which remains inside the cell (Fig. 1). Among other reagents, including BCECF-AM and Carboxy-fluorescein diacetate, Calcein-AM is the most suitable fluorescent probe for staining viable cells because of its low cytotoxicity. Calcein does not inhibit any cellular functions such as proliferation or chemotaxis of lymophocyte. In addition, viability assays using Calcein are reliable and correlate well with the standard ^{51}Cr-release assay. The excitation and emission wavelengths of calcein are 490 nm and 515 nm, respectively.

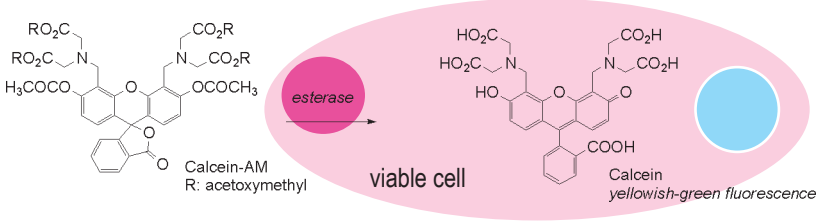


Fig. 1 Cell staining mechanism

Staining Procedure

1. Prepare 1 mM Calcein-AM solution with DMSO and dilute to prepare 1-50 μM Calcein-AM solution with PBS.[a)]
2. Add Calcein-AM solution with 1/10 of the volume of cell culture medium to the cell culture.[b)]
3. Incubate the cell at 37°C for 15-30 minutes.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells under a fluorescence microscope with 490 nm excitation and 515 nm emission filters.

[a)] If you have difficulty loading the Calcein-AM into cells, use a detergent such as Pluronic F127.
[b)] You may replace the culture medium with 1/10 concentration of Calcein-AM buffer solution.

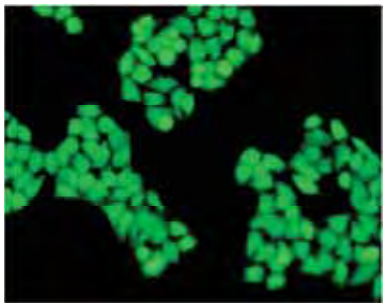


Fig. 2 Cell staining with Calcein-AM
Cell type: HeLa

References

1. K. McGinnes, *et al*., A Fluorescence NK Assay Using Flow Cytometry. *J Immunol Methods*. 1986;**86**:7-15.
2. S. J. Morris, Real-time Multi-wavelength Fluorescence Imaging of Living Cells. *BioTechniques*. 1990;**8**:296-308.
3. S. A. Weston, *et al*., New Fluorescent Dyes for Lymphocyte Migration Studies Analysis by Flow Cytometry and Fluorescent Microscopy. *J Immunol Methods*. 1990;**133**:87-97.
4. D. M. Callewaert, *et al*., Characterization of Effector-Target Conjugates for Cloned Human Natural Killer and Human Lymphokine Activated Killer Cells by Flow Cytometry. *Cytometry*. 1991;**12**:666-676.
5. H. Xie, *et al*., Intercellular Communication Through Gap Junctions Is Reduced in Senescent Cell. *Biophys J*. 1992;**62**:45-47.
6. S. A. Weston, *et al*., Calcein: a Novel Marker for Lymphocytes Which Enter Lymph Nodes. *Cytometry*. 1992;**13**:739-749.
7. X. M. Wang, *et al*., A New Microcellular Cytotoxicity Test Based on Calcein AM Release. *Hum Immunol*. 1993;**37**:264-270.
8. N. G. Papadopoulos, *et al*., An Improved Fluorescence Assay for the Determination of Lymphocyte-Mediated Cytotoxicity Using Flow Cytometry. *J Immunol Methods*. 1994;**177**:101-111.
9. L. S. D. Clerck, *et al*., Use of Fluorescent Dyes in the Determination of Adherence of Human Leucocytes to Endothelial Cells and the Effects of Fluorochromes on Cellular Function. *J Immunol Methods*. 1994;**172**:115-124.
10. H. Ohata, *et al*., Confocal Imaging Analysis of ATP-Induced Ca^{2+} Response in Individual Endothelial Cells of the Artery in Situ. *Am J Physiol*. 1997;**272**:C1980-C1987.

-Cellstain- CFSE

5- or 6-(*N*-Succinimidyloxycarbonyl)-fluorescein 3',6' diacetate [CAS: 150347-59-4]

Application: Viable cell staining
Appearance: White to slightly yellow solid
MW: 557.46, $C_{29}H_{19}NO_{11}$

Storage Condition	Shipping Condition
-20°C	ambient temperature

Ordering Information	
Product code	Unit
C375-10	1 mg

Product Description

CFSE is cell-membrane permeable and readily accumulates inside viable cells where it covalently attaches to intracellular proteins (Fig. 1). Hydrolyzed CFSE emits fluorescence and covalently attached fluorescein molecules do not leak from cells. CFSE-labeled cells can be monitored over several weeks in vivo. Therefore, CFSE is utilized for detection of viable cell as well as for the long-term observation of cell activities by fluorescent microscopy. The excitation and emission wavelengths of CFSE-labeled cells are 500 nm and 520 nm, respectively. CFSE-stained cells are shown in Fig. 2.

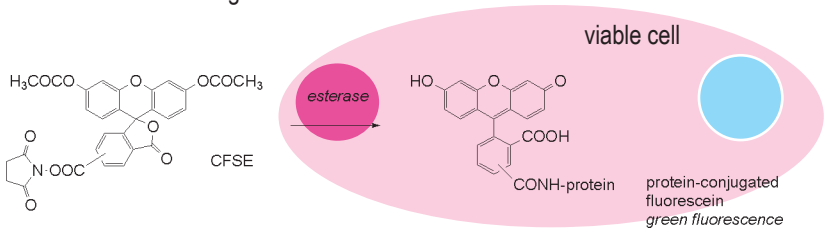


Fig. 1 Cell staining mechanism

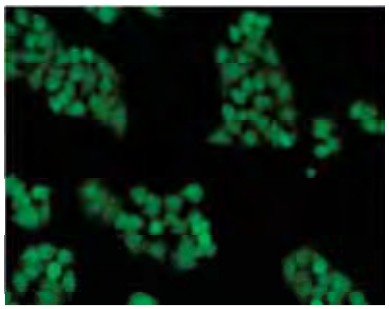


Fig. 2 Cell staining with CFSE
Cell type: HeLa

Staining Procedure

1. Prepare 1 mM CFSE solution with DMSO. Dilute it to prepare 10-50 μM CFSE solution with PBS or an appropriate buffer.
2. Add CFSE solution with 1/10 of the volume of cell culture medium to the cell culture.
3. Incubate the cell at 37°C for 15 to 30 minutes.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells under a fluorescence microscope with 490 nm excitation and 530 nm emission filters.

References

1. M. Bronner-Fraser, Alterations in Neural Crest Migration by a Monoclonal Antibody That Affects Cell Adhesion. *J Cell Biol*. 1985;**101**:610-617.
2. A. Nose, *et al*., A Novel Cadherin Cell Adhesion Molecule:Its Expression Patterns Associated WithImplantation and Organogenesis of Mouse Embryos. *J Cell Biol*. 1986;**103**:2649-2658.
3. S. A. Weston, *et al*., New Fluorescent Dyes for Lymphocyte Migration Studies Analysis by Flow Cytometry and Fluorescent Microscopy. *J Immunol Methods*. 1990;**133**:87-97.
4. C. K. Raymond, *et al*., Molecular Analysis of the Yeast VPS3 Gene and the Role of Its Product in Vacuolar Protein Sorting and Vacuolar Segregation during the Cell Cycle. *J Cell Biol*. 1990;**111**:877-892.
5. G. Radcliff, *et al*., Quantification of Effector/Target Conjugation Involving Natural Killer(NK) or Lymphokine Activated Killer(LAK) Cells by Two-color Flow Cytometry. *J Immunol Methods*. 1991;**139**:281-292.
6. S. A. Weston, *et al*., Calcein: a Novel Marker for Lymphocytes Which Enter Lymph Nodes. *Cytometry*. 1992;**13**:739-749.
7. L. S. D. Clerck, *et al*., Use of Fluorescent Dyes in the Determination of Adherence of Human Leucocytes to Endothelial Cells and the Effects of Fluorochromes on Cellular Function. *J Immunol Methods*. 1994;**172**:115-124.

-*Cellstain*- CytoRed Solution

7-Isobutyloxycarbonyloxy-3*H*-phenoxazin-3-one, solution

Application: Viable cell staining
Product: 1 mM CytoRed DMSO solution
Appearance: Yellowish-orange liquid
MW: 313.31, $C_{17}H_{15}NO_5$

Ordering Information	
Product code	Unit
C410-10	1 ml

Storage Condition	Shipping Condition
-20°C, protect from light	with blue ice or dry ice

Product Description

CytoRed is cell-membrane permeable and accumulates inside viable cells as resorufin (Fig. 1). CytoRed has a much wider spectrum than BCECF or Calcein, so filters for fluorescein and rhodamine can also be used. The excitation and emission wavelengths of resorufin are 560 nm and 590 nm, respectively. Cells stained with CytoRed are shown in Fig. 2.

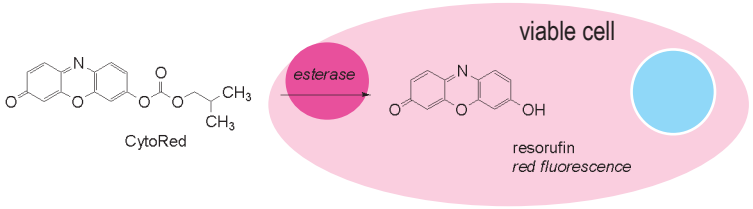


Fig. 1 Cell staining mechanism

Staining Procedure

1. Prepare 1 mM CytoRed solution with DMSO. Dilute it to prepare 10 µM CytoRed solution with culture medium or an appropriate buffer.[a)]
2. Prepare a $1x10^5$-$1x10^6$ cells/ml cell suspension and culture the cells in a chamber slide.
3. Remove culture medium and wash cells with culture medium (PBS-Hanks medium, etc.).
4. Add CytoRed solution to the cells and incubate the chamber at 37°C for 30 minutes to 1 hour.
5. Remove the culture medium from cells and add new medium.[b)]
6. Wash cells twice with PBS or an appropriate buffer.
7. Observe the cells under a fluorescence microscope with 560 nm excitation and 590 nm emission filters.

[a)] Incubate the CytoRed buffer solution at 37° C prior to adding to cells.
[b)] For fixing after washing cells, add 10% formarin buffer and incubate for 15-20 minutes, and then wash with PBS.

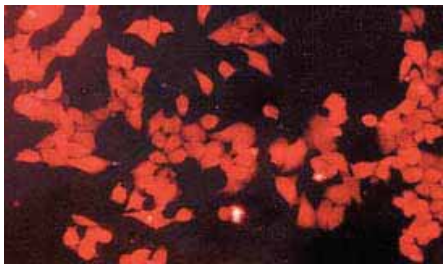


Fig. 2 Cell staining with CytoRed
Cell type: HeLa

Reference

1. M. Ishiyama, *et al*., A Resorufin Derivative as a Fluorogenic Indicator for Cell Viability. *Anal Sci*. 1999;**15**:1025-1028.

-Cellstain- FDA Fluorescein diacetate [CAS: 596-09-8]

Application: Viable cell staining
Appearance: White crystalline powder
MW: 416.38, $C_{24}H_{16}O_7$

Storage Condition	Shipping Condition
-20°C, protect from light	ambient temperature

Ordering Information

Product code	Unit
F209-10	1 mg

Product Description

FDA is cell-membrane permeable and accumulates inside of viable cells as fluorescein (Fig. 1). Since fluorescein is less hydrophilic than BCECF or Calcein, the leakage of fluorescein from cells is rather high. FDA is also utilized for flow cytometry. The excitation and emission wavelengths of fluorescein are 488 nm and 530 nm, respectively. FDA-stained cells are shown in Fig. 2.

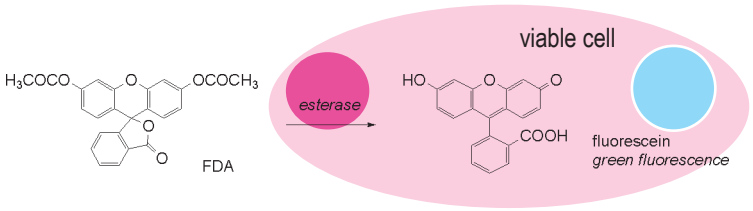


Fig. 1 Cell staining mechanism

Staining Procedure

1. Prepare 0.5 mg/ml FDA stock solution with DMSO. Dilute 10 µl of the stock solution with 5 ml PBS(-).
2. Prepare a cell suspension and wash cells with PBS(-). Prepare $1x10^5$-$1x10^6$ cells/ml cell suspension.
3. Add 15 µl FDA solution to 30 µl cell suspension and incubate at 37°C for 15-30 minutes.
4. Put 10 µl stained cell suspension on a glass slide and cover with a cover glass.
5. Observe the cells under a fluorescence microscope with 488 nm excitation and 530 nm emission filters.

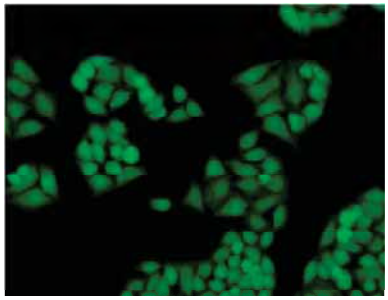


Fig. 2 Cell staining with FDA
Cell type: HeLa

References

1. B. Rotman, *et al*., Membrane Properties of Living Mammalian Cells as Studied by Enzymatic Hydrolysis of Fluorogenic Esters. *PNAS*. 1966;**55**:134-141.
2. H. R. Hulett, *et al*., Cell Sorting: Automated Separation of Mammalian Cells as a Function of Intercellular Fluorescence. *Science*. 1969;**166**:747-749.
3. K. H. Jones, *et al*., An Improved Method to Determine Cell Viability by Simultaneous Staining with Fluorescein Diacetate-Propidium Iodide. *J Histochem Cytochem*. 1985;**33**:77-79.
4. K. McGinnes, *et al*., A Fluorescence NK Assay Using Flow Cytometry. *J Immunol Methods*. 1986;**86**:7-15.
5. W. M. J. Vuist, *et al*., Potentiation by Interleukin 2 of Burkitt's Lymphoma Therapy with Anti-Pan B (Anti-CD19) Monoclonal Antibodies in a Mouse Xenotransplantation Model. *Cancer Res*. 1989;**49**:3783-3788.
6. E. Prosperi, Intracellular Turnover of Fluorescein Diacetate. Influence of Membrane Ionic Gradients on Fluorescein Efflux. *Histochem J*. 1990;**22**:227-233.

-*Cellstain*- MitoRed

9-[2-(4'-Methylcoumarin-7'-oxycarbonyl)phenyl]-3,6-bis(diethylamino)xanthylium chloride

Application: Mitochondrial staining
Appearance: Red purple to purplish-brown solid
MW: 637.17, $C_{38}H_{37}ClN_2O_5$

Storage Condition
0-5°C, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
R237-10	50 µg x 8 vials

Product Description
MitoRed is a cell-membrane-permeable, rhodamine-based dye. It localizes in mitochondria and emits red fluorescence (Fig. 1). The interaction of MitoRed with mitochondria depends on the membrane potential of the mitochondria. Mitochondria can be stained with 20 to 200 nM MitoRed. The excitation and emission wavelengths of MitoRed are 560 nm and 580 nm, respectively.

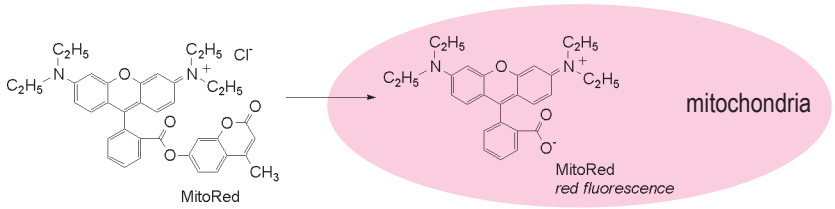


Fig. 1 Cell staining mechanism

Staining Procedure
1. Dissolve 50 µg MitoRed (1 tube) in 78 µl of DMSO to prepare 1 mM MitoRed-DMSO solution.
2. Prepare cells with a glass slide. The cell number should be $5x10^4$ to $5x10^5$ cells per ml.
3. Incubate the slide and wash cells with PBS or Hank's medium.
4. Dilute the 1 mM MitoRed solution with culture medium to prepare 20-200 nM MitoRed buffer solution.
5. Add the MitoRed buffer solution[a)] to the glass slide and incubate at 37°C for 30 minutes to 1 hour.
6. Remove the MitoRed buffer solution and wash cells with culture medium.[b)]
7. Observe the cells under a fluorescence microscope with a rhodamine filter.

[a)] *Incubate the MitoRed buffer solution at 37°C prior to adding to cells.*
[b)] *For fixing after washing cells, add 10% formarin buffer and incubate for 15-20 minutes, then wash with PBS.*

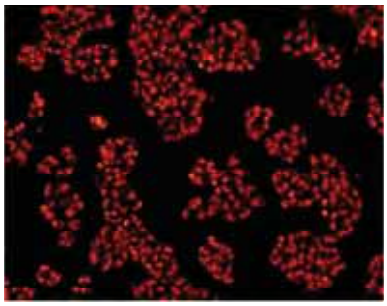


Fig. 2 Cell staining with MitoRed
Cell type: HeLa

References

1. R. Ikeda, *et al*., Effects of Melanin upon Susceptibility of Cryptococcus to Antifungals. *Microbiol Immunol*. 2003;**47**:271-277.

-*Cellstain*- Rh123

Rhodamine 123, 2-(6-Amino-3-imino-3*H*-xanthen-9-yl) benzoic acid methyl ester, hydrochloride [CAS: 62669-70-9]

Application: Mitochondrial staining
Appearance: Red to reddish-brown powder or solid
MW: 380.82, $C_{21}H_{17}ClN_2O_3$

Storage Condition
0-5°C, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
R233-10	1 mg

Product Description

Rhodamine 123 (Rh123) is cell-membrane permeable and localizes in mitochondria of viable cells to emit yellowish-green fluorescence (Fig. 1). Rh123 is utilized for staining a wide variety of cells, including plant cells and bacteria. Since there is a correlation between the amount of ATP in a cell and the fluorescence intensity of Rh123, this compound is used for the detection of intracellular ATP. Rh123 is also used in cancer research.

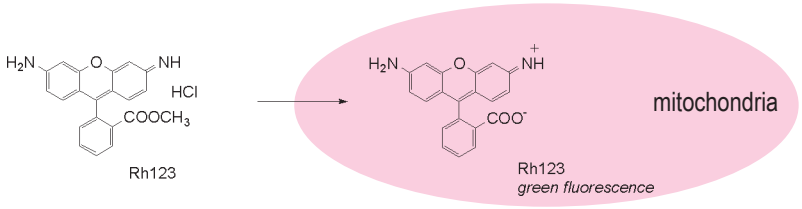


Fig. 1 Cell staining mechanism

Staining Procedure

1. Dissolve 0.4 mg Rh123 in 1 ml DMSO to prepare 1 mM Rh123-DMSO solution.
2. Prepare cells with a glass plate. The cell number will be $5x10^4$ to $5x10^5$ cells per ml.
3. Incubate the slide and wash cells with PBS or Hank's medium.
4. Dilute the 1 mM Rh123 solution with culture medium to prepare 1-20 µM Rh123 buffer solution.
5. Add the Rh123 buffer solution[a)] to the glass slide and incubate at 37°C for 30 minutes to 1 hour.
6. Remove the Rh123 buffer solution and wash cells with culture medium.[b)]
7. Observe the cells under a fluorescence microscope with a fluorescein filter.

[a)] *Incubate the Rh123 buffer solution at 37°C prior to adding to cells.*
[b)] *For fixing after washing cells, add 10% formarin buffer and incubate for 15-20 minutes, then wash with PBS.*

References

1. L. V. Johnson, *et al*., Localization of mitochondria in living cells with rhodamine 123. *PNAS*. 1980;**77**:990-994.
2. C. S. Downes, *et al*., Novobiocin inhibition of DNA excision repair may occur through effects on mitochondrial structure and ATP metabolism, not on repair topoisomerases. *Carcinogenesis*. 1985;**6**:1343-1352.
3. G. Varbiro, *et al*., Direct effect of Taxol on free radical formation and mitochondrial permeability transition. *Free Radic Biol Med*. 2001;**31**:548-558.

-Cellstain- AO 3,6-Bis(dimethylamino)acridine, hydrochloride [CAS: 65-61-2]

Application: DNA, RNA staining
Appearance: Reddish-brown powder or solid
MW: 301.81, $C_{17}H_{20}ClN_3$

Storage Condition	Shipping Condition
0-5°C, protect from light	ambient temperature

Ordering Information

Product code	Unit
A386-10	1 mg

-Cellstain- AO Solution

3,6-Bis(dimethylamino)acridine, hydrochloride, solution [CAS: 65-61-2(AO)]

Application: DNA, RNA staining
Product: 3.3 mM AO aquous solution
(1 mg AO per 1 ml water)
Appearance: Yellow to orange liquid
MW: 301.81, $C_{17}H_{20}ClN_3$

Storage Condition	Shipping Condition
-20°C, protect from light	ambient temperature

Ordering Information

Product code	Unit
A430-10	1 ml

Product Description

Acridine orange (AO) forms a complex with double-stranded DNA to emit green fluorescence (Fig. 1). AO also forms a complex with single-stranded DNA or RNA to emit red fluorescence. One molecule of AO intercalates with three base pairs of double-stranded DNA and emits green fluorescence with the maximum wavelength at 526 nm (excitation 502 nm). One molecule of AO can also interact with one phosphate group of single-stranded DNA or RNA to form an aggregated, or stacked, structure that emits red fluorescence with the maximum wavelength at 650 nm (excitation 460 nm). Therefore, AO is utilized for the detection of both double-stranded DNA and single-stranded DNA or RNA. It enables simultaneous determination of DNA and RNA with argon laser excitation or flow cytometry.

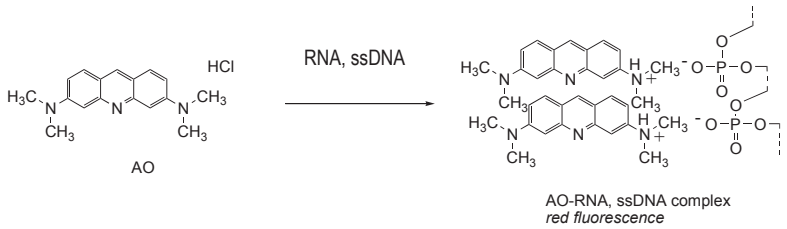


Fig. 1 Cell staining mechanism

Staining Procedure

1. Prepare 10-50 µM AO solution with PBS or an appropriate buffer.[a)]
2. Add AO solution with 1/10 of the volume of cell culture medium to the cell culture.[b)]
3. Incubate the cell at 37°C for 10-20 minutes.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells under a fluorescence microscope with 500 nm excitation and 530 nm emission filters.

[a)] Since AO may be carcinogenic, extreme care is necessary during handling and disposal.
[b)] You may replace the culture medium with 1/10 concentration of AO buffer solution.

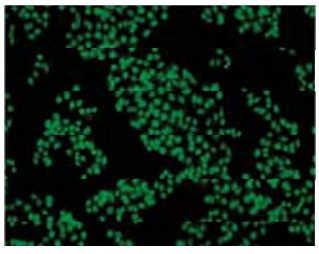


Fig. 2 Cell staining with AO
Cell type: HeLa

References
1. I. W. Taylor, *et al*., An Evaluation of DNA Fluochromes, Staining Techniques, and Analysis for Flow Cytometry. I. Unperturebed Cellpopulations. *J Histochem Cytochem*. 1980;**28**:1224-1232.
2. N. Miyoshi, *et al*., Fluorescence Lifetime of Acridine Orange in Sodiu Dodecyl Sulfate Premicellar Solutions. *Photochem Photobiol*. 1988;**47**:685-688.
3. A. K. El-Naggar, *et al*., Single- and Double-stranded RNA Measurements by Flow Cytometry in Solid Neoplasms. *Cytometry*. 1991;**12**:330-335.
4. Y. Miyakoshi, *et al*., The Frequencies of Micronuclei Induced by Cisplatin in Newborn Rat Astrocytes Are Increased by 50-Hz, 7.5- and 10-mT Electromagnetic Fields. *Environ Health and Prev Med*. 2005;**10**:138-143.

-Cellstain- DAPI

4',6-Diamidino-2-phenylindole, dihydrochloride [CAS: 28718-90-3]

Application: DNA staining
Appearance: Yellow powder or solid
MW: 350.25, $C_{16}H_{17}Cl_2N_5$

Storage Condition: -20°C, protect from light
Shipping Condition: ambient temperature

Ordering Information

Product code	Unit
D212-10	1 mg

-Cellstain- DAPI Solution

4',6-Diamidino-2-phenylindole, dihydrochloride, solution [CAS: 28718-90-3(DAPI)]

Application: DNA staining
Product: 2.9 mM DAPI buffer solution
(1 mg DAPI per 1 ml buffer)
Appearance: Slightly yellow to yellow liquid
MW: 350.25, $C_{16}H_{17}Cl_2N_5$

Storage Condition: 0-5°C, protect from light
Shipping Condition: ambient temperature

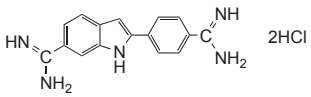
Ordering Information

Product code	Unit
D523-10	1 ml

Product Description

DAPI is an AT-sequence-specific DNA intercalator that attaches to DNA at the minor groove of the double helix like Hoechst dyes. DAPI is not permeable through viable cell membranes, but it passes through disturbed cell membranes to stain the nucleus. DAPI has a high photobleaching tolerance level. DAPI is utilized for the detection of mitochondrial DNA in yeast, chloroplast DNA, virus DNA, micoplasm DNA, and chromosomal DNA. The excitation and emission wavelengths of DAPI-DNA complex are 360 nm and 460 nm, respectively.

Chemical Structure



Staining Procedure

1. Prepare 10-50 µM DAPI solution with PBS or an appropriate buffer.[a)]
2. Add DAPI solution with 1/10 of the volume of cell culture medium to the cell culture.[b)]
3. Incubate the cell at 37°C for 10-20 minutes.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells using a fluorescence microscope with 360 nm excitation and 460 nm emission filters.

[a)] *Since DAPI may be carcinogenic, extreme care is necessary during handling and disposal.*
[b)] *You may replace the culture medium with 1/10 concentration of DAPI buffer solution.*

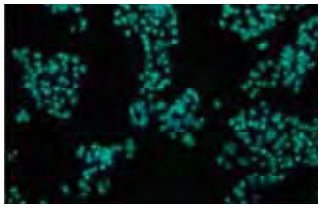


Fig. 1 Cell staining with DAPI

References
1. W. Schnedl, *et al*., DIPI and DAPI: Fluorescence Banding with Only Negligible Fading. *Hum Genet*. 1977;**36**:167-172.
2. I. W. Taylor, *et al*., An Evaluation of DNA Fluochromes, Staining Techniques, and Analysis for Flow Cytometry. I. Unperturebed Cellpopulations. *J Histochem Cytochem*. 1980;**28**:1224-1232.
3. F. Otto, *et al*., A Comparative Study of DAPI, DIPI, and Hoechst 33258 and 33342 as Chromosomal DNA Stains. *Stain Technol*. 1985;**60**:7-11.
4. N. Poulin, *et al*., Quantitative Precision of an Automated Image Cytometric System for the Measurement of DNA Content and Distribution in Cells Labeled with Fluorescent Nucleic Acid Stains. *Cytometry*. 1994;**16**:227-235.
5. M. Kawai, *et al*., Rapid Enumeration of Physiologically Active Bacteria in Purified Water Used in the Pharmaceutical Manufacuturing Process. *J Appl Microbiol*. 1999;**86**:496-504.

-*Cellstain*- Hoechst 33258 Solution

2'-(4-hydroxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1*H*-benzimidazole, trihydrochloride, solution [CAS: 23491-45-4(Hoechst 33258)]

Application: DNA staining
Product: 1 mg/ml aqueous solution
Appearance: Yellow liquid
MW: 533.88, $C_{25}H_{27}Cl_3N_6O$

Storage Condition
0-5°C, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
H341-10	1 ml

-*Cellstain*- Hoechst 33342 Solution

Bisbenzimide H33342, 2'-(4-ethoxyphenyl)-5-(4-methyl-1-piperazinyl)-2,5'-bi-1*H*-benzimidazole, trihydrochloride, solution [CAS: 23491-52-3(Hoechst 33342)]

Application: DNA staining
Product: 1.8 mM Hochest 33342 aqueous solution
(1 mg Hochest 33342 per 1 ml water)
Appearance: Yellow liquid
MW: 561.93, $C_{27}H_{31}Cl_3N_6O$

Storage Condition
0-5°C, protect from light

Shipping Condition
ambient temperature

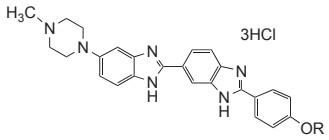
Ordering Information

Product code	Unit
H342-10	1 ml

Product Description (for Hoeches 33258 / 33342)

Hoechst dyes are cell membrane permeable and stain DNA to emit intense blue fluorescence. They bind to DNA in the minor groove of areas rich in poly-AT sequence. Both Hoechst 33342 and Hoechst 33258 are water soluble and stable in aqueous solutions. The excitation and emission wavelengths of Hoechst-DNA complex are 350 nm and 460 nm, respectively.

Chemical Structure



Hoechst 33258: R=OH
Hoechst 33342: R=C_2H_5

Staining Procedure

1. Prepare 10-50 μM Hoechst dye solution with PBS or an appropriate buffer.[a)]
2. Add Hoechst dye solution with 1/10 of the volume of cell culture medium to the cell culture.[b)]
3. Incubate the cell at 37°C for 10-20 minutes.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells under a fluorescence microscope with 350 nm excitation and 460 nm emission filters.

[a)] Since Hoechst dyes may be carcinogenic, extreme care is necessary during handling and disposal.
[b)] You may replace the culture medium with 1/10 concentration of Hoechst dye buffer solution.

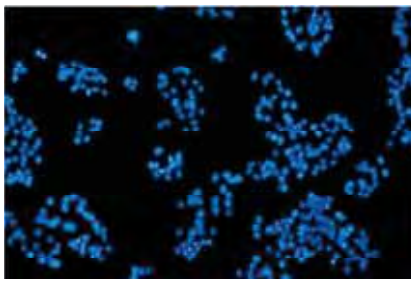


Fig. 1 Cell staining with Hoechst 33258
Cell type: human fetal cell

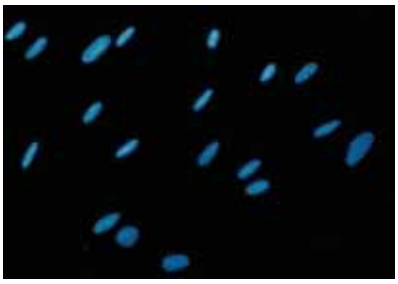


Fig. 2 Cell staining with Hoechst 33342
Cell type: human fetal cell

References

1. M. J. Lydon, *et al*., Vital DNA Staining and Cell Sorting by Flow Microfluorometry. *J Cell Physiol*. 1980;**102**:175-181.
2. M. Sriram, *et al*., Structural Consequences of a Carcinogenic Alkylation Lesion on DNA: Effect of O6-ethylguanine on the Molecular Structure of the d(CGC[e6G]AATTCGCG)-netropsin Complex. *Biochemistry*. 1992;**31**:11823-11834.
3. Y. Tadokoro, *et al*., Characterization of Histone H2A.X Expression in Testis and Specific Labeling of Germ Cells at the Commitment Stage of Meiosis with Histone H2A.X Promoter-Enhanced Green Fluorescent Protein Transgene. *Biol Reprod*. 2003;**69**:1325-1329.
4. F. Wada, *et al*., Analyses of Expression and Localization of Two Mammalian-Type Transglutaminases in Physarum polycephalum, an Acellular Slime Mold. *J Biochem*. 2004;**136**:665-672.
5. T. Ohara, *et al*., FoSTUA, Encoding a Basic Helix-Loop-Helix Protein, Differentially Regulates Development of Three Kinds of Asexual Spores, Macroconidia, Microconidia, and Chlamydospores, in the Fungal Plant Pathogen Fusarium oxysporum. *Eukaryot Cell*. 2004;**3**:1412-1422.

-*Cellstain*- PI

3,8-Diamino-5-[3-(diethylmethylammonio)propyl]-6-phenylphenanthridinium diiodide [CAS: 25535-16-4]

Application: DNA staining
Appearance: Reddish-brown powder or solid
MW: 668.39, $C_{27}H_{34}I_2N_4$

Storage Condition
0-5°C, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
P346-10	1 mg

-*Cellstain*- PI Solution

3,8-Diamino-5-[3-(diethylmethylammonio)propyl]-6-phenylphenanthridinium diiodide, solution [CAS: 25535-16-4(PI)]

Application: DNA staining
Product: 1.5 mM PI aqueous solution (1 mg PI per 1 ml water)
Appearance: Orange to red liquid
MW: 668.39, $C_{27}H_{34}I_2N_4$

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

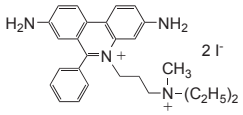
Ordering Information

Product code	Unit
P378-10	1 ml

Product Description

Propidium iodide (PI) is an ethidium bromide analog that emits red fluorescence upon intercalation with double-stranded DNA. PI does not permeate viable cell membranes, but passes through disturbed cell membranes and stains the nuclei. PI is often used in combination with a fluorescein compound, such as Calcein-AM or FDA, for simultaneous staining of viable and dead cells. The excitation and emission wavelengths of PI-DNA complex are 535 nm and 615 nm, respectively.

Chemical Structure



Staining Procedure

1. Prepare 10-50 µM PI solution with PBS or an appropriate buffer.[a)]
2. Add PI solution with 1/10 of the volume of cell culture medium to the cell culture.[b)]
3. Incubate the cells at 37°C for 10-20 minutes.
4. Wash cells twice with PBS or an appropriate buffer.
5. Observe the cells under a fluorescence microscope with 535 nm excitation and 615 nm emission filters.

[a)] Since PI may be carcinogenic, extreme care is necessary during handling and disposal.
[b)] You may replace the culture medium with 1/10 concentration of PI buffer solution.

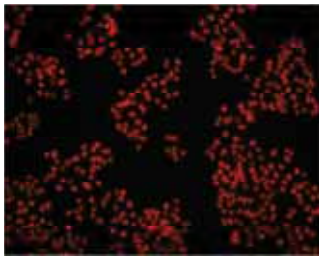


Fig. 1 Cell staining with PI

References

1. I. W. Taylor, *et al*., An Evaluation of DNA Fluochromes, Staining Techniques, and Analysis for Flow Cytometry. I. Unperturebed Cellpopulations. *J Histochem Cytochem*. 1980;**28**:1224-1232.
2. W. M. J. Vuist, *et al*., Potentiation by Interleukin 2 of Burkitt's Lymphoma Therapy with Anti-Pan B (Anti-CD19) Monoclonal Antibodies in a Mouse Xenotransplantation Model. *Cancer Res*. 1989;**49**:3783-3788.
3. A. K. El-Naggar, *et al*., Single- and Double-stranded RNA Measurements by Flow Cytometry in Solid Neoplasms. *Cytometry*. 1991;**12**:330-335.
4. C. Souchier, *et al*., Methods for Cell Proliferation Analysis by Fluorescent Image Cytometry. *Cytometry*. 1995;**20**:203-209.
5. T. Irino, *et al*., Establishment of Real-Time Polymerase Chain Reaction Method for Quantitative Analysis of Asparagine Synthetase Expression. *J Mol Diagn*. 2004;**6**:217-224.

-*Cellstain*- Double Staining Kit

Features: Fluorometric microscope detection
Simultaneous determination of viable and dead cells

Storage Condition	Shipping Condition
-20°C, protect from light	with blue ice or dry ice

Ordering Information	
Product code	Unit
CS01-10	1 set

Kit Components:

Solution A (Calcein-AM) ..... 4 vials
Solution B (PI) ..... 1 vial

Required Equipment and Materials

Microscope with 490 nm excitation filter and 530 nm emission filter, glass slide or glass bottom plate, CO_2 incubator, 10 µl and 200 µl adjustable pipettes, PBS

Product Description

-*Cellstain*-Double Staining Kit is utilized for simultaneous fluorescence staining of viable and dead cells. This kit contains Calcein-AM and Propidium Iodide (PI) solutions, which stain viable and dead cells, respectively (Fig. 1). Calcein-AM, an acetoxymethyl ester of calcein, is highly lipophilic and cell membrane permeable. Although Calcein-AM itself is not a fluorescent molecule, the calcein generated from Calcein-AM by esterase in a viable cell emits a strong green fluorescence (excitation: 490 nm, emission: 515 nm). Therefore, Calcein-AM only stains viable cells. On the other hand, PI, a nuclei staining dye, cannot pass through a viable cell membrane. It reaches the nucleus by passing through disordered areas of dead cell membrane and intercalates with the DNA double helix of the cell to emit red fluorescence (excitation: 535 nm, emmision: 617 nm). Since both calcein and PI-DNA can be excited with 490 nm, simultaneous monitoring of viable and dead cells is possible with a fluorescence microscope. With 545 nm excitation, only dead cells can be observed (Fig. 2). Since optimal staining conditions differ from cell line to cell line, we recommend that a suitable concentration of PI and Calcein- AM be individually determined. Please note that PI is suspected to be highly carcinogenic; careful handling is required.

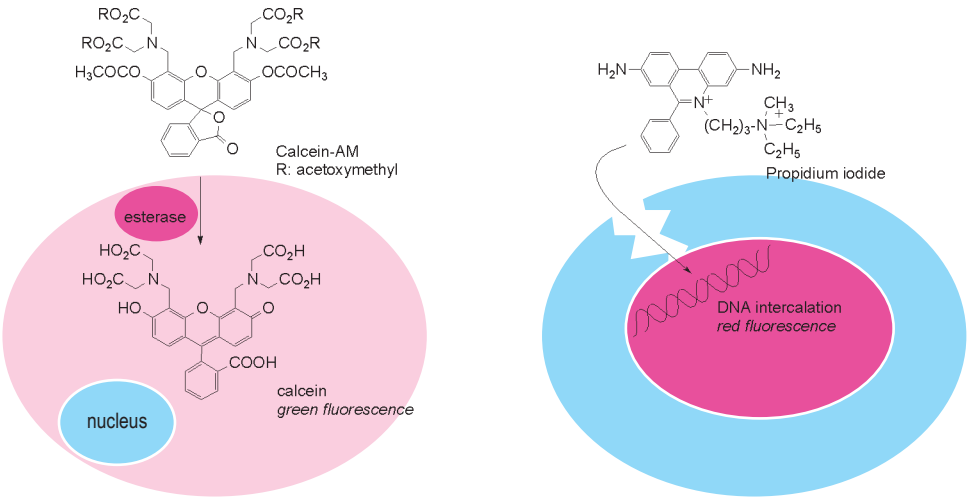


Fig. 1 Assay system to determine viable cells and dead cells

Assay Procedure

1. Add 10 µl Solution A and 5 µl Solution B to 5 ml PBS to prepare assay solution.*
2. Wash cells with PBS several times to remove residual esterase activity.
3. Add 100 µl of assay solution to cells and incubate the mixture at 37°C for 15 minutes.
4. Detect fluorescence using a fluorescence mircoscope with 490 nm excitation for simultaneous monitoring of viable and dead cells. With 545 nm excitation, only dead cells can be observed.

* The concentration of each reagent should be optimized. The following steps may be necessary to determine the suitable concentration of each reagent:

1. Prepare dead cells by 10 minutes incubation in 0.1% saponin or 0.1-0.5% digitonin or by 30 minutes incubation in 70% ethanol.
2. Stain dead cells with 0.1-10 µM PI solution to find a PI concentration that stains the nucleus only, not the cytosol.
3. Stain dead cells with 0.1-10 µM Calcein-AM solution to find a Calcein-AM concentration that does not stain the cytosol. Then stain viable cells with that Calcein-AM solution to check whether the viable cell can be stained.

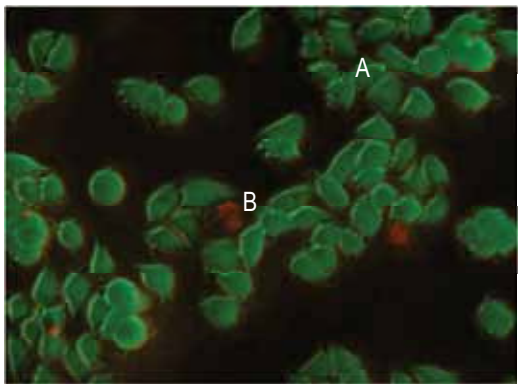


Fig. 2 Cell staining with Double Staining Kit
HeLa cell, incubated with assay solution for 15 minutes.
A) viable cell
B) dead cell

References

1. L. S. D. Clerck, *et al*., Use of Fluorescent Dyes in the Determination of Adherence of Human Leucocytes to Endothelial Cells and the Effects of Fluorochromes on Cellular Function. *J Immunol Methods*. 1994;**172**:115-124.
2. E. S. Kaneshiro, *et al*., Reliability of Calcein Acetoxy Methyl Ester and Ethidium Homodimer or Propidium Iodide for Viability Assessment of Microbes. *J Microbiol Methods*. 1993;**17**:1-16.
3. N. G. Papadopoulos, *et al*., An Improved Fluorescence Assay for the Determination of Lymphocyte-Mediated Cytotoxicity Using Flow Cytometry. *J Immunol Methods*. 1994;**177**:101-111.
4. M. Adler, *et al*., Cytotoxic actions of the heavy metal chelator TPEN on NG108-15 neuroblastoma-glioma cells. *Neurotoxicology*. 1999;**20**:571-582.
5. P. G. Bush, *et al*., Viability and volume of in situ bovine articular chondrocytes-changes following a single impact and effects of medium osmolarity. *Osteoarthritis Cartilage*. 2005;**13**:54-65.

-*Cellstain*- Trypan Blue

3,3'-{[3,3'-Dimethyl(1,1'-biphenyl)-4,4'-diyl]bis(azo)}bis(5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid), tetrasodium salt [CAS: 72-57-1]

Application: Dead cell staining
Appearance: Blackish brown crystalline powder
MW: 960.81, $C_{34}H_{24}N_6Na_4O_{14}S_4$

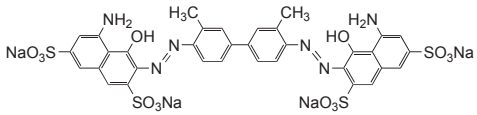
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
T375-10	5 g

Product Description
Trypan Blue is commonly utilized for dead cell staining in what is called the dye exclusion test. Viable cells are not stained by Trypan Blue. Therefore, dead Trypan Blue-stained cells are easily recognized by microscopy and can be counted using a hematocytometer. Erythrosin B, negrosine, eosin Y, AO, and EB are also utilized for this purpose. Although it is hard to detect cells in early to middle stages of apoptosis, Trypan Blue staining is a very simple and widely used method to visualize dead cells.



Chemical Structure

References

1. K. H. Jones, *et al*., An Improved Method to Determine Cell Viability by Simultaneous Staining with Fluorescein Diacetate-Propidium Iodide. *J Histochem Cytochem*. 1985;**33**:77-79.

FSB solution 1-Fluoro-2,5-bis(3-carboxy-4-hydroxystyryl)benzene, 1% DMSO solution

Application: Amyloid staining
Properties: High affinity with β-sheet structure
High detection sensitivity
Appearance: Pale yellow to yellowish brown liquid
Absorbance: 0.6-0.85 (around 370nm)
MW: 420.39, $C_{24}H_{17}FO_6$

Storage Condition
0-5°C, protect from light

Shipping Condition
ambient temperature

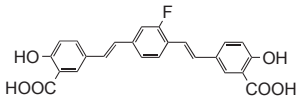
Ordering Information

Product code	Unit
F308-10	100 µl

Product Description
Amyloidosis, a disease which has been identified as a particular disorder by the Japanese Ministry of Health, is an illness that involves an abnormal protein called amyloid that has a β sheet structure, aggregates in fibers, and is deposited on the outside of internal organs and systems, inhibiting the function of those organs and systems. Disorders among many Japanese include immunocytic amyloidosis (AL amylodosis), responsive AA amyloidsis, familial amyloid polyneuropathy (FAP), and dialysis amyloidsis (DRA). It is estimated that there are hundreds of patients throughout Japan. The proteins that cause amyloidosis can be largely divided into two groups: amylids that are deposited in various organs throughout the body (systemic amyloidosis) such as the disorders listed above, and [amyloids that are deposited in a particular organ, such as the brain in the case of Alzheimer's disease (localized amyloidosis). The dye 1-Bromo-2,5-bis(3-carboxy-4-hydroxystyryl)benzene (BSB) has been used for detecting amyloids because of its high affinity with amyloid β peptide (Aβ), the amyloid associated with Alzheimer's disease.
Skovronsky confirmed that the dye accumulates in senile plaque of brain tissue of transgenic mice Tg2576 that express the amyloid precursor protein of Aβ(APP) 18 hours after the intravenous injection of BSB.[1)] Not limited to Aβ, Ando and others have announced that amyloid deposits in various systemic amyloidosis (AA, AL, ATTR, Ascr, Aβ 2M) are stained more sensitively with BSB than Congo red, which is a common dye used for β sheet staining. BSB has twice the fluoresence strength of Congo red. In addition, BSB is not only a staining dye, it is also able to block the amyloid formation FAP's amyloid precursor TTR. Newly developed FSB is also utilized for highly sensitive amyloid staining. This is made possible by the bromine in BSB changing to fluorine and being recovered from the fluorescence quenching caused by the heavy electron effect of bromine. From the results of the stains of Alzheimer patients' brain tissue (Fig. 1) and the heart tissue of AL amyloidosis patients (Fig 2), it appears that FSB detects amyloid deposits better than BSB.

β-sheet Staining

Chemical Structure



Protocol

1. Add 50% EtOH to the product and dilute to concentration of 0.01~0.0001% FSB solution.
2. Soak a slice in FSB stain for 30 minutes. Then soak the slice in saturated lithium carbonate for 30 minutes and wash with 50% EtOH
3. Detect stained area under UV light (V excitation)

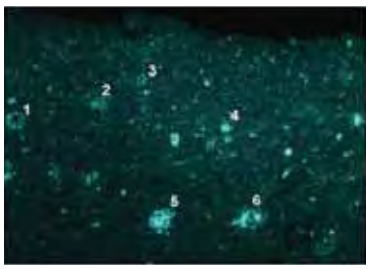


Fig. 1 Tissue staining with FSB

A stained image of a segment of the frontal cortex of a patient with Alzheimer's disease. The tissue was fixed with ethanol. The illuminated portions are amyloids. The numbers in the subadjacent slice figures correspond to each senile plaque. (Image was courteously provided by Dr. Higuchi, Dr. Saido, Laboratory for Proteolytic Neuroscience, RIKEN Brain Science Institute.)

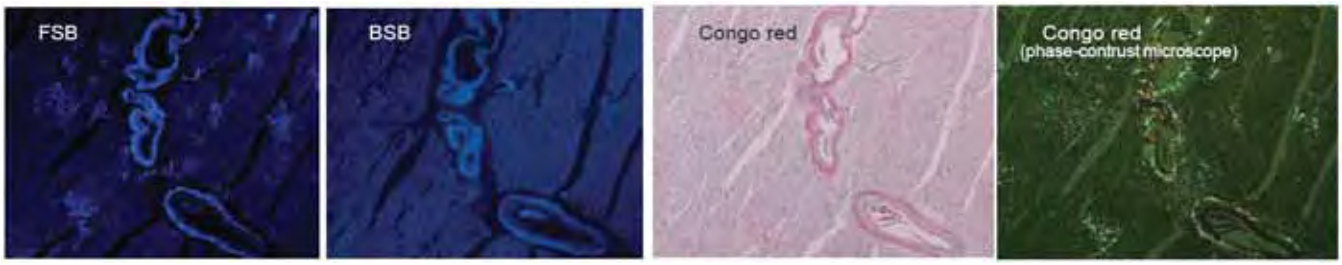


Fig. 2 Tissue staining with FSB, BSA, and Congo red

A slice of heart tissue of a patient with AL amyloidosis (Congo red is auburn colored, the illuminated portions of BSB and FSB are amyloids). These are sub-adjacent slices. It is possible to examine finer portions by FSB, and the contrast with the amyloid deposit portions are clear. (Image was courteously provided by Dr. Andoh: Department of Laboratory Medicine, Kumamoto University School of Medicine.)

References

1. K. Sato, *et al*., Fluoro-substituted and ^{13}C-labeled styrylbenzene derivatives for detecting brain amyloid plaques. *Eur J Med Chem*. 2004;**39**:573-578.
2. M. Higuchi, *et al*., ^{19}F and ^{1}H MRI detection of amyloid β plaques *in vivo*. *Nature Nurosci*. 2005;**8**:527-533.
3. M. Yamamoto, *et al*., Interferon-γ and Tumor Necrosis Factor-α Regulate Amyloid-β Plaque Deposition and β-Secretase Expression in Swedish Mutant APP Transgenic Mice. *Am J Pathol*. 2007;**170**:680-692.
4. J. Maeda, *et al*., Longitudinal, Quantitative Assessment of Amyloid, Neuroinflammation, and Anti-Amyloid Treatment in a Living Mouse Model of Alzheimer's Disease Enabled by Positron Emission Tomography. *J Neurosci*. 2007;**27**:10957-10968.
5. A. Velasco, *et al*., Detection of filamentous tau inclusions by the fluorescent Congo red derivative FSB [(*trans*,*trans*)-1-fluoro-2,5-bis(3-hydroxycarbonyl-4-hydroxy)styrylbenzene]. *FEBS Lett*. 2008;**582**:901-906.
6. B. Ji, *et al*., Imaging of Peripheral Benzodiazepine Receptor Expression as Biomarkers of Detrimental versus Beneficial Glial Responses in Mouse Models of Alzheimer's and Other CNS Pathologies. *J Neurosci*. 2008;**28**:12255-12267..

Intracellular Fluorescent Probes

Introduction

Signal transduction is one of the most important functions carried out by living cells. Information outside of a cell is usually transmitted into the cell in the form of various chemical stimulations. A living cell has many different receptors on its surface by the activation of which information is transferred to the intracellular spaces. The signal then changes to another form to process the information and cause a suitable cellular response. Second messengers play an important role in transferring information to the inside of the cell.

Change in ion concentration is a major step that helps second messengers relay information. Monitoring and control of the intracellular ion concentration is extremely important in understanding second messengers and intracellular signal transduction. Dojindo offers a wide selection of fluorescent intracellular ion probes for calcium, proton, zinc, and chloride ions.

Fluorescent Calcium Indicators

Calcium is one of the most important messengers in living cells. It carries messages that help in muscle contraction, nerve cell signal transmission, hormone secretion, immune cell activation, and other cellular functions. Dr. Tsien first developed fluorescent probes for calcium monitoring in viable cells. Calcium indicators selectively bind to calcium ions. Their fluorescent signal intensities or wavelengths change because each intracellular calcium probe has a calcium-selective chelator and a fluorescent moiety in its molecule. EGTA is a good chelator with selectivity for calcium ions 10^5 times higher than for magnesium ions. However, an EGTA molecule has two highly basic aliphatic amines that are protonated at neutral pH. This means that a deprotonation step is involved in the calcium binding process, which causes a change in the fluorescent signal. To avoid pH dependence of the fluorescent signal in calcium detection, Dr. Tsien changed the aliphatic amines of EGTA to aromatic amines, thereby transforming EGTA into a new calcium chelator, BAPTA. In designing calcium probes, the BAPTA structure is required as the calcium-binding moiety.

To prepare a calcium probe, which shifts its fluorescent wavelength and changes its fluorescent intensity upon calcium chelation, a suitable fluorescent part must be selected. When the fluorophore is a stilbene type molecule, such as Fura 2 and Indo 1, the lone electron pair on the aromatic nitrogen of the BAPTA structure conjugates the fluorophore. If the calcium ion binds to indicators, donation of the lone electron pair on the aromatic nitrogen to the calcium ion causes a change in the fluorescent signaling. In this case, the calcium concentration may be measured by ratiometry. When xanthene is combined with BAPTA, as with Fluo 3 and Rhod 2, the signal wavelength does not change; however, the signal intensity increases dramatically. This is because the conjugation of the lone electron pair on the BAPTA nitrogen is not involved in the excitation state of xanthene, but rather contributes to the resonance form, which is non-fluorescent. Thus, this type of calcium indicator is practically non-fluorescent. Once the calcium complex is formed, the contribution of the lone electron pair disappears and the original xanthene fluorescent signal is markedly recovered (as shown in Fig. 1). Xanthene has a high quantum yield and long wavelength fluorescence, thus Fluo 3 has an emission and excitation maximum of 528 nm and 490 nm, respectively; for Rhod 2, they are 581 nm and 557 nm, respectively. These excitation wavelengths are convenient when using an argon laser microscope. Therefore, this type of probe is gaining popularity with the progress in laser microscopy, especially confocal laser microscopy.

Chelators for Calcium Experiments

Dojindo offers chelators for experiments using fluorescent calcium indicators. Fluorescent calcium probes bind to some transition metal cations very tightly, and this causes a signal artifact. The zinc ion makes a fluorescent complex with calcium probes just as the calcium ion does. TPEN, a transition metal-ion-selective chelator, is often used to cancel such an artifact. We also offer calcium-selective chelators, such as EGTA, BAPTA, and BAPTA-AM, to control the intra- or extra-cellular calcium concentrations. EGTA is convenient for creating the calcium buffer used in calcium calibration.

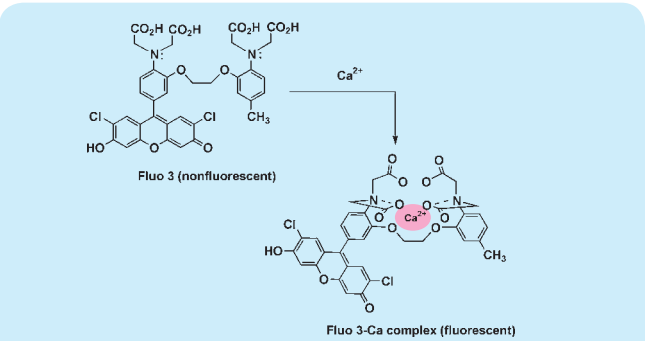


Fig. 1 Calcium complex formation

Fluorescent Zinc Indicators

Zinc is an essential metal ion, and its level is reported to be 28.5 mg per 1 g tissue in humans. However, since most zinc ions are strongly combined with proteins, the concentration of free zinc ions in a cell is at the millimolar level or lower. DNA-binding proteins combine with DNA through several motifs called zinc fingers, zinc twists, ring fingers, or zinc clusters. A shortage of zinc ions influences DNA transcription, and may have some relationship with the development of cancer. Furthermore, zinc ions are thought to have a role in controlling gestation and sexual activity. Zinc ion regulates the activities of several enzymes. Some tissues and organs, such as the brain, spermatozoa, and salivary glands, contain a large amount of zinc ions. Staining of such tissues with TSQ has demonstrated that zinc ions play very important roles in neuronal cell death, endocrine functions, and other physiological phenomena. The role of intracellular zinc ions in apoptosis has also been discussed for several decades. TSQ, Zinquin ethyl ester, and Dansylaminoethylcyclen are useful probes for these studies.

Fluorescent pH Probes

Changes in proton concentration (pH) are often coupled with many cellular events. For example, the Na/H antiporter causes intracellular pH changes. The release of some cellular materials and motility of bacterial flagella are caused by pH fluctuations around the cellular membrane. Monitoring of the intra- or extra-cellular pH is useful in understanding cell signal transduction. If the proton dissociation affects the fluorescent signal of the parent molecule, the molecule can be used to monitor the pH around its pKa value. Thus, to monitor the pH of biological samples, a fluorescent molecule that has a pKa value around 7 is needed. Carboxyfluorescein, with a pKa value of 6.3, is a classical pH indicator for physiological pH fluctuation. However, its pKa value is still a little too low for accurate monitoring of biological pH changes. Carboxyfluorescein also tends to leak from cells. Dojindo offers effective fluorescent pH probes that have good intracellular retention rates and suitable pKa values for this purpose.

Probe Loading (AM Method)

Most intracellular probes have some hydrophilic groups, carboxylates, in their molecules. Their high water solubility does not allow passage through the cell membrane because it is a hydrophobic barrier against the extracellular environment. Dr. Roger Y. Tsien developed acetoxymethyl ester (AM ester) of those probes to deal with this issue. The ester renders the molecules hydrophobic and eases the transfer of the probes into the cell membrane. The AM ester probe is easily and rapidly hydrolyzed into a hydroxymethyl ester by intracellular ubiquitous esterases. The hydroxymethyl ester spontaneously hydrolyzes and produces a parent carboxylate (Fig. 2). Thus, the original ion probe molecules accumulate in the cell simply by incubation with the AM esters since the resulting hydrophilic molecules can no longer pass through the cell membrane. This loading method, called the AM method, is an extremely simple way to load hydrophilic molecules into cells compared to other known loading techniques such as microinjection, osmotic shock, or electroporation. Dojindo offers highly purified AM esters of various intracellular ion probes.

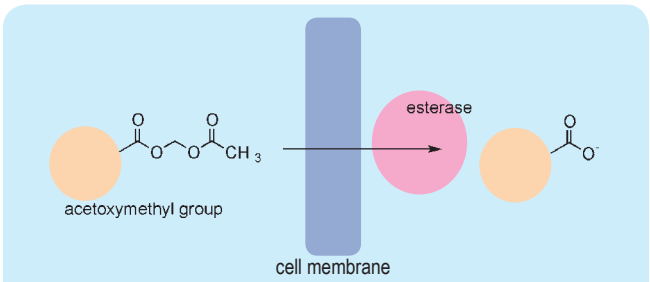


Fig. 2 Acetoxymethyl ester cleavage

Directions for AM Ester Use

AM ester is usually dispersed in dimethylsulfoxide (DMSO) and diluted with a buffer solution or a culture medium for incubation with living cells. However, because of the high hydrophobicity of AM ester, it is difficult to prepare more than 1 mM aqueous solution, even using DMSO. To prepare a high concentration AM solution, to improve its loading efficiency into living cells, surfactants or BSA are required. Such agents help the AM ester disperse into an aqueous phase. Pluronic F127 or Cremophore EL are suitable for this purpose. Although such surfactants have low cytotoxicity, they should be used at concentrations of 0.2% or less. Sometimes, sonication is necessary to obtain a well-dispersed AM ester solution. The loading of a probe is usually accomplished by 10 to 60 minutes incubation at 37°C in the CO_2 incubator. However, in some cases, incubation at a lower temperature may be more desirable to avoid the localization of the probes in cells. Please note that AM ester is sensitive to hydrolysis. Therefore, repeated freezing and thawing of the AM ester stock solution should be avoided because of the risk of humidity absorption by the DMSO solution. One approach to avoid spoiling is to divide 1 to 10 mM of AM ester in DMSO solution into individual use quantities, and keep them frozen. The incubation media should be amine and serum free. Serum contains some esterase activities, and amine tends to accelerate the hydrolysis of AM ester or reacts with AM ester to generate an amide form.

Autofluorescence

Living cells contain pyridine nucleotides and flavin nucleotides, which are fluorescent molecules. Muscle contractions or activation of metabolism in the cell can sometimes increase the fluorescence of these molecules. This autofluorescence often affects estimates of intracellular ion concentrations when fluorescent probe techniques are used. The excitation and emission wavelength of pyridine nucleotides are 340 nm and 470 nm respectively. The fluorescent spectrum of flavin nucleotides is between 300 nm and 580 nm, which can be a serious problem when using Fura 2, which has a similar excitation wavelength. Fluorescent noise from extracellular space causes another distortion. The extracellular fluorescent noise may be estimated by adding Mn^{2+} to quench the fluorescence after the experiment. Two ways to avoid these problems altogether are to use a probe with a longer fluorescent wavelength or to use a ratiometric measurement.

Ratiometry

If the excitation or emission maximum of a probe is shifted by the interaction with its target ion, it is possible to estimate near absolute intracellular ion concentration by measuring the ratio of two separate wavelength fluorescence intensities. The measurement can be independent of the sample thickness, concentration of probes loaded, leakage of probes from cells, photobleaching, or other variables. For Fura 2 and Indo 1, ratiometry may be used to measure the calcium concentration changes, while BCECF may be used for pH determination. For Fura 2 and BCECF, the excitation spectra shift. For Indo 1, the emission spectrum changes. If using digital imaging microscopy, it is more convenient to measure the emission wavelength after measuring the excitation wavelength. Fura 2 is thus the most popular calcium probe. If using a flow cytometer, which has a single excitatory light source, Indo 1 is more convenient. It should be noted that if the shape of cells changes during an experiment, ratiometry may not estimate the exact ion concentration.

Calibration

When a pH indicator is loaded into cells, its pKa value is often changed because of differences in the ionic strengths, viscosities, and other factors. Thus, calibration of the fluorescent signal is needed after the experiment. Take nigericin, for example. Adding this ionophore of monovalent cations (10 mM) and potassium in high concentration (100-150 mM) to the sample keeps the pH value the same as that of the plasma membrane. The extracellular pH is then changed, and the ratio of the two fluorescent signals is measured. Dojindo also offers a probe, BCECF, to monitor the intracellular pH gradient. The probe is useful in monitoring acidic organelles, biogenesis of lysosomes, development of autophagic vacuoles, and so on.

Fluorescent Chloride Indicator

Fluorescent chloride probes such as MQAE allow the monitoring of chloride ion in living cells. The fluorescent intensities of these chloride probes, which are based on quinoline structures, are influenced by counteranions. The chloride anion strongly suppresses fluorescent emission by quinoline. Therefore, a decreasing ratio of total fluorescence indicates an increase in the concentration of chloride ions. MQAE is cell permeable.

Inositols

Inositol phosphates and diacylglycerols are generated from phosphatidyl inositols by phospholipase C. They are involved in the movement of cellular calcium ion as second messengers. Inositol 1,4,5-triphosphate is an extremely important messenger that triggers calcium ion release from endoplasmic and sarcoplasmic reticulum. It is thought to increase calcium sensitivity of the inositol-induced calcium release (IICR) channel. Thus, the IICR channel can be opened by a low level calcium ion concentration, causing a dramatic burst of calcium into the cytosol. The released calcium ions and diacylglycerol then activate protein kinase C, a key enzyme of signal transduction. Dojindo's inositol triphosphate is chemically synthesized, and is fairly stable at room temperature in aqueous solution.

Coelenterazine-WS

Coelenterazine, β-cyclodextrin complex

Application: Luminescent calcium probe
Appearance: Slightly yellowish brown solid
Solubility (in 0.1 mol/l phosphate buffer):
To pass test (clear, slightly yellow)

Ordering Information

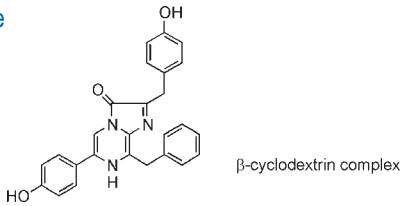
Product code	Unit
C397-10	1 mg

* Coelenterazine content: 2%

Storage Condition
-20°C, protect from light and moisture

Shipping Condition
with blue ice or dry ice

Chemical Structure



β-cyclodextrin complex

Product Description

Aequorin is a luminescent protein that contains coelenterazine as a luminescent compound. Since aequorin emits luminescence by calcium conjugation, it is used for intracellular calcium ion detection. However, coelenterazine has poor water solubility under physiological conditions, and it is adsorbed to cell membranes. Dojindo's Coelenterazine-WS is a β-cyclodextrin complex of coelenterazine with drastically improved water solubility at neutral pH.

Reference

1. K. Teranishi, *et al.*, Solubilizing Coelenterazine in Water with Hydroxypropyl-β-cyclodextrin. *Biosci Biotech Biochem.* 1997;**61**:1219-1220.

Fluo 3

1-[2-Amino-5-(2,7-dichloro-6-hydroxy-3-oxo-9-xanthenyl)phenoxy]-2-(2-amino-5-methylphenoxy)ethane-*N*,*N*,*N*',*N*'-tetraacetic acid
[CAS: 123632-39-3]

Application: Fluorescent calcium probe
Appearance: Reddish brown to dark reddish brown powder
Purity: ≥70.0 % (HPLC)
MW: 769.53, $C_{36}H_{30}Cl_2N_2O_{13}$

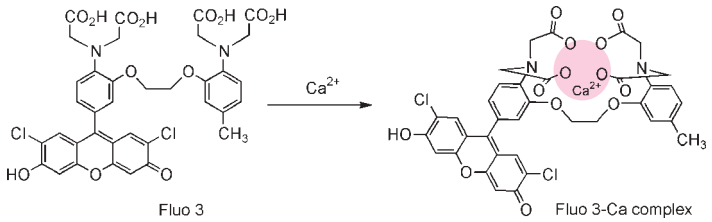
Ordering Information

Product code	Unit
F019-10	1 mg

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

Calcium Chelation



Fluo 3 Fluo 3-Ca complex

Fluo 3-AM

1-[2-Amino-5-(2,7-dichloro-6-hydroxy-3-oxo-9-xanthenyl)phenoxy]-2-(2-amino-5-methylphenoxy)ethane-*N*,*N*,*N*',*N*'-tetraacetic acid, pentaacetoxymethyl ester [CAS: 121714-22-5]

Application: Fluorescent calcium probe
Appearance: Red powder or solid
Purity: ≥85.0 % (HPLC)
MW: 1129.85, $C_{51}H_{50}Cl_2N_2O_{23}$

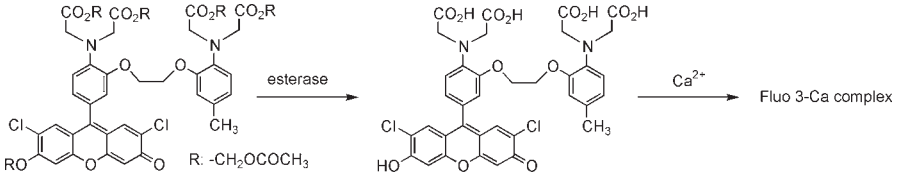
Ordering Information

Product code	Unit
F023-10	1 mg

Storage Condition
-20°C, protect from light and moisture

Shipping Condition
ambient temperature

Hydrolysis of AM ester



Product Description

Fluo 3 is a long wavelength calcium probe that is practically nonfluorescent in its free ligand form, but its fluorescence increases 60-80 times when it forms complexes with calcium. Thus, it has been widely used with confocal laser fluorescent microscopy because the microscope has an argon laser. The long wavelength of the fluorescent signal is also convenient for minimizing photodamage to sample cells. Fluo 3 is also useful for caged calcium and others that are cleaved by the photoirradiation in the UV region. Fluo 3-AM is an acetoxymethyl ester derivative of Fluo 3 that can be easily loaded into cells by incubation.

General Protocol (for Human T cells)*

Reagents:

2 mM Fluo 3-AM/DMSO (1 mg Fluo 3-AM in 442 µl DMSO)
Pluronic F127
Hanks' balanced salt solution (HBSS)
HEPES buffer saline (10 mM HEPES, 1 mM Na_2HPO_4, 137 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM glucose, 0.1% BSA, pH 7.4)

Protocol:

1. Add Pluronic F127 to Fluo 3-AM/DMSO solution to prepare 37.5 mg/ml. Pluronic F127 prevents aggregation of Fluo 3-AM in HBSS and helps uptake with cells.
2. Dilute the Fluo 3-AM solution with HBSS to prepare 4 µM Fluo 3-AM working solution.
3. Add the Fluo 3-AM working solution to the cells and incubate at 37°C for 20 minutes.
4. Add HBSS containing 1% fetal Calf serum equivalent to 5 times the volume of Fluo 3 -AM working solution (step 3).
5. Wash the cells 3 times with HEPES buffer saline. Then resuspend the cells to prepare $1x10^5$ cells per ml solution using HEPES buffer saline.
6. Incubate at 37°C for 10 minutes. Then use the cells for fluorescent calcium ion detection.
7. Monitor the fluorescence at 528 nm (excitation: 490-500 nm).

**Cell staining conditions depend on cell types, so it is necessary to optimize the conditions for each experiment.*

References

1. A. Minta, *et al*., Fluorescent Indicators for Cytosolic Calcium Based on Rhodamine and Fluorescein Chromophores. *J Biol Chem*. 1989;**264**:8171-8178.
2. J. P. Kao, *et al*., Photochemically Generated Cytosolic Calcium Pulses and Their Detection by Fluo-3. *J Biol Chem*. 1989;**264**:8179-8184.
3. M. Eberhard, *et al*., Kinetics of Calcium Binding to Fluo-3 by Stopped-Flow Fluorescence. *Biochem Biophys Res Commun*. 1989;**163**:309-314.
4. A. Hernandez-Cruz, *et al*., Subcellular Calcium Transients Visualized by Confocal Microscopy in a Voltage-clamped Vertebrate Neuron. *Science*. 1990;**247**:858-862.
5. A. H. Cornell-Bell, *et al*., Glutamate Induces Calcium Waves in Cultured Astrocytes: Long-Range Glial Signaling. *Science*. 1990;**247**:470-473.
6. D. A. Williams, Quantitative Intracellular Calcium Imaging with Laser-scanning Confocal Microscopy. *Cell Calcium*. 1990;**11**:589-597.
7. D. A. Williams, *et al*., Confocal Imaging of Ionised Calcium in Living Plant Cells. *Cell Calcium*. 1990;**11**:291-297.
8. P. A. Vandenberghe, *et al*., Flow Cytometric Measurement of Cytoplasmic Free Calcium in Human Peripheral Blood T Lymphocytes with Fluo-3, A New Fluorescent Calcium Indicator. *J Immunol Methods*. 1990;**127**:197-205.
9. M. Iino, *et al*., Visualization of Neural Control of Intracellular Ca^{2+} Concentration in Single Vascular Smooth Muscle Cells in situ. *EMBO J*. 1994;**13**:5026-5031.
10. M. E. Dailey, *et al*., Spontaneous Ca^{2+} Transients in Developing Hippocampal Pyramidal Cells. *J Neurobiol*. 1994;**25**:243-251.

11. M. Burnier, *et al*., Confocal Microscopy to Analyze Cytosolic and Nuclear Calcium in Cultured Vascular Cells. *Am J Physiol*. 1994;**266**:C1118-C1127.
12. E. Donnadieu, *et al*., Ca^{2+} Signaling in Endothelial Cells Stimulated by Bradykinin: Ca^{2+} Measurement in the Mitochondria and the Cytosol by Confocal Microscopy. *Cell Calcium*. 1996;**20**:53-61.
13. M. Ikeda, *et al*., Separate Analysis of Nuclear and Cytosolic Ca^{2+} Concentrations in Human Umbilical Vein Endothelial Cells. *J Cell Biochem*. 1996;**63**:23-36.
14. J. E. Merritt, *et al*., Use of fluo-3 to Measure Cytosolic Ca^{2+} in Platelets and Neutrophils Loading Cells with the Dye, Calibration of Traces, Measurements in the Presence of Plasma, and Buffering of Cytosolic Ca^{2+}. *Biochem J*. 1990;**269**:513-519.

Fura 2

1-[6-Amino-2-(5-carboxy-2-oxazolyl)-5-benzofuranyloxy]-2-(2-amino-5-methylphenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, pentapotassium salt [CAS: 96314-98-6]

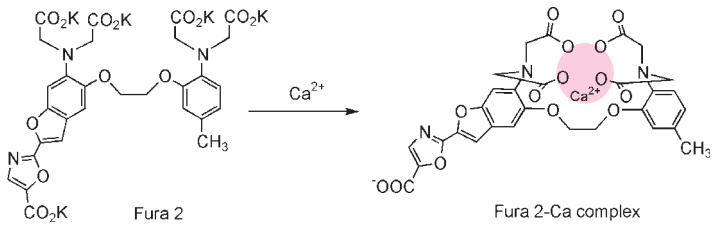
Application: Fluorescent calcium probe
Appearance: Yellow or yellowish-orange powder
Purity: ≥98.0% (HPLC)
MW: 831.99, $C_{29}H_{22}K_5N_3O_{14}$

Ordering Information

Product code	Unit
F014-10	1 mg

Storage Condition: ambient temperature
Shipping Condition: ambient temperature

Calcium Chelation



Fura 2-AM

1-[6-Amino-2-(5-carboxy-2-oxazolyl)-5-benzofuranyloxy]-2-(2-amino-5-methylphenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid, pentaacetoxymethyl ester [CAS: 108964-32-5]

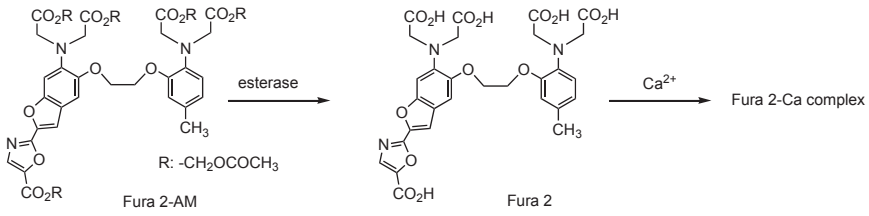
Application: Fluorescent calcium probe
Appearance: Yellow to yellowish orange solid
Purity: ≥98.0% (HPLC)
MW: 1001.85, $C_{44}H_{47}N_3O_{24}$

Ordering Information

Product code	Unit
F015-10	1 mg

Storage Condition: -20°C, protect from light and moisture
Shipping Condition: ambient temperature

Hydrolysis of AM ester



Product Description

Fura 2 was developed to improve the fluorescent properties of Quin 2. The signal intensity in 1 mM of loaded Fura 2 corresponds to that of 30 mM of loaded Quin 2. This allows an experiment at a lower concentration of indicator using Fura 2 instead of Quin 2. Fura 2 is one of the most widely used calcium indicators for ratiometric measurement. Many types of instrumentation are now available for experiments using Fura 2, but Fura 2 is especially suitable for digital imaging microscopy. It is less susceptible to photobleaching than Indo 1. Changes in the cell shape can sometimes affect the fluorescent ratio at 340 nm and 380 nm. For example, fluorescent signal intensities at these wavelengths

sometimes decrease simultaneously with smooth muscle contraction. For blood vessels, however, the increase of the signal intensity at 340 nm tends to be smaller on contraction, while the decrease of the signal intensity at 380 nm tends to be larger with its contraction. Fura 2-AM is an acetoxymethyl ester derivative of Fura 2 that can be easily loaded into cells by incubation.

General Protocol (for NG108-15 neuron cell line)*

Reagents:

1 mM Fura 2-AM/DMSO (1 mg Fura 2-AM in 1 ml DMSO)
Hanks' balanced salt solution (HBSS)
HEPES buffer saline (20 mM HEPES, 115 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgCl_2$, 13.8 mM glucose, pH 7.4)

Protocol:

1. Culture cells on a glass-bottom dish using DMEM containing 5% fetal calf serum.
2. Change the medium to 1 mM dibutyl cAMP/DMEM, and culture the cells for 3-4 days to induce dendrites.
3. Dilute 20 µl of 1 mM Fura 2-AM DMSO solution with 20 ml of HEPES buffer saline to prepare 1 µM Fura 2-AM working solution.
   **Keep the Fura 2-AM working solution warm at 37°C.* Make sure to disperse Fura 2-AM in the solution by a ultrasonication or an addition of Chremophor® EL or Pluronic® F127 (final conc.: 0.02%).
4. Remove the culture medium, and add 0.5 ml of the Fura 2-AM working solution to the cells.
5. Incubate for 20 minutes. Then remove the Fura 2-AM working solution.
6. Wash the cells once with HEPES buffer saline. Then incubate the cells for 1 hour in the HEPES buffer saline.
7. Use the cells for fluorescent calcium ion detection.
8. Monitor the excitation spectra at 380 nm (calcium free) and 340 nm (calcium complex) with fixed emission at 510 nm.
   **Cell staining conditions differ by cell types, so it is necessary to optimize the conditions for each experiment.*

References

1. G. Grynkiewicz, *et al*., A New Generation of Ca^{2+} Indicators with Greatly Improved Fluorescence Properties. *J Biol Chem*. 1985;**260**:3440-3450.
2. D. A. Williams, *et al*., Calcium Gradients in Single Smooth Muscle Cells Revealed by the Digital Imaging Microscope Using Fura-2. *Nature*. 1985;**318**:558-561.
3. R. Y. Tsien, *et al*., Measurement of Cytosolic Free Ca^{2+} in Individual Small Cells Using Fluorescence Microscopy with Dual Excitation Wavelengths. *Cell Calcium*. 1985;**6**:145-157.
4. D. A. Williams, *et al*., Intracellular Calibration of the Fluorescent Calcium Indicator Fura-2. *Cell Calcium*. 1990;**11**:75-83.
5. W. Almers, *et al*., The Ca Signal from Fura-2 Loaded Mast Cells Depends Strongly on the Method of Dye-loading. *FEBS Lett*. 1985;**192**:13-18.
6. G. H. Rao, *et al*., Measurement of Ionized Calcium in Blood Platelets with a New Generation Calcium Indicator", *Biochem Biophys Res Commun*. 1985;**132**:652-657.
7. H. Ozaki, *et al*., Simultaneous Recordings of Calcium Signals and Mechanical Activity Using Fluorescent Dye Fura 2 in Isolated Strips of Vascular Smooth Muscle. *Jpn J Pharmacol*. 1987;**45**:429-433.
8. M, Mitsui, *et al*., Leakage of the Fluorescent Ca^{2+} Indicator Fura-2 in Smooth Muscle. *Jpn J Pharmacol*. 1993;**61**:165-170.

Indo 1

1-[2-Amino-5-(6-carboxy-2-indolyl)phenoxy]-2-(2-amino-5-methylphenoxy)ethane-*N*,*N*,*N*',*N*'-tetraacetic acid, pentapotassium salt [CAS: 96314-96-4 (free acid)]

Application: Fluorescent calcium probe
Appearance: Slightly yellowish-white powder
Purity: ≥95.0% (HPLC)
MW: 840.05, $C_{32}H_{26}K_5N_3O_{12}$

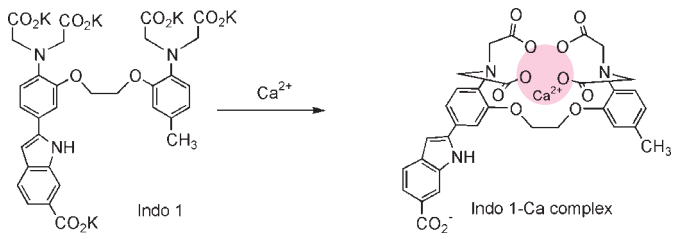
Ordering Information

Product code	Unit
I004-10	1 mg

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

Calcium Chelation



Indo 1-AM

1-[2-Amino-5-(6-carboxy-2-indolyl)phenoxy]-2-(2-amino-5-methylphenoxy)ethane-*N*,*N*,*N*',*N*'-tetraacetic acid, pentaacetoxymethyl ester [CAS: 112926-2-0]

Application: Fluorescent calcium probe
Appearance: Greenish brown solid
Purity: ≥97.0% (HPLC)
MW: 1009.91, $C_{47}H_{51}N_3O_{22}$

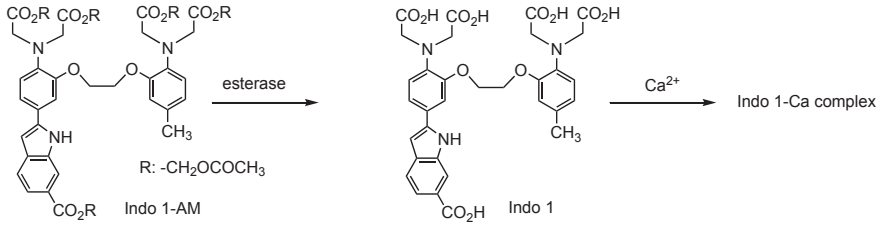
Ordering Information

Product code	Unit
I005-10	1 mg

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

Hydrolysis of AM ester



Product Description

Indo 1 is another type of improved calcium indicator that can be used in ratiometry, including when using a flow cytometer. Two separate wavelengths in the emission spectrum, usually at 410 nm and 480 nm, are measured in the ratiometry of Indo 1. Indo 1 is reported to have better properties for localization than Fura 2 after being loaded into cells. Indo 1-AM is an acetoxymethyl ester derivative of Indo 1 that can be loaded into cells by incubation.

References

1. R. Y. Tsien, New Calcium Indicators and Buffers with High Selectivity against Magnesium and Protons: Design, Synthesis, and Properties of Prototype Structures. *Biochemistry*. 1980;**19**:2396-2404.
2. G. Grynkiewicz, *et al*., A New Generation of Ca^{2+} Indicators with Greatly Improved Fluorescence Properties. *J Biol Chem*. 1985;**260**:3440-3450.
3. A. Luckhoff, Measuring Cytosolic Free Calcium Concentration in Endothelial Cells with Indo-1: the Pitfall of Using the Ratio of Two Fluorescence Intensities Recorded at Different Wavelengths. *Cell Calcium*. 1986;**7**:233-248.
4. K. G. Lazzari, *et al*., Simultaneous Measurement of Stimulus-induced Changes in Cytoplasmic Ca^{2+} and in Membrane Potential of Human Neutrophils. *J Biol Chem*. 1986;**261**:9710-9713.
5. M. K. Bijsterbosch, *et al*., Cross-linking of Surface Immunoglobulin on B Lymphocytes Induces Both Intracellular Ca^{2+} Release and Ca^{2+} Influx:Analysis with Indo-1. *Biochem Biophys Res Commun*. 1986;**137**:500-506.
6. P. S. Rabinovitch, *et al*., Heterogeneity Among T Cells in Intracellular Free Calcium Responses After Mitogen Stimulation with PHA or Anti-CD3. Simultaneous Use of Indo-1 and Immunofluorescence with Flow Cytometry. *J Immunol*. 1986;**137**:952-961.
7. T. Sugiyama, *et al*., The Elevation of the Cytoplasmic Calcium Ions in Vascular Smooth Muscle Cells in SHR Measurement of the Free Calcium Ions in Single Living Cells by Lasermicrofluorospectrometry. *Biochem Biophys Res Commun*. 1986;**141**:340-345.
8. A. P. Jackson, *et al*., The Kinetics of Calcium Binding to Fura-2 and Indo-1. *FEBS Lett*. 1987;**216**:35-39.
9. D. S. Bush, *et al*., Measurement of Cytoplasmic Ca^{2+} and H^+ in Barley Aleurone Protoplasts. *Plant Cell Tissue Organ Cult*. 1988;**12**:159-162.
10. K. Higashi, *et al*., Ethanol Causes Desensitization of Receptor-mediated Phospholipase C Activation in Isolated Hepatocytes. *J Biol Chem*. 1991;**266**:2178-2190.
11. W. J. Scheenen, *et al*., Photodegradation of Indo-1 and Its Effect on Apparent Ca^{2+} Concentraions. *Chem Biol*. 1996;**3**:765-774.

Quin 2

8-Amino-2-[(2-amino-5-methylphenoxy)methyl]-6-methoxyquinoline-*N*,*N*,*N*',*N*'-tetraacetic acid, tetrapotassium salt [CAS: 73630-23-6]

Application: Fluorescent calcium probe
Appearance: Pale yellow powder
Purity: ≥95.0% (HPLC)
MW: 693.87, $C_{26}H_{23}K_4N_3O_{10}$

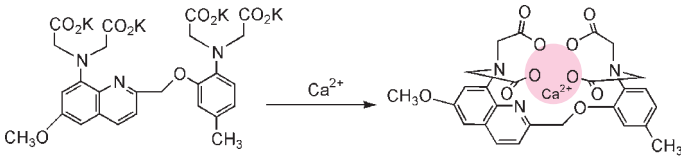
Ordering Information

Product code	Unit
Q001-10	100 mg

Storage Condition
ambient temperature,
protect from light and moisture

Shipping Condition
ambient temperature

Calcium Chelation



Quin 2 Quin 2-Ca complex

Product Description

Quin 2 forms a stable fluorescent complex with calcium (logKCaY=7.1), but not with magnesium (logMgY=2.7). The complex has a high quantum yield (0.14) at emission wavelength 525 nm, excitation wavelength 339 nm, and emission wavelength 492 nm. Quin 2-AM is an acetoxymethyl ester derivative of Quin 2 that is readily permeable through cell membranes. Within the cell, the ester is hydrolyzed to Quin 2.

References

1. R. Y. Tsien, New Calcium Indicators and Buffers with High Selectivity against Magnesium and Protons: Design, Synthesis, and Properties of Prototype Structures. *Biochemistry*. 1980;**19**:2396-2404.
2. R. Y. Tsien, *et al*., Calcium Homeostasis in Intact Lymphocytes: Cytoplasmic Free Calcium Monitored with a New, Intracellularly Trapped Fluorescent Indicator. *J Cell Biol*. 1982;**94**:325-334.
3. M. B. Feinstein, *et al*., The Cytoplasmic Concentration of Free Calcium Inplatelets Is Controlled by Stimulators of Cyclic AMP Production (PGD2, PGE1, FORSKOLIN). *Biochem Biophys Res Commun*. 1983;**113**:598-604.
4. N. Miyoshi, *et al*., A New Method of Determining Intracellular Free Ca^{2+} Concentration Using Quin2-fluorescence. *Photochem Photobiol*. 1991;**53**:415-418.

Rhod 2

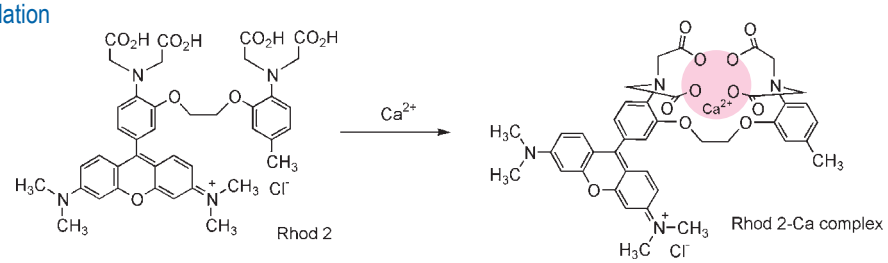
1-[2-Amino-5-(3-dimethylamino-6-dimethylammonio-9-xanthenyl)phenoxy]-2-(2-amino-5-methylphenoxy)ethane-*N*,*N*,*N*',*N*'-tetraacetic acid, chlorid [CAS: 132523-91-2]

Application: Fluorescent calcium probe
Appearance: Dark purple solid
Purity: ≥60.0% (HPLC)
MW: 791.24, $C_{40}H_{43}ClN_4O_{11}$

Ordering Information	
Product code	Unit
R001-10	1 mg

Storage Condition	Shipping Condition
-20°C, protect from light	ambient temperature

Calcium Chelation



Rhod 2 Rhod 2-Ca complex

Rhod 2-AM

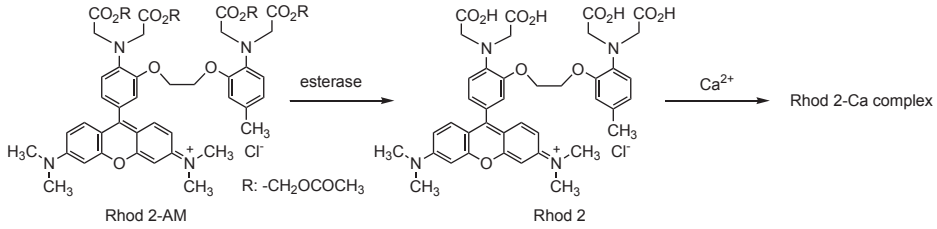
1-[2-Amino-5-(3-dimethylamino-6-dimethylammonio-9-xanthenyl)phenoxy]-2-(2-amino-5-methylphenoxy)ethane-*N*,*N*,*N*',*N*'-tetraacetic acid, tetraacetoxymethyl ester, chloride [CAS: 129787-64-0]

Application: Fluorescent calcium probe
Appearance: Dark purple solid
MW: 1079.49, $C_{52}H_{59}ClN_4O_{19}$

Ordering Information	
Product code	Unit
R002-10	1 mg

Storage Condition	Shipping Condition
-20°C, protect from light and moisture	ambient temperature

Hydrolysis of AM ester



Product Description

Rhod 2 has the longest fluorescent wavelength signal of all the calcium indicators. It has a rhodamine-like fluorophore whose excitation and emission maxima are 557 nm and 581 nm, respectively. This makes it a convenient excitation source for argon and krypton lasers. Although it was thought that the fluorescent signal of Rhod 2 only increases several times with the calcium complex, Dojindo's Rhod 2 increases its signal with calcium 80-100 times because of its high purity. Its signal intensity is the strongest of all the calcium probes. Rhod 2 is thus highly recommended as a probe for intracellular calcium monitoring using laser microscopes. Rhod 2 is reported to have a better loading profile at the point of localization, especially in neural slice cultures. The dissociation constant of Rhod 2 with calcium (Kd=1.0 mM) is the highest of all the fluorescent calcium probes, providing a wider range for monitoring calcium concentration. Rhod 2-AM is an acetoxymethyl ester derivative of Rhod 2 that can be easily loaded into cells using the AM method.

References

1. M. Burnier, *et al*., Confocal Microscopy to Analyze Cytosolic and Nuclear Calcium in Cultured Vascular Cells. *Am J Physiol*. 1994;**266**:C1118-C1127.
2. J. Vergara, *et al*., Imaging of Calcium Transients in Skeletal Muscle Fibers. *Biophys J*. 1991;**59**:12-24.
3. T. Meyer, *et al*., Kinetics of Calcium Channel Opening by Inositol 1, 4, 5-Trisphosphate. *Biochemistry*. 1990;**29**:32-37.
4. A. Minta, *et al*., Fluorescent Indicators for Cytosolic Calcium Based on Rhodamine and Fluorescein Chromophores. *J Biol Chem*. 1989;**264**:8171-8178.
5. D. R. Trollinger, *et al*., Selective Loading of Rhod 2 into Mitochondria Shows Mitochondrial Ca^{2+} Transients during the Contractile Cycle in Adult Rabbit Cardiac Myocytes. *Biochem Biophys Res Commun*. 1997;**236**:738-742.

BAPTA

O,*O*'-Bis(2-aminophenyl)ethyleneglycol-*N*,*N*,*N*',*N*'-tetraacetic acid, tetrapotassium salt, hydrate [CAS: 85233-19-8(free acid)]

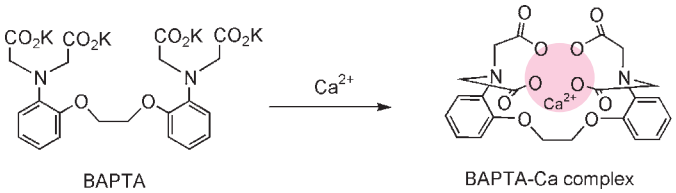
Application: Calcium masking
Appearance: White powder or crystalline powder
Purity: ≥95.0% (Titration)
MW: 628.79, $C_{22}H_{20}K_4N_2O_{10}$

Storage Condition	Shipping Condition
ambient temperature	ambient temperature

Ordering Information

Product code	Unit
B019-10	500 mg

Calcium Chelation



BAPTA-AM

O,O'-Bis(2-aminophenyl)ethyleneglycol-*N,N,N',N'*-tetraacetic acid, tetraacetoxymethyl ester [CAS: 126150-97-8]

Application: Calcium masking
Appearance: White powder
Purity: ≥98.0% (HPLC)
MW: 764.68, $C_{34}H_{40}N_2O_{18}$

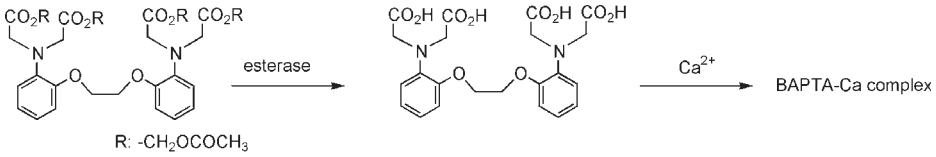
Ordering Information

Product code	Unit
B018-10	25 mg

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

Hydrolysis of AM ester



Product Description

BAPTA is a calcium-selective chelator developed by Dr. Tsien. It has $\log K_{Ca}$=6.97 and $\log K_{Mg}$=1.77. The basic chelating unit resembles that of EGTA, but the two aliphatic nitrogen atoms are replaced by aromatic nitrogen. Thus, BAPTA is not protonated at physiological pH. BAPTA possesses pKa_3=5.47 and pKa_4=6.36. This property indicates that the deprotonation step is not included in its calcium complexation step, and it has a higher complexation rate than EGTA because it is not affected by proton interference. BAPTA-AM is an acetoxymethyl ester derivative of BAPTA that can be easily loaded into cells using the AM method. BAPTA-AM is useful for controlling the intracellular calcium concentration.

References

1. R. Y. Tsien, New Calcium Indicators and Buffers with High Selectivity against Magnesium and Protons: Design, Synthesis, and Properties of Prototype Structures. *Biochemistry*. 1980;**19**:2396-2404.
2. R. Y. Tsien, A Non-disruptive Technique for Loading Calcium Buffers and Indicators into Cells. *Nature*. 1981;**290**:527-528.
3. J. I. Korenbrot, *et al*., The Use of Tetracarboxylate Fluorescent Indicators in the Measurement and Control of Intracellular Free Calcium Ions. *Soc Gen Physiol Ser*. 1986;**40**:347-363.
4. S. M. Harrison, *et al*., The Effect of Temperature and Ionic Strength on the Apparent Ca-affinity of EGTA and the Analogous Ca-chelators BAPTA and Dibromo-BAPTA. *Biochim Biophys Acta*. 1987;**925**:133-143.
5. E. W. Gelfand, *et al*., Dissociation of Unidirectional Influx of External Ca2+ and Release from Internal Stores in Activated Human T Lymphocytes. *Eur J Immunol*. 1990;**20**:1237-1241.
6. J. P. Kao, *et al*., Active Involvement of Ca^{2+} in Mitotic Progression of Swiss 3T3 Fibroblasts. *J Cell Biol*. 1990;**111**:183-196.
7. M. L. Schubert, *et al*., Functionally Distinct Muscarinic Receptors on Gastric Somatostatin Cells. *Am J Physiol*. 1990;**258**:G982-G987.
8. Y. Tojyo, *et al*., Inhibitory Effects of Loading with the Calcium-chelator 1, 2-Bis(o-aminophenoxy)ethane-N, N, N', N'-tetraacetic acid (BAPTA) on Amylase Release and Cellular ATP Level in Rat Parotid Cells. *Biochem Pharmacol*. 1990;**39**:1775-1779.

GEDTA (EGTA)

O,O'-Bis(2-aminoethyl)ethyleneglycol-*N,N,N',N'*-tetraacetic acid [CAS: 67-42-5]

Application: Calcium masking
Appearance: White crystalline powder
Purity: ≥97.0% (Titration)
MW: 380.35, $C_{14}H_{24}N_2O_{10}$

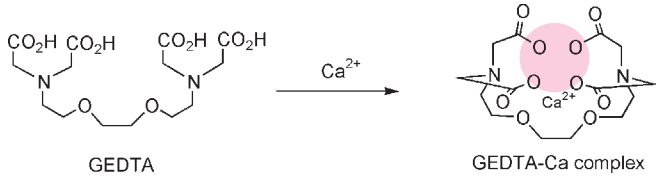
Ordering Information

Product code	Unit
G002-12	100 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Calcium Chelation



Product Description
EGTA is the most widely used calcium- selective chelator. The calcium complex of EGTA is 100,000 times more stable than its Mg complex. It is utilized to prepare calcium buffers and control the calcium ion concentration.

References

1. C. L. Luke, *et al*., Photometric Determination of Magnesium in Electronic Nickel. *Anal Chem*. 1954;**26**:1778-1780.
2. F. S. Sadek, *et al*., Visual EGTA Titration of Calcium in Thepresence of Magnesium. *Talanta*. 1959;**2**:38-51.
3. R. Pribil, *et al*., Contributions to the Basic Problems of Complexometry-XX Determination of Calcium and Magnesium. *Talanta*. 1966;**13**:233-236.
4. T. Tatsumi, *et al*., Hypochlorous Acid Mobilizes Intracellular Zinc in Isolated Rat Heart Myocytes. *J Mol Cell Cardiol*. 1994;**26**:471-479.
5. H. Ohata, *et al*., Confocal Imaging Analysis of ATP-Induced Ca^{2+} Response in Individual Endothelial Cells of the Artery in Situ. *Am J Physiol*. 1997;**272**:C1980-C1987.
6. I. Sakabe, *et al*., Induction of Apoptosis in Neuro-2A Cells by Zn^{2+} Chelating. *Cell Struct Funct*. 1998;**23**:95-99.

Dansylaminoethyl-cyclen

1-[2-[5-(Dimethylamino)-1-naphthalenesulfonamido]ethyl]-1,4,7,10,-tetraazacyclo-dodecane, tetrahydrochloride, dihydrate
[CAS: 184537-03-9]

Application: Fluorescent zinc probe, cell staining
Appearance: Slightly yellow crystalline powder
Purity: ≥95.0% (HPLC)
MW: 630.50, $C_{22}H_{40}Cl_4N_6O_2S.\ 2H_2O$

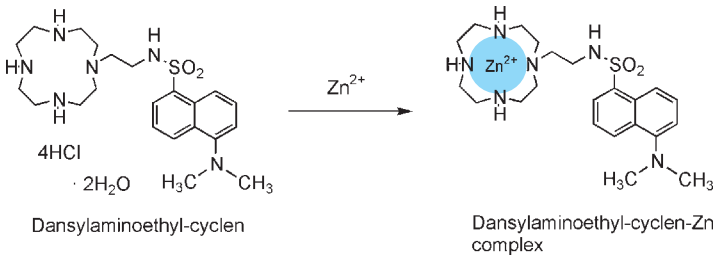
Ordering Information

Product code	Unit
D480-10	5 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Zinc Chelation



Dansylaminoethyl-cyclen

Dansylaminoethyl-cyclen-Zn complex

Product Description
Dansylaminoethyl-cyclen is a water-soluble and cell-membrane-permeable fluorescent zinc indicator developed by Dr. E. Kimura. The aqueous solution of this reagent allows intracellular zinc ion to be monitored. The fluorescence intensity of the complex is 5 times that of the free ligand. Most other cations do not form fluorescent complexes with this reagent. Although the cadmium ion forms a fluorescent complex, the stability constant of this complex is one tenth that of the zinc complex. Thus, cadmium ion does not affect zinc detection in most cases. Copper ion (Cu(II)), which forms a non-fluorescent complex, also does not affect zinc detection because it is extremely rare as a free ion in normal living cells. Recently, the zinc ion has gained a lot of attention; it is thought to play an important role in ischemia and subsequent selective neuronal cell death. Reactive oxygen species might damage cystein-residues in a zinc-finger motif to produce free zinc ions.

References

1. T. Koike, *et al*., A Novel Biomimetic Zinc(II)-fluorohphore, Dansylamidoethyl-pendant Macrocyclic Tetraamine 1, 4, 7, 10-Tetraazacyclododecane (Cyclen). *J Am Chem Soc*. 1996;**118**:12696-12703.
2. E. Kimura, *et al*., A Macrocyclic Zinc(II) Fluorophore as a Detector of Apoptosis. *PNAS*. 2003;**100**:3731-3736.

Zinquin ethyl ester

Ethyl [[2-methyl-8-[[(4-methylphenyl)sulfonyl]amino]-6-quinolinyl]oxy]acetate [CAS: 151606-29-0]

Application: Fluorescent zinc probe, cell staining
Appearance: White or slightly blue solid
Purity: ≥98.0% (HPLC)
MW: 414.48, $C_{21}H_{22}N_2O_5S$

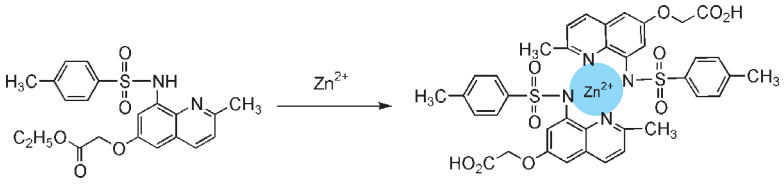
Ordering Information

Product code	Unit
Z215-10	1 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Zinc Chelation



Zinquin ethyl ester　　Zinquin-Zn complex

Product Description

Zinquin ethyl ester is an analog of the widely used indicator TSQ. Although Zinquin ethyl ester itself is fluorescent, its fluorescence intensity is negligibly weak (1/30). Zinquin ethyl ester is membrane permeable, as are acetoxymethyl esters of calcium probes such as Fura 2-AM and Fluo 3-AM. Zinquin ethyl ester is thus useful to detect intracellular zinc ions. It forms a complex with a zinc ion with nitrogen atoms in the structure. This compound also forms a fluorescent complex with cadmium ion; however, detectable amounts of cadmium ions are not contained in normal living cells. Because the water solubility of Zinquin ethyl ester is poor, dimethylsulfoxide (DMSO) or ethanol is required as a solvent for preparing the stock solution.

General Protocol for Lymphoblastoid Cells*

Reagents:

2.4 mM Zinquin ethyl ester stock solution (1 mg Zinquin ethyl ester in 1 ml DMSO)
Zinquin ethyl ester stock solution is stable for 1 month at -20°C.

Protocol:

1. Suspend cells in Hanks' balanced salt solution (HBSS) to prepare 5-10 x 10^6 cells per ml medium.
2. Add Zinquin ethyl ester stock solution to the cell suspension to prepare 2.4 µM Zinquin ethyl ester (1/1000 vol of the cell suspension) as the final concentration.
3. Incubate the cell suspension at 37°C for 30 minutes.
4. Wash the cells 3 times with HBSS and then prepare 2-5 x 10^6 cells per ml of cell suspension.
5. Determine the fluorescence intensity of each cell using a fluorescence microscope or a confocal laser microscope coupled with an image analyzer.

** Cell staining conditions depend on cell type, so it is necessary to optimize the conditions for each experiment.*

References

1. P. Coyle, *et al*., Measurement of Zinc in Hepatocytes by Using a Fluorescent Probe, Zinquin: Relationship to Metallothionein and Intracellular Zinc. *Biochem J*. 1993;**303**:781-786.
2. P. D. Zalewski, *et al*., Correlation of Apoptosis with Change in Intracellular Labile Zn(II) Using Zinquin [(2-Methyl-8-p-Toluensulphonamido-6-Quinolyloxy)-Acetic Acid], a New Specific Fluorescent Probe for Zn(II). *Biochem J*. 1993;**296**:403-408.
3. P. D. Zalewski, *et al*., Video Image Analysis of Labile Zinc in Viable Pancreatic Islet Cells Using a Specific Fluorescent Probe for Zinc. *J Histochem Cytochem*. 1994;**42**:877-884.
4. P. Coyle, *et al*., Measurement of Zinc in Hepatocytes by Using a Fluorescent Probe, Zinquin: Relationship to Metallothionein and Intracellular Zinc. *Biochem J*. 1994;**303**:781-786.
5. P. D. Zalewski, *et al*., Flux of Intracellular Labile Zinc During Apoptosis (Gene-Directed Cell Death) Revealed by a Specific Chemical Probe, Zinquin. *Chem Biol*. 1994;**1**:153-161.
6. I. A. Brand, *et al*., Intercellular Zinc Movement and Its Effect on the Carbohydrate Metabolism of Isolated Rat Hepatocytes. *J Biol Chem*. 1996;**271**:1941-1949.
7. N. Wellinghausen, *et al*., Interaction of Zinc Ions with Human Peripheral Blood Mononuclear Cells. *Cell Immunol*. 1996;**171**:255-261.
8. P. D. Zalewski, *et al*., Changes in Distribution of Labile Zinc in Mouse Spermatozoa During Maturation in the Epididymis Assessed by the Fluorophore Zinquin. *Reprod Fertil Dev*. 1996;**8**:1097-1105.
9. R. D. Palmiter, *et al*., ZnT-2, a Mammalian Protein That Confers Resistance to Zinc by Facilitating Vesicular Sequestration. *EMBO J*. 1996;**15**:1784-1791.
10. D. Berendji, *et al*., Nitric Oxide Mediates Intracytoplasmic and Intranuclear Zinc Release. *FEBS Lett*. 1997;**405**:37-41.
11. M. Tsuda, *et al*., Expression of Zinc Transporter Gene, ZnT-1, Is Induced After Transient Forebrain Ischemia in the Gerbil. *J Neurosci*. 1997;**17**:6678-6684.

TPEN *N,N,N',N'*-Tetrakis(2-pyridylmethyl)ethylenediamine [CAS: 16858-02-9]

Application: Heavy metal ion masking
Appearance: White or slightly yellow crystalline powder
Purity: ≥98.0% (Titration)
MW: 424.54, $C_{26}H_{28}N_6$

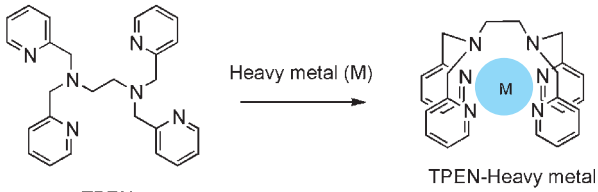
Ordering Information

Product code	Unit
T040-10	100 mg

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Heavy Metal Chelation



TPEN

TPEN-Heavy metal complex

Product Description
TPEN is a highly selective chelator of transition metal cations because the pyridine groups act as soft electron donators. TPEN is thus utilized for masking transition metal cations in fluorescent calcium monitoring. The masking function of the zinc cation is important for canceling the Fluorescent effect. TPEN is water-soluble and cell membrane permeable. Maximum wavelength is 260 nm. The physical properties of TPEN are as follows: pKa_1=10.27, pKa_2=3.32, pKa_3=4.85, and pKa_4=7.19; $LogK_{Mn}$=10.27, $logK_{Fe}$=14.61, $logK_{Zn}$=15.58, $logK_{Mg}$=1.7, and $logK_{Ca}$=4.4.

References

1. G. Anderegg, *et al*., Pyridinderivate Als Komplexbildner VIII Die Herstellung Je Eines Neuen Vier- und Sechszahnigen Liganden. *Helv Chim Acta*. 1967;**50**:2330-2332.
2. G. Anderegg. *et al*., Pyridinderivate als Komplexbildner. XI). Die Thermodynamik der Metallkomplexbildung mit Bis-, Tris- und Tetrakis[(2-pyridyl) methyl]-aminen. *Helv Chim Acta*. 1977;**60**:123-140.
3. P. Arslan, *et al*., Cytosolic Ca^{2+} Homeostasis in Ehrlich and Yoshida Carcinomas A, New Membrane-Permeant Chelator of Heavy Metals Reveals That These Ascites Tumor Cell Lines Have Normal Cytosolic Free Ca^{2+}. *J Biol Chem*. 1985;**260**:2719-2727.
4. T. Tatsumi, *et al*., Hypochlorous Acid Mobilizes Intracellular Zinc in Isolated Rat Heart Myocytes. *J Mol Cell Cardiol*. 1994;**26**:471-479.
5. I. Sakabe, *et al*., Induction of Apoptosis in Neuro-2A Cells by Zn^{2+} Chelating. *Cell Struct Funct*. 1998;**23**:95-99.

BCECF 2',7'-Bis(carboxyethyl)-4 or 5-carboxyfluorescein [CAS: 85138-49-4]

Application: Fluorescent pH probe
Appearance: Reddish-brown or red crystalline powder
Purity: ≥85.0% (HPLC)
MW: 520.44, $C_{27}H_{20}O_{11}$

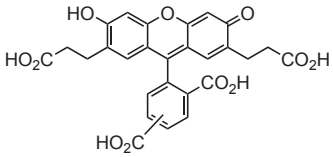
Ordering Information

Product code	Unit
B031-10	5 mg

Storage Condition
ambient temperature, protect from light

Shipping Condition
ambient temperature

Chemical Structure



BCECF-AM 3'-*O*-Acetyl-2',7'-bis(carboxyethyl)-4 or 5-carboxyfluorescein, diacetoxymethyl ester [CAS:117464-70-7]

Application: Fluorescent pH probe
Appearance: Orange or orange-brown solid
Purity: ≥90.0% (HPLC)
MW: 688.59, $C_{35}H_{28}O_{15}$

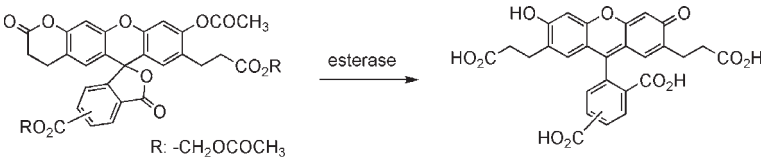
Ordering Information

Product code	Unit
B262-10	1 mg

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

Zinc Chelation



BCECF-AM BCECF

Product Description

BCECF is the most widely used intracellular pH probe. Dr. Tsien and others improved this carboxyfluorescein by introducing two extra carboxylates that allow it to be retained better by the cell. BCECF is highly water-soluble because it has 4 to 5 negative charges at neutral pH; it becomes difficult to pass through the cell membrane after loading. Its pKa value, 6.97, is higher than that of carboxyfluorescein. BCECF has an isosbestic point at 439 nm in the excitation spectra, so it can be used in ratiometry, similar to Fura 2. Wavelengths of 505 nm and 439 nm are usually used for the ratiometric assay, and 490 nm and 450 nm filters are set in front of the excitation light source. The 530 nm filter is used for its fluorescent signal. Please note that the excitation spectrum is slightly different from the absorption spectra. BCECF-AM is an acetoxymethyl ester of BCECF that enables easy loading of BCECF into cells. BCECF-AM accumulates in a cell only by incubation as do the other acetoxymethyl esters. BCECF-AM is very sensitive to moisture; it should be carefully handled. The color of the DMSO solution changes from pale yellow to dark orange with decomposition of the AM form. Therefore, hydrolysis of the AM ester can be monitored by changes in color.

General Protocol for Human Neutrophil*

Reagents:

1 mM BCECF-AM/DMSO solution (1 mg BCECF in 1.45 ml DMSO)
HEPES buffer saline (20 mM HEPES, 153 mM NaCl, 5 mM KCl, 5 mM glucose, pH 7.4)

Protocol:

1. Suspend cells in HEPES buffer solution to prepare $4x10^7$ cells per ml.
2. Add 1 mM BCECF-AM/DMSO solution to the cell suspension to prepare 3 µM BCECF-AM (1/300 vol of cell suspension) as the final concentration.
3. Incubate the cell suspension at 37°C for 30 minutes.
4. Wash the cells 3 times with HEPES buffer saline and then prepare $3x10^6$ cells per ml of the cell suspension.
5. Determine the fluorescence intensity using a fluorescence microscope or a confocal laser microscope coupled with an image analyzer.

** Cell staining conditions depend on cell type, so it is necessary to optimize the conditions for each experiment*

References

1. R. A. Steinhardt, *et al*., Development of K^+-conductance and Membrane Potentials in Unfertilized Sea Urchin Eggs After Exposure to NH_4OH. *Nature*. 1973;*241*:400-401.
2. T. J. Rink, *et al*., Cytoplasmic pH and Free Mg^{2+} in Lymphocytes. *J Cell Biol*. 1982;**95**:189-196.
3. A. M. Paradiso, *et al*., Na^+ -H^+ Exchange in Gastric Glands as Measured with a Cytoplasmic-trapped, Fluorescent pH Indicator. *PNAS*. 1984;**81**:7436-7440.
4. S. Grinstein, *et al*., Phorbol Ester-induces Changes of Cytoplasmic pH in Neutrophils: Role of Exocytosis in Na^+ - H^+ Exchange. *Am J Physiol*. 1985;**248**:C379-C386.
5. G. B. Zavoico, *et al*., Regulation of intracellular pH in human platelets. Effects of thrombin, A23187, and ionomycin and evidence for activation of Na^+/H^+ exchange and its inhibition by amiloride analogs. *J Biol Chem*. 1986;**261**:13160-13167.
6. G. R. Bright, *et al*., Fluorescence Ratio Imaging Microscopy: Temporal and Spatial Measurements of Cytoplasmic pH. *J Cell Biol*. 1987;**104**:1019-1033.
7. C. Aalkjaer, *et al*., Intracellular pH Regulation in Resting and Contracting Segments of Rat Mesenteric Resistance Vessels. *J Physiol*. 1988;**402**:391-410.
8. K. Tsujimoto, *et al*., Intracellular pH of Halobacteria Can Be Determined by the Fluorescent Dye 2', 7'-bis(carboxyethyl)-5(6)-carboxyfluorescein. *Biochem Biophys Res Commun*. 1988;**155**:123-129.

9. M. A. Kolber, *et al*., Measurament of Cytotoxicity by Target Cell Release and Retention of the Fluorescent Dye Bis-carboxyethyl-carboxyfluorescein(BCECF). *J Immunol Methods*. 1988;**108**:255-264.
10. H. Harada, *et al*., cAMP Activates Cl^-/HCO_3^- Exchange for Regulation of Intracellular pH in Renal Epithelial Cells. *Biochim Biophys Acta*. 1991;**1092**:404-407.
11. C. C. Freudenrich, *et al*., Intracellular pH Modulates Cytosolic Free Magnesium in Cultured Chicken Heart Cells. *Am J Physiol*. 1992;**262**:C1024-C1030.
12. K. Khodakhah, *et al*., Functional Heterogeneity of Calcium Release by Inositol Triphosphate in Single Purkinje Neurones, Cultured Cerebellar Astrocytes, and Peripheral Tissues. *PNAS*. 1993;**90**:4976-4980.
13. G. Boyarsky, *et al*., Superiority of in vitro Over in vivo Calibrations of BCECF in Vascular Smooth Muscle Cells. *FASEB J*. 1996;**10**:1205-1212.
14. S. A. Weston, *et al*., New Fluorescent Dyes for Lymphocyte Migration Studies Analysis by Flow Cytometry and Fluorescent Microscopy. *J Immunol Methods*. 1990;**133**:87-97.
15. L. S. De Clerck, *et al*., Use of Fluorescent Dyes in the Determination of Adherence of Human Leucocytes to Endothelial Cells and the Effects of Fluorochromes on Cellular Function. *J Immunol Methods*. 1994;**172**:115-124.

MQAE *N*-Ethoxycarbonylmethyl-6-methoxyquinolinium bromide [CAS: 124505-60-8]

Application: Fluorescent chloride ion probe
Appearance: Pale yellow powder
Purity: ≥95.0% (HPLC)
MW: 326.19, $C_{14}H_{16}BrNO_3$

Ordering Information

Product code	Unit
M024-10	50 mg

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Chemical Structure

MQAE MQAE-Cl complex

Product Description
MQAE with bromide ion as a counteranion has fluorescence excitation at 355 nm and emission at 460 nm. The fluorescent intensity of MQAE decreases in proportion to the chloride increase in cells. Chloride ion concentrations less than 50 mM may be monitored without being affected by pH changes. MQAE has high water solubility and membrane permeability.

General Protocol*
Reagents:
5 mM MQAE/Krebs-HEPES buffer
Krebs-HEPES buffer (20 mM HEPES, 128 mM NaCl, 2.5 mM KCl, 2.7 mM $CaCl_2$, 1 mM $MgCl_2$, 16 mM glucose, pH 7.4)

Protocol:
1. Wash the cells with Krebs-HEPES buffer 3 times.
2. Add 5 mM Cl probe solution to the cells and incubate at 37°C for 1 hour.
3. Wash the cells with Krebs-HEPES buffer 5 times.
4. Determine the fluorescence intensity using a fluorescence microscope coupled with an image analyzer.
** Cell staining conditions depend on cell type, so it is necessary to optimize the conditions for each experiment*

References

1. A. S. Verkman, *et al*., Synthesis and Characterization of Improved Chloride-sensitive Fluorescent Indicators for Biological Applications. *Anal Biochem*. 1989;**178**:355-361.
2. M. Inoue, *et al*., An ATP-driven Cl^- Pump Regulates Cl^- Concentrations in Rathippocampal Neurons. *Neurosci Lett*. 1991;**134**:75-78.
3. T. Nakamura, *et al*., Direct Measurement of Chloride Concentration in Newt Olfactory Receptors with the Fluorescent Probe. *Neurosci Lett*. 1997;**237**:5-8.

$DiBAC_4(3)$ Bis(1,3-dibutylbarbituric acid)trimethine oxonol, sodium salt

Application: Membrane potential sensitive dye
Appearance: Reddish-orange to red powder
Purity: ≥98.0% (HPLC)
MW: 538.61, $C_{27}H_{39}N_4NaO_6$

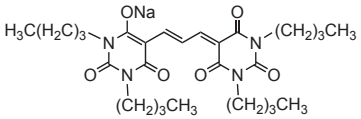
Ordering Information

Product code	Unit
D545-10	25 mg

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Chemical Structure



Product Description
$DiBAC_4(3)$ is a Bis-oxonol-type, membrane-potential, sensitive dye. According to membrane depolarization detection, when DiBAC distribution in cytosol is increased, the fluorescence intensitiy is increased. Since an argon laser (488 nm) can be used for $DiBAC_4(3)$ excitation, it is applicable for flow cytometry and confocal microscopy.

References

1. D. E. Epps, *et al*., Characterization of the Steady-state and Dynamic Fluorescence Properties of the Potential-sensitive Dye Bis-(1,3-dibutylbarbituric acid)trimethine oxonol(Dibac4(3)) in Model Systems and Cells. *Chem Phys Lipids*. 1994;**69**:137-150.
2. T. Brauner, *et al*., Comparative Measurements of Membrane Potentials with Microelectrodes and Voltage-sensitibe Dyes. *Biochim Biophys Acta*. 1984;**771**:208-216.
3. T. T. Rohn, *et al*., Xanthine Affects $[Ca^{2+}]i$ and Contractile Responses of Ventricular Cardiocytes to Electrical Stimulation. *Am J Physiol*. 1997;**273**:C909-C917.
4. D. J. Mason, *et al*., Rapid Estimation of Bacterial Antibiotic Susceptibility with Flow Cytometry. *J Microsc*. 1994;**176**:8-16.
5. U. Langheinrich, *et al*., Hyperpolarization of Isolated Capillaries from Guinea-pig Heart Induced by K^+ Channel Openers and Glucose Deprivation. *J Physiol*. 1997;**502**:397-408.
6. K. S. Schroeder, *et al*., FLIPR: A New Instrument for Accurate, High Throughput Optical Screening. *J Biomol Screen*. 1996;**1**:75-80.

$Ins(1,4,5)P_3$ (synthetic) D-*myo*-Inositol-1,4,5-triphosphate, tripotassium salt [CAS:85166-31-0]

Application: Cell signal transduction research
Appearance: White pellet
Purity: pass test (TLC)
MW: 534.37, $C_6H_{12}K_3O_{15}P_3$

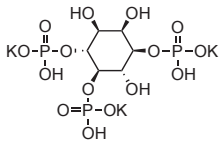
Ordering Information

Product code	Unit
I007-10	100 µg
I007-12	1 mg

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Inositol phosphates and diacylglycerols are generated from phosphatidyl inositols by phospholipase C. They are involved in cellular calcium ion movements as second messengers. Inositol-1,4,5-triphosphate(Ins(1,4,5)P3) is an extremely important messenger that triggers calcium ion release from endoplasmic and sarcoplasmic reticulum. It is thought to increase the calcium sensitivity of the inositol- induced calcium release(IICR) channel in the intracellular calcium source. Thus, the IICR channel can be opened with a low level of calcium ion concentration, causing a dramatic calcium burst into the cytosol. The released calcium ions and diacylglycerol then activate protein kinase C, which is one of the key enzymes in signal transduction.

References

1. M. Hirata, *et al*., Synthetic Inositol Trisphosphate Analogs and Their Effects on Phosphatase, Kinase, and the Release of Ca^{2+}. *J Biol Chem*. 1989;**264**:20303-20308.

-*Bacstain*- Series

Products Description

The Bacstain series can be used for microorganism staining as indicated in Fig. 1. CFDA is used for staining of viable microorganisms. CFDA is bacterial cell wall and cell membrane permeable, and remains in the cell after being hydrolized by esterase. Other Bacstain reagents are used for nucleotide staining and are cell wall permeable, except for PI. Therefore, using one of these nucleotide staining reagents and CFDA or Bacstain-CTC Rapid Staining Kit, it is possible to stain both cells with intact membranes and cells with damaged membranes simultaneously. Since PI can stain only membrane-damaged cells, it does not stain membrane-intact cells. PI is also used for double staining coupled with CFDA.

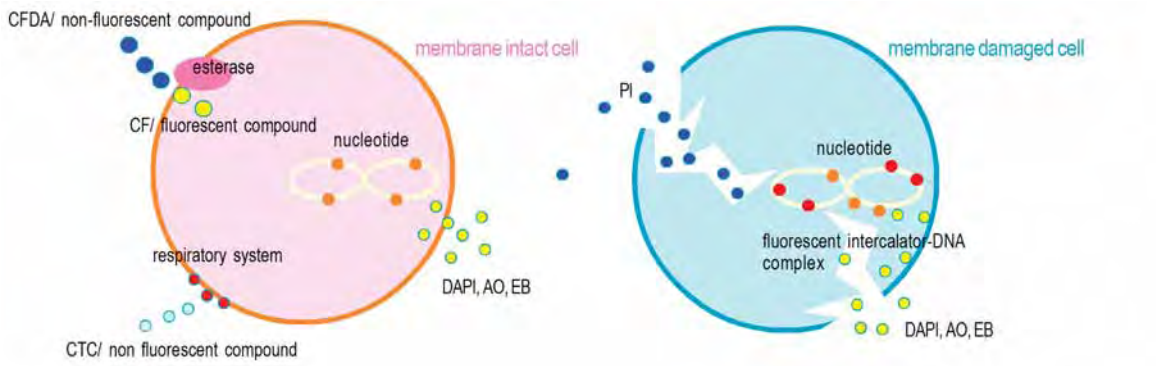


Fig. 1 Microorganism staining mechanism with Bacstain reagents

-*Bacstain*- CTC Rapid Staining Kit

for Flow cytometry [BS01-10]
for Microscopy [BS02-10]

Application: Aerobic bacterial cell staining
Detection of hard-to-culture bacteria

Features: Highly sensitive fluorescence detection
No washing required

Storage Condition
0-5°C, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit *
BS01-10	100 assays
BS02-10	100 assays

* One kit can stain 100 samples when using standard protocol.

Required Equipment and Materials

10 µl and 1000 µl pipettes, incubator, Microscope (blue excitation filter and red emission filter) or flow cytometer (488 nm blue laser)

Product Description

Colony formation using an agar plate is a very common and reliable method for counting bacterial cells. However, it takes quite a long time to form a colony. Therefore, alternative detection methods have been developed. Bacteria-specific gene amplification methods such as PCR, LAMP, and nucleus staining are quite rapid, but these methods count dead bacteria as well. Therefore, detection of live cell functions is essential to determining the actual number of living bacteria in a sample. Tetrazolium salts can be used to detect respiratory activity of bacterial cells or mitochondria.

CTC is a tetrazolium salt and is reduced by this respiratory activity to form fluorescent CTC formazan on the cell surface. Therefore, CTC is used for specific staining of aerobic live bacteria and can be applied to hard-to-culture bacteria (VNC: viable but non-culturable). CTC forms a fluorescent formazan by an electron transfer system. However, CTC alone is not sensitive enough to stain single cells. Therefore, the CTC-Rapid Staining Kit contains an enhancing reagent that improves the CTC staining efficiency. Compared with staining with CTC only, this staining kit enables rapid and sensitive staining of microorganisms. Maximum wavelengths of the CTC formazan dye are 450 nm or 480 nm for excitation and 630 nm for emission.

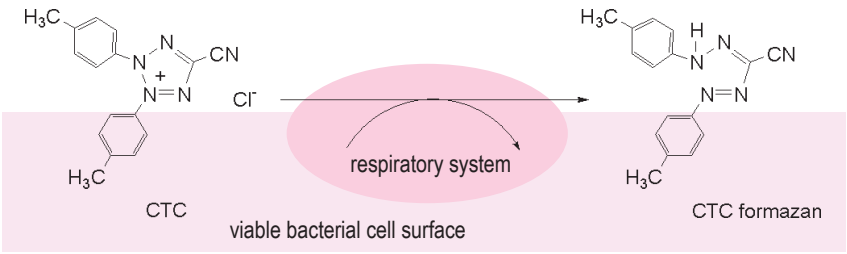


Fig. 1 Bacterial cell viability detection mechanism with CTC

General Staining Protocol

Microscopy detection

1. Centrifuge bacteria culture and remove the supernatant, and then resuspend the bacteria pellet with PBS(-).
2. Add CTC + Enhancing reagent-B. Incubate at 37°C for 1 hour.
3. Prepare a slide and detect fluorescence by B-excitation filter set.

Flow cytometry detection

1. Centrifuge bacteria culture and remove the supernatant, and then resuspend the bacteria pellet with PBS(-).
2. Add CTC + Enhancing reagent-A. Incubate at 37°C for 1 hour.
3. Analyze the cells with a flow cytometry: 488 nm excitation, 630 nm emission.

Experimental Example

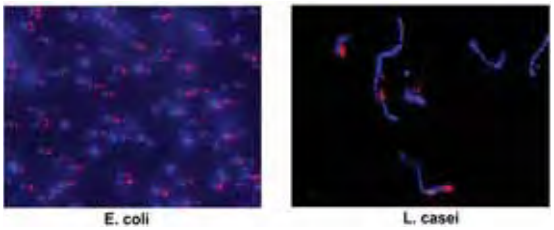


Fig. 2 *E. coli* staining (left) and *L. casei* staining (right) with CTC and DAPI.

Bacterial cells were stained with CTC Rapid Staining Kit first, and then 1 µl of DAPI solution was added. The cells were incubated at room temperature for 5 minutes. Formaldehyde fixation with 1-4% formaldehyde can be performed before DAPI staining.

References

1. A. Hiraishi, *et al*., An Improved Redox Dye-Staining Method Using 5-Cyano-2,3-Ditoryl Tetrazolium Chloride for Detection of Metabolically Active Bacteria in Activated Sludge. *Microbes Environ*. 2004;**19**:61-70.
2. A. Kitaguchi, *et al*., Enumeration of Respiring *Pseudomonas* spp. in Milk within 6 Hours by Fluorescence In Situ Hybridization Following Formazan Reduction. *Appl Environ Microbiol*. 2005;**71**:2748-2752.

CTC 5-Cyano-2,3-ditolyl-2*H*-tetrazolium chloride [CAS: 90217-02-0]

Application: Living bacterial counting
Features: Selective staining possible
Fast results
Fluorescent microscopy and flow cytometry detection
Appearance: White or slightly orange crystalline powder
MW: 311.77, $C_{16}H_{14}ClN_5$

Ordering Information

Product code	Unit
C440-10	100 mg

Storage Condition
0-5°C, protect from light

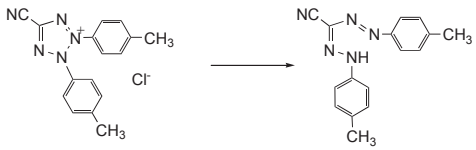
Shipping Condition
ambient temperature

Product Description

CTC can be used in various ways to detect bacteria, depending on the objective. Detection methods include the agar plate cultivation method, which involves counting colonies that arise from bacteria cultivation; staining bacteria using the fluorescent stain method; testing for bacteria that has potential for growth using the DVC method; and detecting particular bacteria using the FISH method-DNA amplification method. CTC is reduced to CTC formazan (CTF) by electron transfer through respiratory activity and builds up as fluorescent sedimentation inside a cell that has become insoluble in water. CTC itself is water-soluble and non-fluorescent in aqueous solution; CTF, however, is not fluorescent in fluids with low viscosity. However, in fluids with high viscosity and in a solid state, it gives off a red fluorescence. It is possible to search for cells with respiratory activity after incubating CTC with reagent, by counting under a fluorescent microscope or analyzing by flow cytometry. By using with nucleic acid staining reagent to count the total cell population and the number of living cells, or using with the FISH

method to selectively count a particular viable cell type, it is possible to collect a higher level of data. Since the existence of VNC (viable but non-culturable) bacteria (has become evident, the demand for a rapid detection method of microorganisms is on the rise and is expected to become a technique in sanitation testing.

Chemical Structure



CTC CTC formazan

E. coli DH5a

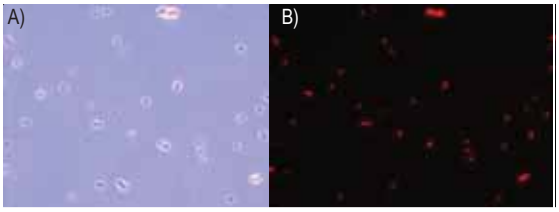
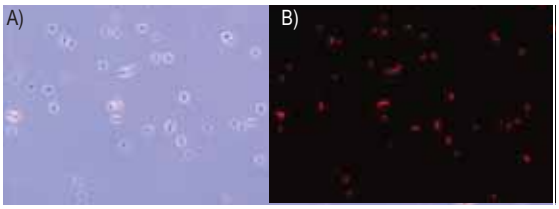


Fig. 1 Microorganism staining with CTC

E. coli staining Condition

E. coli culture was stained with 5 mg/ml CTC for 4 hours at 37°C.
A) Phase-contrast microscope
B) fluorescent microscope (485 nm, 510 nm filters)

B. subtilies JCM1465



B. subtiles staining Condition

B. subtilis culture was stained with 5 mg/ml CTC for 4 hours at 37°C.
A) Phase-contrast microscope
B) fluorescent microscope (485 nm, 510 nm filters)

References

1. A. W. Coleman, Enhanced Detection of Bacteria in Natural Environments by Fluorochrome Staining of DNA. *Limnol Oceanogr.* 1980;**25**:948-951.
2. E. Severin, *et al*., Fluorimetric Assay of Redox Activity in Cells. *Anal Chim Acta*. 1985;**170**:341-346.
3. G. G. Rodriguez, *et al*., Use of a Fluorescent Redox Probe for Direct Visualization of Actively Respiring Bacteria. *Appl Environ Microbiol*. 1992;**58**:1801-1808.
4. G. Schaule, *et al*., Use of 5-Cyano-2,3-ditolyl Tetrazolium Chloride for Quantifying Planktonic and Sessile Respiring Bacteria in Drinking Water. *Appl Environ Microbiol.* 1993;**59**:3850-3857.
5. R. A. Bovill, *et al*., Comparison of the Fluorescent Redox Dye 5-Cyano-2,3-ditolyltetrazolium Chloride with p-Iodonitrotetrazolium Violet to Detect Metabolic Activity in Heat-stressed Listeria monocytogenes Cells. *J Appl Bacteriol.* 1994;**77**:353-358.
6. M. T. E. Suller, *et al*., Flow Cytometric Assesment of the Postantibiotic Effect of Methicillin on *Staphylococcus aureus*. *Antimicrob Agents Chemother. 1998;***42**:1195-1199.
7. M. Kawai, *et al*., Rapid Enumeration of Physiologically Active Bacteria in Purified Water Used in the Pharmaceutical Manufacuturing Process. *J Appl Microbiol.* 1999;**86**:496-504.
8. N. Yamaguchi, *et al*., Rapid Detection of Respiring *Escherichia coli* O157:H7 in Apple Juice, Milk, and Ground Beef by Flow Cytometry. *Cytometry.* 2003;**54A**:27-35.
9. A. Hiraishi, *et al*., An Improved Redox Dye-Staining Method Using 5-Cyano-2,3-Ditoryl Tetrazolium Chloride for Detection of Metabolically Active Bacteria in Activated Sludge. *Microbes Environ*. 2004;**19**:61-70.
10. A. Kitaguchi, *et al*., Enumeration of Respiring - spp. in Milk within 6 Hours by Fluorescence In Situ Hybridization Following Formazan Reduction. *Appl Environ Microbiol*. 2005;**71**:2748-2752.

-*Bacstain*- CFDA solution

5(6)-Carboxyfluorescein diacetate, DMSO solution [CAS: 79955-27-4]

Application: Viable microorganism staining
Features: Fluorescence detection
Ready-to-use solution

Contents of the Kits:
CFDA solution ..... 375 µl x 4 vials

Storage Condition
0-5°C, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
BS03-10	100 assays

Required Equipment and Materials

10 µl and 1000 µl pipettes, incubator, Microscope (blue excitation filter and green emission filter) or flow cytometer (488 nm blue laser)

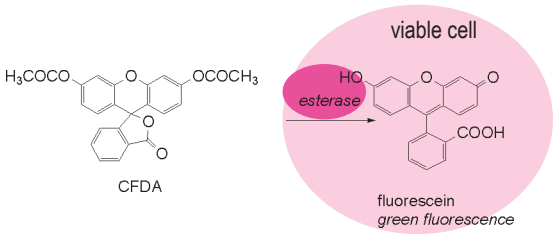


Fig. 1 Cell staining mechanism

Staining procedure

1. Allow CFDA solution to stand at room temperature for 30 minutes to thaw. Solution should be protected from light.
2. Resuspend the organism with an appropriate buffer (phosphate buffer, saline, etc.) [a] and adjust the number of cells to 10^6 cells/mL(flow cytometry) or 10^8-10^9 cells/mL(microscopy).
3. Add CFDA solution into the microbial cell suspension and vortex gently to mix. Use 5 µl for flow cytometry and 15 µl for microscopy analysis. The maximum wavelengths of the dye are 493 nm for excitation and 515 nm for emission.
4. Incubate the microbial cell at 37°C for 5 minutes [b].
5. Fix the microbial cell by addition of formaldehyde (1-4% final concentration).
6. Remove the buffer by filtration or centrifugation, and resuspend with buffer.
7. Analyze the stained-cells by a flow cytometer or a microscope.

[a] *Gram-negative bacteria tend to exhibit lower fluorescence intensity than Gram-positive bacteria because of their cell structure (outer membrane impedes penetration of CFDA). For the staining of Gram-negative bacteria, use 0.1 M phosphate buffer, 0.9 M NaCl, 0.5 mM EDTA, pH 8.5.*

[b] *If CFDA staining is not sufficient with 5 minutes of incubation, increase the incubation time.*

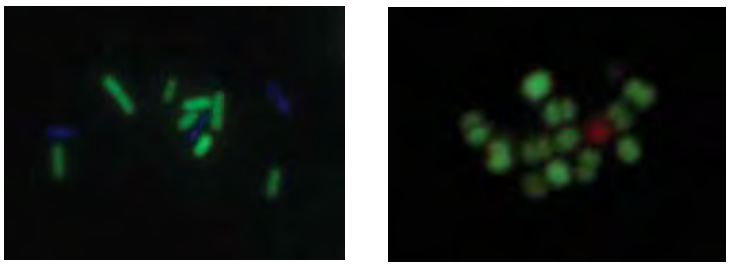


Fig. 2 *B. cereus* stained with CFDA and DAPI (left).
S. epidermidis stained with CFDA and PI (right).

References

1. N. Yamaguchi, *et al*., Flow cytometric analysis of bacterial respiratory enzymatic activity in the natural aquatic environment. *J Appl Microbiol*. 1997;**83**:43-52.
2. M. Kawai, *et al*., Rapid Enumeration of Physiologically Active Bacteria in Purified Water Used in the Pharmaceutical Manufacturing Process. *J Appl Microbiol.* 1999;**86**:496-504.

-Bacstain- DAPI solution

4',6-Diamidino-2-phenylindole, dihydrochloride, solution [CAS: 28718-90-3]

Application: Microorganism staining
Features: Fluorescence detection
Ready-to-use solution

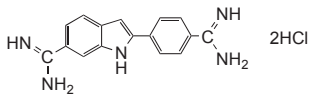
Contents of the Kits:
DAPI solution ..... 25 µl x 4 vials

Storage Condition	Shipping Condition
0-5°C, protect from light	ambient temperature

Ordering Information

Product code	Unit
BS04-10	100 assays

Chemical Structure



Required Equipment and Materials

10 µl and 1000 µl pipettes, incubator, Microscope (UV excitation filter and blue emission filter) or flow cytometer (UV laser)

Staining Procedure

1. Allow DAPI solution[a] to stand at room temperature for 30 minutes. Solution should be protected from light.
2. Resuspend the organisms with PBS(-) or saline and adjust the number of cells to 10^6cells/mL(flow cytometry) or 10^8-10^9cells/mL(microscopy).
3. Add 1 µL of DAPI solution into the microbial cell suspension and vortex gently to mix. Formaldehyde-fixation can be recommended if necessary.
4. Incubate the microbial cells at room temperature for 5 minutes.
5. Analyze the stained-cells by a flow cytometer or a microscope. The maximum wavelengths of the dye are 360 nm for excitation and 460 nm for emission.

[a] *Since DAPI may be carcinogenic, be careful when handling and disposing.*

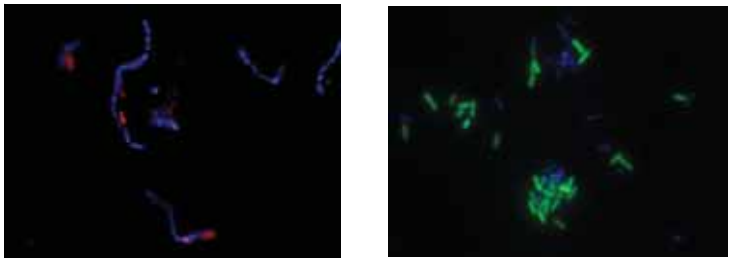


Fig. 1 *L. casei* stained with CTC and DAPI (left).
B. cereus stained with CFDA and DAPI (right).

-*Bacstain*- AO solution

3,6-Bis(dimethylamino)acridine hydrochloride, solution [CAS: 65-61-2 AO]

Application: Microorganism staining
Features: Fluorescence detection
Ready-to-use solution

Contents of the Kits:
AO solution ..... 75 µl x 4 vials

Strage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
BS05-10	100 assays

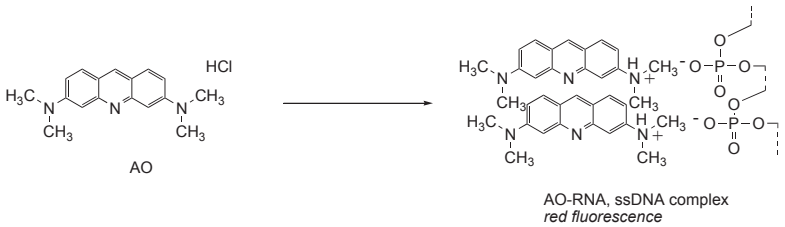


Fig. 1 Cell staining mechanism

Required Equipment and Materials

10 µl and 1000 µl pipettes, incubator, Microscope (blue excitation filter and green emission filter) or flow cytometer (488 nm blue laser)

Staining Protocol

1. Allow AO solution[a)] to stand at room temperature for 30 minutes to thaw. Solution should be protected from light.
2. Resuspend the organisms with PBS(-) or saline and adjust the number of cells to 10^6 cells/mL(flow cytometry) or 10^8-10^9 cells/mL(microscopy).
3. Add 3 µl of AO solution into the 1 mL of microbial cell suspension and vortex gently to mix. Formaldehyde-fixation can be carried out if necessary.
4. Incubate the microbial cells at room temperature for 5 minutes.
5. Analyze the stained-cells with a flow cytometer or a microscope. The maximum wavelengths of the dye with ssDNA are 420-460 nm for excitation and 630-650 nm for emission. The maximum wavelengths of the dye with dsDNA are 500 nm for excitation and 520 nm for emission.

[a)] *Since AO may be carcinogenic, be careful when handling and disposing.*

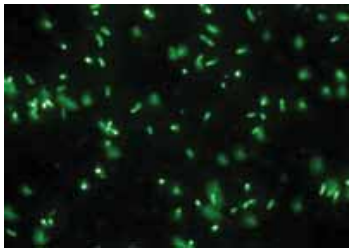


Fig. 1 *B. subtils* stained with AO.

References

1. J. E. Hobbie, *et al*., Use of Nucleopore Filters for Counting Bacteria by Fluorescence Microscopy. *Appl Environ Microbiol*. 1997;**33**:1225-1228.
2. S. F. Nishino, *et al*., Direct Acridine Orange Counting of Bacteria Preserved with Acidified Lugol Iodine. *Appl Environ Microbiol*. 1986;**52**:602-604.

-*Bacstain*- PI solution

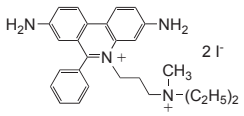
3,8-Diamino-5-[3-(diethylmethylammonio)propyl]-6-phenylphenanthridinium diiodide, solution

Application: Membrane-damaged microorganism staining
Features: Fluorescence detection
Ready-to-use solution

Storage Condition	Shipping Condition
-20°C, protect from light	ambient temperature

Ordering Information	
Product code	Unit
BS07-10	100 assays

Chemical structure



Required Equipment and Materials

10 µl and 1000 µl pipettes, incubator, Microscope (blue excitation filter and red emission filter) or flow cytometer (488 nm blue laser)

Staining Procedure

1. Allow PI solution[a)] to stand at room temperature for 30 minutes to thaw. Solution should be protected from light.
2. Resuspend the organisms with PBS(-) or saline and adjust the number of cells to 10^6 cells/mL(flow cytometry) or 10^8-10^9 cells/mL(microscopy).
3. Add 10 µl of PI solution into 1 mL of microbial cell suspension and vortex gently to mix. Formaldehyde-fixation may be carried out, if necessary.
4. Incubate the microbial cells at room temperature for 5 minutes.
5. Analyze the stained-cells with a flow cytometer or a microscope. The maximum wavelengths of the dye are 530 nm for excitation and 620 nm for emission.

[a)] *Since PI may be carcinogenic, be careful when handling and disposing.*

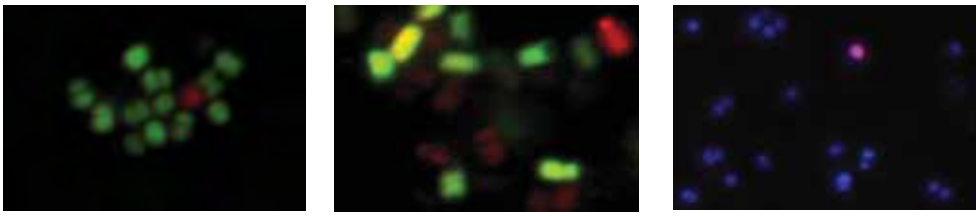


Fig. 1 *S. epidermidis* stained with CFDA and PI (left).
E. coli stained with CFDA and PI (middle).
S. epidermidis stained with DAPI and PI (right).

Reference

1. N. Yamaguchi, *et al*., Flow cytometric analysis of bacterial respiratory and enzymatic activity in the natural aquatic environment. *J Appl Microbiol*. 1997;**83**:43-52.

HilyMax

Application: DNA and RNA transfection to mammalian cells
Features: Best Reagent for Cell Signaling Research
High Transfection Efficiency in Wide Variety of Cells
Optimized Protocol for Maximizing Transfection
Great Result with Insect Cell
Transfection Efficiency: To pass test

Ordering Information

Product code	Unit
H357-10	1 ml x 1

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Kit Contents:
H357-10: HilyMax reagent ..... 1 vial Lipoform buffer .....1 ml x 1 vial

Required Equipment and Materials
micro plate; 10 µl, 100-200 µl, and 1000 µl pipettes; micro tubes; CO_2 incubator

Product Description
Various methods have been developed to express specific proteins in mammalian cells. The first method to introduce DNA to cells was calcium phosphateprecipitation. However, the transfection efficiency was very poor and there was a high rate of cell-to-cell variation. The second method introduced was the DEAEsephadex method. The transfection efficiency drastically improved, but still the method could not be used for all cells and required heavy metalions to enhance transfection efficiency. The cation liposome method was then developed, which proved to be a much better method to transfect DNA and RNA into cells. Other methods used are magnet bead, metal particle shoot, and electroporation. However, the cationic liposome method does not require any special instruments or special skill. Therefore, many researchers are using this method.
HilyMax is a newly developed gene transfection reagent that forms a liposome to be used for highly efficient gene transfection to a wide variety of cells. In addition, in signal transduction research, HilyMax gives better signal because the reagent introduced in cells does not interrupt intracellular signal pathways (Fig. 3). Since serum in the growth medium does not interfere with the transfection using HilyMax, no exchange of the medium during the transfection is required. HilyMax does not contain biological components that might interfere with the transfection.

Principle
HilyMax readily interacts with DNA because cationic liposome(+) and anionic DNA(-) spontaneously form DNAliposome complexes. The overall charge of DNA-HilyMax complex is positive, so that the DNA-HilyMax complex is electrostatically bound on an anionic cell surface and introduces DNA into the cell by endocytosis.

Fig. 1 Transfection principle

Table 1 Transfection efficiency of HilyMax

Cell line	Efficiency	Cell line	Efficiency
3T3-L1	30%	L6	30%
A549	50%	LNCap	30%
CHO	90%	MCF-7	70%
COS7	40%	MDCK	20%
HC	50%	MG63	20%
HEK293	60%	Neuro2a	70%
HeLa	70%	NIH3T3	70%
HepG2	10%	PC3	70%
Jurkat	3%	UtSMC	10%
K562	30%	Vero	40%

General protocol for DNA transfection for 24-well plate*
Adherent cells: Prepare 40-90% confluent cell culture in 0.5 ml of growth medium. Preincubation for 24 hours may be necessary. Non-adherent cells: Adjust the concentration of cells to 0.1-1.6x10^6 cells in 0.5 ml of growth medium and innoculate the cell suspension onto a plate. Preincubation for 24 hours may be necessary.

1. Add 30 µl serum-free medium[a)] to a plastic tube.
2. Add plasmid DNA (0.5-1.5 µg) to the tube and mix gently using a pipette.
3. Add HilyMax to prepared DNA (µg):HilyMax (µl)=1:2-1:6 and mix using a pipette.
4. Incubate the tube at room temperature for 15 minutes.[b)]
5. Add DNA-HilyMax complex to each well,[c)] and incubate the plate at 37°C in a CO_2 incubator.[d)]
6. Measure the reporter gene activity after 24 to 72 hours.

* Protocols for other plates are available in the HilyMax Technical manual.

[a)] Serum and antibiotics in medium interfere with the DNA-HilyMax complex formation. Opti-MEM, DMEM and MEM can be used during transfection. Please determine the transfection efficiency for other media.
[b)] Incubation for more than 30 minutes may cause a low transfection efficiency.
[c)] Serum in cell culture medium does not interfere with the transfection.
[d)] A medium change after transfection is effective for increasing the transfection efficiency and for decreasing the cytotoxicity in some cell lines.

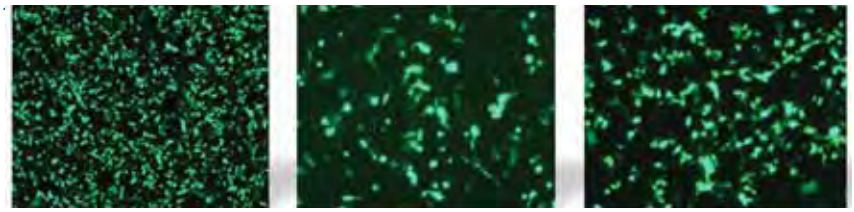


Fig. 2 GFP expressed cells after transfection with HilyMax

Cell Signaling Research

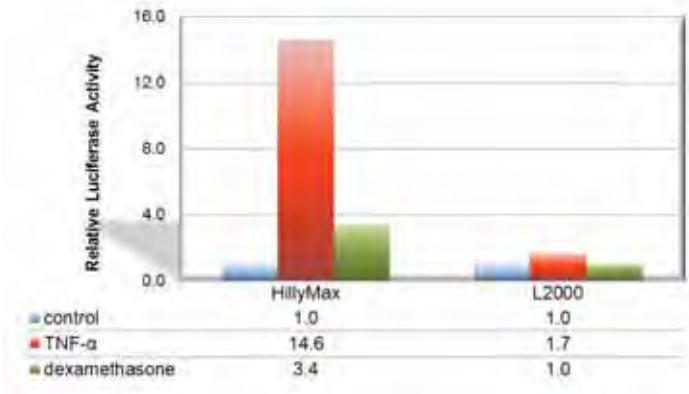


Fig. 3 Suitable signal transduction research using HilyMax.

The signal transduction from the A549 cell was confirmed with TNF-a stimulation. For detection of cellular response,an IL-8 dependent luciferase expression vector was transfected with HilyMax or L2000. The signal transduction response was detected as luciferase activity after stimulation and suppression. The signal response using HilyMax corresponded to the amount of expressed IL-8 in the stimulated cell.

Reference

1. K. Park, *et al*., Regulation of Cathelicidin Antimicrobial Peptide Expression by an Endoplasmic Reticulum (ER) Stress Signaling, Vitamin D Receptor-independent Pathway. *J Biol Chem*. 2011;**286**:34121-34130.
2. M. Taura, *et al*., MEF/ELF4 transactivation by E2F1 is inhibited by p53. *Nucleic Acids Res*. 2011;**39**:76-88.
3. A. Shimahara, *et al*., Acetylation of Lysine 564 Adjacent to the C-terminal Binding Protein-binding Motif in EVI1 Is Crucial for Transcriptional Activation of *GATA2*. *J Biol Chem*. 2010;**285**:16967-16977.
4. M. Koyama, *et al*., Histone Deacetylase Inhibitors and 15-Deoxy-$\Delta^{12,14}$-Prostaglandin J_2 Synergistically Induce Apoptosis. *Clin Cancer Res*. 2010;**16**:2320-2332.
5. A. Ryo, *et al*., A Suppressive Role of the Prolyl Isomerase Pin1 in Cellular Apoptosis Mediated by the Death-associated Protein Daxx. *J Biol Chem*. 2007;**282**:36671-36681.
6. H. Nakajima, *et al*., The Active Site Cysteine of the Proapoptotic Protein Glyceraldehyde-3-phosphate Dehydrogenase Is Essential in Oxidative Stress-induced Aggregation and Cell Death. *J Biol Chem*. 2007;**282**:26562-26574.

CarryMax-R

Application: Protein delivery to viable cells
Features: Simple procedure to optimize delivery conditions
Low toxicity to cells
Antibody and enzyme transfection
Delivery Efficiency: To pass test

Ordering Information

Product code	Unit
C471-10	0.2 ml

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Required Equipment and Materials
10 µl, 100-200 µl, and 1000 µl pipettes; CO_2 incubator, microtubes (sterile)

Contents of the Kit:
PT-R..... 1 tube
Lipoform buffer .....0.2 ml
Enhancer E..... 1 tube

Product Description
CarryMax-R is a protein delivery kit designed to achieve the best performance of protein delivery to various animal cells. CarryMax-R's protein delivery mechanism is similar to gene delivery by cationic liposomes. The protein-cationic liposome complex binds on the cell membrane and then the complex is taken inside cells by endocytosis; upon entry, the protein is released to the cytoplasm. The location of the protein delivered by this method will be determined by various experiments.
Recent studies have shown that the delivered protein in the cell, such as apoptosis related proteins, works in the same manner as a protein synthesized in the cell by gene expression. This delivery method will be very useful in understanding the function of the protein in the cell. The total time to deliver proteins is about 2-4 hours if no further cell culture is required. Therefore, less time-consuming experiments will be possible to check the function of the protein than when using a DNA transfection method. Protein delivery techniques are not an alternative method to DNA transfection Techniques; rather, they are complementary to each other. Both techniques can be used together, depending on the purpose of the experiment.
CarryMax-R can be applied to a wide variety of studies related to intracellular protein functions, such as apoptosis, cell cycle regulation, cell signaling, DNA transcription, protein expression regulation, and RNA splicing. Additionally, since antibodies or other foreign molecules, such as peptides, biomolecules, and charged polymers, can be delivered into a viable cell, therapeutic applications of CarryMax will be possible. In the near future, dual delivery of protein and DNA by CarryMax-R will be another possible application.

Procedure

Mix sample protein and protein delivery reagent.

Add protein-protein delivery reagent complex to cells and incubate for 4 hours.

Analyze the cells with an appropriate method.

General Protocol
Preparation of protein delivery solution
1. Add 0.2 ml of Lipoform Buffer to the PT-R tube and dissolve with a vortex mixer for 30 seconds.
2. Add 40 µl DMSO to Enhancer E and dissolve by pipetting.
3. Mix 100 µl of PT-R solution and 5 µl of Enhancer E solution in a microtube followed by vortexing for 10 seconds to prepare Liposome solution.

Preparation of Protein-Protein delivery reagent complex
1. Add 30 µl of sterilized water solution to the tube.
2. Add 1 µg protein and mix with pipetting.
3. Add 4 µl Protein delivery solution to the tube and mix with pipetting.
4. Incubate for 15 minutes at room temperature.

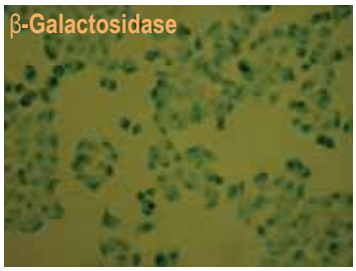
Transfect to cells

1. Change the cell culture media to fresh, serum-free media.
2. Add Protein delivery reagent complex solution to the cells.
3. Change the culture media to media containing serum 4 hours after the addition of the Protein delivery complex solution.
4. Continue to incubate for another 4 hours or appropriate period of time depending on the experiment.

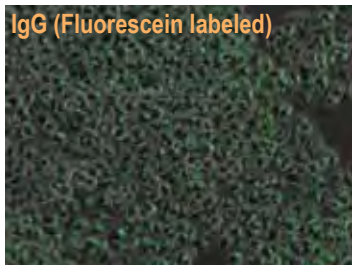
Recommended amount of protein (Amount for 1-well of a 24-well plate)

BSA	: 0.5 -1 μg
R-Phycoerythrin	: 1 μg
beta-Galactosidase	: 1 μg
IgG1	: 1 -2 μg

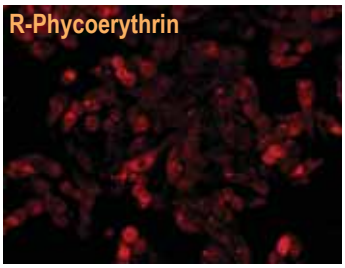
Delivery to Living Cells with CarryMax-R



The β-Galactosidase activity was kept after delivery to living cells.



Whole IgG was delivered to living cells without cytotoxicity.



Delivered R-Phycoerythrin fluorescence was observed in cytosol of CHO cell after 24 hrs incubation.

Introduction

Protein labeling reagents are used for various assays that are coupled with antigen-antibody reaction. Of these, fluorescent, biotin, and enzyme labeling reagents are used most frequently for antibody labeling. Most protein labeling reagents have similar reactive sites, such as succinimidyl ester (NHS) for amino groups and maleimide or bromoacetamide for sulfhydryl groups. Dojindo offers the following protein labeling reagents and kits for protein research and proteomics.

There are several reactive sites on proteins as indicated in Fig. 1. The most common reactive site used is the NH_2 group from lysine residue because of the simple process of labeling an NH_2 terminal under physiological conditions. Sometimes, protein labeling with NH_2-reactive compounds is not favorable because of the potential blockage of the protein's active site. However, activity loss due to blockage of active sites by labeling is rare in the case of antibody labeling with NH_2-reactive reagent. Therefore, labeling at the NH_2 group on a protein is fairly reliable and simple for preparing a conjugate. The second most common labeling method is the use of SH-reactive compounds. Most proteins do not have an SH group, just a disulfide group. Disulfide groups can be converted to SH groups by reducing agents such as dithiothreitol (DTT) or betamercaptoethanol. Then, SH-reactive compounds such as maleimide and bromoacetyl compounds are applied in labeling the protein's SH groups. Because the location of the disulfide group on an antibody is more specific than that of an NH_2 group, activity loss by SH group labeling is minimal. The reduction process may cause activity loss of an antibody because of possible cleavage between a heavy chain and a light chain. However, a conjugate prepared by SH labeling provides a better signal than that prepared by NH_2 labeling because of site-specific labeling. Overall, a conjugate prepared by NH_2 labeling is adequate for general assays while a conjugate prepared by SH labeling can be used for high-sensitivity assays. The sugar chain of a protein is also a possible labeling site with hydrazide compounds. To convert to an aldehyde group before labeling with a hydrazide labeling agent, sugar chains must be reduced.

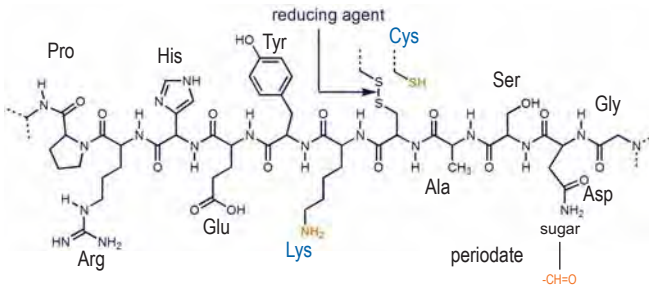


Fig. 1 Reaction site for protein labeling

Labeling Kits

Dojindo offers a wide variety of protein labeling kits. These kits can be used to prepare conjugates with enzyme, fluorophore, and biotin (Table 1). There are two types of kits available: NH_2 labeling (Kit-NH_2) and SH labeling (Kit-SH). Dojindo's labeling kits contain centrifugal filtration tubes that are used for protein purification, conjugation reaction, condensation, and buffer exchange. This single filtration tube format is the most unique feature of the kit (Fig. 2). Labeling requires 1) purification of protein and buffer exchange, 2) labeling reaction, and 3) purification and recovery of the conjugate. Because these three processes can be performed in a single filtration tube, recovery of conjugated proteins is always high, nearly 100% of protein can be recovered from the tube, and reproducibility is high as well. The filtration tube has a membrane that allows smaller molecules to pass through it while larger molecules remain on the membrane. Through this simple centrifuge process, small proteins can be removed, leaving only conjugated proteins to be recovered by pippetting with a buffer solution.

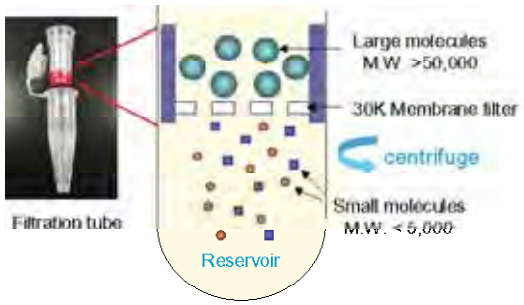


Fig. 2 Filteration tube with a 30K membrane filter and its molecule separation mechanism.

Additionally, the kit's unique buffer system prevents aggregation of protein and conjugate during labeling and storage of the conjugate. The conjugate recovered with the buffer (storage buffer) is stable for several months at 4°C or several years if it is stored at -20°C. A 10K membrane is available for proteins smaller than 50K. The enzyme labeling kits can be used for labeling small molecules, such as organic chemicals, amino acids, peptides, or oligonucleotides. For more detailed information, please review the product information section. Kit-NH_2 and Kit-SH include buffer solutions for washing, labeling reaction, and recovery of proteins; activated compounds; 30K filtration tubes; and an easy-to-follow protocol with photos. Kit-SH additionally contains reducing agents for converting disulfide groups to sulfhydryl groups (SH). The overall protocols for Kit-NH_2 and Kit-SH for labeling macromolecules and small molecules g are indicated in Figs. 3 and 4. Most small molecules, such as buffer components, sodium azide (preservative), amine compounds, thiol compounds, detergents, glycerol, etc.,are removed from proteins by the first step. Then, the appropriate labeling reagent is added directly to the filtration tube and incubated at 37°C for a certain time period. The conjugate is then recovered with storage buffer or WS buffer. The average concentration of protein will be 500-1,000 µg per ml as antibody. In most cases, the conjugates can be applied for downstream experiments such as ELISA, western blotting, tissue staining, and flowcytometry without further purification.

Table 1 Labeling Kits

Category	Labeling Material	Target Group
Biotin Labeling	Biotin	amine, thiol
Enzyme Labeling	Peroxidase	amine, thiol
	Alkaline phosphatase	amine, thiol
Fluorophore Labeling	Phycobiliproteins	amine, thiol
	Fluorescent dyes	amine, thiol

Labeling Process

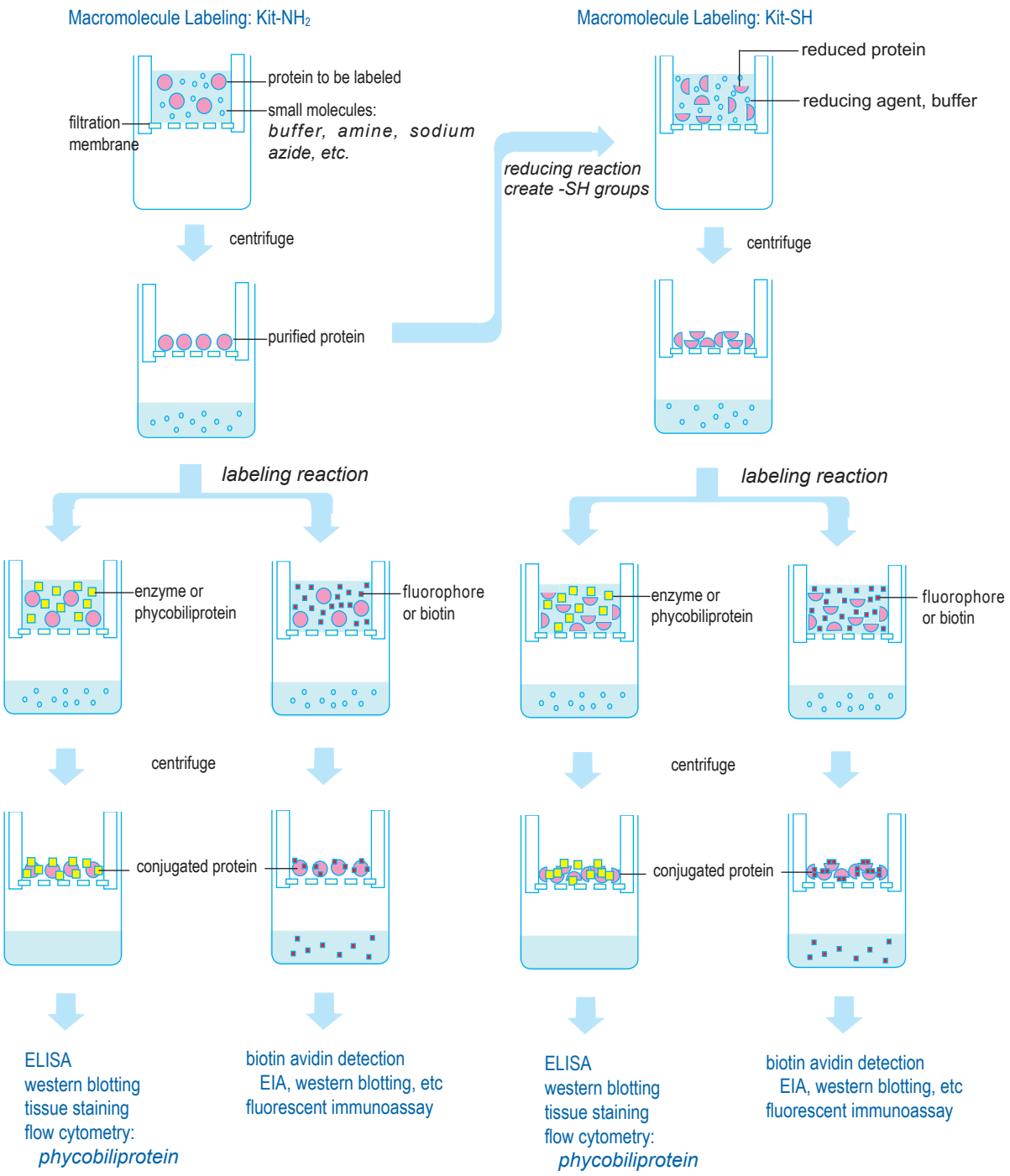


Fig. 3 Macromolecule labeling processes

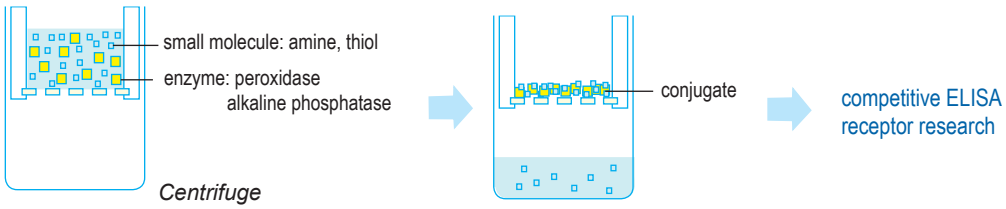


Fig. 4 Small molecule labeling processes

Enzyme Labeling Kits

Peroxidase and alkaline phosphatase are the most frequently used enzymes for enzyme immunoassays (EIA) because they lose little activity by chemical modification, and because of their high sensitivity, high stability, and wide selection of colorimetric, fluorometric, and chemiluminometric substrates. Peroxidase-labeled protein is generally prepared using a reactive periodate-treated peroxidase. An aldehyde group of peroxidase derived from a sugar chain by the periodate oxidation reaction forms Schiff's base with an amine group of the protein to create a covalent bond between the protein and peroxidase, After the conjugation reaction, a reducing agent is usually required to convert the Schiffs base to a C-N single bond, stabilizing the peroxidase conjugate. Dojindo's Peroxidase Labeling Kit-NH_2 and Alkaline Phosphatase Labeling Kit-NH_2 can be used to simply and rapidly label both macromolecules (Fig. 5) and small molecules with amino groups. Conjugates prepared with this kit have higher enzyme activity and are more stable than other commercially available peroxidase or alkaline phosphatase-labeled antibodies or streptavidins. Peroxidase Labeling Kit-SH and Alkaline Phosphatase Labeling Kit-SH are used for labeling molecules with sulfhydryl groups. Except for the reduction of protein before labeling SH groups, the overall procedures for labeling are the same for both kits. Because of site-specific labeling with Kit-SH, enzyme-labeled IgGs prepared using Labeling Kit-SH sometimes yield higher sensitivity than conjugates prepared by Labeling Kit-NH_2. Peroxidase Labeling Kits and Alkaline Phosphatase Labeling Kits are used for labeling small molecules as well as macromolecules. Small molecules with amino group(s) or sulfhydryl group(s) can be labeled with Labeling Kit-NH_2 and Labeling Kit-SH, respectively. The labeling process is indicated in Fig. 4. The average number of small molecules conjugated to an enzyme is 2 per enzyme molecule. The conjugate can be used for competitive ELISA, receptor research, and other assays.

Fluorophore Labeling Kits

Fluorescent compounds such as fluorescein, rhodamine, cyanine dyes, and phycobiliproteins are commonly used for labeling biological molecules, especially proteins and DNA. Detection methods using these fluorescent materials are routine and well established. However, labeling conditions must be individually determined depending on the type of molecule, reactivity of the labeling agents, and the type of functional group to be labeled. Sometimes, pre-treatment of the sample may be necessary to remove molecules and reagents that interfere with the labeling reaction. Fluorophore Labeling Kits, such as Phycoerythrin, Allophycocyanin, Fluorescein, Rhodamine, and HiLyte dyes, are for labeling macromolecules with amino groups or sulfhydryl groups. Labeling processes of Kit-NH_2 and Kit-SH are indicated in Fig. 6. The fluorescent compounds in these kits are water soluble and each vial of fluorescent compound can prepare a labeled IgG with 4 to 7 fluorescent molecules. On average, there are 1 to 2 phycobiliprotins per IgG. These kits include a purification system for the proteins to be labeled so that even protein solutions containing materials that interfere with the reaction, such as sodium azide, Tris buffer, and thiols, can be used with these kits. The fluorescent spectra of these reagents are shown on page 65.

A) Enzyme Labeling-NH_2 B) Enzyme Labeling kit-SH

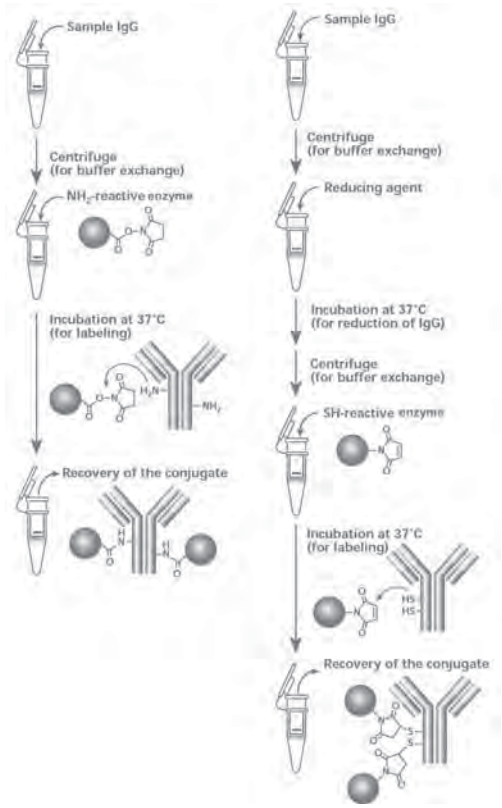


Fig. 5 Enzyme Labeling Kit labeling process

A) Fluorophore or Biotin Labeling Kit-NH_2 B) Fluorophore or Biotin Labeling Kit-SH

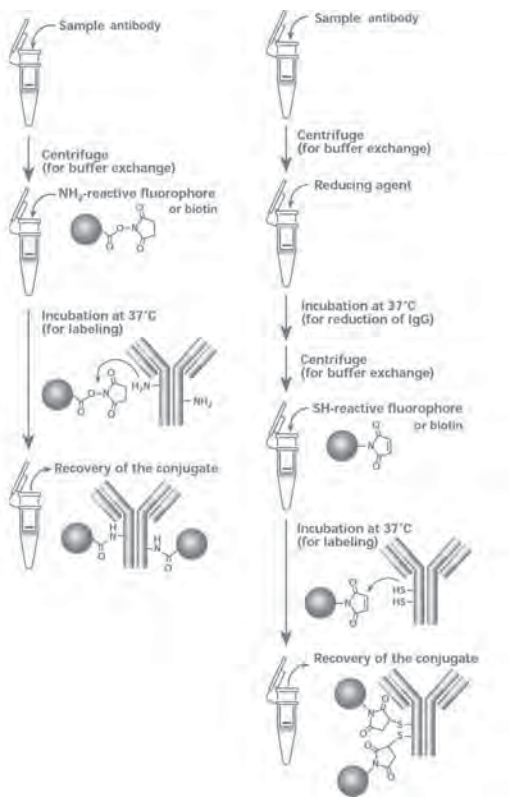


Fig. 6 Fluorophore or Biotin Labeling Kit labeling process

Biotin Labeling Kits and Reagents

Peroxidase-labeled streptavidin and alkaline phosphatase-labeled streptavidin are used for the detection of biotin-tagged macromolecules in EIA. Several types of biotin labeling reagents, such as amine reactive biotins, sulfhydrl reactive biotins, and aldehyde reactive biotins, are available. Spacers between the biotin molecule and the reactive group are also available in various lengths. Biotin Labeling Kit-NH_2 is a ready-to-use kit for labeling amino groups of proteins. Because there is a longer spacer between the biotin and succinimidyl ester (NHS) in this kit's biotin labeling reagent, the binding ability of streptavidin with a conjugated biotin molecule is almost identical to that of a free biotin molecule. Biotin Labeling Kit-SH uses Biotin-PE maleimide for the biotin labeling of sulfhydryl groups. Labeling processes of Kit-NH_2 and Kit-SH are indicated in Fig. 6.

IgG Purification Kits

Commercially available antibody solutions sometimes contain a stabilizer, such as bovine serum albumin or gelatin, which interfere with labeling reaction. Dojindo's IgG Purification Kits are useful for preparing a small amount of a purified antibody that can be applied directly to Dojindo's labeling kits. IgG Purification Kit-A and Kit-G contain protein-A and protein-G coated silica gel, respectively. In using these kits there are four steps in purifying IgG: 1) binding IgG to the gel, 2) removing unbounded materials, 3) washing, and 4) recovery of IgG from gel. Most IgG molecules bind to protein A and protein G in 2 minutes. Because all solution is filtered from silica gel by centrifuging, the sample solution can be recovered without dilution. If the first step in the purification process does not sufficiently recover IgG, the same sample solution can be processed once more to recover additional IgG. Protein A gel and Protein G gel in the kit are capable of isolating IgG from serum with 80 to 90% purity. The gels can be used at least 20 times without diminishing IgG recovery rate.

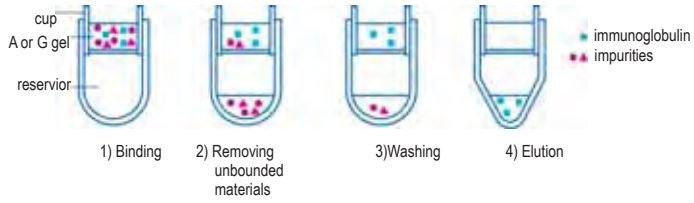


Fig. 7 IgG Purification process

Chelate Labeling Reagents

Chelate labeling reagents are used to bind metal ions to macromolecules. Macromolecules labeled with radioactive metal ions such as ^{99}Tc and ^{111}In are used for radioimaging with a scintillation camera to investigate macromolecule distribution in the body. Meares' reagents are chelate-labeling reagents that have an EDTA structure and a reactive functional group such as isothiocyanate, maleimide, and bromoacetamide. Bromoacetamide-type Meares'reagent, called "BABE" (4-bromoacetamidobenzyl-EDTA), is used for the analysis of proteinprotein or protein-DNA interaction sites.

Bifunctional Cross-Linking Reagents

Cross-linking reagents are used for the conjugation of two or more macromolecules. Cross-linking reagents are classified into two major groups, hetero-bifunctional and homo-bifunctional. Heterobifunctional cross-linking reagents have two types of reactive groups in their molecules, succinimide and maleimide. These two types of reactive groups make it possible, through modification by hetero-bifunctional reagents, for the biological materials to perform different functions, such as amine reactive and thiol reactive function,. These modified materials may then react with other materials through the attached functional groups. In general, conjugated enzymes and monoclonal antibodies for enzyme immunoassay (EIA) are prepared using these heterobifunctional cross-linking reagents. Of the reagents that have succinimide and maleimide as their functional groups, aliphatic compounds such as GMBS and EMCS are superior to aromatic compounds in the stability of maleimide in basic conditions. Four types of maleimide/succinimide cross-linking reagents are available for conjugation, which alters the length of the space between an enzyme and an antibody. Water-soluble cross-linking reagents are also available. Water-soluble cross-linking reagents do not require organic solvents such as DMF or DMSO to prepare a working solution. Therefore, these water-soluble cross-linking agents are useful for proteins sensitive to organic solvents.

Spectra Data

Fig. 8 indicate excitation spectra and emission spectra of IgG conjugates prepared by Fluorophore Labeling Kit-NH_2. The maximum wavelength of the conjugate prepared by Fluorophore Labeling Kit-SH is almost identical to that of the corresponding conjugate prepared by Fluorophore Labeling Kit-NH_2.

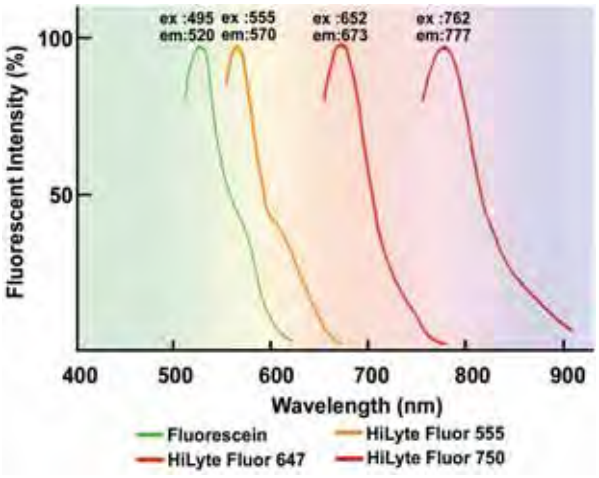


Fig. 8 Emission spectra of Fluorphore conjugated IgG

Peroxidase Labeling Kit-NH_2

Application: Peroxidase labeling of proteins or amine compounds

Features: Only 3 hours to recover conjugates
All processes in a single filtration tube
High recovery of conjugates

Ordering Information

Product code	Unit
LK11-10	3 samples [a]
LK51-10	1 sample [b]

[a] Based on 100 µg IgG sample labeling
[b] Based on 1 mg IgG sample labeling

Contents of the Kit

LK11-10

NH_2-reactive peroxidase ..... 100 µg x 3 tubes
Reaction buffer ..... 200 µl x 1 tube
Filtration tube ..... 3 tubes
Washing buffer .....4 ml x 1 bottle
Storage buffer .....4 ml x 1 bottle

LK51-10

NH_2-reactive peroxidase ..... 1 mg x 1 tube
Reaction buffer .....1.2 ml x 1 tube
Filtration tube ..... 1 tube
Washing buffer ..... 10 ml x 1 bottle
Storage buffer ..... 10 ml x 1 bottle
15 ml tube.....1 tube

Storage Condition

0-5°C

Shipping Condition

with blue ice

Product Description

Peroxidase Labeling Kit-NH_2 is used mainly for the preparation of peroxidase-labeled IgG for enzyme immunoassay (EIA) and for the preparation of peroxidase-labeled antigen for competitive EIA. NH_2-reactive peroxidase, a component of this kit, has succinimidyl groups (NHS) and reacts with proteins or other molecules that have an amino group in their structures (Fig. 1). This kit contains all the reagents necessary for the labeling process, including storage buffer. The labeling process is simple: mix IgG with NH_2-reactive peroxidase and incubate at 37°C for 2 hours. The NH_2-reactive peroxidase forms a covalent link with the target molecule without any activation process. The distance of the NHS from peroxidase is about 1.2 nm, half of the radius of the peroxidase molecule. Therefore, when the peroxidase-labeled IgG is used for EIA, the labeling efficiency of the NH_2-reactive peroxidase is high enough to eliminate the purification process after labeling. Also, peroxidase labeling will not affect the affinity of the target molecule. If a high-purity conjugate is required after labeling, simply use an affinity column or a gel-permeation column. When labeling small molecules, excess molecules can be removed by using the filtration tubes included in this kit. Because the amino groups of NH_2-reactive peroxidase are blocked, no self-conjugation is possible.

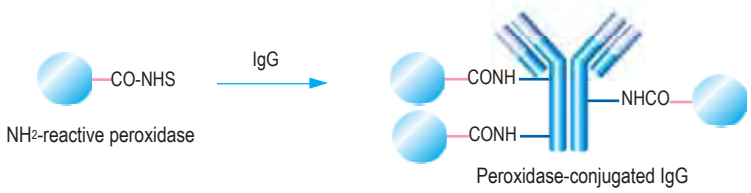


Fig. 1 IgG labeling reaction of NH_2-reactive peroxidase

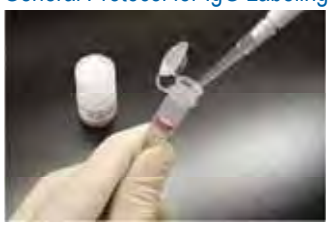
Precaution

- The molecular weight of the protein to be labeled with this kit should be greater than 50,000.
- The molecular weight of the small amine compound to be labeled with this kit should be smaller than 5,000.
- IgG or peroxidase-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with a molecular weight greater than 10,000, such as BSA or gelatin, purify the IgG solution before labeling peroxidase with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials,centrifuge the solution and use the supernatant for labeling.

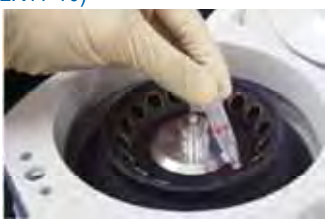
Required Equipment and Materials

LK11-10: microcentrifuge, 10 µl and 50-200 µl adjustable pipettes, 37°C incubator, 0.5 ml microtubes
LK51-10: centrifuge, rotor for 15 ml centrifuge tube, 50-200 µl and 1 ml adjustable pipettes, 37°C incubator, microtubes

General Protocol for IgG Labeling (LK11-10)



Step 1.
Add 100 µl of Washing Buffer and the sample solution containing 50-200 µg protein[a] to the Filtration Tube.



Step 2.
Mix the solution by pipetting several times, and centrifuge at 8,000 x *g* for 10 min.[b]

Step 3.
Add 100 µl of Washing Buffer to the Filtration Tube again.

Step 4.
Mix the solution by pipetting several times, and centrifuge at 8,000 x *g* for 10 min.[b]

Step 5.
Add 10 µl of Reaction Buffer to NH_2-Reactive Peroxidase, and dissolve it by pipetting.[c]

Step 6.
Add NH_2-Reactive Peroxidase solution to the concentrated IgG on the Filtration Tube and pipette to mix.

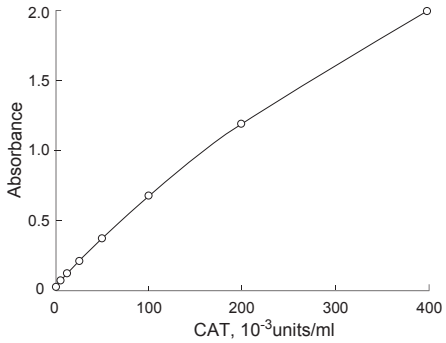
Step 7.
Incubate the tube at 37°C for 2 hours.

Step 8.
Add 100 µl of Washing Buffer to the Filtration Tube.

Step 9.
Centrifuge at 8,000 x *g* for 10 min[b].

Step 10.
Add 200 µl of Storage Buffer and pipette about 10 times to recover the conjugate[d]. Transfer the solution to a microtube (not included in this kit), and store the solution at 0 - 5°C[e].

[a] *The volume of IgG solution should be less than 100 µl. If the IgG concentration is lower than 0.5 mg/ml, repeat Steps 1 and 2 until the total IgG accumulation becomes 50-200 µg.*
[b] *If the solution still remains on the membrane after the centrifugation, spin for another 5 min.*
[c] *NH_2-Reactive Peroxidase is unstable in Reaction Buffer. Proceed to Step 6 immediately after the preparation of the NH_2-Reactive Peroxidase solution.*
[d] *One to three molecules of peroxidase should be introduced onto one IgG molecule. Unconjugated peroxidase does not interfere with immunoassays. For more purification, use a gel permeation column or an affinity column for IgG.*
[e] *Generally, the peroxidase-labeled IgG in Storage Buffer is stable for 2 months at 0-5°C. However, it is important to note that the stability will depend on the sample itself. For longer storage, add equal volume of glycerol to the sample solution and store it at -20°C.*



Experimental Examples

Fig. 2 Sandwich ELISA of CAT (chloramphenicol acetyl transferase) assay.

Plate: 2 µg/ml anti-CAT antibody (rabbit anti sera)-coated high binding plate
CAT: 0-400 x 10^{-3}units/ml PBST
Peroxidase-conjugated anti-CAT antibody:
Prepared by Peroxidase Labeling Kit-NH_2.1µg/ml
PBST+blocking reagent
Substrate: TMB peroxidase substrate

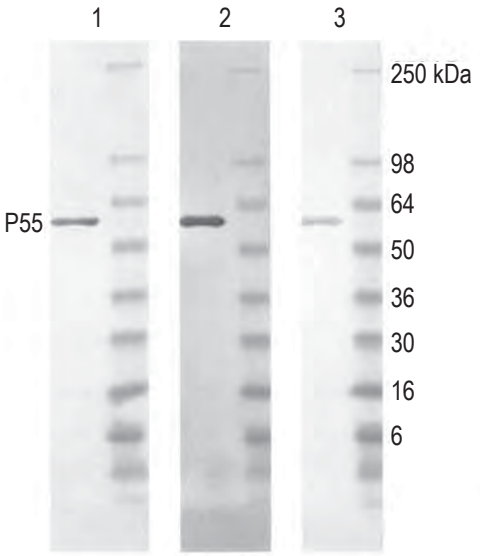


Fig. 3 Western blot using peroxidase-labeled monoclonal antibody to SIV p24 Gag(2F12). SIV P55 and molecular weight markers were analyzed in blots 1, 2, and 3.

Blot 1: conjugate prepared using Peroxidase Labeling Kit-NH_2
Blot 2: conjugate prepared using Peroxidase Labeling Kit-SH
Blot 3: primary antibody and peroxidase-conjugated secondary antibody (commercially available).

Western blotting using peroxidase-labeled primary antibody gives a better result than using peroxidase-labeled secondary antibody. In most cases, because of the site specific conjugation on the antibody the sensitivity of the conjugate prepared with Peroxidase/Alkaline Phosphatase Labeling Kit-SH is higher than that prepared with Labeling Kit-NH_2.

FAQ

♦ Can I use this kit for Fab or Fab'labeling?
Yes, you can label Fab or Fab' using this kit. The recovery rate of the conjugate should be greater than 80%.

♦ Can I use this kit for other proteins?
Yes, if the molecular weight is greater than 50,000 or less than 5,000 and it has a reactive primary or secondary amino group. If the molecular weight is higher than 50,000, follow the labeling protocol for IgG and use 0.5-1 nmol of sample protein for LK11-10. If the molecular weight is less than 5,000, follow the labeling protocol for small molecules. If the molecular weight is higher than 5,000 but lower than 50,000, contact customer service at info@dojindo.com or call 1-877-987-2667 for more information.

♦ Can I use this kit to label an oligonucleotide or oligopeptide?
Yes, if the molecular weight is less than 5,000 and it has a reactive primary or secondary amino group. Follow the labeling protocol for small molecules.

♦ What is the minimum amount of IgG that can be labeled with LK11-10?
The minimum amount is 50 µg. There is no significant difference in sensitivity and background between 50 µg and 200 µg of IgG. Although even 10 µg IgG can be labeled using this kit, the background will be higher.

♦ How many peroxidase molecules per IgG are introduced?
On average, there are 1 to 3 peroxidase molecules per IgG.

♦ Does unconjugated NH_2-reactive peroxidase still have an activated ester after the labeling reaction to IgG?
No. It is completely hydrolyzed during the reaction.

♦ Does NH_2-reactive peroxidase form an oligomer during the labeling reaction?
No. Because all amino groups of NH2-reactive peroxidase are blocked, no oligomerization is possible.

♦ Do I have to use Storage buffer included with the kit?
No, you do not have to use Storage buffer from the kit. You can choose any kind of buffer appropriate for your experiment. However, the Storage buffer helps to increase the stability of the peroxidase conjugate.

♦ Does Storage buffer contain animal products or polymers?
No, Storage buffer does not contain any animal products, polymers, or heavy metal ions.

Peroxidase Labeling Kit-SH

Application: Peroxidase labeling of proteins or thiol compounds

Features: It takes only 3 hours to recover conjugates
All processes in a single filtration tube
High recovery of conjugates

Ordering Information

Product code	Unit
LK09-10	3 samples [a]
LK53-10	1 sample [b]

[a] Based on 100 µg IgG sample labeling
[b] Based on 1 mg IgG sample labeling

Contents of the Kit

LK09-10

SH-reactive peroxidase ..... 100 µg x 3 tubes
Solution A .....4 ml x 1 bottle
Reaction buffer ..... 200 µl x 1 tube
Filtration tube .....3 tubes
Reducing agent .....3 tubes
Solution B .....1 ml x 1 tube
Storage buffer .....4 ml x 1 bottle

LK53-10

SH-reactive peroxidase ..... 1 mg x 1 tube
Solution A .....10 ml x 1 bottle
Reaction buffer ..... 0.6 ml x 1 tube
Filtration tube 1tube
Reducing agent .....1 tube
Solution B .....4 ml x 1 bottle
Storage buffer .....10 ml x 1 bottle
15 ml tube .....1 tube

Storage Condition

0-5°C

Shipping Condition

ambient temperature

Product Description

Peroxidase Labeling Kit-SH is used mainly for the preparation of peroxidase-labeled IgG for enzyme immunoassay (EIA) and for the preparation of peroxidase-labeled antigen for competitive EIA. SH-reactive peroxidase, a component of this kit, can react with the thiol groups of proteins or other molecules (Fig. 1). This kit contains all the necessary reagents for the labeling process, including reducing agent and storage buffer. SH-reactive peroxidase forms a covalent link with the target molecule. Reducing agent can create free thiol groups in the IgG molecule. When peroxidase-labeled IgG is used for EIA, the labeling efficiency of the SH-reactive peroxidase is high enough to eliminate any postlabeling purification process.. If a high-purity conjugate is required after labeling, simply use an affinity column or a gel-permeation column. When labeling small molecules, excess molecules can be removed by using the filtration tubes included in this kit.

Fig. 1 IgG labeling reaction of SH-reactive peroxidase

Precaution

- The molecular weight of the reduced protein to be labeled with this kit should be greater than 50,000.
- The molecular weight of the small thiol compound to be labeled with this kit should be smaller than 5,000.
- IgG or peroxidase-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weight larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to labeling peroxidase with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

Required Equipment and Materials

LK09-10: microcentrifuge, 50-200 µl adjustable pipette, 37°C incubator, 0.5 ml microtubes
LK53-10: centrifuge, rotor for 15 ml centrifuge tube, 50-200 µl and 1 ml adjustable pipettes, 37°C incubator, microtubes

General Protocol for IgG Labeling (LK09-10)

Step 1.
Add 100 µl of Solution A and the sample solution containing 50-200 µg IgG[a] to a Filtration Tube.

Step 2.
Pipette to mix and centrifuge at 8,000 x *g* for 10 min[b].

Step 3.
Add 150 µl of Solution A to Reducing Agent[c], and dissolve by pipetting.

Step 4.
Transfer 100 µl of Reducing Agent solution to the Filtration Tube, and pipette to dissolve the IgG.

Step 5.
Incubate the tube at 37°C for 30 min. Add 100 µl of Solution B and centrifuge at 8,000 x *g* for 10 min[b].

Step 6.
Discard the filtrate, add 200 µl of Solution B and centrifuge at 8,000 x *g* for 10 min again[b].

Step 7.
Add 50 µl of Reaction Buffer to SH-Reactive Peroxidase[d], and dissolve it with pipetting.

Step 8.
Add SH-Reactive Peroxidase solution to the Filtration Tube and pipette to mix.

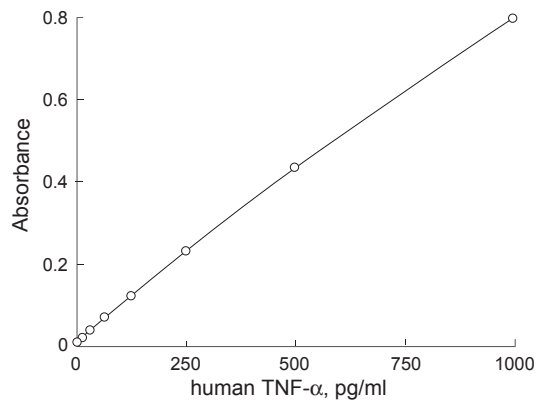
Step 9.
Incubate the tube at 37°C for 1 hour.

Step 10.
Add 100 µl of Solution A to the tube.

Step 11.
Centrifuge at 8,000 x *g* for 10 min[b].

Step 12.
Add 200 µl of Storage Buffer, pipette about 10 times to recover the conjugate[e]. Transfer the solution to a microtube (not included in this kit), and store the solution at 0 - 5°C [f].

[a] The volume of sample solution should be less than 100 µl. If the IgG concentration is lower than 0.5 mg/ml, repeat Steps 1 and 2 until the total IgG accumulation becomes 50-200 µg.
[b] If the solution still remains on the membrane after the centrifugation, spin for another 5 min.
[c] Reducing Agent may be attached on the inside of the cap. Be careful when opening the reducing agent.
[d] SH-Reactive Peroxidase is unstable in Reaction Buffer. Proceed to Step 8 immediately after the preparation of the SH-Reactive Peroxidase solution.
[e] Two to four molecules of peroxidase should be introduced onto one reduced IgG molecule. Unconjugated peroxidase does not interfere with immunoassays. For more purification, use a gel permeation column or an affinity column for IgG.
[f] Generally, the peroxidase-labeled IgG in Storage Buffer is stable for 2 months at 0-5°C. However, it is important to note that the stability will depend on the sample itself. For longer storage, add equal volume of glycerol to the sample solution and store it at -20°C.



Experimental Example

Fig. 2 Sandwich ELISA of human TNF-αdetection

Plate: 2 µg/ml anti-human TNF-αantibody (rabbit, polyclonal)-coated high binding plate
recombinant human TNF-α:
0-1000 pg/ml PBST
Peroxidase-conjugated anti-human TNF-αantibody:
Prepared by Peroxidase Labeling Kit-SH.1µg/ml
PBST+blocking reagent
Substrate: TMB peroxidase substrate

FAQ

- Can I use this kit for F(ab')2?
  Yes, please follow the labeling protocol for IgG. The recovery rate of the conjugate should be greater than 80%.
- Can I use this kit for other proteins or peptides?
  Yes, if the molecular weight of the reduced form is greater than 50,000 or less than 5,000, and it has a reactive SH group, or a disulfide group that can be reduced without losing activity. If the molecular weight is greater than 50,000, follow the labeling protocol for IgG, and use 0.5-1 nmol of sample protein for LK09-10. If the molecular weight is less than 5,000, follow the labeling protocol for small molecules. If the molecular weight is between 5,000 and 50,000, contact our customer service at info@dojindo.com or call 1-877-987-2667 for more information.
- Can I use this kit to label oligopeptides or oligonucleotides?
  Yes, if the molecular weights of the oligonucleotide or the oligopeptide are less than 5,000 and they have at least one SH group. Follow the labeling protocol for small molecule.
- What is the minimum amount of IgG that can be labeled with LK09-10?
  The minimum amount is 50 µg. There is no significant difference in sensitivity and background between 50 µg and 200 µg of IgG. However, even 10 µg IgG can be labeled using 1/5 volume of SH-reactive peroxidase solution at step 8.
- How many peroxidase molecules per reduced IgG are introduced?
  The average number of peroxidase molecule per reduced IgG is 1 to 2.
- Do I have to use a filtration tube prior to labeling the protein?
  If the protein solution does not contain small molecules with reactive SH groups and the concentration of the protein is 10 mg per ml, or about 70 µM, there is no need to use the filtration tube. Just mix the sample solution with Solution B and add the mixture to a vial of the SH-reactive peroxidase.
- Do I have to use Storage buffer included with the kit?
  No, you don't have to use storage buffer from the kit. You can choose any kind of buffer appropriate for your experiment. However, the storage buffer helps to increase the stability of the peroxidase conjugate.
- My sample contains small insoluble material. What should I do?
  Spin the sample and use the supernatant for labeling.
- Does unconjugated SH reactive peroxidase still have a reactive maleimide after the labeling reaction to IgG?
  No. Nearly 100% of SH reactive peroxidase is used for the IgG labeling or the small-molecule labeling.
- Does storage buffer contain animal products or polymers?
  No, storage buffer does not contain any animal products, polymers, or heavy metal ions.

References

1. K. Inoue, A. Sugiyama, P. C. Reid, Y, Ito, K. Miyauchi, S. Mukai, M. Sagara, K. Miyamoto, H. Satoh, I. Kohno, T. Kurata, H. Ota, A. Mantovani, T. Hamakubo, H. Daida and T. Kodama, Establishment of a High Sensitivity Plasma Assay for Human Pentraxin3 as a Marker for Unstable Angina Pectoris, *Arterioscler. Thromb. Vasc. Biol.*, **2007**, 27, 161

Alkaline Phosphatase Labeling Kit-NH_2

Application: Alkaline phosphatase labeling of proteins or amine compounds

Features: Only 3 hours to recover conjugates
All processes in a single filtration tube
High recovery of conjugates

Ordering Information

Product code	Unit
LK12-10	3 samples [a]
LK59-10	1 sample [b]

[a] Based on 100 µg IgG sample labeling
[b] Based on 1 mg IgG sample labeling

Contents of the Kit

LK12-10

NH_2-reactive ALP ..... 100 µg x 3 tubes
Reaction buffer .....200 µl x 1 tube
Filtration tube ..... 3 tubes
Washing buffer ..... 4 ml x 1 bottle
Storage buffer ..... 4 ml x 1 bottle

LK59-10

NH2-reactive ALP ..... 1 mg x 1 tube
Reaction buffer .....1.2 ml x 1 tube
Filtration tube ..... 1 tube
Washing buffer ..... 10 ml x 1 bottle
Storage buffer ..... 10 ml x 1 bottle
15 ml tube ..... 1 tube

* ALP: alkaline phosphatase

Storage Condition

0-5°C

Shipping Condition

with blue ice

Product Description

Alkaline Phosphatase Labeling Kit-NH_2 is mainly used for the preparation of alkaline phosphatase-labeled IgG for enzyme immunoassay (EIA) and for the preparation of alkaline phosphatase-labeled antigen for competitive EIA. NH_2-reactive ALP, a component of this kit, can react with amino groups of proteins or other molecules (Fig. 1). This kit contains all the reagents necessary for the labeling process, including storage buffer. NH_2-reactive ALP forms a covalent link with the target molecule without any activation process. When the alkaline phosphatase-labeled IgG is used for EIA, the labeling efficiency of the NH_2-reactive ALP is high enough to eliminate any post labeling purification process.. If a high purity conjugate is required after labeling, simply use an affinity column or a gel-permeation column. When labeling small molecules, excess molecules can be removed using the filtration tubes included in this kit.

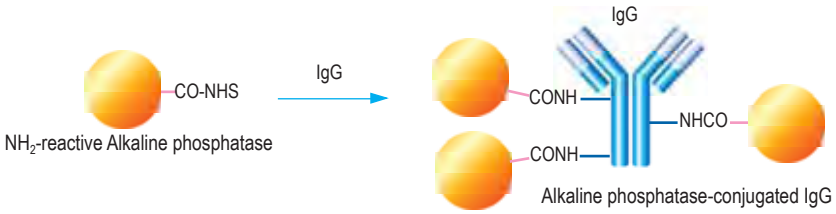


Fig. 1 IgG labeling reaction of NH_2-reactive alkaline phosphatase

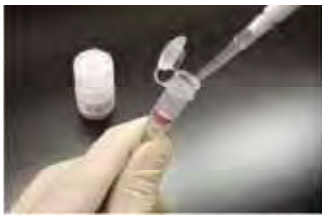
Precaution

- The molecular weight of the protein to be labeled with this kit should be greater than 50,000.
- The molecular weight of the small amine compound to be labeled with this kit should be smaller than 5,000.
- IgG or alkaline phosphatase-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weight larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to labeling alkaline phosphatase with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

Required Equipment and Materials

LK12-10: microcentrifuge, 10 µl and 50-200 µl adjustable pipettes, 37°C incubator, 0.5 ml microtubes
LK59-10: centrifuge, rotor for 15 ml centrifuge tube, 50-200 µl and 1 ml adjustable pipettes, 37°C incubator, microtubes

General Protocol for IgG Labeling (LK12-10)



Step 1.
Add 100 µl of Washing Buffer and the sample solution containing 50-200 µg IgG[a] to a Filtration Tube.

Step 2.
Pipette to mix and centrifuge at 8,000 x g for 10 min[b].

Step 3.
Add 100 µl of Washing Buffer to the Filtration Tube again.

Step 4.
Centrifuge at 8,000 x g for 10 min[b].

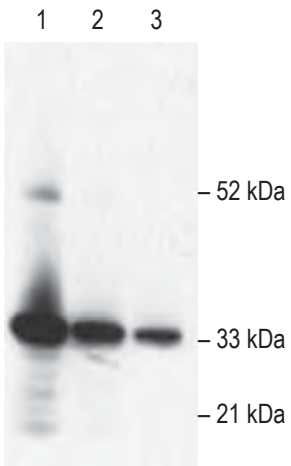
Step 5.
Add 10 µl of Reaction Buffer to NH_2-Reactive Alkaline Phosphatase, and dissolve by pipetting. [c]

Step 6.
Add NH_2-Reactive Alkaline Phosphatase solution to the Filtration Tube and pipette to mix.

Step 7.
Incubate the tube at 37 °C for 2 hour.

Step 8.
Add 190 µl of Storage Buffer, and pipette about 10 times to recover the conjugate[d]. Transfer the solution to a microtube (not included in the kit), and store at 0 - 5 °C[e].

[a] The volume of IgG solution should be less than 100 µl. If the IgG concentration is lower than 0.5 mg/ml, repeat Steps 1 and 2 until the total IgG accumulation becomes 50-200 µg.
[b] If the solution still remains on the membrane after the centrifugation, spin it for another 5 min.
[c] NH_2-Reactive Alkaline Phosphatase is unstable in Reaction Buffer. Proceed to Step 6 immediately after the preparation of the NH_2-Reactive Alkaline Phosphatase solution.
[d] One to three molecules of alkaline phosphatase should be introduced onto one IgG molecule. Unconjugated alkaline phosphatase does not interfere with immunoassays. For more purification, use a gel permeation column or an affinity column for IgG.
[e] Generally, the alkaline phosphatase-labeled IgG in Storage Buffer is stable for 2 months at 0-5°C. However, it is important to note that the stability will depend on the sample itself. For longer storage, store at -20°C.



Experimental Example

Fig. 2 Western blotting of alkaline phosphatase-conjugated antibody prepared by Alkaline Phosphatase Labeling Kit-NH_2.

1: antigen 50 ng
2: antigen 10 ng
3: antigen 2 ng

A target protein (antigen) was detected with ALP-labeled antibody prepared by Alkaline Phosphatase Labeling Kit-NH_2 after it was run with SDS-PAGE and transferred to a nitrocellulose membrane. A target protein was detected with a chemiluminescence substrate for alkaline phosphatase after the treatment with 25,000 times dilution of ALP-labeled primary antibody.

FAQ

- Can I use this kit for Fab or Fab' labeling?
  Yes, you can label Fab and Fab' using this kit. The recovery rate of the conjugate should be greater than 80%.
- Can I use this kit for other proteins or peptides?
  Yes, if the molecular weight is higher than 50,000 or lower than 5,000, and it has a reactive primary or secondary amino group. If the molecular weight is higher than 50,000, follow the labeling protocol for IgG, and use 0.5-1 nmol of sample protein for LK12-10. If the molecular weight is lower than 5,000, follow the labeling protocol for small molecules. If the molecular weight is lower than 50,000 but higher than 5,000, contact our customer service at info@dojindo.com or call 1-877-987-2667 for more information.
- Can I use this kit to label an oligonucleotide or oligopeptide?
  Yes, if the molecular weight is less than 5,000, and it has a reactive primary or secondary amino group. Follow the labeling protocol for small molecules.
- What is the minimum amount of IgG that can be labeled with LK12-10?
  The minimum amount is 50 µg. There is no significant difference in sensitivity and background between 50 µg and 200 µg of IgG. Though 10 µg IgG can still be labeled using this kit, the background will be higher.
- How many alkaline phosphatase molecules are introduced per IgG?
  The average number of alkaline phosphatase molecules per IgG is 1 to 3.
- Does unconjugated NH_2-reactive alkaline phosphatase still have an activated ester after the labeling reaction with IgG?
  No. NHS is completely hydrolyzed during the reaction.
- Does NH_2-reactive alkaline phosphatase form an oligomer during the labeling reaction?
  No. Because all reactive amino groups of NH_2-reactive alkaline phosphatase are blocked, no oligomerization is possible.
- Do I have to use Storage buffer included with the kit?
  No, you do not have to use storage buffer from the kit. You can choose any kind of buffer appropriate for your experiment.
- Does Storage buffer contain animal products or polymers?
  No, storage buffer does not contain any animal products, polymers, or heavy metal ions.

Alkaline Phosphatase Labeling Kit-SH

Application: Alkaline phosphatase labeling of proteins or thiol compounds

Features: Only 3 hours to recover conjugates
All processes in a single filtration tube
High recovery of conjugates

Ordering Information

Product code	Unit
LK13-10	3 samples [a]
LK61-10	1 sample [b]

[a] Based on 100 µg IgG sample labeling
[b] Based on 1 mg IgG sample labeling

Contents of the Kit

LK13-10

SH-reactive ALP ..... 100 µg x 3 tubes
Solution A ..... 4 ml x 1 bottle
Reaction buffer .....200 µl x 1 tube
Filtration tube ..... 3 tubes
Reducing agent .....3 tubes
Solution B ..... 1 ml x 1 tube
Storage buffer ..... 4 ml x 1 bottle

LK61-10

SH-reactive ALP ..... 1 mg x 1 tube
Solution A ..... 10 ml x 1 bottle
Reaction buffer .....0.6 ml x 1 tube
Filtration tube .....1 tube
Reducing agent ..... 1 tube
Solution B .....4 ml x 1 bottle
Storage buffer ..... 10 ml x 1 bottle
15 ml tube ..... 1 tube

* ALP: alkaline phosphatase

Storage Condition

0-5°C

Shipping Condition

ambient temperature

Product Description

Alkaline Phosphatase Labeling Kit-SH is used mainly for the preparation of alkaline phosphatase-labeled IgG for enzyme immunoassay (EIA) and for the preparation of alkaline phosphatase-labeled antigen for competitive EIA. SH-reactive ALP, a component of this kit, can react with the thiol groups of proteins or other molecules (Fig. 1). This kit contains all the reagents necessary for the labeling process, including reducing agent and storage buffer. SH-reactive ALP forms a covalent link with the target molecule. Reducing agent can create free thiol groups in the IgG molecule. The labeling efficiency of the SH-reactive ALP is high enough to eliminate any purification process after labeling when the alkaline phosphatase-labeled IgG is used for EIA. If a high-purity conjugate is required after labeling, simply use an affinity column or a gel-permeation column. When labeling small molecules, excess molecules can be removed by using the filtration tubes included in this kit.

Enzyme Labeling

Fig. 1 IgG labeling reaction of SH-reactive alkaline phosphatase

Precaution

- The molecular weight of the reduced protein to be labeled with this kit should be greater than 50,000.
- The molecular weight of the small amine compound to be labeled with this kit should be smaller than 5,000.
- IgG or alkaline phosphatase-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weight larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to labeling alkaline phosphatase with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

Required Equipment and Materials

LK13-10: microcentrifuge, 50-200 µl adjustable pipette, 37°C incubator, 0.5 ml microtubes
LK61-10: centrifuge, rotor for 15 ml centrifuge tube, 50-200 µl and 1 ml adjustable pipettes, 37°C incubator, microtubes

General Protocol for IgG Labeling (LK13-10)

Step 1.
Add 100 µl of Solution A and the sample solution containing 50-200 µg IgG[a] to a Filtration Tube.

Step 2.
Pipette to mix and centrifuge at 8,000 x g for 10 min[b].

Step 3.
Add 150 µl of Solution A to Reducing Agent[c], and dissolve with pipetting.

Step 4.
Transfer 100 µl of the Reducing Agent solution to the Filtration Tube, and pipette to dissolve the IgG.

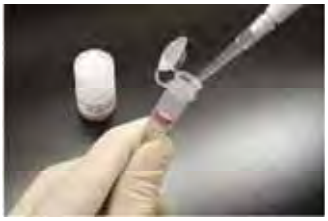
Step 5.
Incubate the tube at 37°C for 30 min. Add 100 µl of Solution B and centrifuge at 8,000 x g for 10 min[b].

Step 6.
Discard the filtrate, add 200 µl of Solution B and centrifuge at 8,000 x g for 10 min again[b].

Step 7.
Add 50 µl of Reaction Buffer to SH-Reactive Alkaline Phosphatase, and dissolve it by pipetting[d].

Step 8.
Add SH-Reactive Alkaline Phosphatase solution to the Filtration Tube and pipette to mix.

Step 9.
Incubate the tube at 37°C for 1 hour.



Step 10.
Add 150 µl of Storage Buffer and pipette about 10 times to recover the conjugate[e]. Transfer the solution to a microtube (not included in the kit), and store the solution at 0 - 5 °C [f].

[a] *The volume of sample solution should be less than 100 µl. If the IgG concentration is lower than 0.5 mg/ml, repeat Steps 1 and 2 until the total IgG accumulation becomes 50-200 µg.*
[b] *If the solution still remains on the membrane after the centrifugation, spin for another 5 min.*
[c] *Reducing Agent may be attached on the inside of the cap. Be careful when opening the reducing agent.*
[d] *SH-Reactive Alkaline Phosphatase is unstable in Reaction Buffer. Proceed to Step 8 immediately after the preparation of the SH-Reactive Alkaline Phosphatase solution.*
[e] *One to two molecules of Alkaline Phosphatase should be introduced onto one reduced IgG molecule. Unconjugated Alkaline Phosphatase does not interfere with immunoassays. For more purification, use a gel permeation column or an affinity column for IgG.*
[f] *Generally, the Alkaline Phosphatase-labeled IgG in Storage Buffer is stable for 2 months at 0-5oC. However, it is important to note that the stability will depend on the sample itself. For longer storage, add equal volume of glycerol to the sample solution and store it at -20oC.*

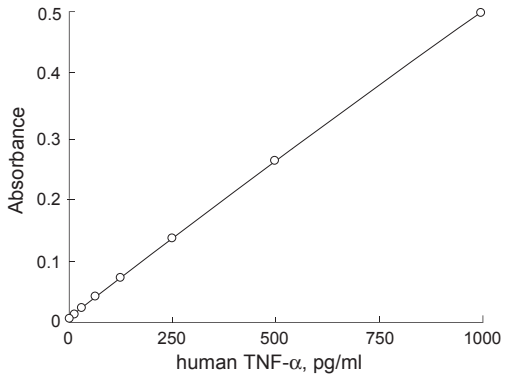


Fig. 3 Sandwich ELISA of human TNF-αdetection

Plate: 2µg/ml anti-human TNF-αantibody (rabbit, polyclonal)-coated high binding plate
recombinant human TNF-α:
0-1000 pg/ml PBST
ALP-conjugated anti-human TNF-αantibody:
Prepared by Alkaline phosphatase Labeling Kit-SH .1µg/ml
PBST+blocking reagent
Substrate: *p*-Nitrophenylphosphate, ALP substrate

FAQ

♦ Can I use this kit for $F(ab')_2$?
Yes, please follow the labeling protocol for IgG. The recovery rate of the conjugate should be greater than 80%.

♦ Can I use this kit for other proteins or peptides?
Yes, if the molecular weight of the reduced form is greater than 50,000 or less than 5,000, and it has a reactive SH group or a disulfide group that can be reduced without losing activity. If the molecular weight is greater than 50,000, follow the labeling protocol for IgG and use 0.5-1 nmol of sample protein for LK13-10. If the molecular weight is less than 5,000, follow the labeling protocol for small molecules. If the molecular weight is between 5,000 and 50,000, contact our customer service at info@dojindo.com or call 1-877-987-2667 for more information.

♦ Can I use this kit to label oligopeptides or oligonucleotides?
Yes, if the molecular weights of the oligonucleotide or the oligopeptide are less than 5,000 and they have at least one SH group. Follow the labeling protocol for small molecule.

♦ What is the minimum amount of IgG that can be labeled with LK13-10?
The minimum amount is 50 µg. There is no significant difference in sensitivity and background between 50 µg and 200 µg of IgG. However, even 10 µg IgG can be labeled, using 1/5 volume of SH-reactive ALP solution at step 8.

♦ How many alkaline phosphatase molecules per reduced IgG are introduced?
The average number of alkaline phosphatase molecules per reduced IgG is 1 to 2.

♦ Do I have to use a filtration tube prior to labeling the protein?
If the protein solution does not contain small molecules with reactive SH groups and the concentration of the protein is 10 mg per ml, or about 70 µM, there is no need to use the filtration tube. Just mix 10 µl of the sample solution with Solution B and add the mixture to a vial of the SH-reactive peroxidase.

♦ Do I have to use storage buffer included with the kit?
No, you don't have to use storage buffer from the kit. You can choose any kind of buffer appropriate for your experiment.

♦ My sample contains small insoluble material. What should I do?
Spin the sample and use the supernatant for labeling.

♦ Does unconjugated SH-reactive ALP still have a reactive maleimide after the labeling reaction to IgG?
No. Nearly 100% of SH-reactive ALP is used for the IgG labeling or the small-molecule labeling.

♦ Does Storage buffer contain animal products or polymers?
No, Storage buffer does not contain any animal products , polymers, or heavy metal ions.

R-Phycoerythrin Labeling Kit-NH_2

Application: R-Phycoerythrin labeling of proteins or amine compounds

Features: Only 3 hours to recover conjugates
All processes in a single filtration tube
High recovery of conjugates
Applicable for 50-200 µg IgG

Ordering Information

Product code	Unit
LK23-10	3 samples [a]

[a] Based on 100 µg IgG sample labeling

Contents of the Kit

NH_2-reactive R-PE ..... 3 tubes
Reaction buffer ..... 200 µl x 1 tube
*R-PE: R-phycoerythrin
WS buffer .....4 ml x 1 bottle
Filtration tube ..... 3 tubes

Storage Condition

0-5°C

Shipping Condition

with blue ice

Product Description

Phycobiliproteins are fluorescent proteins derived from cyanobacteria and eukaryotic algae. Their fluorescence is much higher than chemical fluorescent probes such as fluorescein and rhodamine. R-Phycoerythrin (R-PE) is one of the phycobiliproteins and has a red fluorescence at around 578 nm, and it can be excited at 488nm (Fig. 1). Because of this high fluorescence, phycobiliprotein labeled antibodies, and other molecules can provide greater sensitivity in flow cytometry and immunostaining. R-phycoerythrin Labeling Kit-NH_2 is for simple and rapid preparation of R-PE-labeled IgG (Fig. 2). NH_2-reactive R-PE (a component of this kit) has an activated ester group and can easily make a covalent bond with an amino group of the target molecule without any activation process. The filtration tube in this kit allows a quick buffer exchange and concentration of sample IgG solution. This kit contains all of the reagents necessary for R-PE labeling, including the Storage buffer for conjugates.

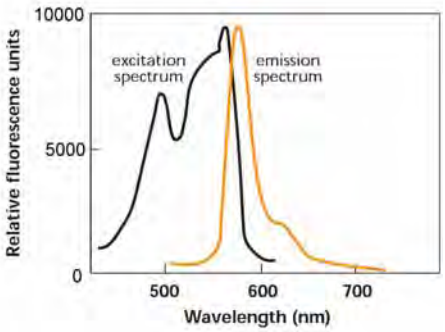


Fig. 1 Fluorescence spectrum of R-PE
Excitation wavelength: 566 nm
Emission wavelength: 578 nm

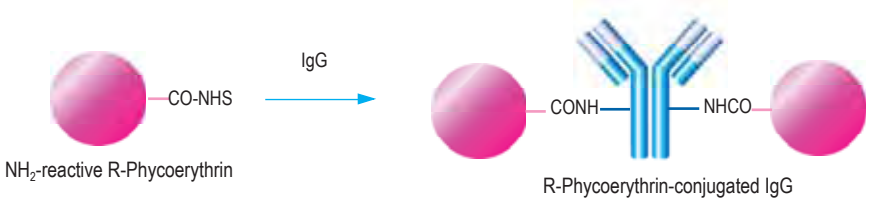


Fig. 2 IgG labeling reaction of NH_2-reactive R-PE

Required Equipment and Materials

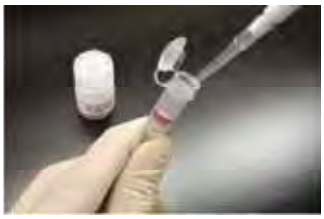
Microcentrifuge, 10 µl and 100-200 µl adjustable pipettes, 37°C incubator, 0.5 ml microtubes

Precaution

- The molecular weight of the protein to be labeled with this kit should be greater than 50,000.
- IgG or R-phycoerythrin-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weight larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to labeling R-phycoerythrin with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

Fluorophore Labeling

General Protocol for IgG Labeling



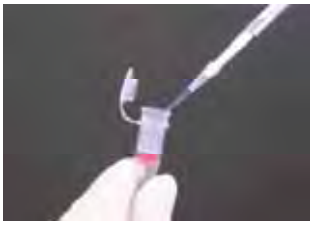
Step 1.
Add 100 µl of WS Buffer and the sample solution containing 50-200 µg protein[a] to a Filtration tube.

Step 2.
Mix the solution with pipetting several times, and centrifuge at 8,000 x g for 10 min.[b]

Step 3.
Add 100 µl of WS Buffer to a Filtration Tube.

Step 4.
Mix the solution with pipetting several times, and centrifuge at 8,000 x g for 10 min.[b]

Step 5.
Add 10 µl of Reaction Buffer to NH_2-Reactive R-Phycoerythrin, and dissolve by pipetting.[c]



Step 6.
Add NH_2-Reactive R-Phycoerythrin solution to the IgG concentrated on the Filtration Tube.

Step 7.
Incubate the tube at 37°C for 2 hours after pipetting to mix.

Step 8.
Add 190 µl of WS Buffer, and pipette about 10 times to recover the conjugate.[d] Transfer the solution to a microtube(not included in this kit), and store at 0-5°C.[e]

[a] The volume of IgG solution should be less than 100 µl. If the IgG concentration is lower than 0.5 mg/ml, repeat Steps 1 and 2 until the total IgG accumulation becomes 50 - 200 µg.

[b] If solution still remains on the membrane after the centrifugation, spin for another 5 min.

[c] NH_2-Reactive R-Phycoerythrin can be hydrolyzed by water. Proceed to Step 6 immediately after the preparation of the NH_2-Reactive R-Phycoerythrin solution.

[d] One to two R-Phycoerythrin should be introduced into one IgG molecule. Unconjugated R-Phycoerythrin remained in the solution might cause background increase with immunoassay. For more purification, use a gel permeation column or an affinity column for IgG.

[e] We recommend using WS Buffer to recover the conjugate. You can choose any kinds of buffers appropriate for your experiment.

FAQ

- **Can I use this kit for other proteins?**
  Yes, if the molecular weight is higher than 50,000, and it has a reactive primary or secondary amino group. Follow the protocol for IgG labeling with 0.5-1 nmol of sample protein.
- **How many R-PE molecules per IgG are introduced?**
  The average number of R-PE molecules per IgG is 1 to 2.
- **Does unconjugated NH_2-reactive R-PE still have an activated ester after the labeling reaction to IgG?**
  No. It is completely hydrolyzed during the reaction.
- **Does NH_2-reactive R-PE form an oligomer during the labeling reaction?**
  No. Since all amino groups of NH_2-reactive R-PE are blocked, no oligomerization is possible.
- **What is the minimum amount of IgG that can be labeled with LK23-10?**
  The minimum amount is 50 µg. There is no significant difference in sensitivity and background between 50 µg and 200 µg of IgG.
- **Do I have to use WS buffer included with the kit?**
  Yes. Use the WS buffer to prepare a stock solution of the conjugate. However, you can choose any kind of buffer appropriate to dilute the conjugate stock solution for your experiment.
- **How long is the conjugate stable?**
  If you store at 4°C, it is stable for more than 2 months. For longer storage, add 100% volume of glycerol, aliquot, and store at -20°C. However, please note that the stability depends on the protein itself.

R-Phycoerythrin Labeling Kit-SH

Application: R-phycoerythrin labeling of proteins

Features: Only 3 hours to recover conjugates
All processes in a single filtration tube
High recovery of conjugates
Applicable for 50-200 µg IgG

Ordering Information

Product code	Unit
LK26-10	3 samples [a]

[a] Based on 100 µg IgG sample labeling

Contents of the Kit

SH-reactive R-PE ..... 3 tubes
WS buffer ..... 4 ml x 1 bottle
Filtration tube .....3 tubes
*R-PE: R-Phycoerythrin

Reducing agent ..... 3 tubes
Reaction buffer ..... 200 µl x 1 tube
RA solution .....1 ml x 1 tube

Storage Condition

0-5°C

Shipping Condition

ambient temperature

Product Description

Phycobiliproteins are fluorescent proteins derived from cyanobacteria and eukaryotic algae. Their fluorescence is much higher than chemical fluorescent probes such as fluorescein and rhodamine. R-phycoerythrin (R-PE) is one of the phycobiliproteins and has an orange fluorescence at around 578 nm, and it can be excited at 488 nm (Fig. 1). Because of this high fluorescence, phycobiliprotein-labeled antibodies or other molecules can give greater sensitivity in flow cytometry and immunostaining. R-phycoerythrin Labeling Kit-SH is for simple and rapid preparation of R-PE-labeled IgG (Fig. 2). SH-reactive R-PE (a component of this kit) has a maleimide group and can easily make a covalent bond with a sulfhydryl group of the target molecule without any activation process. The filtration tube in this kit allows a quick buffer exchange and concentration of sample IgG solution. This kit contains all the reagents necessary for R-PE labeling, including the reducing agent for preparation of reduced IgG that has an SH group and the storage buffer for conjugates.

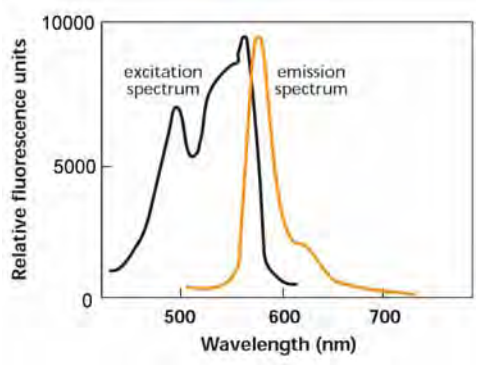


Fig. 1 Fluorescence spectrum of R-PE
Excitation wavelength: 566 nm
Emission wavelength: 578 nm

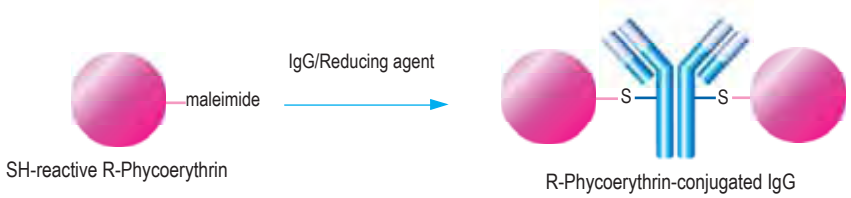


Fig. 2 IgG labeling reaction of SH-reactive R-PE

Required Equipment and Materials

microcentrifuge, 10 µl and 100-200 µl adjustable pipettes, 37°C incubator, 0.5 ml microtubes

Precaution

- The molecular weight of the reduced protein to be labeled with this kit should be greater than 50,000.
- IgG or R-Phycoerythrin-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weight larger than 10,000, such as BSA or gelatin, purify the IgG solution prior to labeling R-phycoerythrin with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

Fluorophore Labeling

General Protocol for IgG Labeling

Step 1.
Add 100 µl WS buffer and the sample solution containing 100 µg IgG to the filtration tube. [a)]

Step 2.
Mix the solution with pipetting several times and centrifuge at 8000-10,000 x g for 10 min.[b)]

Step 3.
Add 150 µl WS buffer to Reducing agent and dissolve it with pipetting several times.

Step 4.
Transfer 100 µl of the solution from step 3 onto the membrane of the filtration tube where IgG is concentrated.

Step 5.
Pipette several times and incubate the tube at 37°C for 30 min.

Step 6.
Add 100 µl RA solution to the tube and centrifuge at 8,000-10,000 x g for 10 min. Discard the filtrate, add 200 µl RA solution, and centrifuge again.[b)]

Step 7.
Add 50 µl Reaction buffer to SH-reactive RPE and dissolve with pipetting.

Step 8.
Transfer the SH-reactive RPE solution onto the membrane of the filtration tube where reduced IgG is concentrated.

Step 9.
Pipette several times and incubate the tube at 37°C for 1 hr.

Step 10.
Add 150 µl WS buffer and pipette 10 to 15 times to recover the conjugate.[c)] Transfer the solution to a 0.5 ml tube and store the solution at 0-5°C.[d)]

[a)] The volume of sample solution should be 100 µl or less. If the antibody concentration is lower than 0.5 mg/ml, repeat step 1 and 2 until the total IgG accumulation becomes 100 µg. If the volume of the filtrate becomes 400 µl or more during the accumulation process, discard the filtrate prior to go to the next centrifuge step.

[b)] If the solution still remains on the membrane after the centrifugation, centrifuge for another 5 min. or increase the centrifuge speed.

[c)] The concentration of the conjugate is 1.0-1.3 mg/ml. Dilute the APC-labeled reduced IgG to prepare a solution with an appropriate concentration prior to using it for flow cytometry, immunoblotting, or immunostaining. One to two APC molecules should be introduced onto one reduced IgG molecule. Unconjugated APC should not interfere with a normal assay. If purification is necessary, use a gel permeation column or an affinity column for IgG.

[d)] Generally, the APC-labeled reduced IgG in WS buffer is stable for at least 2 months at 0-5 °C. For longer storage, add glycerol (final concentration: 50%), aliquot, and store at -20°C. However, it is important to note that the stability will depend on the sample itself.

FAQ

- **Can I use this kit for $F(ab')_2$?**
  Yes, please follow the labeling protocol for IgG. The recovery rate of the conjugate should be greater than 80%.
- **Can I use this kit for other proteins?**
  Yes, if the molecular weight of the reduced form is greater than 50,000 and it has a reactive SH group, or a disulfide group that can be reduced without losing activity. Follow the labeling protocol for IgG, and use 0.5-1 nmol of sample protein.
- **How many R-PE molecules per reduced IgG are introduced?**
  The average number of R-PE molecule per reduced IgG is 1 to 2.
- **Do I have to use the filtration tube prior to labeling the protein?**
  If the protein solution does not contain small molecules with reactive SH groups and the concentration of the protein is 10 mg per ml, or about 70 µM, there is no need to use the filtration tube. Just mix 10 µl of the sample solution with reaction buffer and add the mixture to a vial of the SH-reactive R-PE.
- **Do I have to use WS buffer included with the kit?**
  Yes. Use the WS buffer to prepare a stock solution of the conjugate. However, for your experiment you can choose any kind of buffer that is appropriate for diluting the conjugate stock solution.

Allophycocyanin Labeling Kit-NH_2

Application: Allophycocyanin labeling of proteins

Features: Only 3 hours to recover conjugates
All processes in a single filtration tube
High recovery of conjugates
Applicable for 50-200 µg IgG

Ordering Information

Product code	Unit
LK21-10	3 samples [a]

[a] Based on 100 µg IgG sample labeling

Contents of the Kit

NH_2-reactive APC .....3 tubes
Reaction buffer ..... 200 µl x 1 tube
*APC: Allophycocyanin
WS buffer ..... 4 ml x 1 bottle
Filtration tube ..... 3 tubes

Storage Condition

0-5°C

Shipping Condition

with blue ice

Product Description

Phycobiliproteins are fluorescent proteins derived from cyanobacteria and eukaryotic algae. Their fluorescence is much higher than chemical fluorescent probes such as fluorescein and rhodamine. Allophycocyanin (APC) is one of the phycobiliproteins, and it has a red fluorescence at around 660 nm (Fig. 1). Because of this high fluorescence, phycobiliprotein-labeled antibodies and other molecules can provide greater sensitivity in flow cytometry and immunostaining. Allophycocyanin Labeling Kit-NH_2 is for simple and rapid preparation of APC-labeled IgG (Fig. 2). NH_2-reactive APC (a component of this kit) has an activated ester group and can easily make a covalent bond with an amino group of the target molecule without any activation process. The filtration tube in this kit is used for buffer exchange and concentration of sample IgG solution. This kit contains all the reagents necessary for APC labeling including the storage buffer for conjugates.

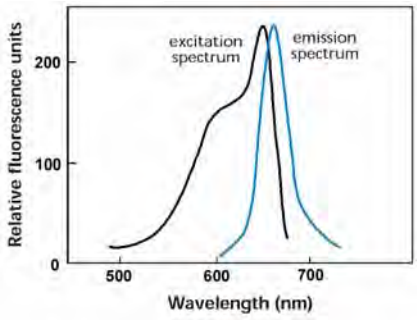


Fig. 1 Fluorescence spectrum of APC
Excitation wavelength: 650 nm
Emission wavelength: 660 nm

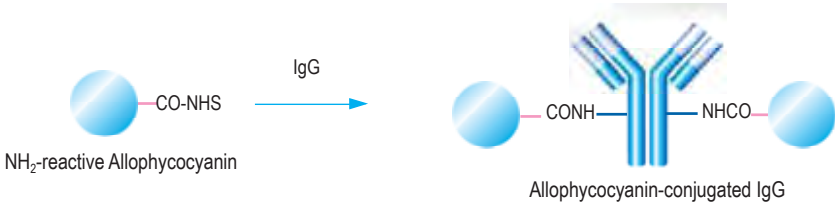


Fig. 2 IgG labeling reaction of NH_2-reactive APC

Required Equipment and Materials

Microcentrifuge, 10 µl and 100-200 µl adjustable pipettes, 37°C incubator, 0.5 ml microtubes

Precaution

- The molecular weight of the protein to be labeled with this kit should be greater than 50,000.
- IgG or allophycocyanin-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weight greater than 10,000, such as BSA or gelatin, purify the IgG solution before labeling allophycocyanin with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

Fluorophore Labeling

General Protocol for IgG Labeling

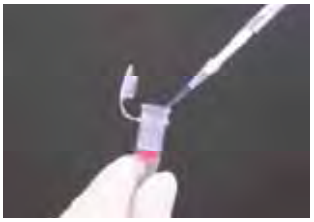
Step 1.
Add 100 µl of WS Buffer and the sample solution containing 50-200 µg protein[a] to a Filtration tube.

Step 2.
Mix the solution by pipetting several times, and centrifuge at 8,000 x *g* for 10 min.[b]

Step 3.
Add 100 µl of WS Buffer to a Filtration Tube.

Step 4.
Mix the solution by pipetting several times, and centrifuge at 8,000 x *g* for 10 min.[b]

Step 5.
Add 10 µl of Reaction Buffer to NH_2-Reactive Allophycocyanin, and dissolve by pipetting.[c]



Step 6.
Add NH_2-Reactive Allophycocyanin solution to the IgG concentrated on the Filtration Tube.

Step 7.
Incubate the tube at 37°C for 2 hours after pipetting to mix.

Step 8.
Add 190 µl of WS Buffer, and pipette about 10 times to recover the conjugate.[d] Transfer the solution to a microtube (not included in this kit), and store at 0-5°C.[e]

[a] *The volume of IgG solution should be less than 100 µl. If the IgG concentration is lower than 0.5 mg/ml, repeat Steps 1 and 2 until the total IgG accumulation becomes 50 - 200 µg.*
[b] *If solution still remains on the membrane after the centrifugation, spin for another 5 min.*
[c] *NH_2-Reactive Allophycocyanin can be hydrolyzed by water. Proceed to Step 6 immediately after the preparation of the NH_2-Reactive Allophycocyanin solution.*
[d] *One to three Allophycocyanin should be introduced into one IgG molecule. Unconjugated Allophycocyanin remained in the solution might cause background increase with immunoassay. For more purification, use a gel permeation column or an affinity column for IgG.*
[e] *We recommend using WS Buffer to recover the conjugate. You can choose any kinds of buffers appropriate for your experiment.*

FAQ

- **Can I use this kit for other proteins?**
  Yes, if the molecular weight is higher than 50,000 and it has a reactive primary or secondary amino group. Follow the protocol for IgG labeling with 0.5-1 nmol of sample protein.
- **How many APC molecules per IgG are introduced?**
  The average number of APC molecules per IgG is 1 to 2.
- **Does unconjugated NH_2-reactive APC still have an activated ester after the labeling reaction to IgG?**
  No. It is completely hydrolyzed during the reaction.
- **Does NH_2-reactive APC form an oligomer during the labeling reaction?**
  No. Because all amino groups of NH_2-reactive APC are blocked, no oligomerization is possible.
- **What is the minimum amount of IgG that can be labeled with LK21-10?**
  The minimum amount is 50 µg. There is no significant difference in sensitivity and background between 50 µg and 200 µg of IgG.
- **Do I have to use the WS buffer included with the kit?**
  Yes. Use the WS buffer to prepare a stock solution of the conjugate. However, for your experiment you can choose any kind of buffer that is appropriate for diluting the conjugate stock solution.
- **How long is the conjugate stable?**
  If you store at 4°C, it is stable for more than 2 months. For longer storage, add 100% volume of glycerol and aliquot and store at -20°C. However, please note that the stability depends on the protein itself.

Reference

1. H. Shinohara, T. Yasuda, Y. Aiba, H. Sanjo, M. Hamadate, H. Watarai, H. Sakurai and T. Kurosaki, PKCβ Regulates BCR-mediated IKK Activatioin by Facilitating the Interaction between TAK1 and CARMA1, *J. Exp. Med*., 2005, **202**, 1423.

Allophycocyanin Labeling Kit-SH

Application: Allophycocyanin labeling of proteins

Features: Only 3 hours to recover conjugates
All processes in a single filtration tube
High recovery of conjugates
Applicable for 50-200 µg IgG

Ordering Information

Product code	Unit
LK24-10	3 samples [a]

[a] Based on 100 µg IgG sample labeling

Contents of the Kit

SH-reactive APC .....3 tubes
WS buffer ..... 4 ml x 1 bottle
Filtration tube .....3 tubes
*APC: Allophycocyanin

Reducing agent ..... 3 tubes
Reaction buffer.....200 µl x 1 tube
RA solution .....1 ml x 1 tube

Storage Condition

0-5°C

Shipping Condition

ambient temperature

Product Description

Phycobiliproteins are fluorescent proteins derived from cyanobacteria and eukaryotic algae. Their fluorescence is much higher than that of chemical fluorescent probes such as fluorescein and rhodamine. Allophycocyanin (APC) is one of the phycobiliproteins, and it has a red fluorescence at around 660 nm (Fig. 1). Because of this high fluorescence, phycobiliprotein-labeled antibodies and other molecules can provide greater sensitivity in flow cytometry and immunostaining. Allophycocyanin Labeling Kit-SH is for simple and rapid preparation of APC-labeled IgG (Fig. 2). SH-reactive APC (a component of this kit) has a maleimide group and can easily make a covalent bond with a sulfhydryl group of the target molecule without any activation process. The filtration tube in this kit allows a quick buffer exchange and concentration of sample IgG solution. This kit contains all the reagents necessary for APC labeling, including a reducing agent for preparation of reduced IgG that has an SH group and a storage buffer for conjugates.

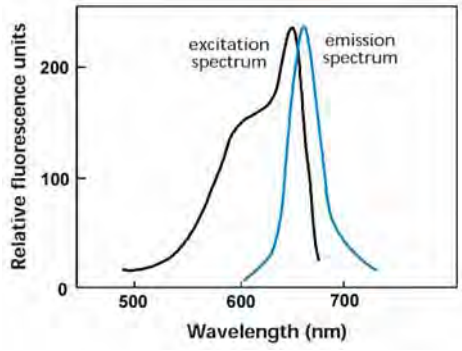


Fig. 1 Comparison of fluorescence intensity of Phycobiliproteins and cyanine dye
Excitation wavelength: 650 nm
Emission wavelength: 660 nm

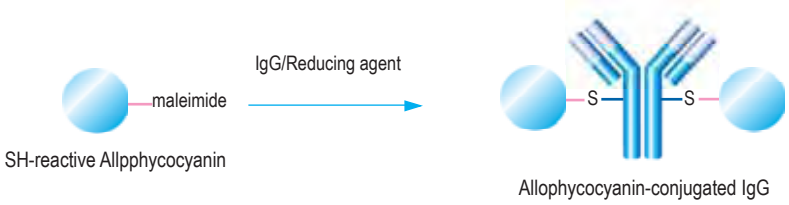


Fig. 2 IgG labeling reaction of SH-reactive APC

Required Equipment and Materials

Microcentrifuge, 10 µl and 100-200 µl adjustable pipettes, 37°C incubator, 0.5 ml microtubes

Precaution

- The molecular weight of the reduced protein to be labeled with this kit should be greater than 50,000.
- IgG or allophycocyanin-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weight larger than 10,000, such as BSA or gelatin, purify the IgG solution before labeling allophycocyanin with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

Fluorophore Labeling

General Protocol for IgG Labeling

Step 1.
Add 100 µl of WS Buffer and the sample solution containing 50-200 µg protein[a] to a Filtration tube.

Step 2.
Mix the solution by pipetting several times, and centrifuge at 8,000 x g for 10 min.[b]

Step 3.
Add 100 µl of WS Buffer to a Filtration Tube.

Step 4.
Mix the solution by pipetting several times, and centrifuge at 8,000 x g for 10 min.[b]

Step 5.
Add 10 µl of Reaction Buffer to SH-Reactive Allophycocyanin, and dissolve by pipetting.[c]

Step 6.
Add SH-Reactive Allophycocyanin solution to the IgG concentrated on the Filtration Tube.

Step 7.
Incubate the tube at 37°C for 2 hours after pipetting to mix.

Step 8.
Add 190 µl of WS Buffer, and pipette about 10 times to recover the conjugate.[d] Transfer the solution to a microtube(not included in this kit), and store at 0-5°C.[e]

[a] The volume of IgG solution should be less than 100 µl. If the IgG concentration is lower than 0.5 mg/ml, repeat Steps 1 and 2 until the total IgG accumulation becomes 50 - 200 µg.
[b] If solution still remains on the membrane after the centrifugation, spin for another 5 min.
[c] NH_2-Reactive Allophycocyanin can be hydrolyzed by water. Proceed to Step 6 immediately after the preparation of the NH_2-Reactive Allophycocyanin solution.
[d] One to three Allophycocyanin should be introduced into one IgG molecule. Unconjugated Allophycocyanin remained in the solution might cause background increase with immunoassay. For more purification, use a gel permeation column or an affinity column for IgG.
[e] We recommend using WS Buffer to recover the conjugate. You can choose any kinds of buffers appropriate for your experiment.

FAQ

- **Can I use this kit for $F(ab')_2$?**
  Yes, please follow the labeling protocol for IgG. The recovery rate of the conjugate should be greater than 80%.
- **Can I use this kit for other proteins?**
  Yes, if the molecular weight of the reduced form is greater than 50,000 and it has a reactive SH group, or a disulfide group that can be reduced without losing activity. Follow the labeling protocol for IgG, and use 0.5-1 nmol of sample protein.
- **How many APC molecules per reduced IgG are introduced?**
  The average number of APC molecules per reduced IgG is 1 to 2.
- **Do I have to use the Filtration tube prior to labeling the protein?**
  If the protein solution does not contain small molecules with reactive SH groups and the concentration of the protein is 10 mg per ml, or about 70 µM, there is no need to use the filtration tube. Just mix 10 µl of the sample solution with reaction buffer and add the mixture to a vial of the SH-reactive APC.
- **Do I have to use WS buffer included with the kit?**
  Yes. Use the WS buffer to prepare a stock solution of the conjugate. However, you can choose any kind of buffer that is appropriate for diluting the conjugate stock solution for your experiment.

Fluorescein Labeling Kit-NH_2

Application: Fluorescein labeling of proteins

Features: Only 1 hour to recover conjugates
All processes in a single filtration tube
High recovery of conjugates
Applicable for 50-200 µg IgG

Ordering Information

Product code	Unit
LK01-10	3 samples [a]

[a] Based on 100 µg IgG sample labeling

Contents of the Kit

NH_2-reactive fluorescein ..... 3 tubes
Reaction buffer .....0.5 ml x 1 tube
WS buffer ..... 4 ml x 1 bottle
Filtration tube .....3 tubes

Storage Condition

0-5°C

Shipping Condition

ambient temperature

Product Description

Fluorescein Labeling Kit-NH_2 is mainly used for the preparation of fluorescein-labeled proteins such as IgG for immunostaining and cellular proteins for tracing. Amine-reactive fluorescein, a component of this kit, has succinimidyl groups (NHS) that react with the amino groups on proteins or other molecules (Fig. 1). This kit contains all the reagents necessary for labeling, including storage buffer. Each vial of fluorescein can label up to 200 µg of IgG, conjugating about 4 to 6 fluorescein molecules per IgG molecule. Because this kit also includes a buffer exchange system, a sample containing amine base buffer can be labeled. Although membrane filtration sometimes causes IgG aggregation, the buffer system in this kit prevents aggregation during the concentration of IgG or fluorescein-labeled IgG solution. A fluorescein-labeled IgG solution prepared using this kit is stable for more than 2 months at 4°C. The excitation and emission wavelengths of the fluorescein-labeled IgG are 495 nm and 520 nm, respectively (Fig. 2).

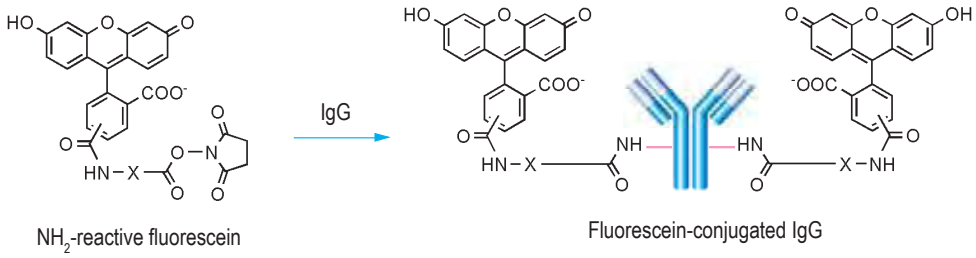


Fig. 1 IgG labeling reaction of NH_2-reactive fluorescein

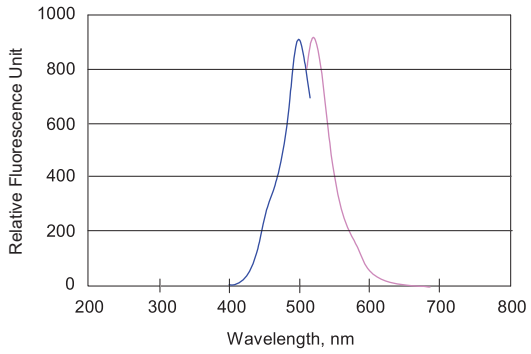


Fig. 2 Fluorescence spectrum of fluorescein-conjugated IgG
excitation spectrum
emission spectrum

Required Equipment and Materials

LK01-10: Microcentrifuge, 10 µl and 100-200 µl adjustable pipettes, 37°C incubator, 0.5 ml microtubes, DMSO, or ethanol

Precaution

- The molecular weight of the protein to be labeled with this kit should be greater than 50,000.
- IgG or fluorescein-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weight greater than 10,000, such as BSA or gelatin, purify the IgG solution before labeling fluorescein with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble material, centrifuge the solution and use the supernatant for the labeling.

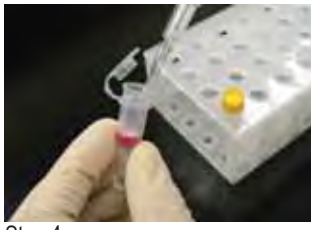
Fluorophore Labeling

General Protocol for IgG Labelin

Step 1.
Add 100 µl WS buffer and the sample solution containing 100 µg IgG to a filtration tube.[a)]

Step. 2
Centrifuge at 8,000-10,000 g for 10 minutes.[b)]

Step 3.
Add 10 µl DMSO to NH_2-reactive fluorescein and dissolve with pipetting.[c)]



Step 4.
Add 100 µl reaction buffer and 8 µl NH_2-reactive fluorescein solution to the filtration tube and pipette to mix.[d)]

Step 5.
Incubate the tube at 37°C for 10 minutes.

Step 6.
Add 100 µl WS buffer to the filtration tube and centrifuge at 8,000-10,000 g for 10 minutes.[b)] Discard the filtrate.

Step 7.
Add 200 µl WS buffer to the filtration tube and centrifuge at 8,000-10,000 g for 10 minutes.[b)] Repeat this step.

Step 8.
Add 200 µl WS buffer and pipette 10 to 15 times to recover the conjugate.[e)] Transfer the solution to a 0.5 ml tube, and store at 0-5°C.

[a)] The volume of IgG solution should be less than 100 µl. If the antibody concentration is less than 1 mg per ml, repeat steps 1 and 2 until the total IgG accumulation becomes 100 µg. If the volume of the filtrate becomes 400 µl or more during the accumulation process, discard the filtrate before going to the next centrifuge step.

[b)] If solution still remains on the filter after the centrifugation, spin for another 5 minutes or increase the centrifuge speed.

[c)] NH_2-reactive fluorescein is on the bottom of the tube. Add 10 µl DMSO to the bottom of the tube, and pipette several times to dissolve. If DMSO is not available, you may use ethanol.

[d)] If the amount of IgG is 200 µg, add the entire NH_2-reactive fluorescein solution at step 4.

[e)] You do not have to use WS buffer to recover fluorescein-conjugated IgG. You can choose any kind of buffer appropriate for your experiment.

Determination of Fluorescein/IgG Ratio

Dilute the fluorescein labeled protein solution with WS buffer or other neutral buffer to a proper volume, then measure the absorbance of the protein solution at 280 nm and 500 nm. If you require the ration of Fluorescein and IgG, calculate the ration using the following equation. When targeted protein is IgG, use 216,000 as the ε of IgG. Molar absorption coefficient of fluorescein in WS buffer is 60,000.

$$\text{Ration (Fluorescein molecules per protein molecule)} = \frac{A_{500}\ /60{,}000}{(A_{280} - A_{555} \times 0.22)/(\varepsilon \text{ of protein})}$$

A_{555}= absorbance at 500 nm
A_{280}= absorbance at 280 nm
ε= molar absorption coefficient of protein at 280 nm

FAQ

- **Can I use this kit for other proteins?**
  Yes, if the molecular weight is greater than 50,000.
- **Do I have to use a filtration tube prior to labeling the protein?**
  If the protein solution does not contain small molecules with an amino group and the concentration of the protein is 10 mg per ml, or about 70 µM, there is no need to use the filtration tube. Just mix 10 µl of the sample solution with 90 µl of reaction buffer, add 8 µl NH_2-reactive fluorescein (prepared at step 3) to the mixture, and follow the protocol starting at step 4.
- **How long is the fluorescein-labeled protein stable?**
  If you store at 4°C, it is stable for more than 2 months. For longer storage, add 100% volume of glycerol and aliquot, and store at -20°C. However, please note that stability depends on the protein itself.
- **What is the minimum amount of IgG that can be labeled by this kit?**
  The minumum amount of IgG is 10 µg. Simply follow the protocol. The labeling ratio remains the same for 10 µg to 100 µg of IgG.
- **Can I use this kit to label oligonucleotides or peptides?**
  No. Oligonucleotides and peptides may be too small to retain on the membrane of the filtration tube.

HiLyte Fluor™ 555 Labeling Kit-NH_2

Application: HiLyte Fluor™ 555 labeling of proteins

Features: Only 1 hour to recover conjugates
All processes in a single filtration tube
High recovery of conjugates
Applicable for 50-200 µg IgG

Ordering Information

Product code	Unit
LK14-10	3 samples [a]

[a] Based on 100 µg IgG sample labeling

Contents of the Kit

NH_2-reactive HiLyte Fluor 555 ..... 3 tubes
Reaction buffer ..... 0.5 ml x 1 tube
WS buffer ..... 4 ml x 1 bottle
Filtration tube .....3 tubes

Storage Condition

0-5°C

Shipping Condition

ambient temperature

Product Description

HiLyte Fluor* 555 Labeling Kit-NH_2 is used mainly for the preparation of red fluorescence-labeled proteins, such as IgG, for immunostaining, and cellular proteins for tracing. NH_2-reactive HiLyteFluor 555, a component of this kit, has a succinimidyl group (NHS) that reacts with amino groups on proteins or other molecules (Fig. 1). This kit contains all the reagents necessary for labeling. Each tube of HiLyte Fluor 555 can label up to 200 µg of IgG, conjugating about 4 to 6 HiLyte Fluor 555 molecules per IgG molecule. The labeling process is simple-add the NH_2-reactive HiLyte Fluor 555 to IgG solution on a membrane and incubate at 37°C for 10 minutes. The excess HiLyte Fluor 555 molecules can be removed by a filtration tube. The excitation and emission wavelengths of the HiLyte Fluor 555-labeled IgG are 555 nm and 570 nm, respectively (Fig. 2).* HiLyte Fluor is a trademark of AnaSpec, Inc.

Fig. 1 Fluorescence Spectrum of HiLyte Fluor 555-conjugated IgG

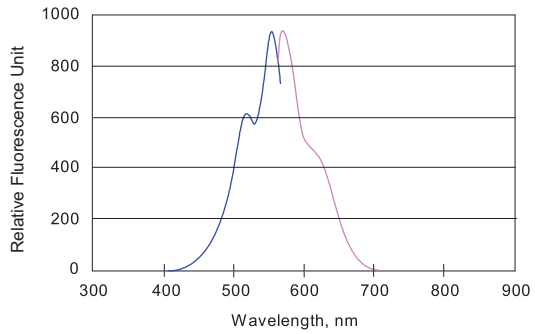


Fig. 2 Fluorescence spectrum of HiLyte Fluor 555-conjugated IgG
excitation spectrum
emission spectrum

Required Equipment and Materials

Microcentrifuge, 10 µl and 100-200 µl adjustable pipettes, 37°C incubator, 0.5 ml microtubes, DMSO

Precaution

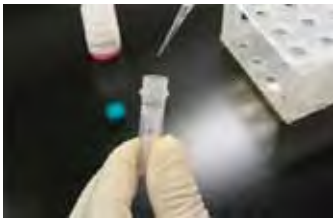
- The molecular weight of the protein to be labeled with this kit should be greater than 50,000.
- IgG or HiLyte Fluor 555-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weights greater than than 10,000, such as BSA or gelatin, purify the IgG solution before labeling HiLyte Fluor 555 with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

Fluorophore Labeling

General Protocol for IgG Labeling

Step 1.
Add 100 µl WS buffer and the sample solution containing 100 µg IgG to a filtration tube. [a)]

Step 2.
Centrifuge at 8,000-10,000 g for 10 minutes. [b)]



Step 3.
Add 10 µl DMSO to NH_2-reactive HiLyte 555 and dissolve with pipetting. [c)]

Step 4.
Add 100 µl reaction buffer and 8 µl NH_2-reactive HiLyte 555 solution to the filtration tube and pipette to mix. [d)]

Step 5.
Incubate the tube at 37°C for 10 minutes.

Step 6.
Add 100 µl WS buffer to the filtration tube and centrifuge at 8,000-10,000 g for 10 minutes. [b)]
Discard the filtrate.

Step 7.
Add 200 µl WS buffer to the filtration tube and centrifuge at 8,000-10,000 g for 10 minutes. [b)]
Repeat this step.

Step 8.
Add 200 µl WS buffer and pipette 10 to 15 times to recover the conjugate. [e)] Transfer the solution to a 0.5 ml tube, and store at 0-5°C.

[a)] The volume of IgG solution should be less than 100 µl. If the antibody concentration is less than 1 mg per ml, repeat steps 1 and 2 until the total IgG accumulation becomes 100 µg. If the volume of the filtrate becomes 400 µl or more during the accumulation process, discard the filtrate before going to the next centrifuge step.
[b)] If solution still remains on the filter after the centrifugation, spin for another 5 minutes or increase the centrifuge speed.
[c)] NH_2-reactive HiLyte Fluor 555 lies on the bottom of the tube. Add 10 µl DMSO to the bottom of the tube, and pipette several times to dissolve.
[d)] If the amount of IgG is 200 µg, add the entire amount of NH_2-reactive HiLyte Fluor 555 solution.
[e)] You do not have to use WS buffer to recover HiLyte Fluor 555-conjugated IgG. You can choose any kind of buffer that is appropriate for your experiment.

Determination of HiLyte Fluor 555 /IgG Ratio

Dilute the HiLyte Fluor 555 labeled protein solution with WS buffer or other neutral buffer to a proper volume, then measure the absorbance of the protein solution at 280 nm and 555 nm. If you require the ration of HiLyte Fluor 555 and IgG, calculate the ration using the following equation. When targeted protein is IgG, use 216,000 as the ε of IgG. Molar absorption coefficient of HiLyte Fluor 555 in WS buffer is 150,000.

$$\text{Ration (HiLyte Fluor 555 per IgG molecule)} = \frac{A_{555} \times 150{,}000}{(A_{280} - A_{555} \times 0.1)/(\varepsilon \text{ of protein})}$$

A_{555}= absorbance at 555 nm
A_{280}= absorbance at 280 nm
ε= molar absorption coefficient of protein at 280 nm

FAQ

- **Can I use this kit for other proteins?**
  Yes, if the molecular weight is greater than 50,000.
- **Do I have to use a filtration tube prior to labeling the protein?**
  If the protein solution does not contain small molecules with an amino group and the concentration of the protein is 10 mg per ml, or about 70 µM, there is no need to use the filtration tube. Mix 10 µl of the sample solution with 90 µl of reaction buffer and add 8 µl NH_2-reactive HiLyte Fluor 555 (prepared at step 3) to the mixture, and follow the protocol beginning with step 4.
- **How long is the HiLyte Fluor 555-labeled protein stable?**
  If you store at 4 °C, it is stable for more than 2 months. For longer storage, add 100% volume of glyceroland aliquot, and store at -20o C. However, please note that stability depends on the protein itself.
- **What is the minimum amount of IgG that can be labeled by this kit?**
  The minimum amount of IgG is 10 µg. For less than 20 µg, follow the manual and add 4 µl NH_2-reactive HiLyte Fluor 555 instead of 8 µl at step 4.
- **Can I use this kit to label oligonucleotides or peptides?**
  No. Oligonucleotides and peptides may be too small to retain on the membrane of the filtration tube.

HiLyte Fluor™ 647 Labeling Kit-NH_2

Application: HiLyte Fluor™ 647 labeling of proteins

Features: Only 1 hour to get conjugates
All processes in a single filtration tube
High recovery of conjugates
Applicable for 50-200 µg IgG

Ordering Information

Product code	Unit
LK15-10	3 samples [a]

[a] Based on 100 µg IgG sample labeling

Contents of the Kit

NH_2-reactive HiLyte Fluor 647 ..... 3 tubes
Reaction buffer ..... 0.5 ml x 1 tube
WS buffer ..... 4 ml x 1 bottle
Filtration tube ..... 3 tubes

Storage Condition

0-5°C

Shipping Condition

ambient temperature

Product Description

HiLyte Fluor* 647 Labeling Kit-NH_2 is mainly used for the preparation of red fluorescence-labeled proteins, such as IgG, for immunostaining, and cellular proteins for tracing. NH_2-reactive HiLyteFluor 647, a component of this kit, has a succinimidyl group (NHS) that reacts with amino groups on proteins or other molecules (Fig. 1). This kit contains all the reagents necessary for labeling. Each tube of HiLyte Fluor 647 can label up to 200 µg of IgG, conjugating about 4 to 6 HiLyte Fluor 647 molecules per IgG molecule. The labeling process is simple-add the NH_2-reactive HiLyte Fluor 647 to IgG solution on a membrane and incubate at 37°C for 10 minutes. The excess HiLyte Fluor 647 molecules can be removed by a filtration tube. The excitation and emission wavelengths of the HiLyte Fluor 647-labeled IgG are 652 nm and 673 nm, respectively (Fig. 2). *HiLyte Fluor is a trademark of AnaSpec, Inc.

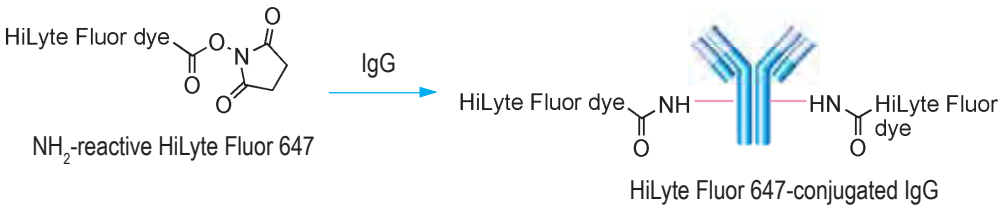


Fig. 1 Fluorescence Spectrum of HiLyte Fluor 647-conjugated IgG

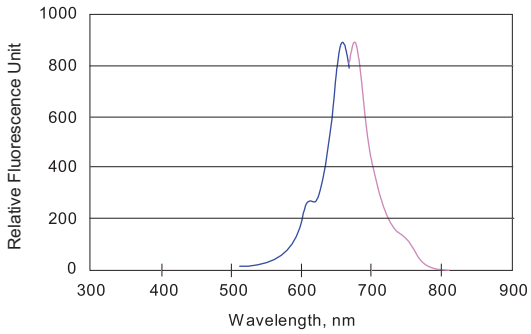


Fig. 2 Fluorescence spectrum of HiLyte Fluor 647-conjugated IgG
excitation spectrum
emission spectrum

Required Equipment and Materials

Microcentrifuge, 10 µl and 100-200 µl adjustable pipettes, 37°C incubator, 0.5 ml microtubes, DMSO

Precaution

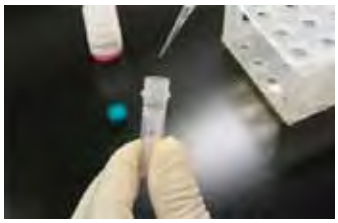
- The molecular weight of the protein to be labeled with this kit should be greater than 50,000.
- IgG or HiLyte Fluor 647-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weights larger than 10,000, such as BSA or gelatin, purify the IgG solution before labeling HiLyte Fluor 647 with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

Fluorophore Labeling

General Protocol for IgG Labeling

Step 1.
Add 100 µl WS buffer and the sample solution containing 100 µg IgG to a filtration tube.[a)]

Step 2.
Centrifuge at 8,000-10,000 g for 10 minutes.[b)]



Step 3.
Add 10 µl DMSO to NH_2-reactive HiLyte Fluor 647 and dissolve by pipetting.[c)]

Step 4.
Add 100 µl reaction buffer and 8 µl NH_2-reactive HiLyte Fluor 647 to the filtration tube and pipette to mix.[d)]

Step 5.
Incubate the tube at 37°C for 10 minutes.

Step 6.
Add 100 µl WS buffer to the filtration tube and centrifuge at 8,000-10,000 g for 10 minutes.[b)]
Discard the filtrate.

Step 7.
Add 200 µl WS buffer to the filtration tube and centrifuge at 8,000-10,000 g for 10 minutes.[b)]
Repeat this step.

Step 8.
Add 200 µl WS buffer and pipette 10 to 15 times to recover the conjugate.[e)] Transfer the solution to a 0.5 ml tube, and store at 0-5°C.

[a)] The volume of IgG solution should be less than 100 µl. If the antibody concentration is less than 1 mg per ml, repeat steps 1 and 2 until the total IgG accumulation becomes 100 µg. If the volume of the filtrate becomes 400 µl or more during the accumulation process, discard the filtrate before going to the next centrifuge step.

[b)] If solution still remains on the filter after the centrifugation, spin for another 5 minutes, or increase the centrifuge speed.

[c)] NH_2-reactive HiLyte Fluor 647 lies on the bottom of the tube. Add 10 µl DMSO to the bottom of the tube and pipette several times to dissolve.

[d)] If the amount of IgG is 200 µg, add entire NH_2-reactive HiLyte Fluor 647 solution.

[e)] You do not have to use WS buffer to recover HiLyte Fluor 647-conjugated IgG. You can choose any kind of buffer that is appropriate for your experiment.

Determination of HiLyte Fluor 647/IgG Ratio

Dilute the HiLyte Fluor 647 labeled protein solution with WS buffer or other neutral buffer to a proper volume, then measure the absorbance of the protein solution at 280 nm and 655 nm. If you require the ration of HiLyte Fluor 647 and IgG, calculate the ration using the following equation. When targeted protein is IgG, use 216,000 as the ε of IgG. Molar absorption coefficient of HiLyte Fluor 647 in WS buffer is 250,000.

$$\text{Ration (HiLyte Fluor 647 per IgG molecule)} = \frac{A_{655} \times 250{,}000}{(A_{280} - A_{655} \times 0.05)/(\varepsilon \text{ of protein})}$$

A_{655}= absorbance at 655 nm
A_{280}= absorbance at 280 nm
ε= molar absorption coefficient of protein at 280 nm

FAQ

- **Can I use this kit for other proteins?**
  Yes, if the molecular weight is greater than 50,000.
- **Do I have to use a filtration tube prior to labeling the protein?**
  If the protein solution does not contain small molecules with an amino group and the concentration of the protein is 10 mg per ml, or about 70 µM, there is no need to use the filtration tube. Mix 10 µl of the sample solution with 90 µl of reaction buffer and add 8 µl NH_2-reactive HiLyte Fluor 647 (prepared at step 3) to the mixture, and follow the protocol beginning with step 4.
- **How long is the HiLyte Fluor 647-labeled protein stable?**
  If you store it at 4°C, it is stable for more than 2 months. For longer storage, add 100% volume of glyceroland aliquot, and store at -20°C. However, please note that stability depends on the protein itself.
- **What is the minimum amount of IgG that can be labeled by this kit?**
  The minumum amount of IgG is 10 µg. For less than 20 µg, follow the manual and add 4 µl NH_2-reactive HiLyte Fluor 647 instead of 8 µl start with step 4.
- **Can I use this kit to label oligonucleotides or peptides?**
  No. Oligonucleotides and peptides may be too small to be retained on the membrane of the filtration tube.

HiLyte Fluor™ 750 Labeling Kit-NH_2

Application: HiLyte Fluor™ 750 labeling of proteins

Features: Only 1 hour to recover conjugates
All processes in a single filtration tube
High recovery of conjugates
Applicable for 50-200 µg IgG

Ordering Information

Product code	Unit
LK16-10	3 samples [a]

[a] Based on 100 µg IgG sample labeling

Contents of the Kit

NH_2-reactive HiLyte Fluor ™750 .....3 tubes
Reaction buffer .....0.5 ml x 1 tube
WS buffer ..... 4 ml x 1 bottle
Filtration tube ..... 3 tubes

Storage Condition

0-5°C

Shipping Condition

ambient temperature

Product Description

HiLyte Fluor* 750 Labeling Kit-NH_2 is used mainly for the preparation of near infrared fluorescence-labeled proteins, such as IgG, for immunostaining, and cellular proteins for tracing. NH_2-reactive HiLyte Fluor 750, a component of this kit, has a succinimidyl group (NHS) that reacts with amino groups on proteins or other molecules (Fig. 1). This kit contains all the reagents necessary for labeling. Each tube of HiLyte Fluor 750 can label up to 200 µg of IgG, conjugating about 3 to 5 HiLyte Fluor 750 molecules per IgG molecule. The labeling process is simple-add the NH_2-reactive HiLyte Fluor 750 to IgG solution on a membrane and incubate at 37°C for 10 minutes. The excess HiLyte Fluor 750 molecules can be removed by a filtration tube. The excitation and emission wavelengths of the HiLyte Fluor 750-labeled IgG are 762 nm and 777 nm, respectively (Fig. 2). *HiLyte Fluor is a trademark of AnaSpec, Inc.

NH_2-reactive HiLyte Fluor 750

HiLyte Fluor 750-conjugated IgG

Fig. 1 IgG labeling reaction of NH_2-reactive HiLyte Fluor 750

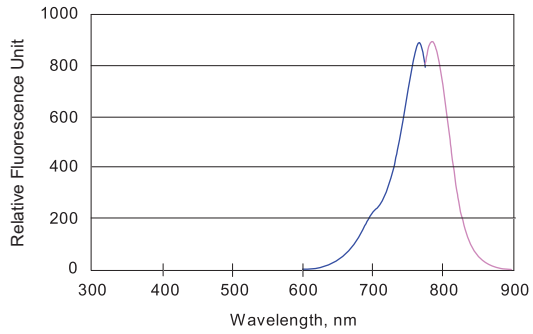


Fig. 2 Fluorescence spectrum of HiLyte Fluor 750-conjugated IgG
excitation spectrum
emission spectrum

Required Equipment and Materials

Microcentrifuge, 10 µl and 100-200 µl adjustable pipettes, 37°C incubator, 0.5 ml microtubes, DMSO

Precaution

- The molecular weight of the protein to be labeled with this kit should be greater than 50,000.
- IgG or HiLyte Fluor 750-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weights larger than 10,000, such as BSA or gelatin, purify the IgG solution before labeling HiLyte Fluor 750 with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for labeling.

Fluorophore Labeling

General Protocol for IgG Labeling

Step 1.
Add 100 µl WS buffer and the sample solution containing 100 µg IgG to a filtration tube.[a)]

Step 2.
Centrifuge at 8,000-10,000 g for 10 minutes.[b)]

Step 3.
Add 10 µl DMSO to NH_2-reactive HiLyte Fluor 750 and dissolve by pipetting.[c)]

Step 4.
Add 400 µl reaction buffer and 8 µl NH_2-reactive HiLyte Fluor 750 solution to the filtration tube and pipette to mix.[d)]

Step 5.
Incubate the tube at 37°C for 10 minutes.

Step 6.
Centrifuge at 8,000-10,000 g for 15 minutes.[b)] Discard the filtrate.

Step 7.
Add 400 µl WS buffer to the filtration tube and centrifuge at 8,000 g for 15 minutes.[b)]

Step 8.
Add 200 µl WS buffer, and pipette 10 to 15 times to recover the conjugate.[e)] Transfer the solution to a 0.5 ml tube and store at 0-5°C.

[a)] The volume of IgG solution should be less than 100 µl. If the antibody concentration is less than 1 mg per ml, repeat steps 1 and 2 until the total IgG accumulation becomes 100 µg. If the volume of the filtrate becomes 400 µl or more during the accumulation process, discard the filtrate before going to the next centrifuge step.

[b)] If solution still remains on the filter after the centrifugation, spin for another 5 minutes, or increase the centrifuge speed.

[c)] NH_2-reactive HiLyte Fluor 750 lies on the bottom of the tube. Add 10 µl DMSO to the bottom of the tube and pipette several times to dissolve.

[d)] If the amount of IgG is 200 µg, add entire amount of NH2-reactive HiLyte Fluor 750 solution.

[e)] You do not have to use WS buffer to recover HiLyte Fluor 750-conjugated IgG. You can choose any kind of buffer that is appropriate for your experiment.

Determination of HiLyte Fluor 750/IgG Ratio

Dilute the HiLyte Fluor 750 labeled protein solution with WS buffer or other neutral buffer to a proper volume, then measure the absorbance of the protein solution at 280 nm and 760 nm. If you require the ration of HiLyte Fluor 750 and IgG, calculate the ration using the following equation. When targeted protein is IgG, use 216,000 as the ε of IgG. Molar absorption coefficient of HiLyte Fluor 750 in WS buffer is 270,000.

$$\text{Ration (HiLyte Fluor 750 per IgG molecule)} = \frac{A_{760} \times 270{,}000}{(A_{280} - A_{655} \times 0.05)/(\varepsilon \text{ of protein})}$$

A_{760}= absorbance at 760 nm
A_{280}= absorbance at 280 nm
ε= molar absorption coefficient of protein at 280 nm

FAQ

- **Can I use this kit for other proteins?**
  Yes, if the molecular weight is greater than 50,000.
- **Do I have to use a filtration tube before labeling the protein?**
  If the protein solution does not contain small molecules with an amino group and the concentration of the protein is 10 mg per ml, or about 70 µM, there is no need to use the filtration tube. Mix 10 µl of the sample solution with 390 µl of reaction buffer, add 8 µl NH_2-reactive HiLyte Fluor 750 (prepared at step 3) to the mixture, and follow the protocol starting at step 4.
- **How long is the HiLyte Fluor 750-labeled protein stable?**
  If you store it at 4°C, it is stable for more than 2 months. For longer storage, add 100% volume of glyceroland aliquot, and store at -20°C. However, please note that stability depends on the protein itself.
- **What is the minimum amount of IgG that can be labeled by this kit?**
  The minumum amount of IgG is 10 µg. There is no significant difference in sensitivity and background between 10 and 200 µg of IgG.
- **Can I use this kit to label oligonucleotides or peptides?**
  No. Oligonucleotides and peptides may be too small to be retained on the membrane of the filtration tube.

ICG Labeling Kit-NH_2

Application: Indo Cyanine Green dye labeling of proteins

Features: Suitable wavelength for in vivo imaging
Quick and Easy Labeling to Antibodies : 1.5hr
High Recovery Rate : more than 90%

Ordering Information

Product code	Unit
LK31-08	1 sample
LK31-10	3 samples

*Based on 100 μg IgG sample labeling

Storage	Shipping Condition
0-5ºC	ambient temperature

Contents of the Kit

LK31-08

NH_2-reactive ICG .....1 tube
WS buffer .....1.5 ml x 1 bottle
Reaction buffer ..... 250 μl x 1 tube
Filtration tube .....1 tube

LK31-10

NH_2-reactive ICG .....3 tubes
WS buffer ..... 4 ml x 1 bottle
Reaction buffer ..... 500 μl x 1 tube
Filtration tube .....3 tubes

Product Description

ICG Labeling Kit - NH_2 is used primarily for the preparation of ICG (Iindocyanin green)-labeled antibody for near-infrared fluorescence imaging. ICG offers two remarkable properties:

1) ICG has a strong near-infrared fluorescence even after a few days under physiological conditions.
The excitation and emission wavelength of the ICG-labeled proteins are 774 nm and 805 nm, respectively.
2) ICG has been used in clinical fields such as a hepatic deficiency testing. Therefore, ICG and ICG conjugates are materials suitable for in vivo imaging.

This kit contains all required compornents required for labeling, including storage buffer for conjugates. The labeling process is simple:. Add NH_2-reactive ICG to protein solution on a filter membrane, and incubate at 37ºC, for 10 minutes. A filtration tube can remove excess ICG molecules.

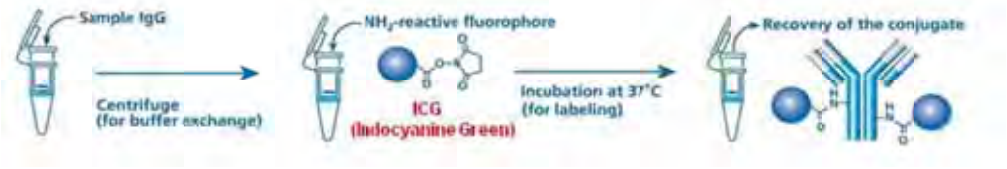
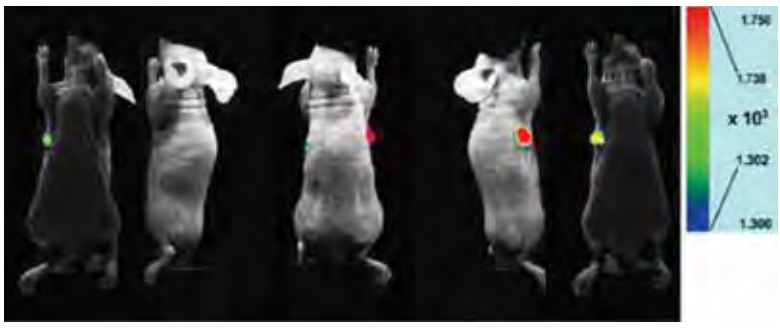


Fig. 1 Fluorescein Labeling Process to IgG



A mouse subcutaneous tumor model at 48 hours postinjection of ICG labeled antibody(50 μg) shows clear evidence of selective accumulation in tumor.

mouse: BALB/c nu/ nu (female, 11 weeks)
tumor cell: HeLa (4 weeks after transplant in subcutaneous right axilla)
antibody: anti-integrin α2 antibody
measurement condition: Ex 785 nm, Em 845/55 nm(center/band), exposure time: 10 sec

Fig. 2 Fluorescent Overlay Imaging

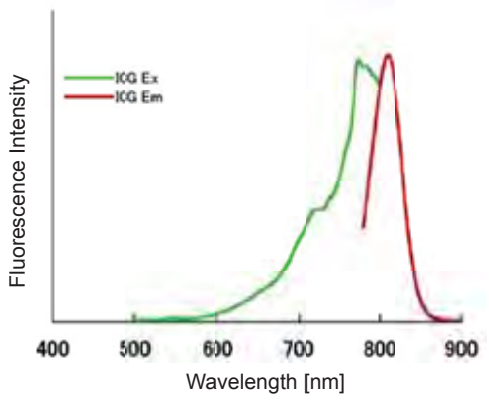


Fig. 3 Fluorescent Property of ICG Dye

Precaution

♦If the IgG solution contains other proteins with molecular weight greater than 10,000, such as serum albumin or gelatin, purify the IgG solution before labeling fluorescein with this kit. Commercially available antibody may contain BSA or gelatin as a stabilizer. Dojindo offers IgG Purification Kit-A (AP01-10) and IgG Purification Kit-G (AP02-10) for the purification of the IgG solution.

Reference

1. N. Muguruma, *et al*., Antibodies Labeled with Fluorescence-agent Excitable by Infrared Rays. *J Gastroenterol*. 1988;**33**:467-471.
2. S. Ito, *et al*., Development of Fluorescence-Emitting Antibody Labeling Substance by Near-Infrared Ray Excitation. *Bioorg Med Chem Lett*. 1995;**5**:2689-2694.
3. S. Ito, *et al*., Development of an Imaging System Using Fluorescent Labeling Substances Excited by Infrared Rays. *Dig Endoscopy*. 1997;**9**:278-282.
4. S. Ito, *et al*., Development of Agents for Reinforcement of Fluorescence on Near-infrared Ray Excitation for Immunohistological Staining. *Bioorg Med Chem*. 1998;**6**:613-618.
5. S. Taoka, *et al*., Reflected Illumination-type Imaging System for the Development of Infrared Fluorescence Endoscopy. *Dig Endoscopy*. 1999;**11**:321-326.
6. N. Muguruma, *et al*., Labeled Carcinoembryonic Antigen Antibodies Excitable by Infrared Rays: a Novel Diagnostic Method for Micro Cancers in the Digestive Tract. *Intern Med*. 1999;**38**:537-542.
7. S. Ito, *et al*., Visualization of Human Gastric Cancer with a Novel Infrared Fluorescent Labeling Marker of Anti-carcinoembryonic Antigen Antibody *in vitro*. *Dig Endoscopy*. 2000;**12**:33-36.
8. S. Ito, *et al*., Detection of Human Ganstric Cancer of Resected Specimens Using a Novel Infrared Fluorescent Anti-Human Carcinoembryonic Antigen Antibody with an Infrared Fluorescence Endoscope in Vitro. *Endoscopy*. 2001;**33**:849-853.
9. T. Bando, *et al*., Basic Study on a Labeled anti-mucin Antibody Detectable by Infrared-fluorescence Endoscopy. *J Gastroenterol*. 2002;**37**:260-269.
10. K. Inayama, *et al*., Basic Study of an Agent for Reinforcement of Near-infrared Fluorescence on Tumor Tissue. *Dig Liver Dis*. 2003;**35**:88-93.
11. M. Ogawa, *et al*., Dual-Modality Molecular Imaging Using Antibodies Labeled with Activatable Fluorescence and a Radionuclide for Specific and Quantitative Targeted Cancer Detection. *Bioconjug Chem*. 2009;**20**:2177-2184.
12. M. Ogawa, *et al*., In vivo Molecular Imaging of Cancer with a Quenching Near-Infrared Fluorescent Probe Using Conjuates of Monoclonal Antibodies and Indocyanine Green. *Cancer Res*. 2009;**69**:1268-1272.
13. N. Kosaka, *et al*., Clinical implications of near-infrared fluorescence imaging in cancer. *Future Oncol*. 2009;**5**:1501-1511.

ICG-Sulfo-OSu

2-[7-[1,3-Dihydro-1,1-dimethyl-3-(4-sulfobutyl)-2*H*-benzo*[e]*indol-2-ylidene]-1,3,5-heptatrienyl]-1,1-dimethyl-3-[5-(3-sulfosuccinimidyl) oxycarbonylpentyl]-*1H*-benzo[e]indolium, inner salt, sodium salt

Application: Indocyanine green labeling of proteins

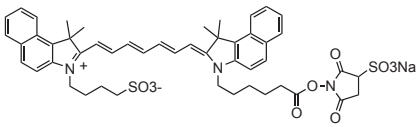
Appearance: Dark green powder
Purity: ≥80% (HPLC)
MW: 930.07, $C_{49}H_{52}N_3O_{10}S_2$

Ordering Information	
Product code	Unit
I254-10	1 mg

Storage Condition
-20°C

Shipping Condition
with dry ice or blue ice

Structural Formula



Product Description

ICG is one of the dyes that is used for determining cardiac output, hepatic function, and liver blood flow, as well as for ophthalmic angiography. It has a long excitation wavelength and emission wavelength of about 780 nm and 800 nm, respectively. Because of its long wavelength near the infrared region and low cytotoxicity, ICG is used to label antibodies for in vivo assay. However, fluorescent intensity after conjugation with protein is quite low because of the formation of H-dimer or an energy transfer to antibody molecules after excitation. Dr. Kobayashi and others reported that the use of SDS and betamecraptoethanol with the conjugate increases fluorescent intensity dramatically by diminishing hydrophobic $\pi-\pi$ interactions and separation of IgG chains. They applied a treated ICG-conjugated daclizumab (Dac.. [?] humanized monoclonal antibody) and humanized anti-HER IgG2 monoclonal antibody for in vivo assay to specifically visualized tumors.

Labeling procedure for IgG

1. Prepare 6.8 nmol antibody solution with pH 8.5 carbonate buffer or bicine buffer (Good's buffer).
2. Add 6.8-68 nmol of ICG-Sulfo-OSu/DMSO solution to the antibody solution and incubate at room temperature for 30 minutes.
3. Purify the reaction mixture with a sephadex G50 column.

IC3-OSu special packaging

N-Ethyl-*N*'-[5-(*N*''-succinimidyloxycarbonyl)pentyl]indocarbocyanine chloride

Application: Indocyanine labeling of proteins

Appearance: Dark red powder
Purity: ≥80% (HPLC)
MW: 604.18, $C_{35}H_{42}ClN_3O_4$

Storage Condition
-20°C

Shipping Condition
with dry ice or blue ice

Ordering Information

Product code	Unit
I271-10	20 µg x 3

IC5-OSu special packaging

N-Ethyl-*N*'-[5-(*N*''-succinimidyloxycarbonyl)pentyl]-3,3,3'',3''-tetramethyl-2,2'-indocarbocyanine chloride

Application: Indocyanine labeling of proteins

Appearance: Dark blue powder
Purity: ≥80% (HPLC)
MW: 630.23, $C_{37}H_{44}ClN_3O_4$

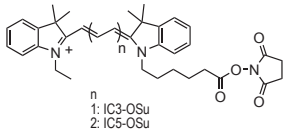
Storage Condition
-20°C

Shipping Condition
with dry ice or blue ice

Ordering Information

Product code	Unit
I272-10	20 µg x 3

Structural Formula



Product Description
IC3 and IC5 are indocyanine dyes that can react with aminegroups on proteins, peptides, and amine-modified oligonucleotides. Because neither IC3 nor IC5 has a sulfonate group in its structure, water solubility of the molecules is very low. An organic solvent is required to dissolve the compound. Over-labeling of proteins with these compounds may precipitate the labeled protein from aqueous solutions as a result of the poor water solubility. However, because no negative charge is added to the protein, the total number of charges of the modified protein is the same before and after labeling with these compounds. The IC dye package contains 100 µg dye. This amount is sufficient to modify 1-2 mg proteins or more depending on the purpose of the downstream experiment.

Biotin Labeling Kit-NH_2

Application: Biotin labeling of protein, amine reactive

Features: Only 1 hour to recover conjugates
All processes in a single filtration tube
High recovery of conjugates
Applicable for 50-200 µg IgG

Ordering Information

Product code	Unit
LK03-10	3 samples [a]
LK55-10	1 sample [b]

[a] Based on 100 µg IgG sample labeling
[b] Based on 1 mg IgG sample labeling

Contents of the Kit

LK03-10

NH_2-reactive biotin ..... 3 tubes
Reaction buffer ..... 0.5 ml x 1 tube
WS buffer ..... 4 ml x1 bottle
Filtration tube ..... 3 tubes

LK55-10

NH_2-reactive biotin ..... 1 tube
Reaction buffer ..... 1.2 ml x 1 bottle
15 ml tube ..... 1 tube
WS buffer .....13 ml x 1bottle
Filtration tube .....1 tube

Storage Condition

0-5°C

Shipping Condition

ambient temperature

Product Description

Biotin Labeling Kit-NH_2 is mainly used for the preparation of biotin-labeled IgG for enzyme immunoassay (EIA). NH_2-reactive biotin, a component of this kit, has succinimidyl groups (NHS) that react with amino groups on proteins or other molecules (Fig. 1). This kit contains all the reagents necessary for the labeling. The labeling process is very simple. Just add the NH_2- reactive Biotin to IgG solution and incubate at 37°C for 10 minutes. An average of 5 to 8 biotin molecules conjugate to each IgG molecule. The number of biotin molecules per protein can be determined by HABA assay. Excess biotin molecules can be removed by a filtration tube.

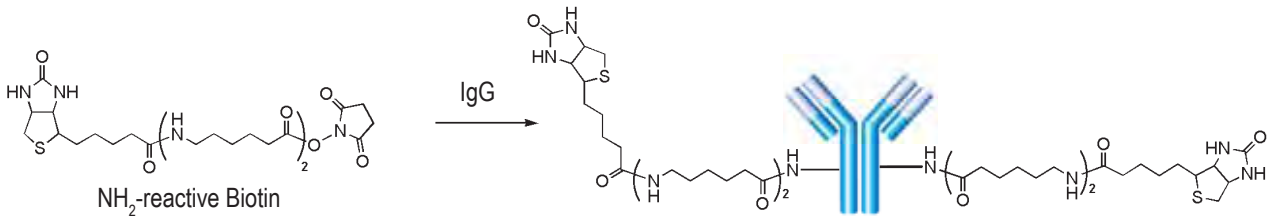


Fig. 1 IgG labeling reaction with NH_2-reactive biotin

Required Equipment and Materials

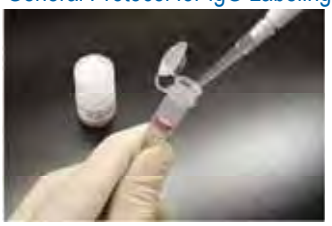
LK03-10: Microcentrifuge, 10 µl and 100-200 µl adjustable pipettes, 37°C incubator, 0.5 ml microtubes, DMSO
LK55-10: Centrifuge, rotor for 15 ml centrifuge tube, 100-200 µl and 1 ml adjustable pipettes, 37°C incubator, microtubes, DMSO

Precaution

- The molecular weight of the protein to be labeled with this kit should be greater than 50,000.
- IgG or biotin-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weights larger than 10,000, such as BSA or gelatin, purify the IgG solution before labeling biotin with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

Biotin Labeling

General Protocol for IgG Labeling



Step 1.
Add 100 µl of WS Buffer and the sample solution containing 50-200 µg IgG[a] to a Filtration Tube.

Step 2.
Mix the solution by pipetting several times, and centrifuge at 8,000 x g for 10 min.[b]

Step 3.
Add 10 µl of DMSO to NH_2-Reactive Biotin, and dissolve by pipetting.[c]

Step 4.
Add 100 µl of Reaction Buffer to the Filtration tube, and then add 8 µl[d] NH_2-Reactive Biotin solution to the Filtration Tube and pipette to mix.

Step 5.
Incubate the tube at 37°C for 10 min.

Step 6.
Add 100 µl of WS Buffer to the Filtration Tube, and centrifuge at 8,000 x g for 10 min.[b] Discard the filtrate.

Step 7.
Add 200 µl of WS Buffer to the Filtration Tube, and centrifuge at 8,000 x g for 10 min.[b] Repeat this step one more time.

Step 8.
Add 200 µl of WS Buffer, and pipette about 10 times to recover the conjugate.[e] Transfer the solution to a microtube(not included in this kit), and store at 0-5°C.

[a] The volume of IgG solution should be less than 100 µl. If the IgG concentration is lower than 0.5 mg/ml, repeat Steps 1 and 2 until the total IgG accumulation becomes 50 - 200 µg.
[b] If the solution still remains on the membrane after the centrifugation, centrifuge for another 5 min.
[c] NH_2-Reactive Biotin is on the bottom of the tube. Add 10 µl DMSO to the bottom of the tube, and pipette several times to dissolve. NH_2-Reactive Biotin can be hydrolyzed by moisture in DMSO. Proceed to Step 4 immediately after the preparation of the NH_2-Reactive Biotin solution.
[d] If the amount of IgG is 200 µg, add entire NH_2-Reactive Biotin solution.
[e] We recommend using WS Buffer to recover the conjugate. You can choose any kinds of buffers appropriate for your experiment.

Determination of Biotin/ Protein Ratio

The average number of biotin molecules per IgG molecule should be in the range of 5 and 8. If you need to determine the precise number of biotin molecules per Protein molecule use HABA assay. The following is a HABA assay protocol.

Reagent solution:

200 µM HABA (4-hydroxyazobenzene-2-carboxylic acid) solution prepared with PBS, pH 7.4	1 ml
0.5 mg avidin/ml solution prepared with PBS, pH 7.4	1 ml
diluted sample solution (55 µl biotinylated protein solution + 110 µl PBS, pH 7.4)	
25 µM biotin prepared with a mixed solution (2 volumes of PBS, pH 7.4 + 1 volume of WS buffer)	200 µl
Prepare solutions of various concentrations (12.5 µM, 6.25 µM, 3.13 µM, 1.56 µM) with serial dilution	200 µl each

1. Mix HABA solution and avidin solution in a plastic tube.
2. Add 100 µl of the HABA-avidin solution to 15 wells for multiple assays (n=3).
3. Add 50 µl biotin solution (12.5 µM, 6.25 µM, 3.13 µM, and 1.56 µM) to 3 wells each and 50 µl of diluted sample solution to the rest of the 3 wells.
4. Read the O.D. at 405 nm with a reference at 492 nm and prepare a calibration curve using the O.D. of various concentrations of biotin solution. Read the O.D. at 280 nm to determine the protein concentration. (e.g. molar absorptivity of IgG at 280 nm: 216,000).
5. Determine the concentration of biotin in the sample solution and calculate the number of biotin molecules per protein.

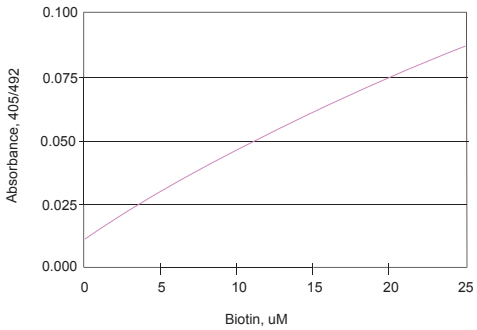


Fig. 2 Typical Calibration Curve of HABA Assay

FAQ

- Can I use this kit for other proteins?
  Yes, if the molecular weight is greater than 50,000.
- Do I have to use a filtration tube before labeling the protein?
  If the protein solution does not contain small molecules with amino groups and the concentration of the protein is 10 mg per ml or about 70 μM, there is no need to use the filtration tube. Just mix 10 μl of the sample solution with 90 μl of reaction buffer and add the mixture to a vial of NH_2-reactive biotin. After the reaction, transfer all the reaction mixture to a filtration tube, and then follow the protocol starting at step 6.
- Do I have to use WS buffer to store the biotin-labeled protein?
  You don't have to use WS buffer. You can choose any kind of buffer according to your experiment.
- My sample contains small insoluble material. What should I do?
  Spin the sample and use the supernatant for the labeling.
- How long is the biotin-labeled protein stable?
  If you store the biotin-labeled protein at 0-5°C, it is stable for 2 months. For longer storage, add 100% volume of glycerol and aliquot, and store at -20°C (if the protein can be frozen). However, please note that stability depends on the protein itself.
- What is the minimum amount of IgG that can be labeled by this kit?
  The minimum amount is 10 μg IgG; simply follow the protocol. The labeling ratio remains the same for 10 μg to 100 μg of IgG.

References

1. Y. Kubota, Y. Oike, S. Satoh, Y. Tabata, Y. Niikura, T. Morisada, M. Akao, T. Urano, Y. Ito, T. Miyamoto, N. Nagai, G. Y. Koh, S. Watanabe and T. Suda, Cooperative Interaction of Angiopoietin-like Proteins 1 and 2 in Zebrafish Vascular Development , *Proc. Natl. Acad. Sci. USA*, 2005, **102**, 13502.
2. T. Yamabuki, A. Takano, S. Hayama, N. Ishikawa, T. Kato, M. Miyamoto, T. Ito, H. Ito, Y. Miyagi, H. Nakayama, M. Fujita, M. Hosokawa, E. Tsuchiya, N. Kohno, S. Kondo, Y. Nakamura, and Y. Daigo, Dikkopf-1 as a Novel Serologic and Prognostic Biomarker for Lung and Esophageal Carcinomas, *Cancer Res.*, 2007, **67**, 2517.
3. H. Kohara, Y. Omatsu, T. Suhiyama, M. Noda, N. Fujii and T. Nagasawa, Development of Plasmacytoid Dendritic Cells in Bone Marrow Stromal Cell niches Requires CXCL12-CXCR4 Chemokine Signaling, *Blood*, 2007, **110**, 4153.
4. N. Ishikawa, A. Takano, W. Yasui, K. Inai, H. Nishimura, H. Ito, Y. Miyagi, H. Nakayama, M. Fujita, M. Hosokawa, E. Tsuchiya, N. Kohno, Y. Nakamura and Y. Daigo, Cancer-testis Antigen Lymphocyte Antigen 6 Complex Locus K is a Serologic Biomarker and a Therapeutic Target for Lung and Esophageal Carcinomas, *Cancer Res.*, 2007, **67**, 11601.

Biotin Labeling Kit-SH

Application: Biotin labeling of protein, sulfhydryl reactive

Features: Only 3 hours to recover conjugates
All processes in a single filtration tube
High recovery of conjugates
Applicable for 50-200 μg IgG

Ordering Information

Product code	Unit
LK10-10	3 samples [a]
LK57-10	1 sample [b]

[a] Based on 100 μg IgG sample labeling
[b] Based on 1 mg IgG sample labeling

Contents of the Kit

LK10-10

SH-reactive biotin ..... 3 tubes
Reducing agent ..... 3 tubes
Filtration tube ..... 3 tubes
WS buffer ..... 4 ml x 1 bottle
Reaction buffer ..... 1 ml x 1 tube

LK57-10

SH-reactive biotin ..... 1 tube
Reducing agent ..... 1 tube
Filtration tube ..... 1 tube
WS buffer ..... 13 ml x 1 bottle
Reaction buffer ..... 6 ml x 1 tube
15 ml tube ..... 1 tube

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Product Description

Biotin Labeling Kit-SH is mainly used for the preparation of biotin-labeled IgG for enzyme immunoassay (EIA). SH reactive biotin, a component of this kit, reacts with sulfhydryl groups of proteins or other molecules (Fig. 1). The kit contains all the reagents necessary for the labeling. The reducing agent included in this kit creates sulfhydryl groups in the IgG molecule. Because the reducing agent is a water-soluble phosphine compound, it can be removed by a filtration tube with a simple process. An average of 5 to 8 biotin molecules conjugate to each IgG molecule. The number of biotin molecules per protein can be determined by HABA assay. Excess biotin molecules can be removed by a filtration tube.

Biotin Labeling

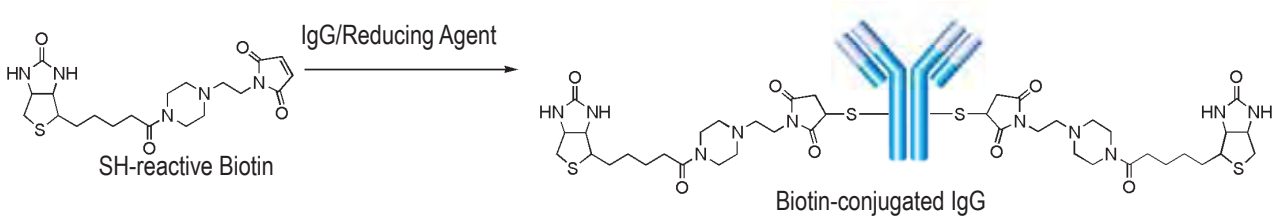


Fig. 1 IgG labeling reaction with SH-reactive biotin

Required Equipment and Materials

Microcentrifuge, 10 µl and 100-200 µl adjustable pipettes, 37 °C incubator, 0.5 ml microtubes, DMSO

Precaution

- The molecular weight of the reduced protein to be labeled with this kit should be greater than 50,000.
- IgG or biotin-conjugated IgG is always on the membrane of the filtration tube during the labeling process.
- If the IgG solution contains other proteins with molecular weights larger than 10,000, such as BSA or gelatin, purify the IgG solution before labeling biotin with this kit. IgG solution can be purified by IgG Purification Kits (not included in this kit).
- If the IgG solution contains small insoluble materials, centrifuge the solution and use the supernatant for the labeling.

General Protocol for IgG Labeling (LK10)

Step 1.
Add 100 µl of WS Buffer and the sample solution containing 50-200 µg IgG[a] to a Filtration Tube.

Step 2.
Centrifuge at 8,000 × g for 10 min[b].

Step 3.
Add 150 µl of WS Buffer to a tube of Reducing Agent[c], and dissolve by pipetting.

Step 4.
Transfer 100 µl of the Reducing Agent solution onto the membrane of Filtration tube, and pipette to dissolve the IgG on the membrane.

Step 5.
Incubate the tube at 37°C for 30 min. Add 100 µl of Reaction Buffer, and centrifuge at 8,000 × g for 10 min[b].

Step 6.
Add 10 µl of DMSO to SH-Reactive Biotin, and dissolve it with pipetting.[d]

Step 7.
Add 100 µl of Reaction Buffer and 8 µl[e] of SH-Reactive Biotin solution to the Filtration Tube, and pipette to mix.

Step 8.
Incubate the tube at 37°C for 30 min. Add 100 µl of WS Buffer to the Filtration Tube, and centrifuge at 8,000 × g for 10 min[b].

Step 9.
Add 200 µl of WS Buffer, and centrifuge at 8,000 × g for 10 min[b]. Repeat this step.

Step 10.
Add 200 µl of WS Buffer, and pipette about 10 times to recover the conjugate.[f] Transfer the solution to a 0.5 ml tube(not included in this kit), and store at 0-5°C.

[a] The volume of IgG solution should be less than 100 µl. If the IgG concentration is lower than 0.5 mg/ml, repeat Steps 1 and 2 until the total IgG accumulation becomes 50-200 µg.
[b] If the solution still remains on the filter after the centrifugation, spin for another 5 min.

[c] Reducing Agent may be attached on the inside of the cap. Be careful when opening the reducing agent.
[d] SH-Reactive Biotin is on the bottom of the tube. Add 10 µl DMSO to the bottom of the tube, and pipette several times to dissolve. SH-Reactive Biotin is unstable in DMSO. Proceed to Step 7 immediately after the preparation of the SH-Reactive Biotin solution.
[e] If the amount of IgG is 200 µg, add entire SH-Reactive Biotin solution.
[f] Since we recommend using WS Buffer to recover conjugate, you can choose any kind of buffers appropriate for your experiment. You do not have to use WS buffer to recover biotin-conjugated IgG; you can choose any kind of buffer that is appropriate for your experiment.

FAQ

♦ Can I use this kit for other proteins?
Yes, if the molecular weight is greater than 50,000.

♦ Do I have to use a filtration tube prior to labeling the protein?
There is no need to use the filtration tube if the following conditions are met:
a) The protein has disulfide groups and its solution does not contain small molecules with SH or disulfide groups. The concentration of the protein is 10 mg per ml or about 70 µM: Mix 10 µl protein solution and 100 µl reducing agent solution, and follow the protocol starting at step 4.
b) The protein has SH groups and its solution does not contain small molecules with SH groups. The concentration of the protein is 10 mg per ml or about 70 µM. Mix 10 µl protein solution and 90 µl Reaction buffer, and add 8 µl SH-reactive biotin solution. Then follow the protocol starting at step 7.

♦ Do I have to use WS buffer to store the biotin-labeled protein?
You don't have to use WS buffer. You can choose any kind of buffer according to your experiment.

♦ My sample contains small, insoluble material. What should I do?
Spin the sample and use the supernatant for the labeling.

♦ How long is the biotin-labeled protein stable?
If you store the biotin-labeled protein at 4°C, it is stable for 2 months. For longer storage, add 100% volume of glycerol and aliquot, and store at -20°C (if the protein can be frozen). Please note that stability depends on the protein itself.

♦ What is the minimum amount of IgG that can be labeled by this kit?
The minimum amount is 10 µg. There is no significant difference in sensitivity and background between 10 and 100 µg of IgG.

Biotin-OSu

Biotin *N*-hydroxysuccinimide ester [CAS: 35013-72-0]

Application: Biotin labeling of molecules with primary and secondary amines

Appearance: White or slightly yellow powder
Purity: ≥95.0% (HPLC)
MW: 341.38, $C_{14}H_{19}N_3O_5S$

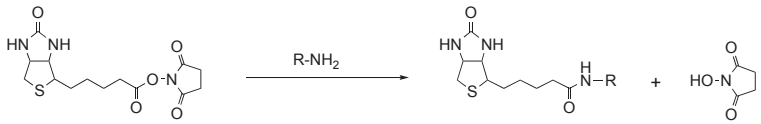
Ordering Information

Product code	Unit
B304-10	10 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Reaction Scheme



Biotin-OSu

Biotin-AC$_5$-OSu

6-(Biotinylamino)hexanoic acid *N*-hydroxysuccinimide ester [CAS: 72040-63-2]

Application: Biotin labeling of molecules with primary and secondary amines

Appearance: White or slightly yellow powder
Purity: ≥95.0% (HPLC)
MW: 454.54, $C_{20}H_{30}N_4O_6S$

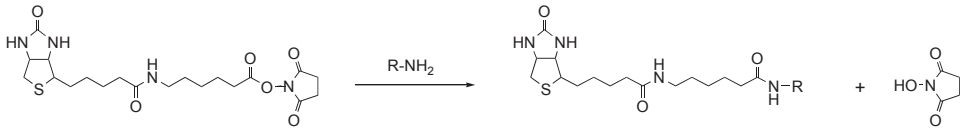
Ordering Information

Product code	Unit
B305-10	10 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Reaction Scheme



Biotin-AC$_5$-OSu

Biotin-(AC$_5$)$_2$-OSu

6-[6-(Biotinylamino)hexanoylamino]hexanoic acid *N*-hydroxysuccinimide ester [CAS: 89889-52-1]

Application: Biotin labeling of molecules with primary and secondary amines

Appearance: White or slightly yellow powder
Purity: ≥90.0% (HPLC)
MW: 567.70, $C_{26}H_{41}N_5O_7S$

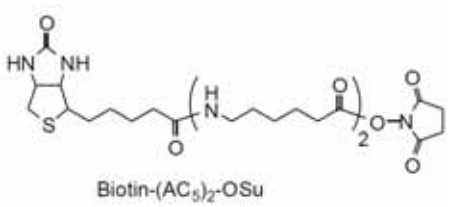
Ordering Information

Product code	Unit
B306-10	10 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Reaction Scheme



Biotin-(AC$_5$)$_2$-OSu

Reference
1. P. Kongtawelert and P. Ghosh, A New Sandwich-ELISA Method for the Determination of Keratan Sulphate Peptides in Biological Fluids Employing a Monoclonal Antibody and Labelled Avidin Biotin Technique., *Clin. Chem. Acta,*, 1990, **195**, 17 .
2. G. Paganelli, S. Pervez, A. G. Siccardi, G. Rowlinson, G. Deleide, F. Chiolerio, M. Malcovati, G. A. Scassllati and A. A. Epenetos, Intraperitoneal Radio-localization of Tumors Pre-targeted by Biotinylated Monoclonal Antibodies, *Int. J. Cancer,* 1990, **45**, 1184.
3. C. Wagener, U. Kruger and J. E. Shively, Selective Precipitation of Biotin-labeled Antigens or Monoclonal Antibodies by Avidin for Determining Epitope Specificities and Affinities in Solution-phase Assays, *Methods Enzymol.*, 1990, **184**, 518 .
4. D. M. Boorsma, J. Van Bommel and E. M. Vander Raaij-Helmer, Simultaneous Immunoenzyme Double Labelling Using Two Different Enzymes Linked Directly to Monoclonal Antibodies or with Biotin-avidin, *J. Microscopy*, 1986, **143**, 197.
5. A. Komura, T. Tokuhisa, T. Nakagawa, A. Sasase, M. chihashi, S. Ferrone and Y.Mishima, Specific Killing of Human Melanoma Cells with an Efficient 10B-compound on Monoclonal Antibodies, *Pigment Cell Res.*, 1989**, 2**, 259.
6. R. Rappuoli, P. Leoncini, P. Tarli and P. Neri, Competitive Enzyme Immunoassay for Human Chorionic Somatomammotropin Using the Avidin-biotin System, *Anal. Biochem.*, 1981, **118**, 168.

Biotin Sulfo-OSu

Biotin *N*-hydroxy-sulfosuccinimide ester [CAS: 119616-38-5]

Application: Biotin labeling of molecules with primary and secondary amines

Appearance: White or pale reddish-brown powder
Purity≥95.0% (HPLC)
MW: 443.43, $C_{14}H_{18}N_3NaO_8S_2$

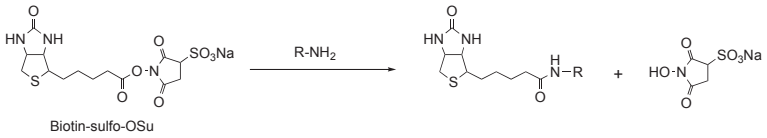
Ordering Information

Product code	Unit
B319-10	10 mg

Storage Condition
-20°C

Shipping Condition
with dry ice or blue ice

Reaction Scheme



Biotin-sulfo-OSu

Biotin-AC$_5$ Sulfo-OSu

6-(Biotinylamino)hexanoic acid *N*-hydroxy-sulfosuccinimide ester [CAS: 109940-19-4]

Application: Biotin labeling of molecules with primary and secondary amines

Appearance: White or pale reddish-brown powder
Purity: ≥95.0% (HPLC)
MW: 556.59, $C_{20}H_{29}N_4NaO_9S_2$

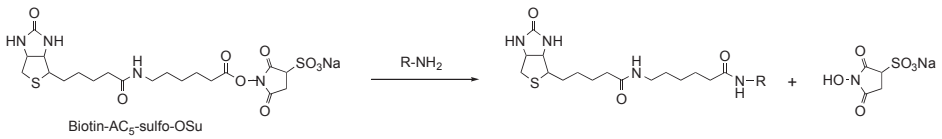
Ordering Information

Product code	Unit
B320-10	10 mg

Storage Condition
-20°C

Shipping Condition
with dry ice or blue ice

Reaction Scheme



Biotin-AC$_5$-sulfo-OSu

Biotin-$(AC_5)_2$ Sulfo-OSu

6-[6-(Biotinylamino)hexanoylamino]hexanoic acid *N*-hydroxy-sulfo succinimide ester [CAS: 180028-78-8(free acid)]

Application: Biotin labeling of molecules with primary and secondary amines

Appearance: White or pale reddish-brown powder
Purity: ≥95.0% (HPLC)
MW: 669.75, $C_{26}H_{40}N_5NaO_{10}S_2$

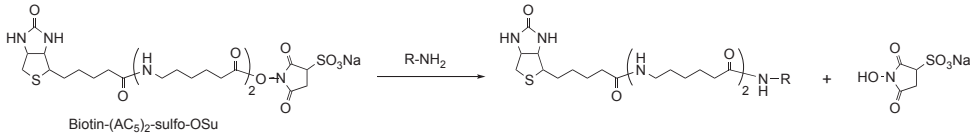
Ordering Information

Product code	Unit
B321-10	10 mg

Storage Condition
-20°C

Shipping Condition
with dry ice or blue ice

Reaction Scheme



Biotin-$(AC_5)_2$-sulfo-OSu

Product Description of Amine Reactive Biotins

The avidin-biotin system has many applications in immunology and histochemistry. The interaction between avidin and biotin is remarkably strong with a dissociation constant on the order of 10^{-15} M. Biotin is usually added to primary or secondary antibodies such as anti-IgG and anti-IgM. After preparing the antigen-antibody complex with the biotin-labeled antibody, colorimetric, or fluorometric detection of the antigen is performed using enzyme or fluorescein-labeled avidin or streptavidin. Succinimidyl ester biotins react with primary and secondary amines, such as amino acids and proteins, at pH 7-9. Succinimidyl ester reacts with free amine groups to create a stable amide bond. Succinimidyl biotin reagents must be dissolved in DMSO, DMF, or alcohol. Stock solutions prepared with DMSO are stable for several months at -20°C. Sulfosuccinimidyl biotin reagents are soluble in water, so there is no need to use organic solvents such as DMF or DMSO. IgG prepared using biotin with a longer spacer such as Biotin-$(AC_5)_2$-OSu or Biotin-$(AC_5)_2$-Sulfo-OSu, has a better signal-to-noise ratio. The longer spacer enables streptavidin or anti-biotin IgG to recognize biotin without structural inhibition. Therefore, Biotin-$(AC_5)_2$-OSu is utilized as the biotin labeling agent in the Biotin Labeling Kit-NH_2.

Labeling Procedure for IgG

1. Prepare 10 mM of the biotin labeling reagent using DMSO.
2. Prepare 100 µl of 1 mg per ml IgG buffer solution (pH 7.5-8.5) that does not contain any large molecules with amine compounds.
3. Add 1-5µl biotin labeling reagent DMSO solution to the IgG buffer solution and incubate at 37°C for 1 hour.
4. Remove excess biotin labeling reagent using gel filtration or dialysis.
5. Prepare solutions for further experiment using an appropriate buffer such as PBST (0.05% Tween 20/PBS).

References

1. J. Wormmeester, F. Stiekema and C. Groot, Immunoselective Cell Separation, *Methods Enzymol.*, 1990, **184**, 314.
2. J. J. Leary, D. J. Brigati and D. C. Ward, Rapid and Sensitive Colorimetric Method for Visualizing Biotin-labeled DNA Probes Hybridized to DNA or RNA Immobilized on Nitrocellulose: Bio-blots, *Proc. Batl. Acad. Sci.* USA, 1983, **80**, 4045.
3. W. T. Lee and D. H. Conrad, The Murine Lymphocyte Receptor for IgE. II. Characterization of the Multivalent Nature of the B Lymphocyte Receptor for IgE, *J. Exp. Med.*, 1984, **159**, 1790.
4. D. R. Gretch, M. Suter and M. F. Stinski, The use of Biotinylated Monoclonal Antibodies and Streptavidin Affinity Chromatography to Isolate Herpesvirus Hydrophobic Proteins or Glycoproteins, *Anal. Biochem.*, 1987, **163**, 270 .
5. M. Shimkus, J. Levy and T.Herman, A Chemically Cleavable Biotinylated Nucleotide: Usefulness in the Recovery of Protein-DNA Complexes from Avidin Affinity Columns, *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 2593.
6. W. J. LaRochelle and S. C. Froehner, Immunochemical Detection of Proteins Biotinylated on Nitrocellulose Replicas, *J. Immunol. Methods*, 1986, **92**, 65.
7. P. S. Anjaneyulu and J. V. Staros, Reactions of N-hydroxysulfosuccinimide Active Esters, Int. *J. Pep. Protein Res.*, 1987, **30**, 117.
8. H. M. Ingalls, C. M. Goodloe-Holland and E. J. Luna, Junctional Plasma Membrane Domains Isolated from Aggregating Dictyostelium Discoideum Amebae, *Proc. Natl. Acad. Sci. USA*, 1986, **83**, 4779.
9. J. L. Guesdon, T. Ternynck and S. Avrameas, The Use of Avidin-biotin Interaction in Immunoenzymatic Techniques, *J. Histochem, Cytochem,* 1979, **27**, 1131.

Biotin-PE-maleimide

N-Biotinyl-N'-[2-(*N*-Maleimido)ethyl]piperazine, hydrochloride

Application: Biotin labeling of molecules with sulfhydryl groups

Appearance: White or slightly yellow powder
Purity: ≥90.0% (HPLC)
MW: 472.00, $C_{20}H_{30}ClN_5O_4S$

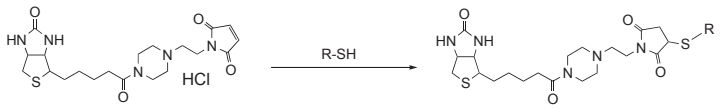
Ordering Information

Product code	Unit
B300-10	10 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Reaction Scheme



Biotin-PE-maleimide

Biotin-PEAC$_5$-maleimide

N-6-(Biotinylamino)hexanoyl-*N'*-[2-(N-maleimido)ethyl]piperazone, hydrochloride

Application: Biotin labeling of molecules with sulfhydryl groups

Appearance: White or slightly yellow powder
Purity: ≥90.0% (HPLC)
MW: 585.16, $C_{26}H_{41}ClN_6O_5S$

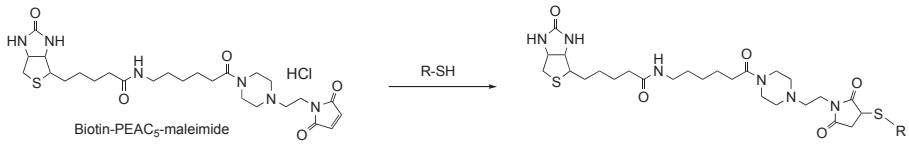
Ordering Information

Product code	Unit
B299-10	10 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Reaction Scheme



Biotin-PEAC$_5$-maleimide

Product Description

The avidin-biotin system has many applications in immunology and histochemistry. The interaction between avidin and biotin is remarkably strong with a dissociation constant on the order of the 10-15 M. Biotin is usually added to primary or secondary antibodies such as anti-IgG and anti-IgM. After preparing the antigen-antibody complex with the biotin-labeled antibody, colorimetric or fluorometric detection of the antigen is performed using enzyme or fluorophore-labeled avidin or streptavidin. Maleimide biotins react with thiol compounds, such as proteins or peptides with sulfhydryl groups, at pH 7-7.5. Maleimide reacts with sulfhydryl group to create a thioether bond. Although other maleimide biotin reagents must be dissolved in DMSO, DMF, or alcohol, Biotin-PE-maleimide can be solubilized in PBS at pH 7.4 to prepare 2 mM solution without using an organic solvent. The reactivity of maleimide with sulfhydryl groups is higher than that of bromoacetamide, therefore the required concentration of maleimide biotin is much lower than that of bromoacetamide biotins. Stock solutions of Biotin-PE-maleimide and Biotin-PEAC$_5$-maleimide in DMSO are stable for one year at -20°C.

Labeling Procedure for Reduced IgG

1. Prepare 10 mM of the biotin labeling reagent using DMSO.
2. Prepare 100 µl of 1 mg per ml reduced IgG/ml buffer solution that does not contain any large molecules with SH groups.
   Reduced IgG can be prepared by TCEP (tricarboxyethylphosphine), DTT, or 2-mercaptoethylamine.
3. Add 1-5 µl of biotin labeling reagent DMSO solution to the IgG buffer solution and incubate at 37°C for 1 hour.
4. Remove excess biotin labeling reagent using a gel column or a Filtration tube.
5. Prepare solutions for further experiments using an appropriate buffer, such as PBST (0.05% Tween 20/PBS).

References

1. Hashida, M. Imagawa, S. Inoue, K. H. Ruan and E. Ishikawa , Mor Useful Maleimide Compounds for the Conjugation of Fab' to Horseradish Peroxidase throuth Thiol Groups in the Hinge, *J. Appl. Biochem.*, 1984, **6**, 56.

2. E. Ishikawa, M. Imagawa, S. Hashida, S. Toshitake, Y. Hamaguchi and T. Ueno, Enzyme-labeling of Antibodies and their Fragments for Enzyme Immunoassay and Immunohistochemical Staining, *J. Immunoassay*, **1983**, 4, 209.
3. H. -J. Friesen, P. Hermentin and P. Gronski, Novel Maleimido-Biotins for the Selective Biotinylation of Sulfhydrils, *Protides Biol. Fluids,* **1987**, 34, 43.
4. E. Ishikawa, S. Hashida, T. Kohno, T. Kotani and S. Ohtani, Modification of Monoclonal Antibodies with Enzymes, Biotin, and Fluorochromes and Their Applications, *Immunol. Ser.*, **1987**, 33, 113.
5. R. B. del Rosalio and R. L. Wahl, Disulfide Bond-targeted Radiolabeling : Tumor Specificity of a Streptavidine-biotinylated Monoclonal Antibody Complex, *Cancer Res.(Suppl.)*, **1990**, 50, 804S.

ARP (Aldehyde Reactive Probe)

N-(Aminooxyacetyl)-N'-biotinyl-hydrazine [CAS: 139585-03-8]

Application: Biotin labeling of molecules with aldehyde or ketone groups

Appearance: White powder
Purity:≥ 95.0% (HPLC)
MW: 331.39, $C_{12}H_{21}N_5O_4S$

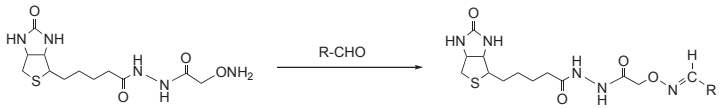
Ordering Information

Product code	Unit
A305-10	10 mg
A305-12	25 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Reaction Scheme



ARP

Product Description

Aldehyde reactive probe (ARP) is used to detect abasic sites (AP sites, depurine/depyrimidine sites) in DNA because of its aldehyde-specific reactivity. ARP reacts with aldehyde and ketone groups, and it adds biotin to AP sites in DNA. The biotin-tagged DNA is then detected using peroxidase-labeled avidin and oxidative chromogenic dyes. The ARP method has been used to detect less than 1 AP site in $1x10^4$ nucleotides of DNA. Dojindo offers a DNA Damage Quantification Kit (Product Code: DK02) containing all the reagents and components necessary to determine 1 to 40 ARP sites per $1x10^5$ base pairs. ARP is highly soluble in water and the stock solution can be stored at 4°C for one year without significant loss of reactivity.

Labeling Procedure for DNA Abasic Site

1. Prepare 10 mM ARP with water.
2. Adjust the DNA concentration to 100 µg per ml with TE buffer (pH 7.4).
3. Mix equal volumes of the DNA and ARP solutions and incubate at 37°C for 1 hour.
4. Isolate the ARP-labeled DNA using either ethanol precipitation or membrane filtration tube.
5. Dissolve the DNA pellet in TE to prepare 10-100 µg per ml solution.

References

1. R. Rago, J. Mitchen and G. Wilding, DNA Fluorometric Assay in 96-well Tissue Culture Plates Using Hoechst 33258 after Cell Lysis by Freezing in Distilled Water, *Anal. Biochem.*, 1990, **191**, 31.
2. K. Kubo, H. Ide, S. S. Wallace and Y. W. Kow, A Novel, Sensitive, and Specific Assay for Abasic Sites, The Most Commonly Produced DNA Lesion, *Biochemistry*, 1992, **31**, 3703.
3. H. Ide, K. Akamatsu, Y. Kimura, K. Michiue, K. Makino, A. Asaeda, Y. Takamori and K. Kubo, Synthesis and Damage Specificity of a Novel Probe for the Detection of Abasic Sites in DNA, *Biochemistry*, 1993, **32**, 8276.
4. M. Yao and Y. W. Kow, Strand-specific Cleavage of Mismatch-containig DNA by Deoxyinosine 3'-Endonuclease from Escherichia coli, *J. Biol. Chem.*, 1994, **269**, 31390-6.
5. T. Shida, M. Noda and J. Sekiguchi, The recognition of DNA containning AP-site by E. coli endonuclease IV(exonuclease), *Nucleic Acids Symposium Series*, 1995, **34**, 87.
6. H. B. Sun, L. Qian and H. Yokota, Detection of abasic sites on individual DNA molecules using atomic force microscopy, *Anal. Chem.*, 2001, **73**, 2229.
7. J. Nakamura, J. A. Swenberg, Endogenous Apurinic/apyrimidinic Sites in Genomic DNA of Mammalian Tissues, *Cancer Res.*, 1999, **59**, 2522-2526.
8. Y. W. Kow and A. Dare, Detection of Abasic Sites and Oxidative DNA Base Damage using an ELISA-like Assay, *METHODS*, 2000, **22**, 164.
9. L. Yan, A. Bulgar, Y. Miao, V. Mahajan, J. R. Donze, S. L. Gerson and L. Liu, Combined Treatment with Temozolomide and Methoxyamine: Blocking Apurininc/Pyrimidinic Site Repair Coupled with Targeting Topoisomerase II, *Clin. Cancer Res.*, 2007, **13**, 1532.
10. P. D. Chastain, II, J. Nakamura, J. Swenberg and D. Kaufman, Nonrandom AP site distribution in highly proliferative cells, *FASEB J.*, 2006, **20**, 2612.

AB-NTA free acid

N-(5-Amino-1-carboxypentyl)iminodiacetic acid [CAS: 129179-17-5]

Application: Chelate labeling
Appearance: White or pale yellowish white powder
Purity: ≥97.0% (HPLC)
MW: 262.26, $C_{10}H_{18}N_2O_6$

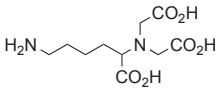
Ordering Information

Product code	Unit
A459-10	100 mg

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description

Aminobutyl-NTA (AB-NTA) free acid replaces AB-NTA (disodium salt form; former Product Code: A296-10). The new free acid form is less hygroscopic than the disodium salt form. Dissolving the new acid form in a buffer involves the same procedure as dissolving disodium salt in a buffer. The new acid form can also be dissolved in water with sonication (up to 3%). Aminobutyl-NTA was first used by Dr. Hochuli in 1987 to purify recombinant proteins (the "Histag" technique). Since then, the AB=NTA has become an indispensable tool for immobilizing proteins with high specificity on solid surfaces, such as glass or the gold electrode. A solid surface is modified by AB-NTA and bio-functionalized via Ni (II) with a genetically expressed protein bearing a hexahistidine extension at its terminus. The His-tag technique has become increasingly important, particularl in surface plasmon resonance and structural analyses of proteins by x-ray interference. Using the His-tag technique, Dr. Noji was able to directly observe the rotation of F1-ATPase by fluorescence microscope.

References

1. E. Hochuli, H. Doeli and A. Schacher, New Metal Chelate Adsorbent Selective for Proteins and Peptides Containing Neighbouring Histidine Residues, *J. Chromatogr.*, 1987, **411**, 177.
2. E. Hochuli, Large-scale Chromatography of Recombinant Proteins, *J. Chromatogr.*, 1988, **444**, 293.
3. Y. C. Sasaki, Y. Suzuki and T. Ishibashi, Fluorescent X-ray Interference from a Protein Monolayer, *Science*, 1994, **263**, 62.
4. G. B. Sigal, C. Bamdad, A. Barberis, J. Strominger and G. M. Whitesides, A Self-assembled Monolayer for the Binding and Study of Histidine-tagged Proteins by Surface Plasmon Resonance, *Anal. Chem.*, 1996, **68**, 490.
5. E. L. Schmid, T. A. Keller, Z. Dienes and H. Vogel, Reversible Oriented Surface Immobilization of Functional Proteins on Oxide Surface, *Anal. Chem.*, 1997, **69**, 1979.
6. R. Yasuda, H. Noji, K. Kinosita, and M. Yoshida, F1-ATPase is a Highly Efficient Molecular Motor that Rotates with Discrete 120°Steps, *Cell*, 1998, **93**, 1117.

Isothiocyanobenzyl-EDTA

1-(4-Isothiocyanatobenzyl)ethylenediamine-*N,N,N',N'*-tetraacetic acid [CAS: 105394-74-9]

Application: Chelate labeling of proteins

Appearance: Slightly yellowish-brown powder
Purity: ≥ 90.0% (HPLC)
MW: 439.44, $C_{18}H_{21}N_3O_8S$

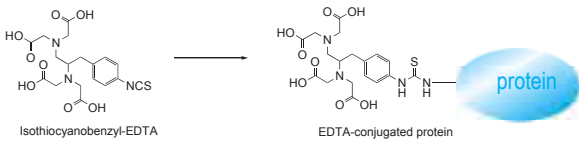
Ordering Information

Product code	Unit
M030-10	10 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Chemical Structure



Product Description

Isothiocyanobenzyl-EDTA adds EDTA moieties to proteins through their amine groups. The EDTA-tagged protein can then be labeled with heavy metal ions and radioactive metals, such as ^{111}In and ^{99}Tc, and used for immunoimaging.

References
1. C. F. Meares and T. G. Wensel, Metal Chelates as Probes of Biological Systems, *Acc. Chem. Res.*,1984, **17**, 202.
2. S. V. Deshpande, R. Subramanian, M. J. McCall, S. J. Denardo, G. L. Denardo and C. F. Meares, Metabolism of Indium Chelates Attached to Monoclonal Antibody: Minimal Transchelation of Indium form Benzyl-EDTA Chelate *in vivo*, *J. Nucl. Med.*, 1990, **31**, 218.
3. S. Mirzadeh, M. W. Brechbiel and R. W. Atcher, Otto A. Gansow Radiometal Labeling of Immunoproteins: Covalent Linkage of 2-(4-Isothiocyanatobenzyl) diethylenetriaminepentaacetic acid Ligands to Immunoglobulin, *Bioconjugate Chem.*, 1990, **1**, 59.

DTPA anhydride

Diethylenetriamine-*N,N,N',N",N"*-pentaacetic acid dianhydride [CAS: 23911-26-4]

Application: Chelate labeling of proteins

Appearance: Slightly yellow or slightly yellowish-brown powder
Purity:≥ 99.0% (Titration)
MW: 357.32, $C_{14}H_{19}N_3O_8$

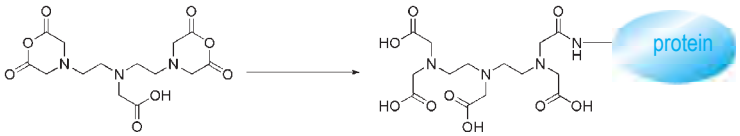
Ordering Information

Product code	Unit
D033-10	1 g
D033-12	5 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



DTPA anhydride　　DTPA-conjugated protein

Product Description
DTPA anhydride is utilized to add chelate functions to molecules or surfaces with amine groups. After adding chelate functions, metal ions such as radioisotopes can be introduced to the molecule. According to Dr. Hnatowich, IgG may be labeled with radioactive indium using DTPA-anhydride. DTPA is first linked to IgG through a covalent bond, then In(III) is tightly chelated to DTPA. Such radioactive antibodies are known to bind with antigen *in vitro* and *in vivo*.

References
1. W. C. Eckelman, S. M. Karesh and R. C. Reba, New Compounds: Fatty Acid and Long Chain Hydrocarbon Derivatives Containing a Strong Chelating Agent, *J. Pharm. Sci.*, 1975, **64**, 704.
2. B. A. Khaw, J. T. Fallon, H. W. Strauss and E. Haber, Myocardial Infarct Imaging of Antibodies to Canine Cardiac Myosin with Indium-111-Diethylenetriamine pentaacetic acid, *Science*, 1980, **209**, 295.
3. D. J. Hnatowich, W. W. Layne and R. L. Childs, The Preparation and Labeling of DTPA-Coupled Albumin, *J. Appl. Radiat. Isot.*, 1982, **33**, 327.
4. D. J. Hnatowich, W. W. Layne, R. L. Childs, D. Lanteigne, M. A. Davis, T. W. Griffin and P. W. Doherty, Radioactive Labeling of Antibody: A Simple and Efficient Method, *Science*, 1983, **220**, 613.
5. C. F. Meares and D. A. Goodwin, Linking Radiometals to Proteins with Bifunctional Chelating Agents, *J. Protein Chem.* , 1984, **3**, 215.
6. A. Canfi, M. P. Bailey and B. F. Rocks, Fluorescent Terbium Chelates Derived from Diethylenetriaminepentaacetic Acid and Heterocyclic Compounds, *Analyst*, 1989, **114**, 1405.
7. C. H. Paik, K. Yokoyama, J. C. Reynolds, S. M. Quadri, C. Y. Min, S. Y. Shin, P. J. Maloney, S. M. Larson and R. C. Reba, Reduction of Background Activities by Introduction of a Diester Linkage between Antibody and a Chelatein Radioimmunodetection of Tumor, *J. Nuclear Medicine*, 1989, **30**, 1693.
8. D. J. Hnatowich, Recent Developments in the Radiolabeling of Antibodies with iodine, Indium, and Technetium, *Semi. Nucl. Medicine*, 1990, **20**, 80.

Maleimido-C_3-NTA

N-(5-(3'-Maleimidopropylamido)-1-carboxy pentyl)iminodiacetic acid, disodium salt, monohydrate

Application: Chelate labeling of proteins
surfaces with sulfhydryl groups

Appearance: White or pale yellowish white powder
Purity: ≥97.0% (HPLC)
MW: 489.38, $C_{18}H_{23}N_3Na_2O_9 \cdot H_2O$

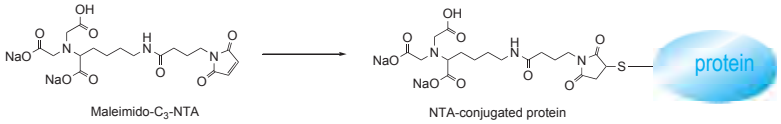
Ordering Information

Product code	Unit
M035-10	10 mg

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Chemical Structure



Maleimido-C_3-NTA NTA-conjugated protein

Product Description
Maleimido-C_3-NTA is utilized to modify the surfaces on which thiol groups are attached. Through the NTA moiety attached to the surface, genetically expressed protein, bearing a hexahistidine extension at its terminus, can be immobilized via Ni (II) (His-Tag method). Using this technique, Dr. Noji and co-workers were able to directly observe the rotation of F1-ATPase with a fluorescence microscope.

References

1. E. Hochuli, H. Doeli and A. Schacher, New Metal Chelate Adsorbent Selective for Proteins and Peptides Containing Neighbouring Histidine Residues, *J. Chromatogr.*, 1987, **411**, 177.
2. E. Hochuli, Large-Scale Chromatography of Recombinant Proteins, *J. Chromatogr.*, 1988, **444**, 293.
3. Y. C. Sasaki, Y. Suzuki and T. Ishibashi, Fluorescent X-ray Interference from a Protein Monolayer, *Science*, 1994, **263**, 62.
4. G. B. Sigal, C. Bamdad, A. Barberis, J. Strominger and G. M. Whitesides, A Self-Assembled Monolayer for The Binding and Study of Histidine-Tagged Proteins by Surface Plasmon Resonance, *Anal. Chem.*, 1996, **68**, 490.
5. E. L. Schmid, T. A. Keller, Z. Dienes and H. Vogel, Reversible Oriented Surface Immobilization of Functional Proteins on Oxide Surface, *Anal. Chem.*, 1997, **69**, 1979.

Isothiocyanobenzyl-NTA

N-[5-(4-Isothiocyanatobenzyl)amido-1-carboxypentyl]iminodiacetic acid

Application: Chelate labeling of proteins

Appearance: White or slightly yellow powder
Purity: ≥ 90.0% (HPLC)
MW: 437.47, $C_{19}H_{23}N_3O_7S$

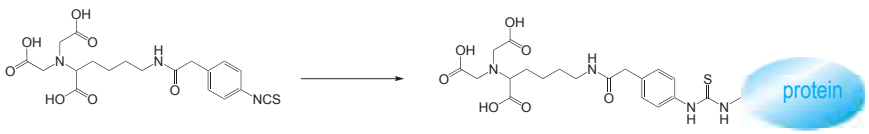
Ordering Information

Product code	Unit
I279-10	10 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Isothiocyanobenzyl-NTA is utilized to modify the surfaces on which amine groups are attached. Through the NTA moiety attached on the surface, genetically expressed protein, bearing a hexahistidine extension at its terminus, can be immobilized via Ni (II) (His-Tag method). Using this technique, Dr. Noji and co-workers were able to directly observe the rotation of F1-ATPase with a fluorescence microscope.

EMCS *N*-(6-Maleimidocaproyloxy)succinimide [CAS: 55750-63-5]

Application: Amine and sulfhydryl group cross-linking

Appearance: White or slightly yellowish white powder
Purity: ≥90.0% (HPLC)
MW: 308.29, $C_{14}H_{16}N_2O_6$

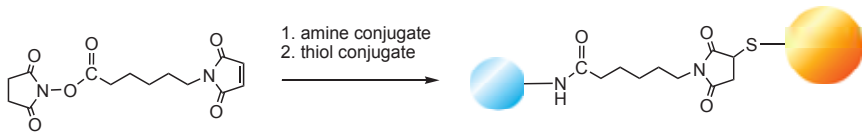
Ordering Information

Product code	Unit
E018-10	50 mg
E018-12	100 mg

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Cross-linking Reaction



GMBS *N*-(4-Maleimidobutyryloxy)succinimide [CAS: 80307-12-6]

Application: Amine and sulfhydryl group cross-linking

Appearance: White powder
Purity: ≥95.0% (HPLC)
MW: 280.23, $C_{12}H_{12}N_2O_6$

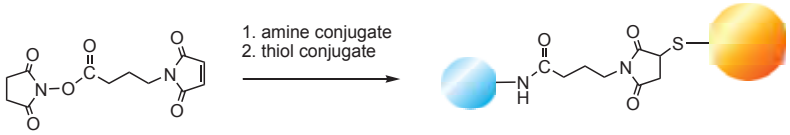
Ordering Information

Product code	Unit
G005-10	50 mg
G005-12	100 mg

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Cross-linking Reaction



HMCS *N*-(8-Maleimidocapryloxy)succinimide

Application: Amine and sulfhydryl group cross-linking

Appearance: White powder or cystaline powder
Purity:≥90.0% (HPLC)
MW: 336.34, $C_{16}H_{20}N_2O_6$

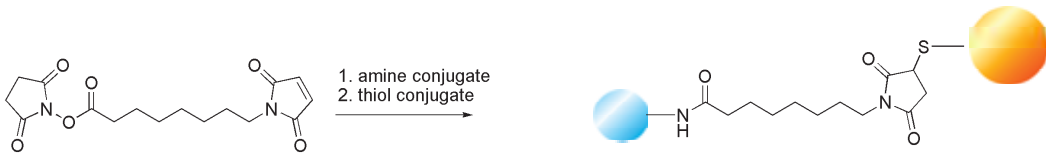
Ordering Information

Product code	Unit
H257-10	50 mg

Storage Condition
0-5°C, protect from moisture

Shipping Condition
with dry ice or blue ice

Cross-linking Reaction



KMUS *N*-(11-Maleimidoundecanoyloxy)succinimide [CAS: 87981-04-2]

Application: Amine and sulfhydryl group cross-linking

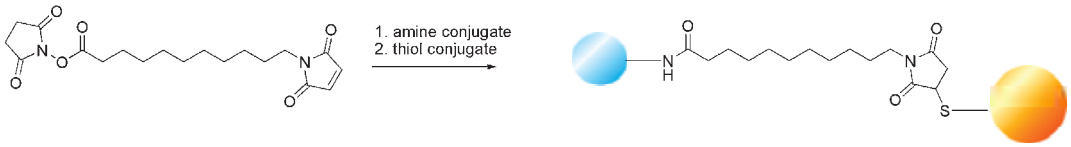
Appearance: White powder or crystalline powder
Purity: ≥90.0% (HPLC)
MW: 378.42, $C_{19}H_{26}N_2O_6$

Ordering Information	
Product code	Unit
K214-10	50 mg

Storage Condition
0-5°C, protect from moisture

Shipping Condition
ambient temperature

Cross-linking Reaction



Sulfo-EMCS *N*-(6-Maleimidocaproyloxy)sulfosuccinimide, sodium salt [CAS: 103848-61-9 (free acid)]

Application: Amine and sulfhydryl group cross-linking, water-soluble

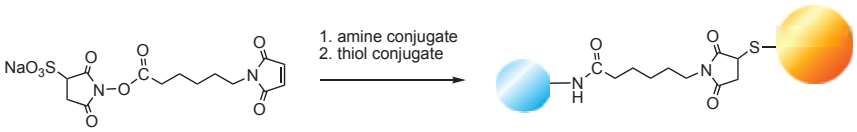
Appearance: White or slightly yellowish-pink powder
Purity: ≥90.0% (HPLC)
MW: 410.33, $C_{14}H_{15}N_2NaO_9S$

Ordering Information	
Product code	Unit
S024-10	50 mg

Storage Condition
0-5°C

Shipping Condition
with dry ice or blue ice

Cross-linking Reaction



Sulfo-GMBS *N*-(4-Maleimidobutyryloxy)sulfosuccinimide, sodium salt

Application: Amine and sulfhydryl group cross-linking, water-soluble

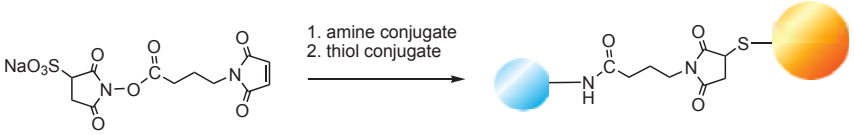
Appearance: White or slightly yellowish-pink powder
Purity: ≥90.0% (HPLC)
MW: 382.28, $C_{12}H_{11}N_2NaO_9S$

Ordering Information	
Product code	Unit
S025-10	50 mg

Storage Condition
0-5°C

Shipping Condition
with dry ice or blue ice

Cross-linking Reaction



Sulfo-HMCS

N-(8-Maleimidocapryloxy)sulfosuccinimide, sodium salt [CAS: 211236-35-0]

Application: Amine and sulfhydryl group cross-linking, water-soluble

Appearance: White or slightly yellowish-pink powder
Purity: ≥90.0% (HPLC)
MW: 438.39, $C_{16}H_{19}N_2NaO_9S$

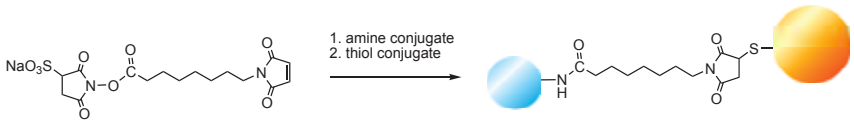
Ordering Information

Product code	Unit
S026-10	50 mg

Storage Condition
0-5°C, protect from moisture

Shipping Condition
with dry ice or blue ice

Cross-linking Reaction



Sulfo-KMUS

N-(11-Maleimidoundecanoyloxy)sulfosuccinimide, sodium salt [CAS: 211236-68-9]

Application: Amine and sulfhydryl group cross-linking, water-soluble

Appearance: White or slightly yellowish-pink powder
Purity:≥90.0% (HPLC)
MW: 480.47, $C_{19}H_{25}N_2NaO_9S$

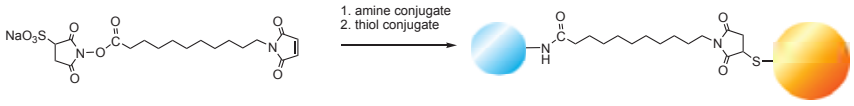
Ordering Information

Product code	Unit
S250-10	50 mg

Storage Condition
0-5°C, protect from moisture

Shipping Condition
with dry ice or blue ice

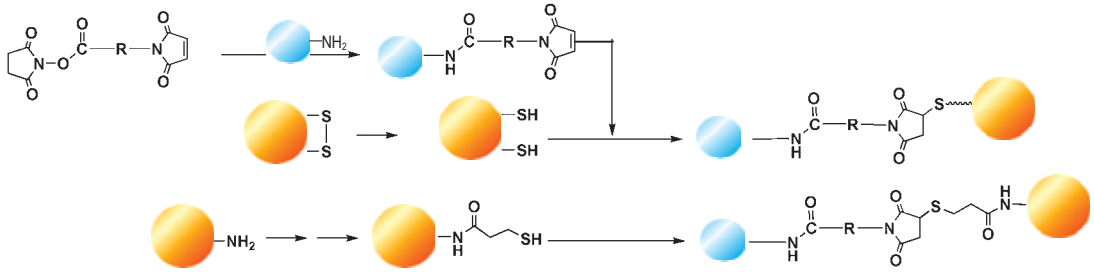
Cross-linking Reaction



Product Description

Hetero-bifunctional cross-linking reagents have activated esters and maleimide reactive groups. These functional groups react with amines and sulfhydryl groups of proteins, respectively. Enzyme-labeled haptens are prepared using hetero-bifunctional cross-linking reagents such as EMCS or GMBS. The cross-linking reaction requires neutral pH and mild temperature because it is necessary to maintain enzyme activity and antibody titers in the cross-linking reaction. Hetero-bifunctional cross-linking reagents with 3, 5, 7, or 10 linear carbon chains are available. These linear aliphatic chains act as spacers between the two reactive sites and their water-soluble reagents. They are more stable than the aromatic cross-linking reagents such as succinimidyl-4-*N*-maleimidobenzoate in a wider pH range.

Conjugation of Macromolecules with Hetero-Bifunctional Cross-Linking Reagent



References

E018

1. S. Yoshitake, Y. Yamada, E. Ishikawa and R. Masseyeff, Conjugation of Glucose Oxidase from Aspergillus Niger and Rabbit Antibodies Using N-Hydroxysuccinimide Ester of N-(4-Carboxycyclohexylmethyl)-Maleimide, Eur. *J. Biochem.*, 1979, **101**, 395.
2. T. Kitagawa, T. Shimozono, T. Aikawa, T. Yoshida and H. Nishimura, Preparation and Characterization of Hetero-bifunctional Cross-linking Reagents for Protein Modifications, *Chem. Pharm. Bull.*, 1981, **29**, 1130.
3. S. Yoshitake, M. Imagawa, E. Ishikawa, Y. Niitsu, I. Urushizaki, M. Nishimura, R. Kanazawa, H. Kurosaki, S. Tachibana, N. Nakazawa and H. Ogawa, Mild and Efficient Conjugation of Rabbit Fab' and Horseradish Peroxidase Using a Maleimide Compound and Its Use for Enzyme Immunoassay, *J. Biochem.*, 1982, **92**, 1413.

4. E. Ishikawa, M. Imagawa, S. Hashida, S. Yoshitake, Y. Hamaguchi and T. Ueno, Enzyme-Labelling of Antibodies and Their Fragments for Enzyme Immunoassay and Immunohistochemical Staining, *J. Immunol.*, 1983, **4**, 209.
5. I. Weeks, A. K. Campbell and J. S. Woodhead, Two-site Immunochemiluminometric Assay for Human α1-Fetoprotein, *Clin. Chem.*, 1983, **29**, 1480.
6. S. Hashida, M. Imagawa, S. Inoue, K. Ruan and E. Ishikawa, More Useful Maleimide Compounds for the Conjugation of Fab' to Horseradish Peroxidase through Thiol Groups in the Hinge, *J. Appl. Biochem.*, 1984, **6**, 56.
7. S. Inoue, M. Imagawa, S. Hashida, K. Ruan and E. Ishikawa, A Small Scale Preparation of Affinity-Purified Rabbit Fab'-Horseradish Peroxidase Conjugate for Enzyme Immunoassay, *Anal. lett.*, 1984, **17**, 229.
8. M. Koizumi, K. Endo, M. Kunimatsu, H. Sakahara, T. Nakashima, Y. Kawamura, Y. Watanabe, T. Saga, J. Konishi, T. Yamamura, S. Hosoi, S. Toyama, Y. Arano and A. Yokoyama, Ga-Labeled Antibodies for Immunoscintigraphy and Evalution of Tumor Targeting of Drug-Antibody Conjugatesin Mice, *Cancer Res.*, 1988, **48**, 1189.
9. T. Kohno and E. Ishikawa, Novel Enzyme Immunoassay(Immune Complex Transfer Enzyme Immunoassay) for Anti-insulin IgG in Guinea Pig Serum, *Anal. lett.*, 1988, **21**, 1019.

G005

1. S. Yoshitake, Y. Yamada, E. Ishikawa and Rene Masseyeff, Conjugation of Glucose Oxidase from Aspergillus Niger and Rabbit Antibodies Using N-Hydroxysuccinimide Ester of N-(4-Carboxycyclohexylmethyl)-Maleimide, *Eur. J. Biochem.*, 1979, **101**, 395.
2. H. Tanimori, T. Kitagawa, T. Tsunoda and R. Tsuchiya, Enzyme Immunoassay of Neocarzinostain Using β-Galactosidase as Label, *J. Pharm. Dyn.*, 1981, **4**, 812.
3. K. Fujiwara, M. Yasuno and T. Kitagawa, Novel Preparation Method of Immunogen for Hydrophobic Hapten, Enzyme Immunoassay for Daunomycin and Adriamycin, J. Immunol. Methods, *1981*, **45**, 195.
4. T. Miura, H. Kouno and T. Kitagawa, Detection of Residual Penicillin in Milk by Sensitive Enzyme Immunoassay, *J. Pharm. Dyn.*, 1981, **4**, 706.
5. S. Yoshitake, M. Imagawa, E. Ishikawa, Y. Niitsu, I. Urushizaki, M. Nishiura, R. Kanazawa and H. Ogawa, Mild and Efficient Conjugation of Rabbit Fab' and Horseradish Peroxidase Using a Maleimide Compound and Its Use for Enzyme Immunoassay, *J. Biochem.* ,1982, **92**, 1413.
6. T. Kitagawa, H. Tanimori, K. Yoshida, H. Asada, T. Miura and K. Fujiwara, Studies on Viomycin. XV. Comparative Study on the Specificities of Two Anti-viomycin Antisera by Enzyme Immunoassay, *Chem. Pharm. Bull.*, 1982, **30**, 2487.
7. T. Kitagawa, T. Kawasaki and H. Munechika, Enzyme Immunoassay of Blasticidin S with High Sensitivity:A New and Convenient Method for Preparation of Immunogenic(Hapten-Protein) Conjugates, *J. Biochem.*,1982, **92**, 585.

S024, S025, S026, S250

1. J. V. Staros, N-Hydroxysulfosuccinimide Active Esters:Bis(N-hydroxysulfosuccinimide) Esters of Two Dicarboxylic acids Are Hydrophilic, Membrane-impermeant, Protein Cross-linkers, *Biochemistry*, 1982, **21**, 3950.
2. P. S. R. Anjaneyulu, James V. Staros, Reactions of N-Hydroxysulfosuccinimide Active Esters, Int. *J. Peptide Protein Res.*, 1987, **30**, 117.
3. Y. Fukami, K. Sato, K. Ikeda, K. Kamisango, K. Koizumi and T. Matsuno, Evidence for Autoinhibitory Regulation of the c-src Gene Product a Possible Interaction Between the Src Homology 2 Domain and Autophosphorylation Site, *J. Biol. Chem.*, 1993, **268**, 1132.

SPDP *N*-Succinimidyl 3-(2-pyridyldithio)propionate [CAS: 68181-17-9]

Application: Amine and sulfhydryl group cross-linking

Appearance: White or slightly yellow powder
Purity: ≥98.0% (HPLC)
MW: 312.37, $C_{12}H_{12}N_2O_4S_2$

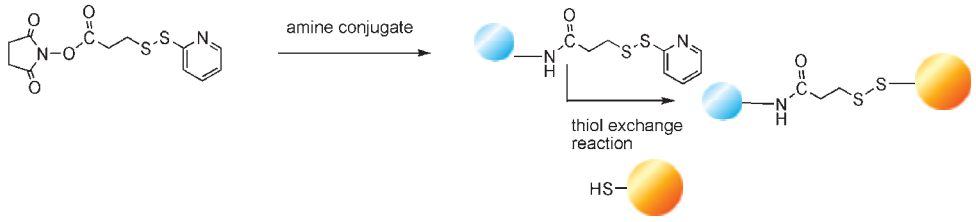
Ordering Information

Product code	Unit
S291-10	100 mg

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Cross-linking Reaction



Product Description

SPDP, a hetero-bifunctional reagent, is useful for bridging two different proteins, such as enzymes and antibodies. SPDP first reacts with a protein molecule through its amino groups. SPDP introduces a pyridyl disulfide moiety to the protein, which is then reduced by DTT to form thiol groups. These thiol groups then form a disulfide bond with another protein molecule thus yielding a heterodimer protein.

References

1. P. Walden, Z. A. Nagy and J. Klein, Major Histocompatibility Complex-Restricted and Unrestricted Activation of Helper T Cell Lines by Liposome-bound Antigens, *J. Mol. Cell. Immunol*, 1986, **2**, 191.
2. R. D. Gordon, W. E. Fieles, D. L. Schotland, R. Hogue-Angeletti and R. L. Barchi, Topographical Localization of the C-terminal Region of the Voltage-dependent Sodium Channel from Electrophorus Electricus Using Antibodies Raised Against a Synthetic Peptide, *Proc. Natl. Acad. Sci.* USA, **1987**, *84*, 308.

IgG Purification Kit-A

Application: Immunoglobulin G purification, isolation

Features: All processes take place in a single tube
IgG recovery is 70-90%
Purity of the IgG from serum is greater than 80%
Purified IgG is available in 30 minutes
High reproducibility and no affinity loss

Ordering Information

Product code	Unit
AP01-10	1 set

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Contents of the Kit
Protein A pack ..... 1 pack
Elution buffer .....4 ml x 1 bottle
Washing buffer ..... 10 ml x 1 bottle
Catching buffer .....1.5 ml x 1 bottle

IgG Purification Kit-G

Application: Immunoglobulin G purification, isolation

Features: All processes take place in a single tube
IgG recovery is 70-90%
Purity of the IgG from serum is over 80%
Purified IgG is available in 30 minutes
High reproducibility and no affinity loss

Ordering Information

Product code	Unit
AP02-10	1 set

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Contents of the Kit
Protein G pack .....1 pack
Elution buffer .....4 ml x 1 bottle
Washing buffer ..... 10 ml x 1 bottle
Catching buffer .....1.5 ml x 1 bottle

Required Equipment and Materials
Microcentrifuge, 100-200 µl adjustable pipettes, voltex mixer, 0.5 ml microtubes

Product Description
IgG Purification Kits are used for isolation and purification of immunoglobulin G of goats, mice, rabbits, and other animals. The kit contains immobilized protein A or G and buffer solutions for maximum recovery of IgG. The total time to isolate and purify IgG from serum or other solutions containing IgG is about 30 minutes (Fig. 1). Because the support of protein A or G is silica-base gel, the volume of the retained solution on the gel after the centrifugation is very small. Therefore, proteins or other materials that do not bind to protein A can be removed by two washings. SDS-PAGE of purified IgG from various animals are shown in Fig. 2. Additionally, denaturing of IgG during the elution process is minimal because IgGs bound to the gel are released with a very quick process. The gel in this kit can be used repeatedly 20 times or more with an equal performance. Used gel is also stable for one year in washing buffer at 0-5°C .

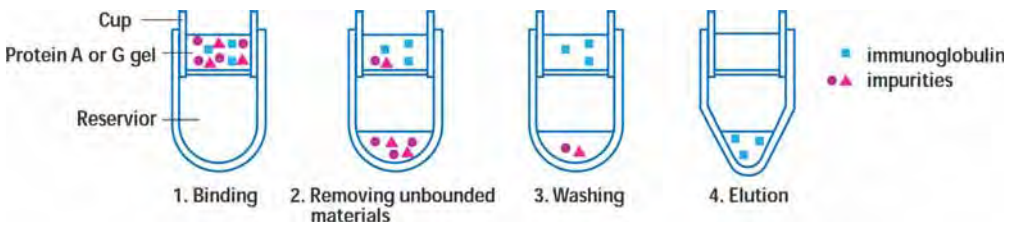


Fig. 1 IgG Isolation Process

Precaution
If the IgG solution contains gelatin,before applying the solution to the kit, enzyme digestion may be required.

IgG Purification

General Protocol for IgG Isolation

Step 1.
Mix 50 µl sample solution containing IgG and 50 µl Washing buffer.

Step 2.
Add the mixture prepared at step 1 to a cup of a cartridge tube in Protein A pack. Do not close the cap.

Step 3
Rotate the cup with a finger several times to mix the gel.[a] Leave the tube at room temperature for 2 minutes.

Step 4.
Close the cap, and centerifuge the tube at maximum speed for 30 seconds.

Step 5.
Add the filtrate to the cup again and repeat steps 3 and 4.[b] Transfer the filtrate to a 0.5 ml tube and store it at 0-5°C.[c]

Step 6.
Add 200 µl washing buffer to the cup. Do not close the cap. Rotate it with a finger several time to mic the gel.[a]

Step 7.
Close the cap and centrifuge at maximum speed for 30 sec. Discard the filtrate of the reservoir.

Step 8.
Repeat step 6 and 7.

Step 9.
Add 60 µl catching buffer to a 1.5 ml tube in protein A pack. Transfer the cup to this tube.[d]

Step 10.
Add 70 µl elution buffer to the cup. Do not close the cap Do not close the cap Rotate the cup several times with a finger to mix the gel.[a]

Step 11.
Close the cap and centrifuge at maximum speed for 30 seconds.

Step 12.
Repeat step 10 and 11.

Step 13.
Remove the cup.[e] Close the cap and vortex to mix. Store the solution at 0-5°C.[f]

[a] Slant the cartridge tube and rotate the cup with a finger 10 to 20 times.
[b] The IgG recovery increases 25 to 30% more by this process. You may repeat this process to get a maximum IgG recovery.
[c] If the recovery rate of the IgG is low, use this filtrate to recover the IgG. Follow the protocol starting at step 2.
[d] Do not discard the reservoir. It will be used in step 13.
[e] Return the cup to the reservoir kept in step 9. Wash gel with washing buffer by following steps 6 and 7. Add 200 µl washing buffer and keep the protein A gel cartridge tube at 0-5°C. Centrifuge the cartridge tube to remove washing buffer before using it.
[f] You may add an equal volume of glycerol and store at -20°C.

Table 1 IgG Recovery from 50 µl Serum

Species	Kit-A	Kit-G	Species	Kit-A	Kit-G
Goat	50-100 µg	150-250 µg	Sheep	50-100 µg	150-250 µg
Rabbit	200-300 µg	150-250 µg	Mouse	150-250 µg	100-150 µg
Hamster	150-250 µg	100-150 µg	Rat	50-100 µg	100-200 µg
Human	200-300 µg	200-300 µg	Dog	200-300 µg	100-200 µg
Cat	150-250 µg	100-200 µg	Porcine	200-300 µg	150-250 µg
Calf	200-300 µg	250-350 µg	Horse	150-250 µg	200-300 µg
Guinea Pig	150-200 µg	150-250 µg	Donkey	200-300 µg	200-300 µg
Chicken	25-50 µg	10-20 µg			

IgG Purification

IgG Purity

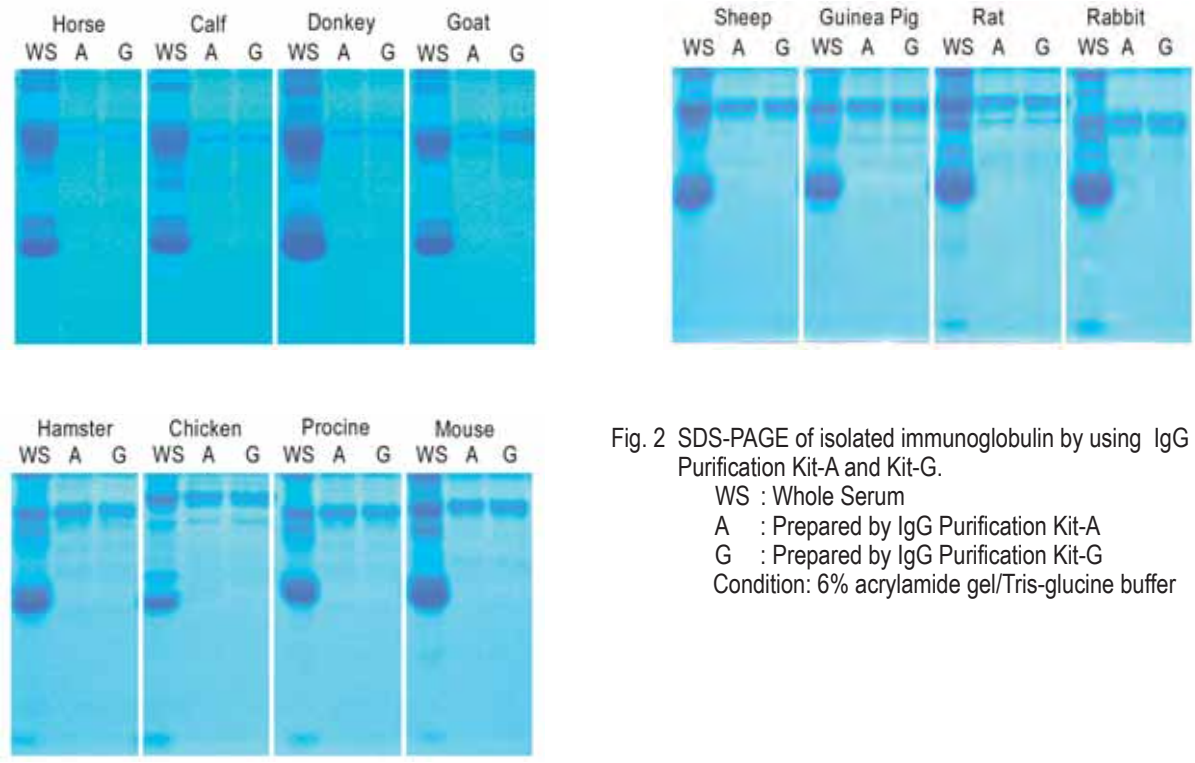


Fig. 2 SDS-PAGE of isolated immunoglobulin by using IgG Purification Kit-A and Kit-G.
WS : Whole Serum
A : Prepared by IgG Purification Kit-A
G : Prepared by IgG Purification Kit-G
Condition: 6% acrylamide gel/Tris-glucine buffer

FAQ

- **What is the recovery of IgG with this kit?**
  It depends on the type of IgG and type of animal. In the case of High-affinity IgG for protein A or protein G, about 70-80% IgG is recovered from 100-200 µg IgG or IgG solution containing other proteins or macromolecules.
- **What is the purity of IgG with this kit?**
  The purity of the IgG from various serums is indicated in the figure above. Highly purified IgG is available with just a one-time purification process.
- **How much IgG can be recovered from serum?**
  About 150-350 µg IgG can be recovered from 50 µl serum.
- **How many times can a protein A gel be used?**
  At least 20 times.
- **Can I use the used protein A gel or protein G gel for the purification of different IgG solutions?**
  Use a new gel for a different sample to avoid contamination.
- **Is a used protein A gel or protein G gel stable?**
  A used protein A gel or protein G gel in washing buffer is stable at 0-5°C for one year.

BABE 1-(*p*-Bromoacetamidobenzyl) ethylenediamine *N,N,N',N'*-tetraacetic acid [CAS: 81677-64-7]

Application: Chelate labeling of molecules with sulfhydryl groups

Appearance: White to grayish white powder
Purity: ≥90.0% (HPLC)
MW: 518.32, $C_{19}H_{24}BrN_3O_9$

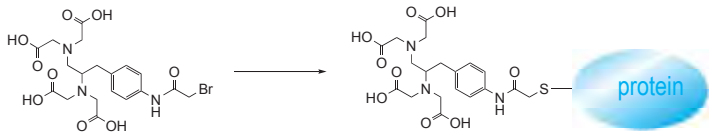
Ordering Information

Product code	Unit
B437-10	10 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Reaction Scheme



BABE EDTA-conjugated protein

FeBABE 1-(*p*-Bromoacetamidobenzyl) ethylenediamine *N,N,N',N'*-tetraacetic acid, acid iron(III)

Application: Chelate labeling of molecules with sulfhydryl groups

Appearance: Yellowish-brown powder
Purity: ≥95.0%(HPLC)
MW: 571.14, $C_{19}H_{21}BrFeN_3O_9$

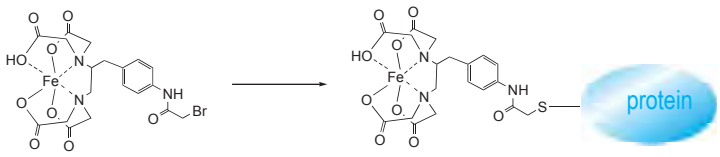
Ordering Information

Product code	Unit
F279-10	1 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Reaction Scheme



FeBABE FeBABE-conjugated protein

Product Description of BABEs

Bromoacetamidobenzyl-EDTA (BABE) is a chelate labeling reagent that conjugates with sulfhydryl groups. The iron chelate of BABE (FeBABE) is a unique tool for determining the three-dimensional structure of proteins and the binding structures of protein-protein or protein-DNA complexes. BABE adds EDTA moieties to proteins through their sulfhydryl groups. Once attached to a protein, FeBABE cuts a nearby peptide or DNA chain. The cleavage site is within 12 angstroms of the FeBABE binding site. Iron (II)-chelate cleaves a peptide or DNA chain in the presence of hydrogen peroxide. The cleavage reaction completes quickly: 10 seconds to 20 minutes of incubation is sufficient. The size of the cleaved fragment is analyzed with gel electrophoresis such as SDS-PAGE.

Labeling Procedure

1. Dialyze the protein solution in conjugation buffer (10-20 mM MOPS, 0.2 M NaCl, 2 mM EDTA, 5% glycerol, pH 8.0) at 4°C overnight.
2. After dialysis, adjust the protein concentration to 15-30 mM.
3. Add 15 μl of 20 mM FeBABE DMSO solution to 1 ml of the protein solution and incubate it at 37°C for 1 hour. The final concentration of FeBABE is 0.3 mM (10-20X excess to the protein).
4. Dialyze the reaction mixture in protein storage buffer (10-20 mM Tris, 0.1-0.2 M KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 50% glycerol, pH 7.6) at 4°C overnight.

References

B437

1. L. H. DeRiemer, C. F. Meares, D. A. Goodwin and C. I. Diamanti, BLEDTA II: Synthesis of a New Tumer-Visualizing Derivative of Co(III)-bleomycin, *J. Labelled Compd. Radiopharm.*, 1981, **18**, 1517.
2. T. M. Rana and C. F. Meares, Specific Cleavage of a Protein by an Attached Iron Chelate, *J. Am. Chem. Soc.*, 1990, **112**, 2457.
3. T. M. Rana and C. F. Meares, Transfer of Oxigen from an artificial protease to peptide carbon during proteolysis, *Proc. Natl. Acad. Sci. USA*, 1991, **88**, 10578.
4. D. P. Greiner, R. Miyake, J. K. Moran, A. D. Jones, T. Negishi, A. Ishihama, and C. F. Meares, Synthesis of the Protein Cutting Reagent Iron (S)-1-(p-Bromoacetamidobenzyl)ethylebediaminetetraacetate and Conjuation to cysteine Sie Cahins, *Bioconjugate Chem.*, **1997**, 8, 44.
5. E. Platis, M. R. Ermacora and R. O. Fox, Oxidative Polypeptide Cleavage Mediated by EDTA-Fe Covalently Linked to Cysteine Residue, *Biochemistry*, 1993, **32**, 12761.
6. S. L. Traviglia, S. A. Datwyler, D. Yan, A. Ishihama and C. F. Meares, Targeted Protein Footprinting: Where Different Transcription Factors bind to RNA Polymerase, *Biochemistry*, 1999, **38**, 4259.
7. J. B. Ghaim, D. P. Greiner, C. F. Meares and R. B. Gennis, Proximity Mapping the suface of Membrane Protein Using an Artificial Protease: Demonstration That the Quinone-Binding Domain of Subunit I Is near the N-Terminal Region of Subunit II of Cytochrome bd, *Biochemistry,* 1995, **34**, 11311.
8. R. Miyake, K. Murakami, J. T. Owens, D. P. Greiner, O. N. Ozoline, A. Ishihama and C. F. Meares, Dimeric Association of Escherichia coli RNA Polymerase alfa subunits, studied by Cleavage of Single-Cysteine alfa Sununits Conjugated to Iron-(S)-1-(p-(Bromoacetamido)benzyl)ethylenediaminetetraacetate, *Biochemistry*, 1998, **37**, 1344.
9. J. T. Owens, R. Miyake, K. Murakami, A. J. Chmura, N. Fujita, A. Ishihama and C. F. Meares, Mapping the sigma70 subunits contact sites on Escherichia coli RNA polymerase with a sigma70-conjugated chemical protease, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 6021.
10. J. A. Bown, J. T. Owens, C. F. Meares, N. Fujita, A. Ishihama, S. J. Busby and S. D. Minchin, Organization of open complexes at Escherichia coli promoters. Location of promoter DNA sites close to region 2.5 of the sigma70 subunit of RNA polymerase, *J. Biol. Chem.*, 1999, **274**, 2263.
11. F. Colland, N. Fujita, D. Kotlarz, J. A. Bown, C. F. Meares, A. Ishihama and A. Kolb, Positioning of sigma(S), the stationary phase sigma factor, in Escherichia coli RNA polymerase-promoter open complexes, *EMBO J.*, 1999, **18**, 4049.
12. G. M. Heilek, R. Marusak, C. F. Meares and H. F. Noller, Directed hydroxyl radical probing of 16S rRNA using Fe(II) tethered to ribosomal protein S4, *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 1113.
13. G. M. Heilek and H. F. Noller, Site-directed hydroxyl radical probing of the rRNA neighborhood of ribosomal protein S5, *Science*, 1996, **272**,

F279

1. L. H. DeRiemer, C. F. Meares, D. A. Goodwin and C. I. Diamanti, BLEDTA II: Synthesis of a New Tumer-Visualizing Derivative of Co(III)-bleomycin, *J. Labelled Compd. Radiopharm.*, 1981, **18**, 1517.
2. T. M. Rana and C. F. Meares, Specific Cleavage of a Protein by an Attached Iron Chelate, *J. Am. Chem. Soc.*, 1990, **112**, 2457.
3. T. M. Rana and C. F. Meares, Transfer of Oxigen from an artificial protease to peptide carbon during proteolysis, *Proc. Natl. Acad. Sci. USA,* 1991, **88**, 10578.
4. D. P. Greiner, R. Miyake, J. K. Moran, A. D. Jones, T. Negishi, A. Ishihama, and C. F. Meares, Synthesis of the Protein Cutting Reagent Iron (S)-1-(p-Bromoacetamidobenzyl)ethylebediaminetetraacetate and Conjuation to cysteine Sie Cahins, *Bioconjugate Chem.*, 1997, **8**, 44.
5. E. Platis, M. R. Ermacora and R. O. Fox, Oxidative Polypeptide Cleavage Mediated by EDTA-Fe Covalently Linked to Cysteine Residue, *Biochemistry*, 1993, **32**, 12761.
6. S. L. Traviglia, S. A. Datwyler, D. Yan, A. Ishihama and C. F. Meares, Targeted Protein Footprinting: Where Different Transcription Factors bind to RNA Polymerase, *Biochemistry,* 1999, **38**, 4259.
7. J. B. Ghaim, D. P. Greiner, C. F. Meares and R. B. Gennis, Proximity Mapping the suface of Membrane Protein Using an Artificial Protease: Demonstration That the Quinone-Binding Domain of Subunit I Is near the N-Terminal Region of Subunit II of Cytochrome bd, *Biochemistry,* 1995, **34**, 11311.
8. R. Miyake, K. Murakami, J. T. Owens, D. P. Greiner, O. N. Ozoline, A. Ishihama and C. F. Meares, Dimeric Association of Escherichia coli RNA Polymerase alfa subunits, studied by Cleavage of Single-Cysteine alfa Sununits Conjugated to Iron-(S)-1-(p-(Bromoacetamido)benzyl)ethylenediaminetetraacetate, *Biochemistry*, 1998, **37**, 1344.
9. J. T. Owens, R. Miyake, K. Murakami, A. J. Chmura, N. Fujita, A. Ishihama and C. F. Meares, Mapping the sigma70 subunits contact sites on Escherichia coli RNA polymerase with a sigma70-conjugated chemical protease, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 6021.
10. J. A. Bown, J. T. Owens, C. F. Meares, N. Fujita, A. Ishihama, S. J. Busby and S. D. Minchin, Organization of open complexes at Escherichia coli promoters. Location of promoter DNA sites close to region 2.5 of the sigma70 subunit of RNA polymerase, *J. Biol. Chem.*, 1999, **274**, 2263.
11. F. Colland, N. Fujita, D. Kotlarz, J. A. Bown, C. F. Meares, A. Ishihama and A. Kolb, Positioning of sigma(S), the stationary phase sigma factor, in Escherichia coli RNA polymerase-promoter open complexes, *EMBO J.*, 1999, **18**, 4049.
12. G. M. Heilek, R. Marusak, C. F. Meares and H. F. Noller, Directed hydroxyl radical probing of 16S rRNA using Fe(II) tethered to ribosomal protein S4, *Proc. Natl. Acad. Sci. USA*, 1995, **92**, 1113.
13. G. M. Heilek and H. F. Noller, Site-directed hydroxyl radical probing of the rRNA neighborhood of ribosomal protein S5, *Science*, 1996, **272**, 1659.
14. K. R. Lieberman and H. F. Noller, Ribosomal protein L15 as a probe of 50 S ribosomal subunit structure., *J. Mol. Biol.*, 1998, **284**, 1367.

Introduction

The phospholipid bilayer is the basic structure of the cell membrane. The most important functions of cells include transportation of substances, energy exchange, and transmission of information. These functions are conducted at the cell membrane by membrane proteins.

In membrane biochemistry research, membrane proteins are solubilized and purified to study their structure and function. Proteins bound to cell membranes have hydrophobic sites buried within the phospholipid bilayers and hydrophilic sites facing toward the water layer. Detergents are used to isolate large insoluble molecules such as proteins. Detergents interact with the hydrophobic sites of proteins, which are then solubilized in the water layer, thus separating membrane proteins. It is important to choose a detergent that does not disrupt the bioactivities of target proteins. A detergent requires the following characteristics to be suitable for isolation of membrane proteins:

1. Sufficient protein solubilization capability
2. No denaturing or inactivation of proteins
3. No interference with protein activities
4. No precipitation at 4°C
5. Appropriate critical micelle concentrations (CMC) and micelle size
6. No absorption in the UV region
7. No toxicity
8. Availability of detergent detection methods
9. Non-ionic detergent if ion exchange chromatography is used

In the past, polyoxyethylene ether non-ionic detergents were widely used. These detergents, however, had several problems, such as denaturation of proteins and low CMC value, which cannot be separated easily by dialysis. n-Octyl-β-D-glucoside, n-Octyl-β-D-thioglucoside, CHAPS, and CHAPSO eliminate these problems and are widely used today. Most of the current detergents are non-ionic and easily applied to ion exchange chromatography purification. deoxy-BIGCHAP is a non-ionic detergent possessing deoxycholic acid and a gluconamide polar group. It has a high CMC value of 1.4 mM and can be easily separated by dialysis. Because its UV absorbance is low, it can be used for the determination of proteins. deoxy-BIGCHAP has been used for the extraction of opioid receptors from neuroblastoma or hybrid cells of glyoma. It has also been applied to adenylate cyclase or acetyltransferase. These detergents are also widely used to solubilize chromophores or to stabilize enzymes in diagnostic analyses and biochemical assays.

Trials of various kinds of detergents are needed to find the appropriate detergent for each study. Dojindo's Detergent Screening Sets, which contain assorted packages of detergents, are available for use in these trials.

Critical Micelle Concentration (CMC)

Detergents are amphipathic compounds, with both lipophobic and lipophilic sites, that will form micelles above a critical concentration, CMC, that is specific to each detergent. The solubilizing abilities of detergents increase dramatically above their CMC values. After they extract membrane proteins, detergents can be easily removed by dilution and then dialysis.

Table 1 Molecular Weight and Critical Micelle Concentration of Detergents

Detergent	MW	CMC
CHAPS	614.88	8 mM
CHAPSO	630.88	8 mM
BIGCHAP	878.06	2.9 mM
DeoxyBIGCHAP	862.06	1.4 mM
Octylglucoside	292.37	25 mM
Heptylthioglucoside	294.41	30 mM
Octylthioglucoside	308.44	9 mM
Decylmaltoside	482.57	1.8 mM
Dodecylmaltoside	510.62	0.17 mM
Nonylthiomaltoside	484.60	2.4 mM
MEGA-8	321.41	-
MEGA-9	335.44	25 mM
MEGA-10	349.46	7 mM
Sucrose monocholate	732.85	4.7 mM
Sodium cholate	448.57	14 mM

BIGCHAP

N,*N*-Bis(3-D-gluconamidopropyl)cholamide [CAS: 86303-22-2]

Appearance: White powder
Purity: ≥95.0% (HPLC)
MW: 878.06, $C_{42}H_{75}N_3O_{16}$

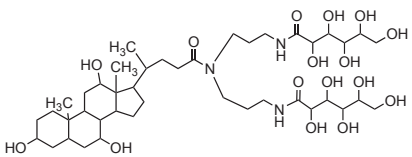
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
B043-10	1 g
B043-12	5 g

Chemical Structure



deoxy-BIGCHAP

N,*N*-Bis(3-D-gluconamidopropyl)deoxycholamide [CAS: 86303-23-3]

Appearance: White powder
Purity: ≥95.0% (HPLC)
MW: 862.06, $C_{42}H_{75}N_3O_{15}$

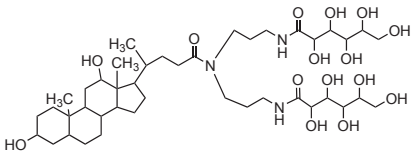
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
D045-08	500 mg

Chemical Structure



Product Description of BIGCHAPs

BIGCHAP and deoxy-BIGCHAP are non-ionic detergents based on a cholic acid and a bisglucon-amidopropyl group. The CMC values are 2.9 mM and 1.4 mM, respectively. BIGCHAP and deoxy-BIGCHAP are easily removed by dialysis, and their absorption in the UV region is very low.

References

B043

1. L. M. Hjelmeland, et al., A New Class of Nonionic Detergents with a Gluconamide Polar Group. *Anal Biochem.* 1983;**130**:485-490.
2. V. Lakshmi, et al., Extraction of 11β-Hydroxysteroid Dehydrogenase from Rat Liver Microsomes by Detergents. *J Steroid Biochem*. 1985;**22**:331-340.

D045

1. L. M. Hjelmeland, et al., A New Class of Nonionic Detergents with a Gluconamide Polar Group. *Anal Biochem.* 1983;**130**:485-490.
2. A. Aigner, et al., Purification and Characterization of Cysteine-S-conjugate N-Acetyltransferase from Pig Kidney. *Biochem J*. 1996;**317**:213-218.

CHAPS

3-[(3-Cholamidopropyl)dimethylammonio]propanesulfonate [CAS: 75621-03-3]

Appearance: White powder
Purity: ≥97.0% (HPLC)
MW: 614.88, $C_{32}H_{58}N_2O_7S$

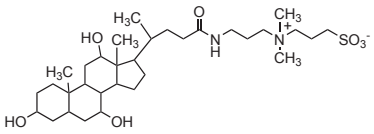
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
C008-10	1 g
C008-12	5 g
C008-14	25 g

Chemical Structure



CHAPSO

3[(3-Cholamidopropyl)dimethylammonio]-2-hydroxypropanesulfonate [CAS: 82473-24-3]

Appearance: White powder
Purity: ≥96.0% (HPLC)
MW: 630.88, $C_{32}H_{58}N_2O_8S$

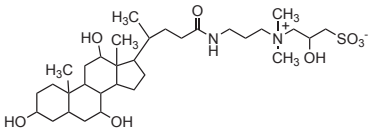
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
C020-10	1 g
C020-12	5 g

Chemical Structure



Product Description of CHAPS and CHAPSO

CHAPS and CHAPSO are zwitter ionic detergents and have cholic acid and sulfobetaine moieties in their structures. Their low background absorbance in the UV region is an attractive feature for the UV monitoring of membrane proteins. The CMC values of both CHAPS and CHAPSO are 8 mM.

References

C008

1. L. M. Hjelmeland, A Nondenaturing Zwitterionic Detergent for Membrane Biochemistry: Design and Synthesis. *PNAS*. 1980;**77**:6368-6370.
2. W. F. Simonds, et al., Solubilization of Active Opiate Receptors. *PNAS*. 1980;**77**:4623-4627.
3. A. J. Bitonti, et al., Resolution and Activity of Adenylate Cyclase Components in Azwitterionic Cholate Derivative[3-[(3-Cholamidopropyl) Dimethylammonio]-1-Propanesulfonate]. *Biochemistry.* 1982;**21**:3650-3653.
4. B. Rivnay, et al., Phospholipids Stabilize the Interaction Between the α and β Subunits of the Solubilized Receptor for Immunoglobulin *E. Biochemistry.* 1982;**21**:6922-6927.
5. G. H. Perdew, et al., The Use of Zwitterionic Detergent in Two-dimensional Gel Electrophoresis of Trout Liver Microsomes. *Anal Biochem.* 1983;**135**:453-455.
6. T. Matoh, et al., Sodium, Potassium, Chloride, and Betaine Concentrations in Isolated Vacuoles from Salt-grown Atriplex gmelini Leaves. *Plant Physiol.* 1987;**84**:173-177.
7. R. Horiuchi, et al., Purification and Characterization of 55-kDa Proteinwith 3, 5, 3'-Triiodo-L-thyronine-binding Activity and Protein Disulfide-isomerase Activity from Beef Liver Membrane. *Eur J Biochem.* 1989;**183**:529-538.
8. N. Funasaki, et al., Odd-even Alternation in the Aggregation Nember Dependence of Stepwise Aggregation Constants. *J Phys Chem.* 1991;**95**:1847-1850.

C020

1. D. S. Liscia, et al., Solubilization of Active Prolactin Receptors by a Nondenaturing Zwitterionic Detergent. *J Biol Chem.* 1982;**257**:9401-9405.

Sodium cholate (purified)

Cholic acid, sodium salt, monohydrate [CAS: 361-09-1]

Appearance: White powder
Purity: ≥98.0% (HPLC)
MW: 448.57, $C_{24}H_{39}NaO_5 \cdot H_2O$

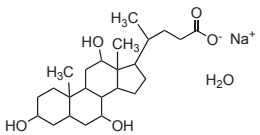
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
C321-10	5 g
C321-12	25 g

Chemical Structure



Product Description

Sodium cholate is an anionic detergent with 14 mM CMC. Since three hydroxy groups are located on a steroid ring and one carboxylic group is at the terminus of the molecular structure, there are no definitive lipophilic or hydrophilic regions in the molecule. In general, cholate detergents can be easily removed by dialysis because of their fairly small micelle sizes.

n-Decyl-β-D-maltoside *n*-Decyl-β-D-maltopyranoside [CAS: 82494-09-5]

Appearance: White or pale yellowish white powder
Purity: ≥98.0% (GC)
MW: 482.57, $C_{22}H_{42}O_{11}$

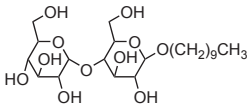
Storage Condition
0-5°C

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
D382-10	1 g
D382-12	5 g

Chemical Structure



n-Dodecyl-β-D-maltoside *n*-Dodecyl-β-D-maltopyranoside [CAS: 69227-93-6]

Appearance: White powder
Purity: ≥98.0% (GC)
MW: 510.62, $C_{24}H_{46}O_{11}$

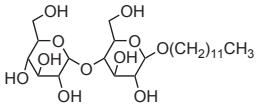
Storage Condition
0-5°C

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
D316-10	1 g
D316-12	5 g

Chemical Structure



Product Description
n-Dodecyl-β-D-maltoside is a non-ionic detergent. It has a glyco-chain in its lipophilic site that is similar to the one in n-Octyl-β-D-glucoside. Using this detergent, Dr. Van Aken and others succeeded in solubilizing the active cytochrome oxidase from mitochondria. Its CMC value is 0.17 mM.

References
D382
1. T. Tsukihara, et al., Structures of Metal Sites of Oxidized Bovine Heart Cytochrome c Oxidase at 2.8Å. *Science*. 1995;**269**:1069-1074.
2. S. Iwata, et al., Structure at 2.8Å resolution of cytochrome c oxidase from Paracoccus denitrificans. *Nature*. 1995;**376**:660-669.

D316
1. T. VanAken, et al., Alkyl Glycoside Detergents: Synthesis and Applications to the Study of Membrane Proteins. *Methods Enzymol*. 1986;**125**:27-35.

n-Heptyl-β-D-thioglucoside *n*-Heptyl-β-D-thioglucopyranoside [CAS: 85618-20-8]

Appearance: White powder or waxy solid
Purity: ≥98.0% (GC)
MW: 294.41, $C_{13}H_{26}O_5S$

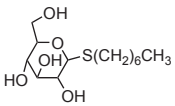
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
H015-10	1 g
H015-12	5 g

Chemical Structure



Product Description
n-Heptyl-β -D-thioglucoside is an effective non-ionic detergent for the solubilization of membrane proteins, similar to *n*-Octyl-β -D-glucoside. Though *n*-Octyl-β -D-glucoside is degraded by β -glycosidase, *n*-Heptyl-β -D-thioglucoside is not. This means that *n*-Heptyl-β -D-thioglucoside is effective for the solubilization of samples with β -glycosidase activity. The CMC value of *n*-Heptyl-β -D-thioglucoside is 30 mM. It is soluble in aqueous solutions at 4°C making it suitable for the solubilization of membrane proteins at low temperatures.

n-Nonyl-β-D-thiomaltoside

n-Nonyl-β -D-thiomaltopyranoside [CAS: 148565-55-3]

Appearance: White power
Purity: ≥98.0% (GC)
MW: 484.60, $C_{21}H_{40}O_{10}S$

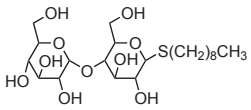
Storage Condition
0-5°C

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
N373-10	1 g

Chemical Structure



Product Description
n-Nonyl-β-D-thiomaltoside is a newly developed detergent whose lipophilic moiety is maltose. This reagent is suitable for isolating proteins inside of cell membranes. The activity of proteins isolated using *n*-Nonyl- β -D-thiomaltoside is higher than those isolated using *n*-Octyl-β -Dthioglucoside or *n*-Heptyl-β-D-thioglucoside. As the CMC value of this detergent is 2.4 mM, it is easy to remove from protein solutions by dialysis.

n-Octyl-β-D-glucoside

n-Octyl-β -D-glucopyranoside [CAS: 29836-26-8]

Appearance: White powder
Purity: ≥98.0% (GC)
MW: 292.37, $C_{14}H_{28}O_6$

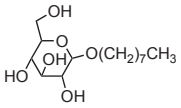
Storage Condition
0-5°C

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
O001-10	1 g
O001-12	5 g

Chemical Structure



Product Description
n-Octyl-β-D-glucoside has definite advantages over the conventional non-ionic detergents as a membrane protein solubilizer. It can be easily separated by dialysis and has very little interaction with membrane proteins. Its CMC value is 25 mM. It is strongly recommended for studies of ATPase or NADH dehydrogenase.

References
H015
1. S. Saito, et al., Characterization of n-Octyl-β-D-Thioglucopyranoside, a Newnon-Ionic Detergent Useful for Membrane Biochemistry. *Biochem J.* 1984;**222**:829-832.
2. T. Tsuchiya, et al., Use of n-Octyl-β-D-Thioglucoside, a New Nonionic Detergent, for Solubilization and Reconstitution of Membrane Proteins. *J Biochem.* 1984;**96**:1593-1597.
3. T. Shimamoto, et al., Value of Heptyl-β-D-Thioglucoside, a New Nonionic Detergent, in Studies on Membrane Proteins. *J Biochem*. 1985;**97**:1807-1810.
4. H. Itami, et al., Purification and Characterization of Membrane-bound 5'-Nucleotidase of Vibrio parahaemolyticus. *J Biochem.* **1989**;105:785-789.
5. M. Kai, et al., Thiobacillus ferrooxidans Cytochrome c Oxidase: Purification, and Molecular and Enzymatic Features. *J Biochem.* 1992;**112**:816-821.

N373
1. S. Izawa, et al., Introduction of a Series of Alkyl Thiomaltosides, Useful New Non-Ionic Detergents to Membrane Biochemistry. *J Biochem.* 1993;**113**:573-576.

O001
1. A. Levitzki, Reconstitution of Membrane Receptor Systems. *Biochim Biophys Acta.* 1985;**822**:127-153.
2. S. Horiuchi, et al., Characterization of a Membrane-Associated Receptor from Ratsinusoidal Liver Cells That Binds Formaldehyde-Treated Serum Albumin. *J Biol Chem.* 1985;**260**:475-481.
3. H. Tokuda, et al., Reconstitution of Translocation Activity for Secretory Proteins from Solubilized Components of Escherichia coli. *Eur J Biochem.* 1990;**192**:583-589.
4. K. Kameyama, et al., Micellar Properties of Octylglucoside in Aqueous Solutions. *J Colloid Interface Sci.* 1990;**137**:1-10.
5. M. Nishikawa, et al., Decreased Expression of Type II Protein Kinase C in HL-60 Variant Cells Resistant to Induction of Cell Differentiation by Phorbol Diester. *Cancer Res.* 1990;**50**:621-626.
6. H. Tokuda, et al., Purification of SecE and Reconstitution of SecE-dependent Protein Translocation Activity. *FEBS Lett.* 1991;**279**:233-236.

O003
1. S. Saito, et al., Characterization of n-Octyl-β-D-Thioglucopyranoside, a Newnon-Ionic Detergent Useful for Membrane Biochemistry. *Biochem J.* 1984;**222**:829-832.
2. T. Tsuchiya, et al., Use of n-Octyl-β-D-Thioglucoside, a New Nonionic Detergent, for Solubilization and Reconstitution of Membrane Proteins. *J Biochem.* 1984;**96**:1593-1597.
3. T. Shimamoto, et al., Value of Heptyl-β-D-Thioglucoside, a New Nonionic Detergent, in Studies on Membrane Proteins. *J Biochem.* 1985;**97**:1807-1810.
4. H. Itami, et al., Purification and Characterization of Membrane-bound 5'-Nucleotidase of Vibrio parahaemolyticus. *J Biochem.* 1989;**105**:785-789.
5. M. Kai, et al., Thiobacillus ferrooxidans Cytochrome c Oxidase: Purification, and Molecular and Enzymatic Features. *J Biochem.* 1992;**112**:816-821.

O393
1. T. Tsukihara, et al., Structures of Metal Sites of Oxidized Bovine Heart Cytochrome c Oxidase at 2.8Å. *Science.* 1995;**269**:1069-1074.
2. S. Iwata, et al., Structure at 2.8Å resolution of cytochrome c oxidase from Paracoccus denitrificans. *Nature*. 1995;**376**:660-669.

n-Octyl-β-D-thioglucoside

n-Octyl-β-D-thioglucopyranoside [CAS: 85618-21-9]

Appearance: White powder
Purity: ≥98.0% (GC)
MW: 308.44, $C_{14}H_{28}O_5S$

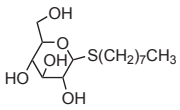
Ordering Information

Product code	Unit
O003-10	1 g
O003-12	5 g

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Chemical Structure



Product Description
n-Octyl-β-D-thioglucoside is similar to *n*-Octyl-β -D-glucoside. After solubilization, it can be separated from phospholipid or protein by dialysis. It does not interfere with enzyme activities. It is more stable than *n*-Octyl-β-D-glucoside and is not affected by esterase. Its CMC value is 9 mM.

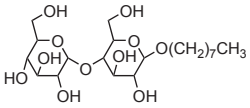
n-Octyl-β-D-maltoside *n*-Octyl-β-D-maltopyranoside [CAS: 82494-08-4]

Appearance: White powder
Purity: ≥98.0% (GC)
MW: 454.51, $C_{20}H_{38}O_{11}$
Storage Condition
0-5°C
Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
O393-10	500 mg

Chemical Structure



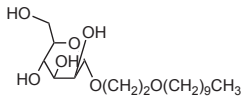
3-Oxatridecyl-α-D-mannoside 3-Oxatridecyl-α-D-mannopyranoside

Appearance: White powder
Purity: ≥95.0% (GC)
MW: 364.47, $C_{18}H_{36}O_{7}$
Storage Condition
0-5°C
Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
O401-10	500 mg

Chemical Structure



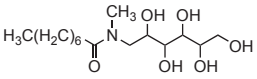
MEGA-8 *n*-Octanoyl-*N*-methyl-D-glucamine [CAS: 85316-98-9]

Appearance: White powder
Purity: ≥98.0% (HPLC)
MW: 321.41, $C_{15}H_{31}NO_{6}$
Storage Condition
ambient temperature
Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
M014-10	1 g
M014-12	5 g

Chemical Structure



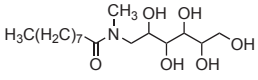
MEGA-9 *n*-Nonanoyl-*N*-methyl-D-glucamine [CAS: 85261-19-4]

Appearance: White powder
Purity: ≥98.0% (HPLC)
MW: 335.44, $C_{16}H_{33}NO_{6}$
Storage Condition
ambient temperature
Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
M015-10	1 g
M015-12	5 g

Chemical Structure



MEGA-10 *n*-Decanoyl-*N*-methyl-D-glucamidne [CAS: 85261-20-7]

Appearance: White powder
Purity: ≥98.0% (HPLC)
MW: 349.46, $C_{17}H_{35}NO_6$

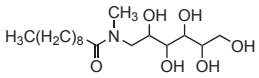
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
M016-10	1 g
M016-12	5 g

Chemical Structure



Product Description
Glucamide detergents are non-ionic, are transparent in the UV region, and possess relatively high CMC values. Therefore, they are ideal for use as membrane protein solubilizers. The CMC values for these detergents are between 7 mM and 25 mM.

References
M014, M015, M016

1. J. E. Hildreth, N-D-gluco-N-methylalkanamide Compounds, a New Class of Non-ionic Detergents for Membrane Biochemistry. *Biochem J.* **1982**;207:363-366.
2. M. Okawauchi, et al., A Light-Scattering Study of Temperature Effect on Micelle Formation of N-alkanoyl-N-methylglucamines in Aqueous Solution. *Bull Chem Soc Jpn*. **1987**;60:2719-2725.
3. G. Sugihara, et al., The CMC of the Mixed System of MEGA-9 with MEGA-10 in Water at 30°C. *J Colloid Interface Sci.* **1988**;123:544-545.
4. V. De Pinto, et al., Interaction of Non-Classical Detergents with the Mitochondrial Porin A New Purification Procedure and Characterization of the Pore-forming Unit. *Eur J Biochem.* 1989;**183**:179-187.
5. E. Malinowska, et al., Potentiometric response of magnesium-selective membrane electrode in the presence of nonionic surfactants. *Anal Chim Acta.* **1999**;382:265-275.

Sodium deoxycholate (for protein crystallization)

Deoxycholic acid, sodium salt, monohydrate [CAS: 145224-92-6]

Appearance: White powder
Purity: ≥98.0% (HPLC)
MW: 432.57, $C_{24}H_{39}NaO_4 \cdot H_2O$

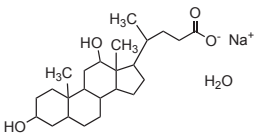
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
D520-10	1 g
D520-12	5 g

Chemical Structure



References

1. H. Hirata, et al., Solubilization and Partial Purification of Alanine Carrier from Membranes of a Thermophilic Bacterium and its Reconstitution into Functional Vesicles. *Biochem Biophys Res Commun.* 1976;**69**:665-671.
2. D. L. Foster, et al., Energy-transducing H^+-ATPase of Escherichia coli. Purification, Reconstitution, and Subunit Composition. *J Biol Chem.* 1979;**254**:8230-8236.
3. J. C. Norton, et al., Enhanced Detection of Human Telomerase Activity. *DNA Cell Biol.* 1998;**17**:217-219.
4. M. B. Jones, et al., Instability of the G-Protein -β-5-subunit in Detergent. *Anal Biochem.* 1999;**268**:126-133.

Detergent Screening Set (first choice-II)

Contents of the Set

CHAPS ..... 200 mg x 1
n-Dodecyl-β-D-maltoside .....200 mg x 1
n-Octyl-β-D-glucoside .....200 mg x 1
Sodium cholate (purified) .....200 mg x 1
MEGA-8 ..... 200 mg x 1

Ordering Information

Product code	Unit
DS06-10	1 set

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Detergent Screening Set (for crystallization)

Contents of the Set

n-Decyl-β-D-maltoside ..... 200 mg x 1
n-Dodecyl-β-D-maltoside .....200 mg x 1
n-Octyl-β-D-glucoside ..... 200 mg x 1
n-Octyl-β-D-maltoside ..... 200 mg x 1
MEGA-10 .....200 mg x 1

Ordering Information

Product code	Unit
DS05-10	1 set

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Product Description

Detergent Screening Sets are used to find the best detergent for an application, such as enzyme stabilization, specific membrane protein isolation, assay development, protein crystallization, protein refolding, and so on. Detergent Screening Set (first choice-II) contains commonly used detergents for membrane protein isolation. Detergent Screening Set (for crystallization) contains detergents commonly used for protein crystallization.

Oxidative Stress Detection

Introduction

Oxygen is a very important molecule for the synthesis of biologically active materials such as hormones and ATP. Acquisition of the ability to utilize oxygen was a significant driving force for the evolution of life. Oxygen activates various enzymes in cells and activated oxygen species are involved in the operation of cell functions. Although oxygen itself is an essential element of life, molecules in cells, such as DNA and proteins, are sometimes damaged by reactive oxygen species (ROS) in what is called oxidative stress. ROS can be created by metabolism, ionizing radiation, and carcinogenic compounds that directly interact with DNA. During metabolism, a small portion of oxygen is converted to superoxide anion by one electron reduction; superoxide anion is then converted to oxygen and hydrogen peroxide by superoxide dismutase (SOD). Hydrogen peroxide is reduced to water by catalase or glutathione peroxidase. However, if hydrogen peroxide is not completely reduced by these enzymes, it can generate an extremely reactive hydroxy radical when oxidized by iron (Fenton reaction). Hydroxy radical is also generated by UV irradiation or directly from water by ionizing radiation. Hydroxy radical reacts with lipid to generate lipid peroxide. However, not all ROS are unwanted. Hypochlorite ion, an ROS derived from hydrogen peroxide by myeloperoxidase in neutrophils, has germicidal activity. Nitric oxide, also known as endothelial-derived relaxation factor, is generated by NO synthetase. However, NO and superoxide anion may react to generate peroxynitrite, which is cytotoxic.

The ROS and reactive nitrogen compounds have many different activities in biological systems. In response, aerobic organisms created defense mechanisms to avoid oxidative stress. Oxidative stress has recently become the focus of many studies seeking to understand these defense mechanisms and the relationships between oxidative damage and disease or aging processes. To this end, many assay methods have been developed for the detection of ROS-related or ROS-derived substances such as superoxide anion, superoxide dismutase, glutathione, glutathione reductase, glutathione peroxidase, DNA lesions, 8-oxoguanine, 8-nitroguanosine, and protein carbonyl.

DNA Damage by Oxidative Stress

Oxidative damage to DNA results from the interaction of DNA with ROS, in particular, hydroxy radicals. Hydroxy radicals, which are produced from superoxide anion and hydrogen peroxide by the Fenton reaction, cause multiple modifications in DNA. Oxidative attacks by hydroxy radicals on the deoxyribose moiety lead to the release of free bases from DNA, generating strand breaks with various sugar modifications and simple abasic sites. These are one of the major types of DNA damage generated by ROS. Measurement of these modifications is important for understanding the mechanisms of oxidative DNA damage and its biological repercussions. Most of the time, the DNA repair system eliminates such damage by severing the disease process and maintaining the integrity of the form of life. However, clinical data clearly indicate that oxidative DNA damage is related to a number of disease processes such as carcinogenesis and neurodegenerative diseases.

DNA Damage Quantification Kit is utilized for determining the number of simple abasic sites (AP sites) in genomic DNA isolated from cell and tissue samples. The DNA damage level can be monitored in the range of 1 to 40 AP sites per 105 base pairs. Therefore, DNA Damage Quantification Kit is useful for the detection of carcinogen toxicity and for the detection of DNA repair activities.

8-Nitroguanosine, another oxidatively modified molecule, is a nitrated base of DNA or RNA. It is known that a large amount of nitric oxide and superoxide molecules, generated by inflammation, can cause nitration of guanosine. Since chemically modified nucleotides cause mutation during DNA replication, 8-nitroguanosine is thought to be a marker of DNA damage related to mutation and cancer. 8-Nitroguanosine is involved in controlling cell functions and is related to oxidizing and reducing reactions. 8-Nitroguanosine antibodies and assay kits are available from Dojindo.

Oxidative Stress Related Components

Superoxide dismutase (SOD) is one of the most important antioxidative enzymes for scavenging toxic superoxides in vivo. SOD catalyzes the dismutation of the superoxide anion into molecular oxygen and hydrogen peroxide, which are much less oxidatively reactive. SOD is a potential therapeutic agent in diseases related to oxidative stress and as a material for moderating the aging process, because of its high antioxidant efficiency (much higher than that of glutathione or other reducing agents) and early action in the initial stages of ROS metabolism. Since glutathione also has superoxide-scavenging activity, it contributes to a reductive atmosphere in cells and tissues to avoid oxidative damage.

SOD Assay Kit-WST is used for the determination of superoxide quenching ability, based on an inhibition assay, and is suitable for evaluating SOD or SOD-like activities in samples. Cytochrome C is a commonly utilized agent for SOD activity detection. However, its reactivity with superoxide anion is too high to determine low SOD levels. Since the reactivity of WST-1 with superoxide anion is much lower than that of cytochrome C, low levels of SOD activity can be determined.

Total Glutathione Quantification Kit is used for measuring the amount of glutathione in samples. The combination of DTNB (Ellman's Reagent) and glutathione reductase in this kit enables highly sensitive detection of total glutathione. DTNB generates an intense yellow dye upon reaction with the reduced form of glutathione. The assay range of this kit is from 1 µM to 100 µM glutathione.

Nitric Oxides

Nitric oxide (NO) has been identified as an endothelial-derived relaxation factor and antiplatelet substance. It serves as a neurotransmitter when derived from a neutrophil, and as a cytotoxic substance when derived from an activated macrophage. NO reacts with superoxide anion to generate highly toxic peroxynitrite. The reaction rate of NO with superoxide is three times that of SOD. In some cases, NO also activates cyclooxygenase. The most important role of NO is thought to be the activation of guanylate cyclase. Recently, published NO research has reported many contradictory results, which are due to NO's unique chemical properties. Since NO is a free radical, it is very reactive and unstable. NO changes its form in a complex manner immediately after appearing in a biological environment. Each of NO's metabolites might have different bioactivities from NO itself. For this reason, it is vital to separately investigate each function of the NO-related metabolites.

Oxidative Stress Detection

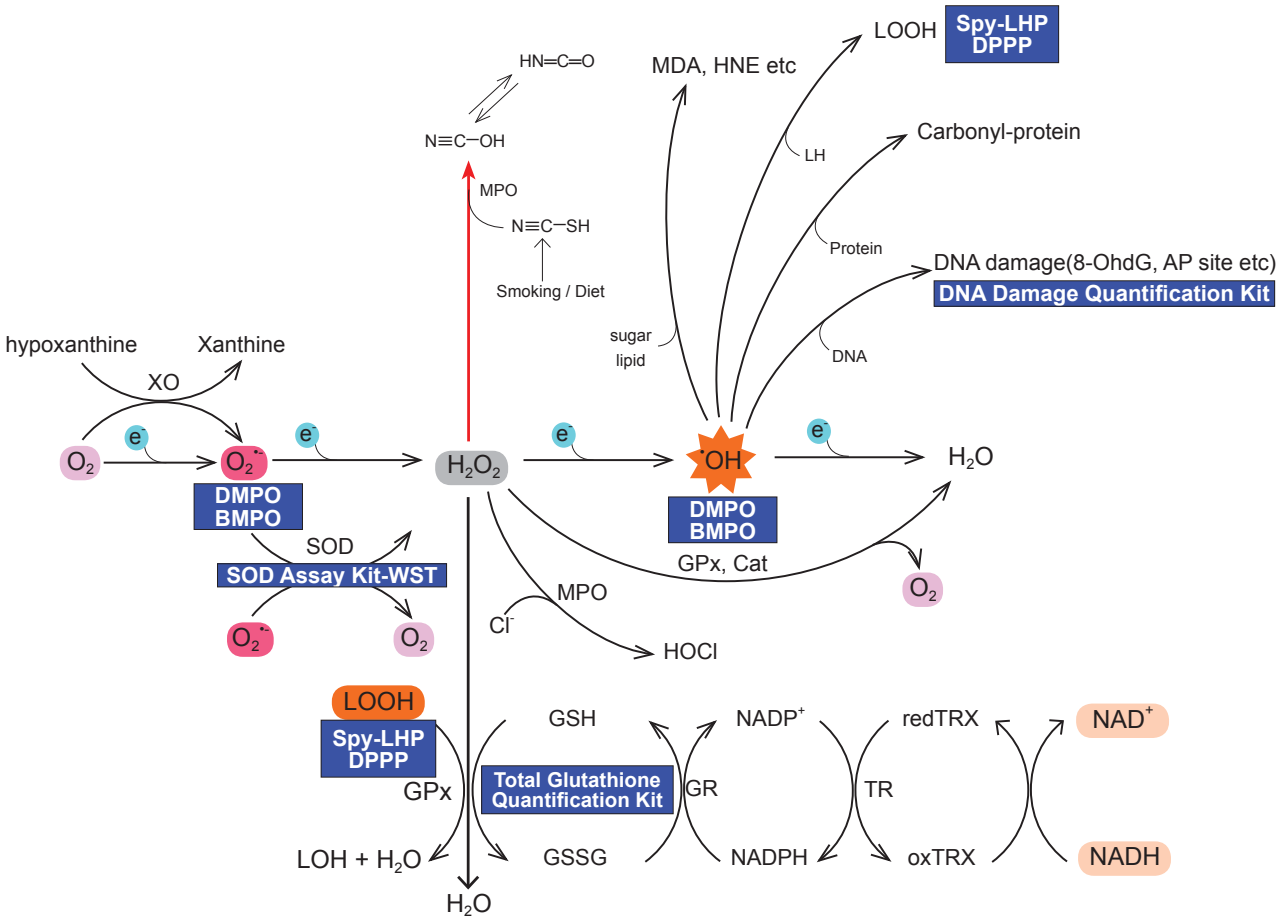


Fig. 1 O_2 and NO Metabolism Chart

DNA Damage Quantification Kit
-AP Site Counting-

Application: Abasic site quantification in genomic DNA
Features:
- Determine the number of abasic sites in genomic DNA samples
- Colorimetric microplate assay
- Detection range: 1-40 abasic sites per 1x10^5 base pairs DNA

Ordering Information

Product code	Unit
DK02-10	5 sample
DK02-12	20 samples

Contents of the Kit: 5 samples

ARP Solution .....100 µl x 1 tube
ARP-DNA standard solution.....250 µl each
Filtration Tube ..... 5 tubes
Washing Buffer ..... 1 packet
96-well Microplate ..... 1 plate

DNA Binding Solution .....10 ml x 1 bottle
Substrate Solution..... 10 ml x 1 bottle
TE Buffer..... 15 ml x 1 bottle
HRP-Streptavidin .....25 µl x 1 tube

Contents of the Kit: 20 samples

ARP Solution ..... 250 µl x 1 tube
ARP-DNA Standard Solution.....250 µl each
Filtration Tube ..... 20 tubes
Washing Buffer ..... 1 packet
96-well Microplate ..... 1 plate

DNA Binding Solution ..... 10 ml x 1 bottle
Substrate Solution.....10 ml x 1 bottle
TE Buffer..... 40 ml x 1 bottles
HRP-Streptavidin ..... 25 µl x 1 tubes

Storage Condition
0-5°C

Shipping Condition
ambient temperature or with blue ice

Required Equipment and Materials
microplate reader with 650 nm filter, 0.5 ml and 1.5 ml tube, 10 µl and 200 µl pipettes, 50-250 µl multi-channel pipette, Centrifuge, Incubator

Product Description
Oxidative damage to DNA is a result of its interaction with reactive oxygen species (ROS), in particular, the hydroxy radical. Hydroxy radicals, which are produced from superoxide anion and hydrogen peroxide by the Fenton reaction, produce multiple modifications in DNA. Oxidative attacks by hydroxy radicals on the deoxyribose moiety will lead to the release of free bases from DNA, generating strand breaks with various sugar modifications and simple abasic sites (AP sites). In fact, AP sites are one of the major types of damage generated by ROS. Aldehyde Reactive Probe (ARP; *N*'-aminooxymethylcarbonylhydrazin-D-biotin) reacts specifically with an aldehyde group present on the open ring form of the AP sites (Fig. 1). This reaction makes it possible to detect DNA modifications that result in the formation of an aldehyde group. After treatment with excess ARP reagent, all of the AP sites on DNA are tagged with a biotin residue. These biotin-tagged AP sites can be quantified using the avidin-biotin assay, followed by colorimetric detection with either peroxidase or alkaline phosphatase conjugated to the avidin. DNA Damage Quantification Kit contains all the necessary solutions for detecting between 1 to 40 AP sites per 1 x 10^5 base pairs.

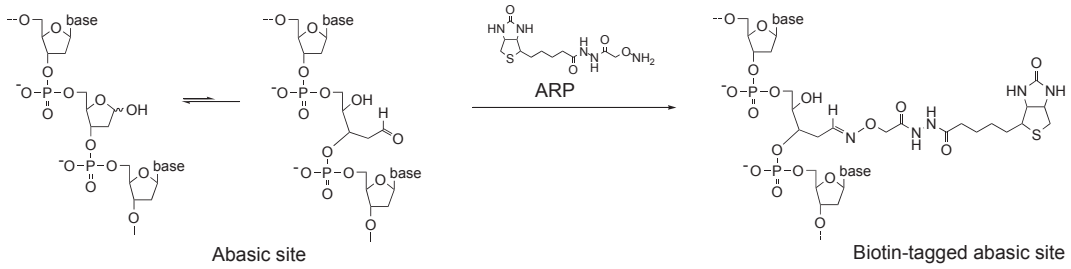


Fig. 1 Mechanism of ARP Tagging at an Abasic Site

DNA Damage Quantification

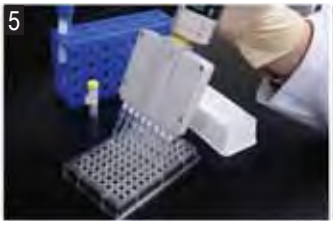
Assay Procedure

Add ARP solution to a sample DNA solution.Incubate at 37°C for 1 hour.

Transfer the ARP reaction mixture to a filtration tube and spin the tube to purify ARP-labeled DNA.

Add the ARP-DNA standard solution or ARP-labeled sample DNA solution to each well. Add DNA binding solution, and leave the plate at room temperature overnight.

Discard the solutions and wash wells with washing buffer. Tap the plate on a paper towel several times to remove as much buffer as possible.



Add HRP-streptavidin solution to each well and incubate at 37°C for 1 hour.

Discard the solutions and wash well with washing buffer. Tap the plate on a paper towel to remove as much buffer as possible.

Add Substrate solution to each well and incubate at 37°C for 1 hour.

Read the O.D. at 650 nm.

ARP Reaction (Preparation of ARP-labeled DNA)

1. Mix 10 µl of purified genomic DNA solution (100 µg per ml) and 10 µl ARP Solution in a 0.5 ml tube, and incubate at 37°C for 1 hour.
2. Wash the inside of the Filtration Tube with 100 µl of TE twice.
3. Add 380 µl TE to the reaction solution, and transfer the solution to a Filtration Tube.
4. Centrifuge the filtration tube at 2,500 g for 15 minutes, and discard the filtrate solution.
5. Add 400 µl TE to the filtration tube using a pipette to re-suspend the DNA on the filter with a pipette.
6. Centrifuge the Filtration Tube at 2,500 g for 15 minutes.[a)]
7. Add 200 µl TE to the Filtration Tube, using a pipette to re-suspend the DNA on the filter with a pipette.
8. Transfer the DNA solution to a 1.5 ml tube, and again add 200 µl of TE to the Filtration Tube to completely transfer the ARP-labeled DNA from the filter to the 1.5 ml tube.[b)]
9. Store the ARP-labeled genomic DNA solution at 0-5°C.

a) If the DNA solution still remains on the filter after centrifuging, centrifuge for another 5 minutes, and then proceed to step 7.

b) The recovery rate of DNA using a filtration tube is 90%, so the approximate concentration of ARP-labeled DNA is 2.25 µg per ml. For a more accurate determination of the number of abasic sites in sample DNA, we recommend measuring the actual DNA concentration.

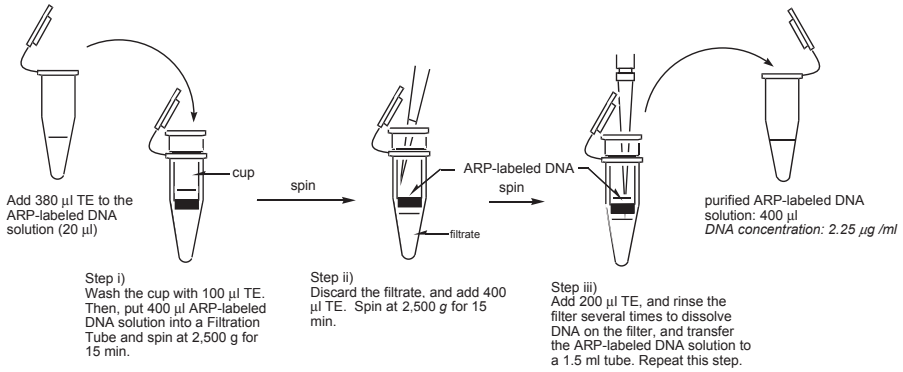


Fig. 2 Isolation Process of ARP-labeled genomic DNA.

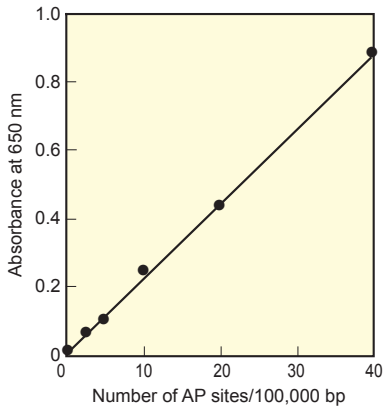
Determination of the Number of Abasic Sites in DNA

Day 1:

1. Dilute 90 µl of the ARP-labeled genomic DNA with 310 µl TE.
2. Add 60 µl of ARP-DNA Standard Solution per well. Use 3 wells per 1 standard solution.
3. Add 60 µl of the diluted ARP-labeled genomic DNA solution per well. Use at least 3 wells per sample.
4. Add 100 µl DNA Binding Solution to each well and mix. Leave the plate at room temperature overnight.

Day 2:

5. Preparation of Solutions
   Washing Buffer : Dissolve the contents of the Washing Buffer packet in 1 L of deionized or distilled water. Store this Washing Buffer at room temperature.
   HRP-Streptavidin solution: Dilute HRP-Streptavidin with Washing Buffer to prepare 1/4000 diluted working solution.
6. Discard the DNA Binding Solution from all the wells and wash the wells five times with 250 µl of Washing Buffer.
   After discarding the Washing Buffer, invert the plate and tap it on a paper towel several times to completely remove the solution.
7. Add 150 µl of diluted HRP-Streptavidin working solution to each well and incubate the plate at 37° C for 1 hour.
8. Discard the solution in all wells, and wash the well five times with 250 µl of Washing Buffer.
9. Add 100 µl Substrate solution to each well and incubate at 37° C for 1 hour.
10. Measure the O.D. at 650 nm, and prepare a calibration curve using the data obtained from the ARP-DNA Standard Solution wells.
11. Determine the number of abasic sites in the genomic DNA using the calibration curve.



How to Prepare a Calibration Curve

1. Calculate the average O.D. of each ARP-DNA standard solution.
2. Subtract the blank O.D. from the average O.D.[a)]
3. Plot the O.D. corresponding to the number of AP sites of the standard solution. X-axis is the number of AP sites and Y-axis is the O.D.
4. Determine the number of AP sites in the sample using this calibration curve.

a) The blank O.D. is about 0.04-0.06 and the O.D. of the 40 ARP DNA standard solution is about 0.8-1.0. The O.D. value depends on HRP-Streptavidin activity.

Fig. 3 Typical calibration curve of DNA Damage Quantification Kit

FAQ

♦Can I use single-stranded DNA or RNA?
No, you cannot use this kit to determine the number of abasic sites in single-stranded DNA or RNA. The O.D. reading of single-stranded DNA will be nearly twice that of double-stranded DNA because of the binding efficiency on the microplate.

♦How should genomic DNA be stored?
Prepare a DNA pellet and store at -20°C or -80°C if the DNA cannot be labeled with ARP immediately after isolation. After ARP labeling, the sample can be stored at 4°C in TE Buffer for several months.

♦How should I prepare the DNA?
You can use general protocols or commercially available DNA isolation kits. Between 2 to 4 abasic sites per 1 x 10^5 base pairs will be created during the DNA isolation process. Therefore, use the same isolation method to prepare each DNA sample.

♦How can I determine the number of abasic sites if there are more than 40 per 1 x 10^5 base pairs?
Simply dilute the ARP-labeled sample DNA with 0.5 µg per ml double-stranded genomic DNA, such as calf thymus or salmon sperm DNA, using TE Buffer.

♦What should I do if the sample DNA concentration is less than 100 µg per ml?
You can either use a filtration tube to concentrate your sample DNA or ethanol precipitation to recover DNA as a pellet and then re-dissolve it to prepare a 100 µg per ml solution.

♦What should I do if the sample DNA is less than 1 µg?
Add the same volume of ARP Solution and follow the manual. The recovery of the ARP-labeled DNA may be lower than the usual reactions, so measure the ARP-labeled DNA solution. The average recovery rate of the 0.5 µg DNA and 0.25 µg DNA is 70% and 50%, respectively.

References

1. T. Lindahl, *et al*., Rate of Depurination of Native Deoxyribonucleic Acid. *Biochemistry*. 1972;**11**:3610-3618.
2. M. Liuzzi, *et al*., A New Approach to the Study of the Base-excision Repair Pathway Using Methoxyamine. *J Biol Chem*. 1985;**260**:5252-5258.
3. A. Sancar, *et al*., DNA Repair Enzymes. *Annu Rev Biochem*. 1988;**57**:29-67.
4. M. Weinfeld, *et al*., Response of Phage T4 Polynucleotide Kinase Toward Dinucleotides Containing Apurinic Sites: Design of a 32P-postlabeling Assay for Apurinic Sites in DNA. *Biochemistry*. 1990;**29**:1737-1743.

5. B. X. Chen, *et al*., Properties of a Monoclonal Antibody for the Detection of Abasic Sites, a Common DNA Lesion. *Mutat Res*. 1992;**273**:253-261.
6. J. A. Gralnick, *et al*., The YggX Protein of Salmonella enterica Is Involoved in Fe(II) Trafficking and Minimizes the DNA Damage Cause by Hydroxyl Radicals:Residue CYS-7 is Essential for YggX Function. *J Biol Chem*. 2003;**278**:20708-20715.
7. J. W. Pippin, *et al*., DNA Damage is a Novel Response to Sublytic Complement C5b-9 Induced Injury in Podocytes. *J Clin Invest*. 2003;**111**:877-885.
8. S. Watanabe, *et al*., Methylated DNA-binding Domain 1 and Methylpurine DNA Glycosylase Link Transcriptional Repression and DNA Repair in Chromatin. *PNAS*. 2003;**100**:12859-12864.
9. M. Endres, *et al*., Folate Deficiency Increases Postischemic Brain Injury. *Stroke*. 2005;**36**:321-325.
10. J. Li, *et al*., Angiotensin II-Induced Neural Differentiation via Angiotensin II Type 2 (AT_2) Receptor-MMS2 Cascade Involving Interaction between AT_2 Receptor-Interacting Protein and Src Homology 2 Domain-Containing Protein-Tyrosine Phosphatase 1. *Mol Endocrinol*. 2007;**21**:499-511.
11. D. R. McNeill, *et al*., A Dominant-Negative Form of the Major Human Abasic Endonuclease Enhances Cellular Sensitivity to Laboratory and Clinical DNA-Damaging Agents. *Mol Cancer Res*. 2007;**5**:61-70.
12. C. A. Downs, *et al*., Cellular pathology and histopathology of hypo-salinity exposure on the coral Stylophora pistillata. *Sci Total Environ*. 2009;**407**:4838-4851.
13. C. A. Downs, *et al*., Symbiophagy as a cellular mechanism for coral bleaching. *Autophagy*. 2009;**5**:211-216.

SOD Assay Kit-WST

Application: SOD or SOD-like activity detection
Features: WST-1 based SOD inhibition assay
Colorimetric microplate measurement
Measures 100% inhibition by SOD
pH-independent IC_{50} determination
Low background noise measurement

Ordering Information

Product code	Unit *
S311-10	500 tests

*One test corresponds to one well of the 96 well plate.

Contents of the Kit: 500 tests

WST solution ..... 5 ml, 1 bottle
Buffer solution..... 100 ml, 1 bottle
Enzyme solution .....100 µl, 1 vial
Dilution buffer.....50 ml,1 bottle

Storage Condition

0-5°C

Shipping Condition

ambient temperature or with blue ice

Required Equipment and Materials

Microplate reader with 450 nm filter, 37°C incubator, 96-well clear plate, 10 µl and 200 µl adjustable pipettes, multi-channel pipette

Product Description

Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. In mammals, cytosolic SOD has a greenish color and consists of two subunits, one containing copper and the other zinc (Cu/Zn-SOD). Mitochondrial and bacterial SOD has a reddish-purple color and contains manganese (Mn-SOD). *E. coli* has Mn-SOD and SOD containing iron (Fe-SOD). Several direct and indirect methods have been developed to determine SOD activity. An indirect method using nitrotetrazolium blue is often used because of its convenience. However, there are several disadvantages to this method, such as poor water solubility of the formazan dye and its reaction with the reduced form of xanthine oxidase. Although cytochrome C is also commonly used for SOD activity detection, its reactivity with superoxide is too high to determine low levels of SOD activity.

SOD Assay Kit-WST allows a very convenient and highly sensitive SOD assay by utilizing Dojindo's highly water-soluble tetrazolium salt, WST-1 (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo-phenyl)-2*H*-tetrazolium, monosodium salt), which produces a water-soluble formazan dye upon reduction with a superoxide anion (Fig. 1). The absorption spectrum is shown in Fig. 2. WST-1 is 70 times less reactive with superoxide anion than cytochrome C; therefore, highly sensitive SOD detection is possible and samples can be diluted with buffer to minimize background problems. WST-1 does not react with the reduced form of xanthine oxidase; therefore, even 100% inhibition with SOD is detectable. The rate of WST-1 reduction by superoxide anion is linearly related to the xanthine oxidase activity and is inhibited by SOD (see figure below). Therefore, the IC_{50} (50% inhibition concentration) of SOD or SOD-like materials can be determined using colorimetric methods.

SOD Activity Detection

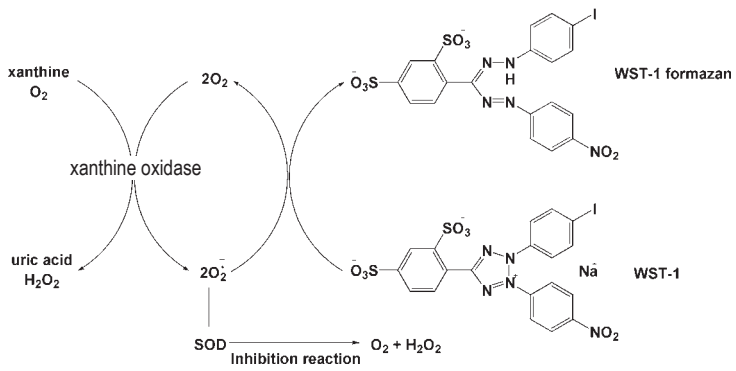


Fig. 1 SOD Inhibition assay mechanism

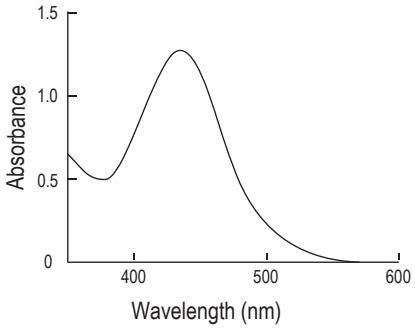


Fig. 2 Absorption spectrum of WST-1 formazan

Preparation of Working Solutions

WST working solution:

Dilute 1 ml WST Solution with 19 ml buffer solution.

Enzyme working solution:

Dilute 15 µl Enzyme Solution with 2.5 ml dilution solution.

SOD solution (if necessary):

Sample Solution

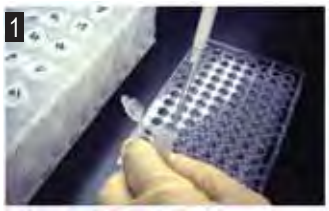
Dilute Sample Solution with Dilution Buffer or saline to prepare Sample solution as follows.

Dilution ratio : 1, 1/5, $1/5^2$, $1/5^3$, $1/5^4$, $1/5^5$, $1/5^6$

Table 1 Solution and Buffer Volume in Each Well

Solution	Sample	Blank 1	Blank 2	Blank 3
sample solution	20 µl	-	20 µl	-
ddH_2O	-	20 µl	-	20 µl
WST working sol	200 µl	200 µl	200 µl	200 µl
Enzyme working sol	20 µl	20 µl	-	-
Dilution buffer	-	-	20 µl	20 µl

Assay Procedure



Add 20 µl Sample solution or H_2O to each well

Add WST working solution, dilution buffer, and Enzyme working solution to each well as indicated in Table 1.[a)]

Incubate the plate at 37°C for 20 minutes.[b)]

Read O.D. at 450 nm.

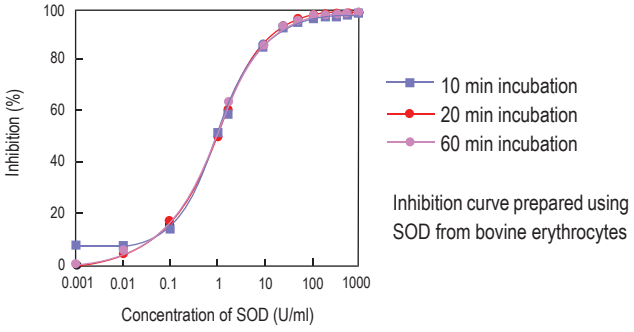
[a)] After the addition of enzyme working solution, the mixed solution generates superoxide. Use a multi-channel pipette to add the enzyme working solution to minimize the reaction time lag.

[b)] If the microplate reader has a temperature control function, incubate the plate on the microplate holder at 37°C.

Inhibition Rate Calculation

$$\text{Inhibition rate (\%)} = \frac{(A_{blank\,1} - A_{blank\,3}) - (A_{sample} - A_{blank\,2})}{A_{blank\,1} - A_{blank\,3}} \times 100$$

$A_{blank\,1}$: absorbance of blank 1
$A_{blank\,2}$: absorbance of blank 2
$A_{blank\,3}$: absorbance of blank 3
A_{sample}: absorbance of sample well



Inhibition curve prepared using SOD from bovine erythrocytes

Fig. 3 Inhibition curve prepared by different data acquisition times.

SOD Activity Detection

Difinition of SOD activity

One unit of SOD is defined as the amount of the enzyme in 20 µl of sample solution that inhibits the reduction reaction of WST-1 with superoxide anion by 50%.

Determination of SOD activity

1) Read the dilution ratio at 50% inhibition (IC_{50}) from inhibition curve.
2) Multiply the dilution ratio at IC_{50} and at the sample preparation to obtain the SOD activity.

Determination of Mn-SOD activity

Mn-SOD activity can be measured by adding potassium cyanide (final concentration: 1 mmol/l) or diethyldithiocarbomate (final concentration: 1 mmol/l) to the sample solution. These reagents inactive Cu, Zn-SOD and extracellular-SOD activities.

Preparation of Sample Solution

Cells (Adherent cells: $9x10^6$ cells, Leukocytes: $1.2 x10^7$ cells)

1. Harvest cells with a scraper, centrifuge at 2,000 *g* for 10 minutes at 4°C, and discard the supernatant.
2. Wash the cells with 1 ml PBS and centrifuge at 2,000 *g* for 10 minutes at 4°C. Discard the supernatant. Repeat this step.
3. Break cells using the freeze-thaw method (-20°C for 20 minutes, then 37°C bath for 10 minutes, repeat twice).
4. Add 1 ml PBS. If necessary, sonicate the cell lysate on an ice bath (60 W with 0.5 second interval for 15 minutes).
5. Centrifuge at 10,000 *g* for 15 minutes at 4°C.
6. Remove the supernatant and dilute it with PBS to prepare sample solution.

Plant or Vegetable (200 mg)

1. Add 1 ml distilled water and homogenize the sample using a homogenizer with beads.
2. Filter the homogenate with paper filter and lyophilize the filtrate.
3. Measure the weight of the lyophilized sample and dissolve with 0.1 M phosphate buffer (pH 7.4) to prepare sample solution.

Tissue (100 mg)

1. Wash the tissue with saline to remove as much blood as possible. Blot the tissue with paper towels and then measure its weight.
2. Add 400-900 µl sucrose buffer (0.25 M sucrose, 10 mM Tris, 1 mM EDTA, pH 7.4) and homogenize the sample using Teflon homogenizer. If necessary, sonicate the homogenized sample on an ice bath (60W with 0.5 second intervals for 15 minutes).
3. Centrifuge the homogenized sample at 10,000 g for 15 minutes at 4°C, and transfer the supernatant to a new tube.
4. Dilute the supernatant with distilled water to prepare sample solution.

Tea (antioxidant activity detection)

1. Add 60 ml boiled water to 10 g of tea, and leave it for 2.5 minutes.
2. Filter the extract with paper filter and then filter again with a 0.45 µm membrane filter.
3. Dilute the filtrate with distilled water to prepare sample solution.

Erythrocytes or Plasma

1. Centrifuge 2-3 ml of anticoagulant-treated blood (such as heparin 10 U/ml final concentration) at 600 g for 10 minutes at 4°C.
2. Remove the supernatant and dilute it with saline to use as a plasma sample. Add saline to the pellet to prepare the same volume and suspend the pellet.
3. Centrifuge the pellet suspension at 600 g for 10 minutes at 4°C, and discard the supernatant.
4. Add the same volume of saline and repeat Step 3 twice.
5. Suspend the pellet with 4 ml distilled water, then add 1 ml ethanol and 0.6 ml chloroform.
6. Shake the mixture vigorously with a shaker for 15 minutes at 4°C.
7. Centrifuge the mixture at 600 g for 10 minutes at 4° C and transfer the upper water-ethanol phase to a new tube.
8. Mix 0.1 ml of the upper phase with 0.7 ml distilled water and dilute with 0.25% ethanol to prepare sample solution.

Extracellular SOD (EC-SOD)

1. Prepare a 0.5 ml volume of Con A-sepharose column equilibrated with PBS.
2. Apply supernatant of a tissue homogenate on the column, and leave the column for 5 minutes at room temperature.
3. Add total 10 ml PBS to wash the column.
4. Add 1 ml of 0.5 M α-methylmannoside/PBS and collect the eluate. Repeat 5 times.
5. Use the eluate for the SOD assay without dilution. If the SOD activity is high enough, dilute the eluate with PBS.

Wine (antioxidant activity detection)

1. Filter wine with a 0.45 µm membrane filter.
2. Dilute the filtrate with distilled water to prepare sample solution.

FAQ

♦Can I use standard SOD to determine SOD activity in sample solutions?

Yes, you can. Prepare an inhibition curve (typical inhibition Curve), and determine SOD activity in the sample solution. SOD bovine erythrocytes (CAS# 9054-89-1, EC 1.15.1.1) can be purchased from Sigma (catalog# S7571).

♦Can I use a kinetic method to determine SOD activity?

Yes, you can use a kinetic method for SOD assay. Since the rate of the color development remains the same for up to 20 minutes, measure the slope for 5 minutes during this linear phase.

♦The sample has color. Can I still use this sample?

Yes, you can still use it for SOD assay. Diluting the sample will minimize the interference. Subtract the O.D. of blank 2 from the O.D. of the sample to cancel out the background color. However, if the SOD activity in the sample is low, it may not be measurable.

♦How do I prepare more Dilution buffer?

Dilution buffer is PBS. Please prepare the Dilution buffer with the following concentrations: 137 mM NaCl, 2.7 mM KCl, 1.47 mM KH_2PO_4, 8.1 mM Na_2HPO_4, pH 7.4.

♦How long can I store the sample?

A sample stored in a freezer at -80°C is stable for 1 month.

♦Can I measure the levels of superoxide anion using this kit?

You could simply use WST-1, instead of this kit, to measure superoxide. However, you would need a standard to determine the amount of superoxide in sample solution. Since superoxide is not stable and reacts with various materials, it might be difficult to determine the total amount of superoxide generated in the system. The xanthine-xanthine oxidase system in this kit can be used as a standard for measuring the relative amount of superoxide production in each sample.

References

1. J. M. McCord, *et al*., An Enzymic Function for Erythrocuprein(hemocuprein). *J Biol Chem*. 1969;**244**:6049-6055.
2. B. L. Geller, *et al*., A Method for Distinguishing Cu,Zn- and Mn-Containing Superoxide Dismutases. *Anal Biochem*. 1983;**128**:86-92.
3. S. Goldstein, *et al*., Comparison Between Different Assays for Superoxide Dismutase-like Activity. *Free Rad Res Commun*. 1991;**12**:5-10.
4. R. H. Burdon, *et al*., Reduction of a Tetrazolium Salt and Superoxide Generation in Human Tumor Cells (HeLa). *Free Rad Res Commun*. 1993;**18**:369-380.
5. M. W. Sutherland, *et al*., The Tetrazolium Dyes MTS and XTT Provide New Quantitative Assays for Superoxide and Superoxide Dismutase. *Free Radic Res*. 1997;**27**:283-289.
6. H. Ukeda, *et al*., Flow-Injection Assay of Superoxide Dismutase Based on the Reduction of Highly Water-Soluble Tetrazolium. *Anal Sci*. 1999;**15**:353-357.
7. H. Ukeda, *et al*., Spectrophotometric Assay for Superoxide Dismutase Based on the Reduction of Highly Water-soluble Tetrazolium Salts by Xanthine-Xanthine Oxidase. *Biosci Biotechnol Biochem*. 1999;**63**:485-488.
8. H.Ukeda, *et al*., Spectrophotometric Assay of Superoxide Anion Formed in Maillard Reaction Based on Highly Water-soluble Tetrazolium Salt. *Anal Sci*. 2002;**18**:1151-1154.
9. N. Tsuji, *et al*., Enhancement of Tolerance to Heavy Metals and Oxidative Stress in *Dunaliella Tertiolecta* by Zn-induced Phytochelatin Synthesis. *Biochem Biophys Res Commun*. 2002;**293**:653-659.
10. A. Sakudo, *et al*., Impairment of Superoxide Dismutase Activation by N-Terminally Truncated Prion Protein (PrP) in PrP-deficient Neuronal Cell Line. *Biochem Biophys Res Commun*. 2003;**308**:660-667.

Total Glutathione Quantification Kit

Application: Total glutathione detection

Features: Colorimetric microplate measurement
Highly sensitive DTNB-based recycling system
Wide detection range of 1 µg to 100 µg

Ordering Information

Product code	Unit
T419-10	100 tests

Contents of the Kit:

Enzyme solution ..... 50 µl, 1 vial
Substrate..... 2 vials
Buffer solution ..... 50 ml, 1 bottle
Co-enzyme (lyophilized) .....2 vials
Standard GSH (lyophilized) ..... 1 vial

Storage Condition	Shipping Condition
0-5°C	ambient temperature or with blue ice

Required Equipment and Materials

Microplate reader with 405 or 415 nm filter, 37°C incubator, 96-well clear plate, 20 µl and 200 µl adjustable pipettes, multi-channel pipette

Glutathione Quantification

Product Description

Glutathione (GSH) is the most abundant thiol compound in animal tissues, plant tissues, bacteria, and yeast. GSH has many different roles, including protection against reactive oxygen species and the maintenance of protein thiol groups. During these processes, GSH is converted into its oxidized form, glutathione disulfide (GSSG). Since GSSG is then enzymatically reduced by glutathione reductase, GSH is the dominant form in organisms. DTNB (5,5'-Dithiobis(2-nitrobenzoic acid)), known as Ellman's Reagent, was developed to detect thiol compounds. In 1985, Dr. M. E. Anderson suggested that the glutathione recycling system involving DTNB and glutathione reductase could be used as a highly sensitive glutathione detection method. DTNB and GSH react to generate 2-nitro-5-thiobenzoic acid and GSSG (Fig. 1). Since 2-nitro-5-thiobenzoic acid is yellow, the GSH concentration in a sample solution can be determined by O.D. measurement at 412 nm absorbance (Fig. 2). GSH is regenerated from GSSG by glutathione reductase and will again react with DTNB to produce 2-nitro-5-thiobenzoic acid. This recycling reaction improves the sensitivity of total glutathione detection. Total Glutathione Quantification Kit contains all of the necessary reagents for total glutathione measurement, except for those used in sample preparation. 5-Sulfosalicylic acid is recommended for the removal of proteins from sample solutions and for the prevention of GSH oxidation and γ-glutamyl transpeptidase reactions. However, the optimum method for sample preparation differs from sample to sample, so please review the references. This kit can be used to quantify total glutathione concentrations from 1 µM to 100 µM using the standard method. For lower glutathione concentrations, such as in blood samples, longer incubation times are required.

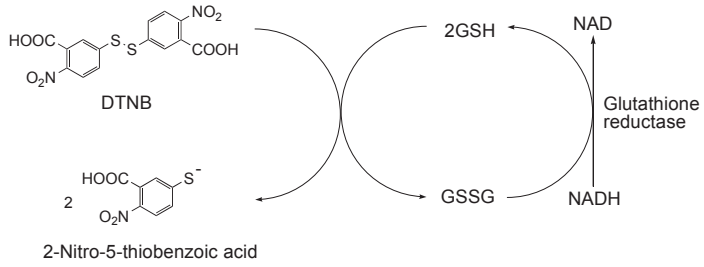


Fig. 1 Mechanism of total glutathione quantification

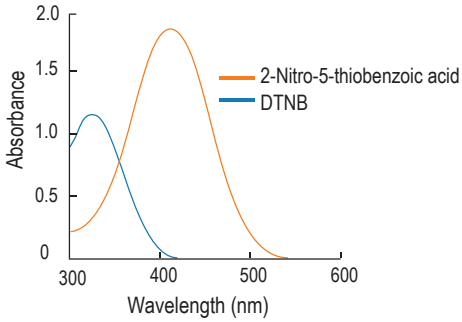
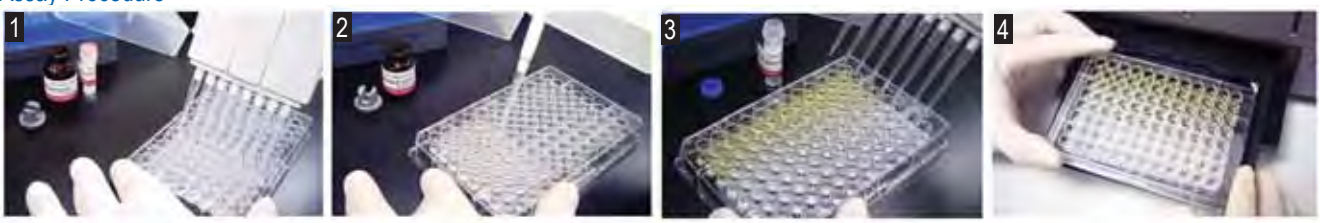


Fig. 2 Absorption spectrum of 2-Nitro-5-thiobenzoic acid

Assay Procedure



1. Add enzyme and co-enzyme solutions to each well.
2. Add GSH standard solution or sample solution to each well and incubate at 37°C for 10 minutes.
3. Add substrate solution to each well and incubate at 37°C for 10 minutes.
4. Read the O.D. at 405 or 415 nm.

Preparation of 5% 5-Sulfosalicylic Acid (SSA) Solution

Note: SSA is not included in this kit.

1. Dissolve 1 g SSA in 19 ml water.
2. Store the solution at 4°C (stable for 6 months at 4°C).

Preparation of Sample Solution

Cells (Adhesive cells: $5x10^5$ cells; Leukocyte cells: $1x10^6$ cells)

1. Collect cells by centrifugation at 200 g for 10 minutes at 4°C. Discard the supernatant.
2. Wash the cells with 300 µl PBS and centrifuge at 200 *g* for 10 minutes at 4°C. Discard the supernatant.
3. Add 80 µl 10 mM HCl, and lyse the cells by freezing and thawing twice.
4. Add 20 µl 5% SSA and centrifuge at 8,000 *g* for 10 minutes.
5. Transfer the supernatant to a new tube, and use it for the assay. If the final concentration of SSA is over 1%, add ddH_2O to reduce the concentration of SSA from 0.5 to 1%.

Tissue (100 mg)

1. Homogenize the tissue in 0.5-1.0 ml 5% SSA.
2. Centrifuge the homogenized tissue sample at 8,000 *g* for 10 minutes.
3. Transfer the supernatant to a new tube and add ddH_2O to reduce the concentration of SSA from 0.5 to 1%. Use it for the assay.

Glutathione Quantification

Plasma
1. Centrifuge anticoagulant-treated blood at 1,000 *g* for 10 minutes at 4°C.
2. Transfer the top plasma layer to a new tube and add 5% SSA equivalent to half of the volume of the plasma.
3. Centrifuge at 8,000 *g* for 10 minutes at 4°C
4. Transfer the supernatant to a new tube, and add ddH_2O to reduce the concentration of SSA from 0.5 to 1%. Use it for the assay.

Erythrocytes
1. Centrifuge anticoagulant-treated blood at 1,000 *g* for 10 minutes at 4°C.
2. Discard the supernatant and the white buffy layer.
3. Lyse the erythrocytes with 5% SSA equivalent to 4 times the volume of the erythrocytes.
4. Centrifuge at 8,000 *g* for 10 minutes at 4°C.
5. Transfer the supernatant to a new tube, and add ddH_2O to reduce the concentration of SSA from 0.5 to 1%. Use it for the assay. Erythrocytes can be isolated from the remaining sample solution after the plasma sample isolation.

Preparation of Assay Solutions
Substrate Working Solution
Add 1.2 ml buffer solution to 1 vial of substrate and dissolve. Substrate Working Solution is stable for 2 months at -20°C.

Enzyme Working Solution
Mix enzyme solution using a pipette before using. Remove 20 µl enzyme solution and mix it with 4 ml buffer solution. Enzyme Working Solution is stable for 2 months at 4°C.

Coenzyme Working Solution
Add 1.2 ml ddH_2O to the Coenzyme vial and dissolve. The coenzyme vial is under vacuum pressure; carefully open the cap or use a syringe to add buffer solution. The Coenzyme Working Solution prepared with ddH2O is stable for 2 months at -20°C.

GSH Standard Solutions
To prepare 200 µM GSH Standard Solution, add 2 ml of 0.5-1% SSA to the standard GSH vial and dissolve. Dilute 100 µl of the 200 µM GSH Standard Solution with 100 µl of 0.5% SSA. Repeat using serial dilution to prepare the following GSH Standard Solutions:
100 µM, 50 µM, 25 µM, 12.5 µM, 6.25 µM, 3.13 µM, 1.56 µM and 0.
The Standard GSH vial is under vacuum pressure; carefully open the cap or use a syringe to add SSA. GSH powder is difficult to see. The GSH Standard Solutions are stable for 2 months at -20 °C.

Total Glutathione Detection - Standard Method
Detection Range: 5-100 µM
1. To each well, add 20 µl of Coenzyme Working Solution, 120 µl of Buffer Solution, and 20 µl of Enzyme Working Solution.
2. Incubate the plate at 37°C for 5 minutes.
3. Add 20 µl of either one of the GSH Standard Solutions or the sample solutions a), and incubate the plate at 37°C for 10 minutes.
4. Add 20 µl of Substrate Working Solution, and incubate the plate at room temperature for 10 min.
5. Read the absorbance at 405 nm or 415 nm using a microplate reader.
6. Determine the concentration of GSH in the sample solution using a calibration curve.b)

[a] Adjust the concentration of SSA in the sample solution to 0.5-1% with ddH_2O before the assay. High concentrations of SSA (>1 %) interfere with the assay.

[b] Since the colorimetric reaction is stable and the O.D. increases linearly over 30 minutes, GSH concentration can be determined by kinetic or pseudo-endpoint (no stopping reaction, quick measurement of O.D. at certain time periods between 5 and 10 min.) method.

Total Glutathione Detection - High Sensitivity Method
Detection Range: 0.5-25 µM
1. To each well, add 20 µl of Enzyme Working Solution, 140 µl of Coenzyme working solution, and 20 µl of either one of the GSH Standard Solutions[a] or the sample solution[b].
2. Incubate the plate at 30°C for 10 minutes.
3. Add 20 µl of Substrate Working Solution, and incubate the plate at 37°C for 20-40 min.
4. Read the absorbance at 405 nm or 415 nm using a microplate reader.
5. Determine the concentration of GSH in the sample solution using a calibration curve.

[a] Prepare 50 mM GSH standard solution, and then prepare different concentrations of GSH Standard Solutions by serial dilution with 0.5% SSA as follows: 25 µM, 12.5 µM, 6.25 µM, 3.13 µM, 1.56 µM, 0.78 µM, 0.39 µM and 0.

[b] Adjust the concentration of SSA in the sample solution to 0.5-1% with ddH2O before the assay. Higher concentrations of SSA (>1%) interfere with the assay.

Glutathione Quantification

Calculation of Total Glutathione (GSH and GSSG) Concentration

Determine the total glutathione concentration in the sample solution using the following equations. Since the values obtained by these equations are the amount fo total glutathione in treated sample solutions, further calculations are necessary if the actual concentration of glutathione in cells or tissues need to be determined.

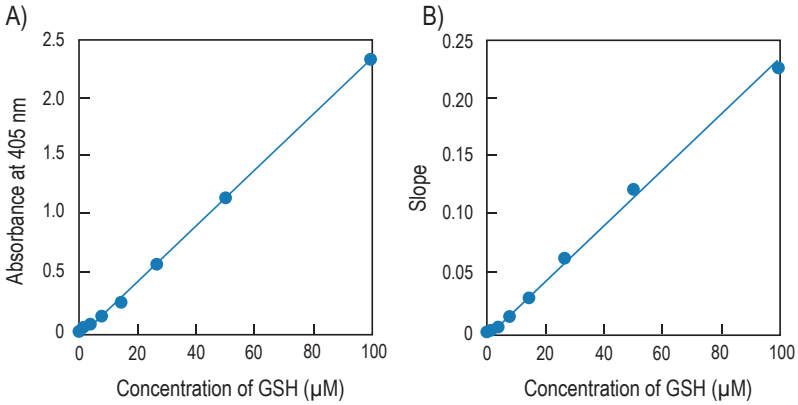


Fig. 3 Calibration curves prepared using pseudo-endpoint method and kinetic method
A) Calibration curve prepared using pseudo-endpoint method. 10 minute incubation at room temperature.
B) Calibration curve prepared using kinetic method.

Pseudo-endpoint method: Total glutathione = $(O.D._{sample} - O.D._{blank}) / slope$ [a)]

Kinetic method: Total glutathione = $(Slope_{sample}$ [b)] $- Slope_{blank}$ [b)]$) / slope$ [b)]

[a)] *The slope of the calibration curve prepared by the pseudo-endpoint or kinetic method.*
[b)] *The slope of the kinetic reaction.*

FAQ

♦Do I have to dilute the sample solution prior to the assay?

If you do not know the total glutathione level of your sample, multiple dilutions may be necessary. If the total glutathione level of your sample is less than 100 μM, no dilution is necessary.

♦What interferes with the assay?

Reducing agents (such as ascorbic acid, β-mercaptoethanol, dithiothreitol, and cysteine) and thiol reactive compounds (such as maleimides) interfere with the glutathione assay. Therefore, they should be avoided during sample preparation.

References

1. G. L. Ellman, Tissue sulfhydryl groups. *Arch Biochem Biophys*. 1959;**82**:70-77.
2. O. W. Griffith, Determination of Glutathione and Glutathione Disulfide Using Glutathione Reductase and 2-Vinylpyridine. *Anal Biochem*. 1980;**106**:207-212.
3. M. E. Anderson, Determination of Glutathione and Glutathione Disulfide in Biological Samples. *Methods Enzymol*. 1985;**113**:548-555.
4. M. A. Baker, *et al*., Microtiter Plate Assay for the Measurement of Glutathione and Glutathione Disulfide in Large Numbers of Biological Samples. *Anal Biochem*. 1990;**190**:360-365.
5. C. Vandeputte, *et al*., A Micrototer Plate Assay for Total Glutathione and Glutathione Disulfide Contents in Cultured/isolated Cells: Performance Study of a New Miniaturized Protocol. *Cell Biol Toxicol*. 1994;**10**:415-421.
6. S. A. McGrath-Morrow, *et al*., Inhibition of Glutamine Synthetase in A549 Cells During Hyperoxia. *Am J Respir Cell Mol Biol*. 2002;**27**:99-106.
7. T. Sato, *et al*., Senescence Marker Protein-30 Protects Mice Lungs from Oxidative Stress, Aging, and Smoking. *Am J Respir Crit Care Med*. 2006;**174**:530-537.
8. M. L. Mulhern, *et al*., The Unfolded Protein Response in Lens Epithelial Cells from Galactosemic Rat Lenses. *Invest Ophthalmol Vis Sci*. 2006;**47**:3951-3959.

DTNB 5,5'-Dithiobis(2-nitrobenzoic acid) [CAS: 69-78-3]

Application: SH detection, colorimetric
Appearance: Pale yellow crystalline powder
Molar absorptivity: ≥12,000 (around 305 nm)
MW: 396.35, $C_{14}H_8N_2O_8S_2$

Ordering Information

Product code	Unit
D029-10	1 g

Storage Condition: ambient temperature
Shipping Condition: ambient temperature

Product Description

DTNB is known as Ellman's reagent. It is used for the colorimetric determination of thiol groups in biological samples. It is fairly soluble in the water. Colorless DTNB is converted to yellow 5-Mercapto-2-nitrobenzoic acid in the presence of thiol compounds (Fig. 1). As 5-Mercapto-2-nitrobenzoic acid has an absorption maximum at 412 nm, the absorption spectrum of DTNB does not interfere with thiol detection (Fig. 2).

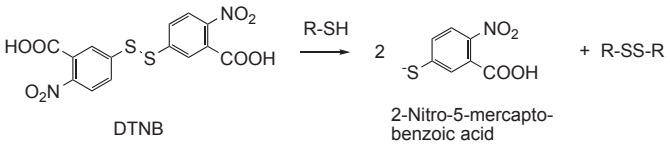


Fig. 1 DTNB reaction with thiol compound

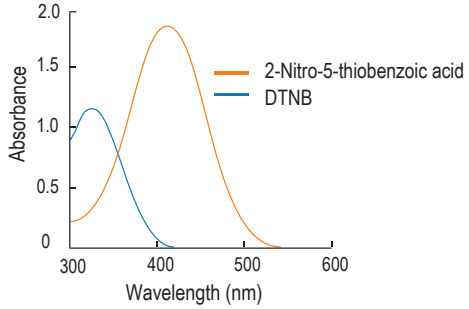


Fig. 2 Absorption spectra of DTNB and reduced DTNB

References

1. G. L. Ellman, Tissue sulfhydryl groups. *Arch Biochem Biophys*. 1959;**82**:70-77.
2. D. R. Jenke, *et al*., Determination of Cysteine in Pharmaceuticals via Liquid Chromatography with Postcolumn Derivatization. *Anal Chem*. 1987;**59**:1509-1512.

2-PDS 2,2'-Dithiodipyridine [CAS: 2127-03-9]

Application: SH detection, UV absorption
Appearance: White or pale yellow crystalline powder
Molar absorptivity: ≥14,000 (around 235 nm)
MW: 220.32, $C_{10}H_8N_2S_2$

Ordering Information

Product code	Unit
P016-10	1 g

Storage Condition: ambient temperature
Shipping Condition: ambient temperature

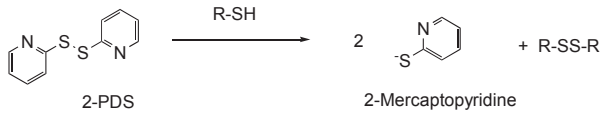


Fig. 1 2-PDS reaction with thiol compound

4-PDS 4,4'-Dithiodipyridine [CAS: 2645-22-9]

Application: SH detection, UV absorption
Appearance: White or pale yellow crystalline powder
Molar absorptivity: ≥15,000 (around 246 nm)
MW: 220.32, $C_{10}H_8N_2S_2$

Ordering Information

Product code	Unit
P017-10	1 g

Lipid Peroxide

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

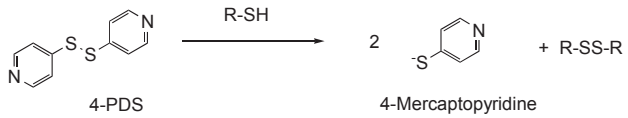


Fig. 2 4-PDS reaction with thiol compound

Product Description
2-PDS and 4-PDS are used for the photometric determination of thiol groups in biological samples. These compounds react with the thiol groups at pH 5 to form mercaptopyridine (Fig. 1 and 2). The maximum wavelengths of 2-Mercaptopyridine and 4-Mercaptopyridine are 343 nm and 324 nm, respectively.

References

1. D. R. Grassetti, *et al*., Determination of Sulfhydryl Groups with 2, 2'- or 4, 4'-Dithiodipyridine. *Arch Biochem Biophys*. 1967;**119**:41-49.
2. R. E. Humphrey, *et al*., Spectrophotometric Determination of Sulfite with 4, 4'-Dithiodipyridine and 5, 5'-Dithiobis-(2-nitrobenzoic acid). *Anal Chem*. 1970;**42**:698-702.
3. R. E. Humphrey, *et al*., Spectrophotometric Determination of Cyanide with Organic Disulphides. *Talanta*. 1971;**18**:491-497.
4. T. Uete, *et al*., Spectrophotometric Micromethod for Measuring Cholinesterase Activity in Serum or Plasma. *Clin Chem*. 1972;**18**:454-458.

DPPP Diphenyl-1-pyrenylphosphine [CAS: 110954-36-4]

Application: Phospholipid peroxide detection
fluorescent HPLC analysis
Appearance: Pale yellow powder
Purity: ≥97.0% (HPLC)
MW: 386.42, $C_{28}H_{19}P$

Ordering Information

Product code	Unit
D350-10	10 mg

Storage Condition
ambient temperature, protect from light

Shipping Condition
ambient temperature

Product Description
DPPP is a non-fluorescent triphenylphosphine compound. It reacts with hydroperoxide to generate DPPP oxide that emits fluorescence at 352 nm excitation and 380 nm emission wavelengths (Fig. 1). Post-column HPLC method is used to determine phospholipid peroxide in sample solutions.

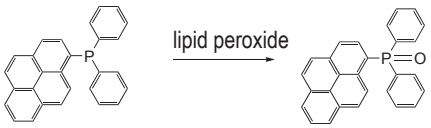


Fig. 1 DPPP reaction with thiol compound

References

1. K. Akasaka, *et al*., Study on Aromatic Phosphines for Novel Fluorometry of Hydroperoxides(II) - the Determination of Lipid Hydroperoxides with Diphenyl-1-Pyrenylphosphine-. *Anal Lett*. 1987;**20**:797-807.
2. K. Akasaka, *et al*., An Aromatic Phosphine Reagent for the HPLC-fluorescence Determination of Hydroperoxides -Determination of Phosphatidylcholine Hydroperoxides in Human Plasma. *Anal Lett*. 1988;**21**:965-975.
3. K. Akasaka, *et al*., A Simple Fluorometry of Hydroperoxides in Oils and Foods. *Biosci Biotech Biochem*. 1992;**56**:605-607.
4. K. Akasaka, *et al*., High-performance Liquid Chromatography and Post-Column Derivatization with Diphenyl-1-Pyrenylphosphine for Fluorimetric Determination of Triacylglycerol Hydroperoxides. *J Chromatogr*. 1992;**596**:197-202.
5. K. Akasaka, *et al*., Simultaneous Determination of Hydroperoxides of Phosphatidylcholine, Cholesterol Esters and Triacylglycerols by Column-Switching High-Performance Liquid Chromatography with a Post-column Detection system. *J Chromatogr*. 1993;**622**:153-159.
6. K. Akasaka, *et al*., Normal-phase High-performance Liquid Chromatograohy with a Fluorimetric Postcolumn Detection System for Lipid Hydroperoxides. *J Chromatogr A*. 1993;**628**:31-35.
7. Y. Okimoto, *et al*., A Novel Fluoresceint Probe Diphenyl-pyrenylphosphine to Follow Lipid Peroxidation in Cell Membranes. *FEBS Lett*. 2000;**474**:137-140.

Spy-LHP

2-(4-Diphenylphosphanyl-phenyl)-9-(1-hexyl-heptyl)-anthra[2,1,9-def,6,5,10-d'e'f']diisoquinoline-1,3,8,10-tetraone

Application: Phospholipid peroxidase detection
Selective to lipid hydroperoxidase
Features: Fluometric detection
Appearance: Reddish black crystalline powder or solid
Purity: ≥90.0% (HPLC)
MW: 832.96, $C_{55}H_{49}N_2O_4P$

Storage Condition
ambient temperature, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
S343-10	1 mg

Product Description

Spy-LHP is a newly developed fluorescent probe for live cell imaging of phospholipid peroxide. There are several detection methods available for lipid peroxides, such as iodide titration method, colorimetric method, or chemiluminometric method to determine malondialdehyde or 4-hydroxynonenal. Malondialdehyde and 4-hydroxynonenal are derivatives from lipid hydroperoxide prepared by oxidation with reactive oxygen species. Thiobarbituriic acid and 1-Methyl-2-phenylindole are used for deriving malondialdehyde for the colorimetric or fluorometric analysis.

Spy-LHP is a low-fluorescent compound, but is oxidized with lipid hydroperoxide to become a high fluorescent compound as indicated in Fig. 1. A similar product, DPPP, is oxidized by a lipid hydroperoxide and becomes a fluorescence compound that can be excited at 352 nm to emit fluorescence at 380 nm. However, the UV excitation for DPPP significantly damages a live cell. Since the oxidized Spy-LHP emits strong fluorescence (quantum yield: ~1) with maximum wavelength at 535 nm when excited at 524 nm, damage to live cells is very small. Spy-LHP has two alkyl chains to improve the affinity to the lipid bilayer. Spy-LHP is highly selective to lipid hydroperoxide and does not react with hydrogen peroxide, hydroxy radicals, superoxide anion, nitric oxides, peroxynitrite, and alkylperoxy radicals.

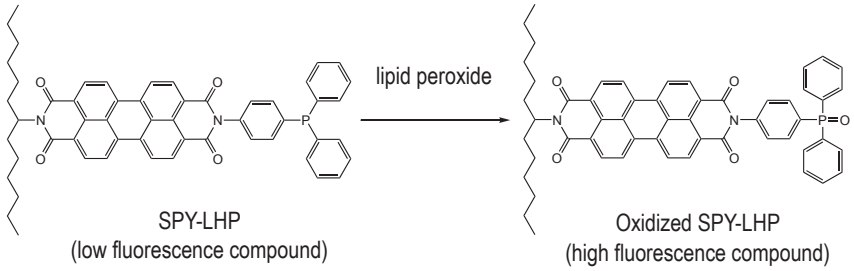


Fig. 1 Reaction of Spy-LHP with lipid hydroperoxide

References

1. N. Soh, *et al*., Novel fluorescent probe for detecting hydroperoxides with strong emission in the visible range. *Bioorg Med Chem Lett*. 2006;**16**:2943-2946.
2. N. Soh, *et al*., Swallow-tailed perylene derivative: a new tool for fluorescent imaging of lipid hydroperoxides. *Org Biomol Chem*. 2007;**5**:3762-3768.

Anti-Nitroguanosine monoclonal antibody

clone#: NO_2G52

Application: 8-Nitroguanosine, 8-Nitroguanine detection
Appearance: Colorless to pale yellow sligtly turbid liquid
Antibody titer: pass test Subtype: IgG1 (mouse BALB/c)
Concentration: 1 mg/ml PBS solution; 0.1% ProClin as a preservative

Storage Condition
-20°C

Shipping Condition
with blue ice or dry ice

Ordering Information

Product code	Unit
AB02-10	50 µg

Anti-Nitroguanosine polyclonal antibody

Application: 8-Nitroguanosine, 8-Nitroguanine detection
Appearance: Colorless or Pale yellow sligtly turbid liquid
Antibody titer: pass test Type: IgG
Concentration: 200 µg per ml PBS solution;
0.1% ProClin as a preservative
Host: Japanese rabbit

Ordering Information	
Product code	Unit
AB01-10	50 µg

Storage Condition	Shipping Condition
-20°C	with blue ice or dry ice

Product Description

8-Nitroguanosine is a nitrated base of DNA and RNA. It is formed by peroxynitrite, which is generated from nitric oxide and superoxide anion radical. It is known that a large amount of nitric oxide molecules and superoxide anion, generated by inflammation, causes nitration of guanosine. Since chemically modified nucleotides cause mutation during DNA replication, 8-nitroguanosine is thought to be one of the markers of DNA damage related to mutation and cancer. Because of its very high specificity, monoclonal antibody NO_2G52 recognizes 8-nitroguanine and 8-nitroguanosine, but it does not cross-react with normal nucleotide bases, 8-hydroxyguanine, 8-hydroxydeoxyguanosine, 3-nitrotyrosine, xanthine, or 2-nitroimidazole (Fig. 1). The specificity of NO_2G52 was determined by a competitive ELISA using an 8-nitroguanosine-BSA-coated plate. As shown in the figures below, NO2G52 has very high affinity for 8-nitroguanine and 8-nitroguanosine, and it slightly cross-reacts with 8-bromoguanosine, 8-bromoguanine, and 8-chloroguanine. Anti-Nitroguanosine polyclonal antibody also recognizes 8-nitroguanine and 8-nitroguanosine, but it does not cross-react with normal guanosine, guanine, 8-hydroxyguanine, or 3-nitrotyrosine. Since this antibody was prepared using rabbits, it can be used for immuno-histostaining of rodent tissues such as mice or rats.

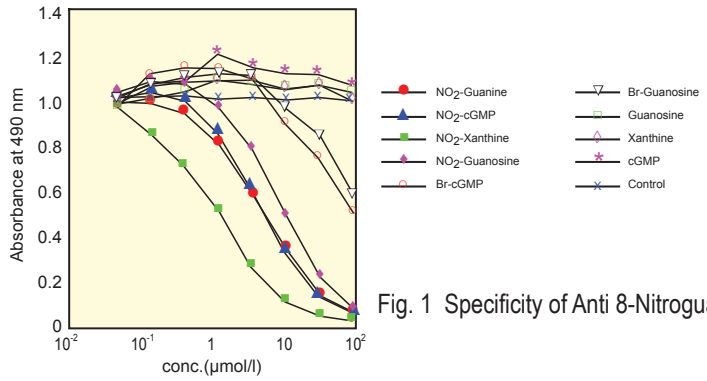
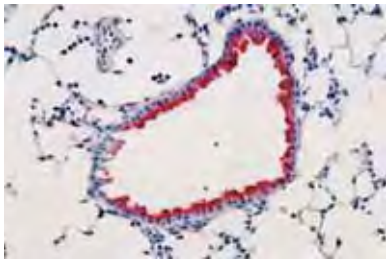


Fig. 1 Specificity of Anti 8-Nitroguanosine monoclonal antibody

A)



B)

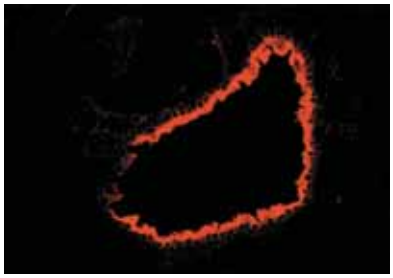


Fig 2 Tissue Staining with Anti-Nitroguanosine Antibody
A) Tissue section (8 days postinfection, influenza) stained with Anti-Nitroguanosine Antibody.
B) The same section viewed using a confocal laser scanning microscope (Fluoroview FV300, Olympus, Nagano, Japan). Strong fluorescence, due to emission of Vector red, is evident in the cytosol.

References

1. T. Akaike, *et al*., 8-nitroguanosine formation in viral pneumonia and its implication for pathogenesis, *PNAS*. 2003;**100**:685-690.
2. J. Yoshitake, *et al*., Nitric oxide as an endogenous mutagen for Sendai virus without antiviral activity. *J Virol*. 2004;**78**:8709-8719.
3. T. Sawa, *et al*., Protein S-guanylation by the biological signal 8-nitroguanosine 3',5'-cyclic monophosphate. *Nat Chem Biol*. 2007;**3**:727-735.

4. M. H. Zaki, *et al*., Cytoprotective function of heme oxygenase 1 induced by a nitrated cyclic nucleotide formed during murine salmonellosis. *J Immunol*. 2009;**182**:3746-3756.
5. Y. Terasaki, *et al*., Guanine nitration in idiopathic pulmonary fibrosis and its implication for carcinogenesis. *Am J Respir Crit Care Med*. 2006;**174**:665-673.
6. T. Sawa, *et al*., Analysis of urinary 8-nitroguanine, a marker of nitrative nucleic acid damage, by high-performance liquid chromatography-electrochemical detection coupled with immunoaffinity purification: association with cigarette smoking. *Free Radic Biol Med*. 2006;**40**:711-720.

8-Nitroguanine (lyophilized)

Application: Blocking agent for immunostaining with Anti-Nitroguanosine Antibody, standard of 8-Nitroguanosine EIA
Appearance: Pale yellow powder
Purity: ≥90.0% (HPLC)
MW: 196.12, $C_5H_4N_6O_3$

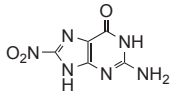
Ordering Information

Product code	Unit
N455-10	100 µg

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Chemical Structure



Product Description
8-Nitroguanine is a nitrated base of DNA and RNA. It is formed by peroxynitrite, which is generated from nitric oxide and superoxide anion radical. It is known that a large amount of nitric oxide molecules and superoxide anion, generated by inflammation, causes nitration of guanosine. Since chemically modified nucleotides cause mutation during DNA replication, 8-Nitroguanine is thought to be a marker of DNA damage related to mutation and cancer. 8-Nitroguanine (lyophilized) is made by the lyophilization of its phosphate-buffered saline solution and is used in immunohistochemistry for absorption testing. Adding 0.4 ml of distilled water to the 8-Nitroguanine powder produces a 1.2 mmol / l of 8-Nitroguanine solution, which is stable for one month at 4°C. If an antibody pre-treated with excessive 8-Nitroguanine shows negative staining, then the subsequent positive staining with this antibody will be specific for 8-nitroguanine or 8-nitroguanosine formed in DNA or RNA.

3-Deoxyglucosone 3-Deoxy-D-erythro-hexos-2-ulose [CAS: 4084-27-9]

Application: Standard for 3-DG analysis, intermediate of AGE
Appearance: White to pale yellow solid
Purity: ≥99.0% (HPLC)
MW: 162.14, $C_6H_{10}O_5$

Ordering Information

Product code	Unit
D535-08	1 mg

Storage Condition
-20°C, protect from moisture

Shipping Condition
with blue ice or dry ice

3-Deoxyglucosone Detection Reagents

Application: 3-DG HPLC assay, fluorometric

Contents of the kit
DAN ..... 10 mg x 1
3-DG/DAN adduct ..... 1 mg x 1

Ordering Information

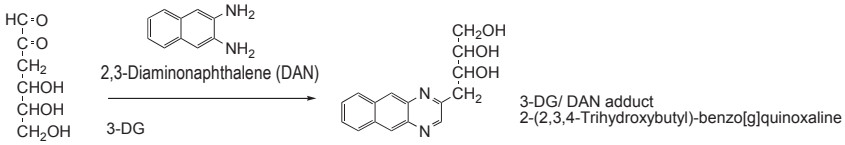
Product code	Unit
D536-10	1 set

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

3-Deoxygluicosone

3-DG Detection



Product Description

Advanced glycation end-products (AGEs) have been studied as one of the causes of diabetic complications. Several compounds have been identified as AGEs, including pyralline, pentosidine, imidazolone, and pyropyridine. Glyoxal and methylglyoxal are reactive dicarbonyl compounds generated by glucose self-oxidation that are known to be AGE precursors. Another dicarbonyl compound, 3-Deoxyglucosone (3-DG), is also known to be one of the AGE precursors. 3-DG is derived from the Amadori rearrangement products of proteins and sugars in early stages of the Maillard reaction. 3-DG is also derived from fructose, which is present in high levels in diabetic patients, by a self-condensation reaction. Fructose-3-phosphate has been found to enhance cross-linking reactions of lens proteins in a diabetic rat model. Therefore, 3-DG derived from fructose-3-phosphate has been studied as a possible cause of cataracts.

Dr. Miyata and others reported that the 3-DG serum level in a diabetic rat model was 918 nM (normal level: 379 nM) and it was suppressed to 695 nM after 3 weeks of feeding aminoguanidine (50 mg/kg/day), an inhibitor of protein glycation. This suggests that compounds with 3-DG quenching activity may have clinical uses. 3-DG may be involved in other diseases as well. Dr. Niwa and others reported that uremia patients had elevated 3-DG levels, and that the 3-DG levels of diabetic uremia patients were even higher. There is also evidence that 3-DG inhibits DNA synthesis, suppressing cell proliferation as a consequence. Though several roles of 3-DG have become clear, many remain unknown. Glyoxal and methylglyoxal are other reactive dicarbonyl compounds generated by glucose self-oxidation that are known to be AGE precursors.

There are two methods for determining 3-DG levels: HPLC and mass spectrometry (MS). However, there is some discrepancy between the HPLC and MS methods when measuring 3-DG levels *in vivo*. HPLC analysis is based on a fluorescent compound, 2-(2,3,4-trihydroxybutyl)-benzo[g]quinoxaline, generated by a coupling reaction between 3-DG and 2,3-diaminonaphthalene. Analogs of 2,3-diaminonaphthalene, such as 1,2-diamino-4,5-dimethoxy-benzene and 1,2-diamino-4,5-methylenedioxybenzene, can also be used. 3-DG can be utilized for AGE production or as a standard for 3-DG level detection in plasma or serum samples.

3-DG Assay Protocol

HPLC Method: Human Serum

1. Add 60% perchloric acid solution to 1 ml human serum and spin at 3,000 *g* for 20 minutes at 4°C.
2. Dilute the supernatant with bicarbonate buffer, then add 0.1 ml of 2,3-Diaminonaphthalene/methanol solution and 25 µl of 1 ppm 3,4-hexanedion as an internal standard.
3. Incubate the mixture at 4°C overnight.
4. Extract the mixture with 4 ml ethyl acetate, and add 4 ml methanol to the extract.
5. Analyze the mixture with reverse-phase HPLC at 267 nm excitation and 503 nm emission for fluorescent detection or at 268 nm for UV detection. Data correlates well with HbA1c level.

   The normal serum 3-DG level: 12.8+5.2 ng per ml

   The serum 3-DG level of diabetic patient: 31.8+11.3 ng per ml

References

1. K. J. Knecht, *et al*., Detection of 3-Deoxyfructose and 3-Deoxyglucosone in Human Urine nd Plasma: Evidence for Intermediate Stages of the Maillard Reaction in Vivo. *Arch Biochem Biophys*. 1992;**294**:130-137.
2. T. Niwa, *et al*., Presence of 3-Deoxyglucosone, a Potent Protein Crosslinking Intermediate of Maillard Reaction, in Diabetic Serum. *Biochem Biophys Res Commun*. 1993;**196**:837-843.
3. H. Yamada, *et al*., Increase in 3-deoxyglucosone levels in diabetic rat plasma. Specific in vivo determination of intermediate in advanced Maillard reaction. *J Biol Chem*. 1994;**269**:20275-20280.
4. Y. Hamada, *et al*., Effects of Glycemic Control on Plasma 3-Deoxyglucosone Levels in NIDDM Patients. *Diabetes Care*. 1997;**20**:1466-1469.
5. T. Niwa, *et al*., Amyloid Beta 2-Microglobulin is Modified with Imidazolone, a Novel Advanced Glycation end Product, in Dialysis-related Amyloidosis. *Kidney Int*. 1997;**51**:187-194.
6. S. Lal, *et al*., Quantitation of 3-Deoxyglucosone Levels in Human Plasma. *Arch Biochem Biophys*. 1997;**342**:254-260.
7. T. Niwa, *et al*., Modification of β_2m with Advanced Glycation End Products as Observed in Dialysis-related Amyloidosis by 3-DG Accumulating in Uremic Serum. *Kidney Int*. 1996;**49**:861-867.

DMPO

5,5-Dimethyl-1-pyrroline *N*-oxide [CAS: 3317-61-1]

Application: Spin Trap Reagent, EPR (ESR) Detection
Superoxide anion radical, Hydroxyl Radical
Features: Ultra high purity
Greater S/N ratio
No pre-purification required
Appearance: Colorless liquid
Purity: ≥99.0%(GC)
MW: 113.16, $C_6H_{11}NO$

Ordering Information

Product code	Unit
D048-10	1 ml

Storage Condition

-20°C, protect from light and metal

Shipping Condition

with blue ice

Product Description

Because of potential cancer risks and their age-promoting effects, free radicals in living bodies have become a frequently studied subject. DMPO is the most frequently used spin-trapping reagent for the study of free radicals. It is suitable for trapping oxygen radicals, especially superoxides, and for producing adducts with characteristic EPR (ESR) patterns. However, most commercially available DMPO contains impurities that cause high backgrounds. Thus, DMPO requires further purification for running experiments on EPR. The quality of Dojindo's DMPO is well controlled and Dojindo's DMPO doesn't require any pre-purification process. There are no impurities to cause a background problem.

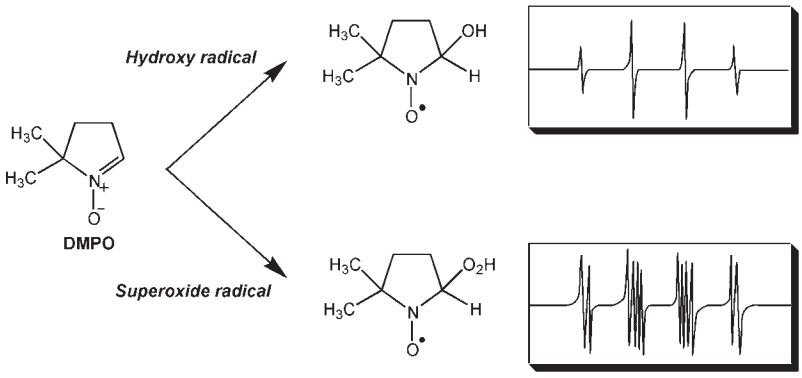


Fig. 1 ESR spectra of DMPO adducts

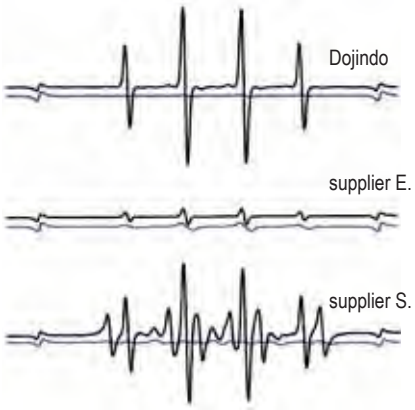


Fig. 2 Purity comparison of ESR spectra

General Protocol

Evaluation of superoxide scavenging activities

1. Add 15 µl of DMPO and 50 µl of 5 mM hypoxanthine to 35 µl of 0.1 M Phosphate buffer(pH 7.8).
2. Add 50 µl of SOD standard or samples to be tested and voltex for 1-2 seconds.
3. Add 50 µl of 0.4 U/ml xanthine oxidase and voltex immediately.
4. Transfer the solution to ESR sample tube and measure ESR spectra after certain time of period, *e.g.* 1minutes.
5. Calculate relative intensity(DMPO-O_2^-/Mn^{2+}) from the peak height.

References

1. S. Sankarapandi, *et al*., Evidence against the generation of free hydroxyl radicals from the interaction of copper,zinc-superoxide dismutase and hydrogen peroxide. *J Biol Chem.* 1999;**274**:34576-34583.
2. H. Li, *et al*., A pyrroline derivative of mexiletine offers marked protection against ischemia/reperfusion-induced myocardial contractile dysfunction. *J Pharmacol Exp Ther.* 2000;**295**:563-571.
3. H. P. Souza, *et al*., Quantitation of superoxide generation and substrate utilization by vascular NAD(P)H oxidase. *Am J Physiol Heart Circ Physiol.* 2002;**282**:H466-H474.
4. S. Kaewpila, *et al*., Manganese superoxide dismutase modulates hypoxia-inducible factor-1 alpha induction via superoxide. *Cancer Res.* 2008;**68**:2781-2788.
5. M. L. T. Teoh, *et al*., Overexpression of extracellular superoxide dismutase attenuates heparanase expression and inhibits breast carcinoma cell growth and invasion. *Cancer Res*. 2009;**69**:6355-6363.
6. Y. Song, *et al*., Nonenzymatic displacement of chlorine and formation of free radicals upon the reaction of glutathione with PCB quinones. *PNAS.* 2009;**106**:9725-9730.

Spin Trap Reagents

BMPO 5-*tert*-Butoxycarbonyl-5-methyl-1-pyrroline-*N*-oxide

Application: Spin trap reagent, EPR (ESR) detection
Superoxide anion radical, Hydroxyl radical
Features: Long half-life
High solubility in water
Greater S/N ratio
Appearance: White powder
Purity: ≥99.0%
MW: 199.25, $C_{10}H_{17}NO_3$

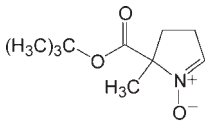
Ordering Information

Product code	Unit
B568-10	50 mg

Storage Condition
0-5°C, protect from moisture

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Spin trapping analysis is one of the most reliable techniques for detecting and identifying short-lived free radicals. The EPR (ESR) spin trap reagent detects both superoxide and hydroxyl radicals produced by systems in vitro and in vivo. BMPO was developed as a spin-trapping reagent that adducts superoxide and shows a much longer half-life ($t_{1/2}$=23 min) than other spin trap reagents. It gives us reproducible and steady results. Because BMPO is highly soluble in water, hydrophilic sample is applicable to analyze the free radicals.

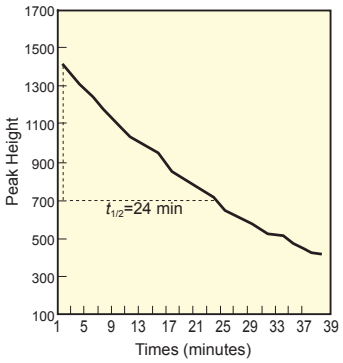


Fig. 1 Half-life of BMPO

General Protocol

Measuring hydroxyl radical from Fenton reaction
1. Dissolve 1.5 mg of BMPO with 5 ml of ddH_2O.
2. Add 15 µl of the BMPO solution, 75 µl of 1 mM H_2O_2 and 75 µl of 100 µM $FeSO_4$ to 50 µl of ddH_2O.
3. Transfer the solution to ESR sample tube and measure ESR spectra after certain time of period, *e.g.* 1 minute.
4. Calculate relative intensity from the peak height.

Measuring superoxide radical from xanthine oxidase(XO) reaction
1. Dissolve 1 mg of BMPO with 1 ml of 50 mM Phosphate buffer(pH7.4). <Solution A>
2. Prepare 50 mM Phosphate buffer(pH 7.4) containing 1 mM DTPA and 0.4 mM Xanthine. <Solution B>
3. Prepare 50 mM Phosphate buffer(pH 7.4) containing 0.1 U/ml xanthine oxidase. <Solution C>
4. Mix 15 µl of Solution A, 135 µl of solution B and 10 µl of Solution C.
5. Transfer the solution to ESR sample tube and measure ESR spectra after certain time of period, *e.g.* 8 minutes.
6. Calculate relative intensity from the peak height.

References
1. H. Zhao, J. Joseph, H. Zhang, H. Karoui and B. Kalyanaraman, Synthesis and biochemical applications of a solid cyclic nitrone spin trap: a relatively superior trap for detecting superoxide anions and glutathiyl radicals. *Free Radic Biol Med*. 2001;**31**:599-606.
2. G.M. Rosen, P. Tsai, J. Weaver, S. Porasuphatana, L. Roman, A. A. Starkov, G. Fiskum and S. Pou, The Role of Tetrahydrobiopterin in the Regulation of Neuronal Nitric-oxide Synthase-generated Superoxide. *J Biol Chem*. 2002;**277**:40275-40280.

Nitric Oxide

Introduction

Nitric oxide (NO) is a unique molecule in biological systems: it can directly interact with target molecules without the involvement of receptors. Since NO is an electrically neutral gaseous molecule, it freely crosses through cells, tissues, and organs. However, its half-life is 3 to 6 seconds, so its active period as NO is fairly short. Therefore, NO activity occurs in the NO-generating cell itself or an adjacent cell. NO is generated by NO synthetase (NOS) from L-arginine. It has been identified as an endothelial-derived relaxation factor (EDRF) and antiplatelet substance. NO also has a number of other activities, including suppression of superoxide anion production from nucleophils, suppression of cell adhesive molecule expression such as VCAM-1 and secretin, suppression of cytokine secretion such as IL-8, suppression of LDL oxidation, and activation of PGI2 synthetase. The most important role of NO is considered to be the activation of guanylate cyclase. The field of NO research has grown dramatically since the discovery of NOS. Despite this rapid growth, NO is still shrouded in mystery. There are many contradictory reports. For example, NO has been found to cause tissue damage in ischemiareperfusion systems; however, there is also evidence that NO is generated to protect tissues from such damage. Another example is glutamate toxicity in a neural system: some researchers find that NO mediates the toxicity, while others report that NO protects from such damage.

There are two reasons why NO research has led to so many contradictory results. First, NO has unique chemical properties that differ from other chemical messengers and hormones. Since NO is a free radical, it is very reactive and unstable. NO is metabolized into various compounds almost immediately after it appears in a biological environment. These metabolites have different activities from NO. For this reason, it is vital to separately investigate the function of each NO metabolite in the NO pathway. Such research should specify the specific NO metabolite that is causing the observed phenomenon. Thus, reagents for NO research must be able to add pure NO or its metabolite to an experimental system without significant side effects or reactions. Second, the timing, rate, amount, and period of NO exposure are also important. The controversial results mentioned above might be caused by differences in NO exposure. The use of a pure NO solution may be the most reliable method in an experimental system. However, it is technically difficult to make a pure NO aqueous solution. The high volatility of NO makes it difficult to produce an exact solution. Moreover, even if a pure NO solution is added to a system, the NO concentration decreases very rapidly with various reactions. Therefore, it is impossible to create a continuous NO exposure state similar to *in vivo* NO synthesis by NOS.

For these reasons, NO donors, reagents that can be used to generate NO, have often been used for the addition of NO into an observed system. Several popular NO donors include nitroglycerin (GTN), isosorbide dinitrate (ISDN), sodium nitroprusside (SNP), and *S*-nitrosothiols. However, these NO donors have not yet resolved the difficulties mentioned above because they are imperfect for the generation of NO. GTN, ISDN, and SNP do not release NO spontaneously; they require thiols as cofactors for NO generation. Furthermore, the favorable conditions for these reagents are very limited, and the amount of NO released from these donors is relatively small. The release conditions might also be affected by the spatial position of samples and their kinds. Nitrate tolerance is another problem for these reagents. SNP also has the disadvantage of cyanoferrin 1 toxicity and the bioactivity of the SNP molecule itself. In contrast to GTN, ISDN, and SNP, nitrosothiol is a spontaneous NO donor. However, NO release of these NO donors accelerates on the surface of the cellular membrane. A more serious issue concerning the use of nitrosothiol as the NO donor is that it may have its own NO-like activity.

Scavenging NO is another important pharmacological method for examining the NO pathway. The most commonly used NO scavengers are NOS inhibitors, including arginine derivatives such as *N*-methylarginine and *N*-nitroarginine methyl ester. These NOS inhibitors, however, scavenge the other NO-related systems as well. This makes a detailed examination of NO action difficult because it may have been caused by another NO metabolite. An ideal NO scavenger would not cut off the arginine-NO pathway by NOS inhibition, but would scavenge the NO molecule directly.

Dojindo has produced a wide variety of reagents for thorough investigation of NO bioactivity, including NO-related metabolite donors such as SIN-1, , NOCs, and NORs. NOCs and NORs can be used for the spontaneous, rate-controlled release of NO. Carboxy-PTIO scavenges the NO molecule without affecting its metabolites. Photoactivatable caged NO donors are also available. Dojindo also offers MGD and 2,3-Diaminonaphthalene for the detection of NO or NO_2^- and NO_3^- as metabolites of NO. Fe(II)-MGD complex easily traps NO *in vivo* or *in vitro*, and can be used to detect NO by electron spin resonance (ESR). Since 2,3-Diaminonaphthalene generates a fluorescent compound by the reaction with NO_2^-, the sensitivity of this fluorescence method is considerably higher than that of the Griess assay.

Nitric Oxide Chemistry

Properties of Nitric Oxide

NO is a free radical that has a lone electron in the 2p-p orbital. It is a highly volatile, hydrophobic, and colorless gaseous material. NO dissolves in water at a maximum of 1.9 mM, and the aqueous NO solution is fairly stable in oxygen-free conditions. NO rapidly reacts with oxygen and produces NO_2^- and NO_3^- in aqueous conditions. The half-life of NO is shorter if the solution contains salts. Under physiological conditions, its half-life is reported to be 3 to 6 seconds. To obtain highly purified NO, the commercial NO gas must be distilled; otherwise NO_2 and NO_3 will remain as contaminants. In the atmosphere, NO generates a brown gas, N_2O_4.

Reactions of Nitric Oxide

Since NO is a highly reactive free radical, several metabolites are produced under physiological conditions. NO reacts with oxygen (eq. 1), and the resulting NO_2 generates N_2O_4 (eq. 2) and N_2O_3 (eq.4). In an aqueous solution, N_2O_4 is hydrolyzed to NO_2^- and NO_3^- (eq.3), and N_2O_3 is hydrolyzed to NO_2^- (eq. 5). In most cases, the reaction ratio of equations 4 and 5 are ten times that of equation 3. Thus, most NO becomes NO_2 in a simple aqueous solution.

$$NO + 1/2O_2 \rightarrow NO_2 \quad \text{(eq. 1)}$$

$$2NO_2 \leftrightarrow N_2O_4 \quad \text{(eq. 2)}$$

$$N_2O_4 + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+ \quad \text{(eq. 3)}$$

$$NO + NO_2 \leftrightarrow N_2O_3 \quad \text{(eq. 4)}$$

$$N_2O_3 + H_2O \rightarrow 2NO_2^- + 2H^+ \quad \text{(eq. 5)}$$

Nitric Oxide

NO, NO^+, and NO^- are physiologically important substances similar to oxygen molecules, O_2, O_2^-, and O_2^{2-} (H_2O_2). NO coordinates with metal ions such as hemoglobin. The coordination rate constant of NO with deoxyhemoglobin is $5x10^7$ $M^{-1}s^{-1}$, and its dissociation constant is $1x10^{-5}$ s^{-1}. An interesting characteristic of NO is its ability to bind to Fe(III), unlike oxygen and carbon monoxide. However, this complex is not stable; attack with a nucleophile will release NO^+. NO also binds to many transitional metals such as Fe-S in the center of the mitochondrial electron carrier and Fe in a macrophage. Since the NO-transitional metal complex has NO^+ characteristics, highly nucleophilic material will be transformed into a nitroso compound by this complex. A NO complex with Fe(II) containing metalloenzyme has NO^- characteristics.

Metabolites of Nitric Oxide

NO^+

It is possible for NO^+ to exist in an aqueous solution, but in most cases, NO^+ exists as nitroso compounds or metal-nitrosyl compounds. These compounds behave like NO^+ in aqueous solutions. They are produced by NO_2, derived from N_2O_4 or N_2O_3 or by the neutrophilic reaction of thiols with Fe(II)-NO^+. There is evidence that the resulting NO^+ equivalents have important roles as NO^+ carriers under physiological conditions. Nitrosothiols are the most common NO^+ equivalents. Although amines may also act as nitroso compounds, they are protonated under physiological conditions and do not react with NO^+. Nitrosothiols are not produced from the reaction between thiols and NO. Thiols are first oxidized and the resulting disulfides then react with NO as shown below.

$$R\text{-}SH \rightarrow RS^- + H^+$$
$$RS^- + \cdot NO > RS\text{-}NO^- + H^+ \rightarrow RS\text{-}N\text{-}OH$$
$$2RS\text{-}N\text{-}OH \rightarrow RSN(OH)N(SR)OH \rightarrow R\text{-}SS\text{-}R + HON{=}NOH$$

On the other hand, NO_2 may generate nitrosothiols from thiols as shown below.

$$RSH + \cdot NO_2 \rightarrow RS\text{-}N(O)OH \rightarrow RSNO + H_2O$$

However, this reaction competes with the following reaction, which produces disulfides.

$$RSH + \cdot NO_2 \rightarrow RS\text{-}N(O)OH \rightarrow RS\text{-}N(OH)_2 + RS\cdot$$
$$2RS\cdot \rightarrow R\text{-}SS\text{-}R$$

Furthermore, nitrosothiols generate NO by decomposition or reaction with thiol radicals.

$$RSNO \rightarrow RS\cdot + \cdot NO$$
$$RSNO + RS\cdot \rightarrow R\text{-}SS\text{-}R + \cdot NO$$

NO^-

NO^- becomes N_2O in aqueous solution with a rate constant of $2x10^9$ $M^{-1}s^{-1}$, and rapidly forms a complex with Fe(III)heme. There are reports that NO^- is produced by Cu(I)-SOD from NO, and also by four electron oxidizations of guanidino-nitrogen by NO synthase in certain conditions.

$ONOO^-$

NO reacts with superoxide anion ($O_2^{\cdot-}$) to produce peroxynitrite ($ONOO^-$) with a rate constant $3.7x10^7$ $M^{-1}s^{-1}$. It is reported that the half-life of $ONOO^-$ at pH 7.5 is 1.9 sec.

$$\cdot NO + O_2^{\cdot-} > ONOO^-$$

This anion decomposes upon protonation to produce NO_2 and .OH.

$$ONOO^- + H > NO_2 + \cdot OH$$

·OH is also produced by the Fenton reaction as shown below.

$$2O_2^{\cdot-} + 2H^+ > H_2O_2 + O_2$$
$$H_2O_2 > \cdot OH + OH^-$$

There are reports that $ONOO^-$ and ·OH are related to epithelial cell damage and neuronal toxicity. However, in oxygen-related toxicity to the central nerve system, the toxicity decreases as NO is trapped by $O_2^{\cdot-}$. This is supported by the observation that toxicity is increased in transgenic mice that express excessive amounts of extracellular SOD. Another possible reason for the decreasing toxicity is that $ONOO^-$ is decomposed to NO^+ by transitional metals in high levels of SOD. The rate of $ONOO^-$ generation is proportional to the concentration of NO and $O_2^{\cdot-}$. Thus, the amount of $ONOO^-$ generated is dependent on rather small changes in the concentration of NO and $O_2^{\cdot-}$. There are two types of $ONOO^-$ decomposition, one generates ·OH and the other HNO_3. The difference between these two reactions is explained by variations in molecular conformation. In the *cis*-conformation, NO_3 is produced by an intramolecular transposition, in the trans-conformation, ·OH is produced by a homolytic cleavage. The intramolecular transposition is dominant in high pH conditions.

$$ON(O)OH > H^+ + NO_3^-$$
$$ONO\text{-}OH > \cdot NO_2 + \cdot OH$$

Experimentally, $ONOO^-$ is produced by NO and $O_2^{\cdot-}$; however, it is reported that this chemical process does not contribute to the generation of $ONOO^-$ *in vivo*. NO is stabilized in the presence of SOD. There are several possible explanations, such as the inhibition of the NO reaction by SOD quenching of $O_2^{\cdot-}$, the competitive binding of $O_2^{\cdot-}$ to the NO binding site of a guanylate cyclase, or the direct reaction of NO as a substrate of SOD.

S-Nitrosothiols

Nitrosothiols are produced from thiols as they react with NO_2 or NO_2^-. They have several different biological activities including a vasorelaxant activity just like NO. Previous research found that the vasorelaxant properties of endothelium-derived relaxation factor (EDRF) are more similar to *S*-nitrosocysteine than to NO; however, this does not seem to be the current majority view. Although nitrosothiol is one of the most important factors for the study of the NO pathway, only a few nitrosothiols, such as SNAP and *S*-nitrosoglutathione (GSNO), are stable enough for use as NO donors. Unfortunately, SNAP is insoluble in water. Thus, GSNO and *S*-nitrosocysteine (SNC) are the only commercially available water-soluble nitrosothiols. Nitrosothiols release nitric oxide and form disulfides as shown below.

$$2RSNO > RSSR + 2NO$$

This reaction is accelerated by light and heat. If GSNO is incubated at 37°C without light, NO will not spontaneously release. Metal ions, such as Cu(II), Cu(I), and Hg(II), also accelerate the reaction. Thus, masking reagents such as EDTA prevent the releasing reaction. Another important characteristic of nitrosothiols is their ability to carry out nitrosation. This reaction is faster than the decomposition of RSNO itself and proceeds readily at physiological pH levels. The reaction rate depends on the pKa of the thiol.
The vasorelaxant activities of nitrosothiols in rat aortic rings have been reported as follows:

SNAP > GSNO = SNAC (S-Nitroso-N-acetylcysteine) > CoAsNO (S-Nitroso-coenzyme A) > CYCNO (S-Nitrosocysteine)

The inhibitory potencies of nitrosothiols for the platelet aggregation have been reported as follows:

GSNO > NO > SNAP > SIN-1

Denitrosation of *S*-nitrosothiol is not spontaneous, and it needs to be catalyzed on the surface of external vascular membranes. *S*-nitroso-*L*-cysteine raises the intracellular calcium level of a PC12 cell by modifying the thiol group of a caffeine-sensitive moiety of the calcium-induced calcium release (CICR) channel. GSNO has been shown to reduce the blood pressure of anesthetized dogs (0.2 mg/kg) and monkeys (10 mg/kg) through the inhibition of platelet aggregation.

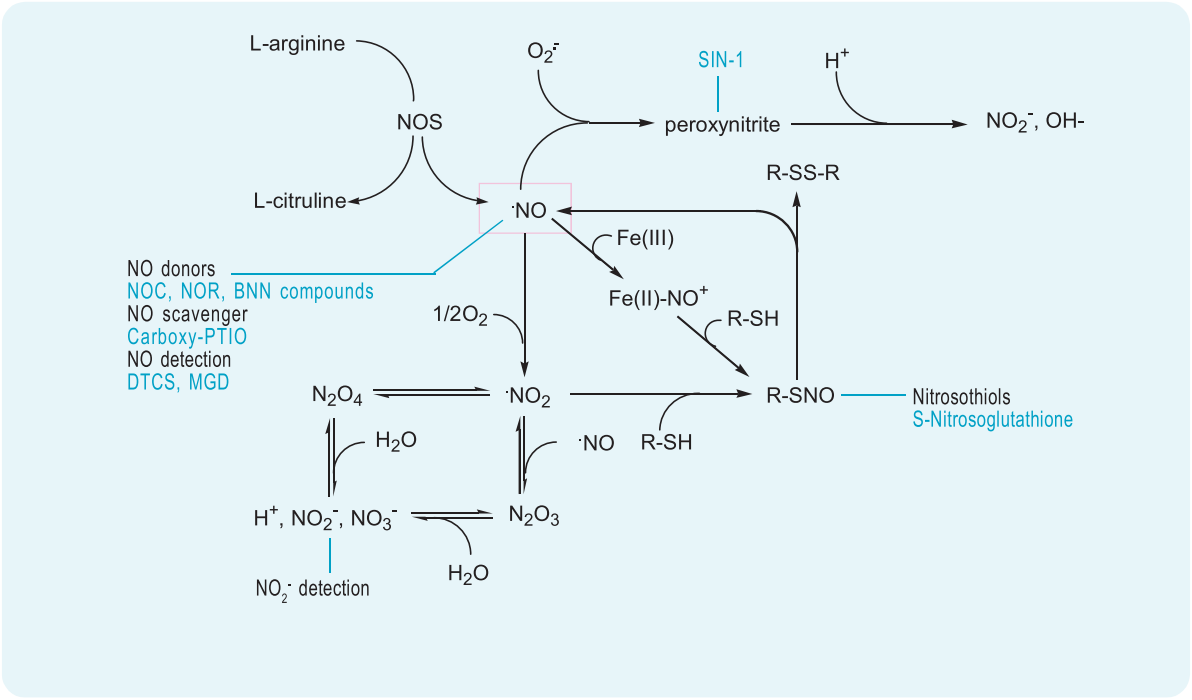


Fig. 6-1 NO metabolism

Nitric Oxide

NOC 5 1-Hydroxy-2-oxo-3-(3-aminopropyl)-3-isopropyl-1-triazene [CAS: 146724-82-5]

Application: Spontaneous NO donor
Appearance: White powder
Purity: ≥90.0% (HPLC)
MW: 176.22, $C_6H_{16}N_4O_2$

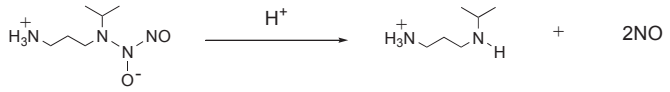
Storage Condition
-20°C, protect from light and moisture

Shipping Condition
with blue ice or dry ice

Ordering Information

Product code	Unit
N380-10	10 mg
N380-12	50 mg

Reaction of NO release



NOC 7 1-Hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene [CAS: 146724-84-7]

Application: Spontaneous NO donor
Appearance: White powder
Purity: ≥90.0% (HPLC)
MW: 162.19, $C_5H_{14}N_4O_2$

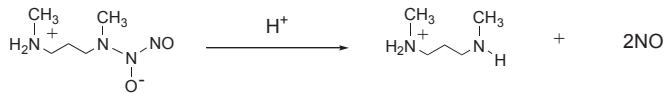
Storage Condition
-20°C, protect from light and moisture

Shipping Condition
with blue ice or dry ice

Ordering Information

Product code	Unit
N377-10	10 mg
N377-12	50 mg

Reaction of NO release



NOC 12 1-Hydroxy-2-oxo-3-(*N*-ethyl-2-aminoethyl)-3-ethyl-1-triazene [CAS: 146724-89-2]

Application: Spontaneous NO donor
Appearance: White powder
Purity: ≥90.0% (HPLC)
MW: 176.22, $C_6H_{16}N_4O_2$

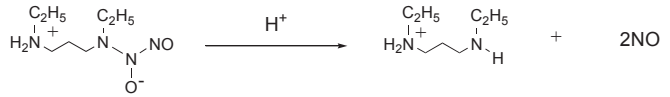
Storage Condition
-20 °C, protect from light and moisture

Shipping Condition
with blue ice or dry ice

Ordering Information

Product code	Unit
N378-10	10 mg
N378-12	50 mg

Reaction of NO release



NOC 18 1-Hydroxy-2-oxo-3,3-bis(2-aminoethyl)-1-triazene [CAS: 146724-94-9]

Application: Spontaneous NO donor
Appearance: White powder
Purity: ≥90.0% (HPLC)
MW: 163.18, $C_4H_{13}N_5O_2$

Ordering Information

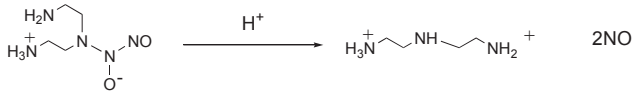
Product code	Unit
N379-10	10 mg
N379-12	50 mg

Nitric Oxide

Storage Condition
-20 °C, protect from light and moisture

Shipping Condition
with blue ice or dry ice

Reaction of NO release



Product Description of NOC Compounds
NOCs are stable NO-amine complexes that spontaneously release NO, without cofactors, under physiological conditions. The rate of NO release depends on the chemical structure of the NOC. The mechanism of spontaneous NO generation by NOCs is very simple compared to other classical NO donors, such as nitroglycerin and nitroprusside, and the by-products do not interfere with cell activities. A single NOC molecule releases two NO molecules (as indicated in the reaction scheme); the release rate of the second NO molecule is very slow. NOCs can be used to add controlled amounts of pure NO to experimental systems at controlled rates with minimal side effects. The amount of NO released can be easily manipulated by altering the concentration and selection of NOC reagents. Dojindo offers four different NOCs (NOC 5, 7, 12, and 18) with different half-lifes. Stock solutions of NOC prepared in alkaline solutions, such as aqueous NaOH, are relatively stable. However, the NOC stock solution should be used within one day because it degrades about 5% per day, even at -20°C. The release of NO begins immediately after adding the stock solution to a sample solution.

Nitric Oxide Release
1. Prepare 10 mM NOC stock solution using 0.1 M NaOH. Since the NOC stock solution is not stable, keep it on an ice bath and use it in one day.
2. Add an appropriate volume of the NOC stock solution to the sample solution in which NO is to be released. To maintain the pH of the sample solution, the volume of the NOC stock solution should not exceed 1/50 of the sample volume. The sample solution should have sufficient buffering action. NO will be released immediately after the addition of the NOC stock solution.

Table 1 pH Dependency of NO Release at 37°C

NO Donor	pH	Half-life	pH	Half-life	pH	Half-life	pH	Half-life	pH	Half-life
NOC 5	7.0	12 min	7.2	20 min	7.4	25 min	7.6	42 min	7.8	66 min
NOC 7	7.0	2.2 min	7.2	3.8 min	7.4	5 min	7.6	8.2 min	7.8	12.4 min
NOC 12	7.0	40 min	7.2	1.2 hours	7.4	100 min	7.6	3 hours	7.8	4.6 hours
NOC 18	7.0	13 hours	7.2	18 hours	7.4	21 hours	7.6	34 hours	7.8	45 hours

FAQ
♦How do I prepare a stock solution?

Prepare 10-50 mM NOC solution with 0.1 M NaOH solution. Then add enough NOC solution to the cell culture to obtain a suitable concentration of NOC in cell culture. If the pH of the culture solution changes, use higher concentration of NOC.

♦What is the solubility of the NOC compounds?

NOC 5: 40 mg per 100 ml 0.1 M NaOH (2.2 M NOC 5)
NOC 7: 70 mg per 100 ml 0.1 M NaOH (4.3 M NOC 7)
NOC 12: 27 mg per 100 ml 0.1 M NaOH (1.5 M NOC 12)
NOC 18: 20 mg per 100 ml 0.1 M NaOH (1.2 M NOC 18)

♦Is the stock solution stable?

The stock solution will lose 5% of its NOC activity per day, even when stored at -20°C. Please prepare fresh solution prior to use and keep the solution on an ice bath during the experiment.

♦How is the half-life of NOC determined?

Prepare 20 mM NOC stock solution with 0.1 M NaOH. Warm PBS at 37°C. Add 100 ml NOC solution to 1.9 ml PBS. Using a UV spectrophotometer, immediately start measuring its absorbance at the maximum wavelength of the NOC. Continue measuring until no further spectra changes are observed.

♦Can I use NOC for in vivo experiments?

Yes. Please review the papers by Shibuta and colleagues (1995, 1996).

♦Is the amount of NO released in vitro the same as in vivo?

The amount of NO released in the solution should be the same if the pH and temperature are the same. However, the activity of NO may be different *in vivo* because of other reactive components such as thiol compounds and heme.

References
1. K. Hayashi, *et al*., Action of Nitric Oxide as a Antioxidant Against Oxidation of Soybean Phosphatidylcholine Liposomal Membrane. *FEBS Lett*. 1995;**370**:37-40. (NOC 12)
2. S. Shibuta, *et al*., Intracerebroventricular Administration of a Nitric Oxide-releasing Compound, NOC-18, Produces Thermal Hyperalgesia in Rats. *Neurosci Lett*. 1995;**187**:103-106. (NOC 18)

Nitric Oxide

3. S. Shibuta, *et al*., A new nitric oxide donor, NOC-18, exhibits a nociceptive effect in the rat formalin model. *J Neurol Sci*. 1996;**141**:1-5. (NOC 18)
4. N. Yamanaka, *et al*., Nitric Oxide Released from Zwitterionic Polyamine/NO Adducts Inhibits Cu^{2+}-induced Low Density Lipoprotein Oxidation. *FEBS Lett*. 1996;**398**:53-56. (NOC 5, NOC 7)
5. D. Berendji, *et al*., Nitric Oxide Mediates Intracytoplasmic and Intranuclear Zinc Release. *FEBS Lett*. 1997;**405**:37-41.
6. T. Ohnishi, *et al*., The Effect of Cu^{2+} on Rat Pulmonary Arterial Rings. *Eur J Pharmacol*. 1997;**319**:49-55. (NOC 7)
7. Y. Adachi, *et al*., Renal Effect of a Nitric Oxide Donor, NOC 7, in Anethetized Rabbits. *Eur J Pharmacol*. 1997;**324**:223-226. (NOC 7)
8. Y. Minamiyama, *et al*., Effect of Thiol Status on Nitric Oxide Metabolism in the Circulation. *Arch Biochem Biophys*. 1997;**341**:186-192. (NOC 7)

NOR 1 (±)-(*E*)-4-Methyl-2-[(*E*)-hydroxyimino]-5-nitro-6-methoxy-3-hexenamide [CAS: 163032-70-0]

Application: Spontaneous NO donor
Appearance: White or slightly yellow powder
Purity: ≥98.0% (HPLC)
MW: 231.21, $C_8H_{13}N_3O_5$

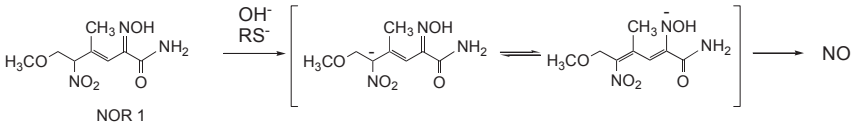
Ordering Information

Product code	Unit
N388-10	10 mg

Storage Condition
-20°C, protect from moisture

Shipping Condition
ambient temperature

Reaction of NO release



NOR 1

NOR 3 (±)-(*E*)-4-Ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexenamide [CAS: 163180-49-2]

Application: Spontaneous NO donor
Appearance: White crystalline powder
Purity: ≥98.0% (HPLC)
MW: 215.21, $C_8H_{13}N_3O_4$

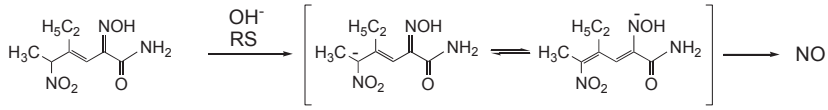
Ordering Information

Product code	Unit
N390-10	10 mg

Storage Condition
-20°C, protect from moisture

Shipping Condition
ambient temperature

Reaction of NO release



NOR 3

NOR 4 (±)-N-{(*E*)-4-Ethyl-2-[(*Z*)-hydroxyimino]-5-nitro-3-hexene-1-yl}-3-pyridinecarboxamide [CAS: 162626-99-5]

Application: Spontaneous NO donor
Appearance: White or slightly yellow powder
Purity: ≥98.0% (HPLC)
MW: 306.32, $C_{14}H_{18}N_4O_4$

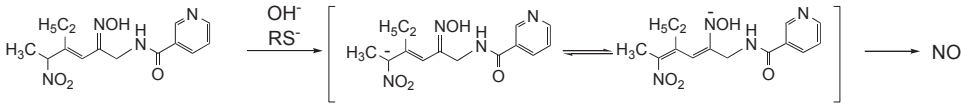
Ordering Information

Product code	Unit
N391-10	10 mg

Storage Condition
-20°C, protect from moisture

Shipping Condition
ambient temperature

Reaction of NO release



NOR 4

NOR 5 (±)-*N*-{(*E*)-4-Ethyl-3-[(*Z*)-hydroxyimino]-6-methyl-5-nitro-3-heptenyl}-3-pyridinecarboxamide

Application: Spontaneous NO donor
Appearance: White or slightly yellow powder
Purity: ≥98.0% (HPLC)
MW: 334.37, $C_{16}H_{22}N_4O_4$

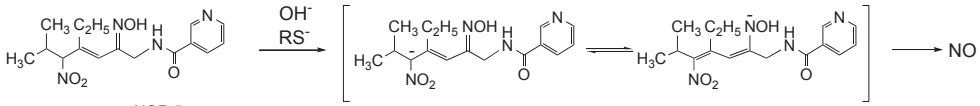
Ordering Information

Product code	Unit
N448-10	10 mg

Storage Condition
-20°C, protect from moisture

Shipping Condition
ambient temperature

Reaction of NO release



NOR 5

Product Description of NOR Compounds

NORs are ideal NO donors with completely different chemical structures from the other NO donors. Although NORs do not have any ONO_2 or ONO moiety, they spontaneously release NO at a steady rate. Even though the NO release mechanism of NOR has not been completely determined, it is confirmed that the byproducts do not possess any significant bioactivities. NOR 3, isolated from Streptomyces genseosporeus, is reported to have strong vasodilatory effects on rat and rabbit aortas and dog coronary arteries. Its activity (ED_{50}=1 nM) is 300 times that of isosorbide dinitrate (ISDN). NOR 3 also increases the plasma cyclic GMP levels, whereas ISDN does not. NOR is a potent inhibitor of platelet aggregation and thrombus formation. NOR 3 (IC_{50}=0-7 mM) effectively inhibits 100% of ADP-initiated human platelet Aggregation, whereas ISDN inhibits only 32% of the total aggregation, even at 100 mM concentrations. NOR 3 has also been reported to have antianginal and cardioprotective effects in the ischemia/reperfusion system. In the rat methacholin-induced coronary vasospasm model, NOR 3 suppressed the elevation of the ST segment dose-dependently and significantly at 1 mg per kg. On the other hand, ISDN suppressed it significantly at 3.2 mg per kg. The difference in the NO release rate of NOR reagents was reflected even on the *in vivo* hypotensive effects. NOR may also be used orally in a 0.5% methylcellulose suspension. NOR is relatively stable in DMSO solution. NOR 1, which has the shortest half-life, is a promising reagent for making NO standard solutions for calibration. For the preparation of the standard solution, a precisely diluted NOR 1/DMSO solution is added to the buffer solutions.

Nitric Oxide Release

1. Prepare 10 mM NOR stock solution using DMSO. Since the NOR stock solution is not stable, keep it on an ice bath and use it in one day.
2. Add an appropriate volume of the NOR stock solution to the sample solution in which NO is to be released. In order to avoid possible damage to cells by DMSO, the volume of the NOR stock solution should not exceed 1/50 of the sample volume. The sample solution should have sufficient buffering action. NO will be released immediately after the addition of the NOR stock solution.

FAQ

♦How do I prepare a stock solution?
Prepare 10-50 mM NOR solution with DMSO. The DMSO should be dried. Then add enough NOR solution to the cell culture to obtain a suitable concentration of NOR.

♦What is the solubility of NOR compounds?
NOR 1: 100 mg per 100 ml DMSO (4.3 M)
NOR 3: 137 mg per 100 ml DMSO (6.4 M)
NOR 4: 30 mg per 100 ml DMSO (1.0 M)
NOR 5: 30 mg per 100 ml DMSO (0.9 M)

♦Is oral administration possible?
Yes. Please review the article by Kita and colleagues (*Eur J Pharmacol*. 1994;**257**:123-130.

♦How many NO molecules does each NOR molecule release in physiological conditions? What are the by products?
On average, each NOR molecule releases from 1 to 1.5 NO molecules in physiological conditions. Unfortunately, the structure of NOR byproducts remains unclear. However, the NOR byproducts have no cytotoxicity at the normal concentration for NO release experiments.

References

1. S. Shibata, *et al*., Characteristics of the Vasorelaxing Action of (*3E*)-4-Ethyl-2-hydroxyimino-5-nitro-3-hexamide FK409, a New Vasodilator Isolated from Microbial Sources, in Isolated Rabbit Arteries. *J Cardiovasc Pharmacol*. 1991;**17**:508-518.
2. Y. Kita, *et al*., Antianginal Effects of FK409, a New Spontaneous NO Releaser. *Br J Pharmacol*. 1994;**113**:1137-1140.
3. Y. Kita, *et al*., Antiplatelet Activities of FK409, a New Spontaneous NO Releaser. *Br J Pharmacol*. 1994;**113**:385-388.
4. Y. Kita, *et al*., Spontaneous Nitric Oxide Release Accounts for the Potent Pharmacological Actions of FK409. *Eur J Pharmacol*. 1994;**257**:123-130.
5. M. Hino, *et al*., FK409, a Novel Vasodilator Isolated from the Acid-treated Fermentation Broth of *Streptomyces Griseosporeus* I. Taxonomy, Fermentation, Isolation, and Physico-chemical and Biological Characteristics. *J Antibiot*. 1989; **42**:1578-1583.
6. J. Decout, *et al*., Decomposition of FK409, a New Vasodilator: Identification of Nitric Oxide as Metaborite. *Bioorg Med Chem Lett*. 1995;**5**:973-978.
7. S. Fukuyama, *et al*., A New Nitric Oxide (NO) Releaser: Spontaneous NO Release from FK409. *Free Radic Res*. 1995;**23**:443-452.
8. Y. Kita, *et al*., FR144420, a Novel, Slow, Nitric Oxide-releasing Agent. *Eur J Pharmacol*. 1995;**275**:125-130.
9. M. Kato, *et al*., New Reagents for Controlled Release of Nitric Oxide. Structure-stability Relationships. *Bioorg Med Chem Lett*. 1996;**6**:33-38.
10. Y. Kita, *et al*., FK409, a Novel Spontaneous NO Releaser: Comparative Pharmacological Studies with ISDN. *Cardiovasc Drug Rev*. 1996;**14**:148-165.
11. Y. Hirasawa, *et al*., Antianginal Effects of FR144420, a Novel Nitric Oxide-releasing Agent. *Eur J Pharmacol*. 1996;**303**:55-59.
12. M. Sato, *et al*., Nitric Oxide Raises Cytosolic Concentrations of Ca^{2+} in Cultured Nodose Ganglion Neurons from Rabbits. *Neurosci Lett*. 1996;**206**:69-72.
13. Y. Kita, *et al*., Oral Biological Activities of Spontaneous Nitric Oxide Releaser are Accounted for by their Nitric Oxide-releaseing Rates and Oral Absorption Manners. *J Pharmacol Exp Ther*. 1996;**276**:421-425.
14. S. Fukuyama, *et al*., Structure-activity Relationships of Spontaneous Nitric Oxide Releasers, FK409 and its Derivatives. *J Pharmacol Exp Ther*. 1997;**282**:236-242.
15. Y. Kita, *et al*., Comparison of Hemodynamic Effects of Nitric Oxide (NO) Donors with Different NO-releasing Properties in Rats. *J Cardiovasc Pharmacol*. 1997;**30**:223-228.
16. Y. Hirasawa, *et al*., Comparison of Antiplatelet Effect of two Nitric Oxide-donating Agents, FR146801 and FK409. *Thromb Haemost*. 1998;**79**:620-624.

S-Nitrosoglutathione

N-(*N*-L-γ-Glutamyl-*S*-nitroso-L-cysteinyl)glycine [CAS: 57564-91-7]

Application: Spontaneous NO donor, nitrosothiol compound
Appearance: Pink powder
Purity: ≥90.0% (HPLC)
MW: 336.32, $C_{10}H_{16}N_4O_7S$

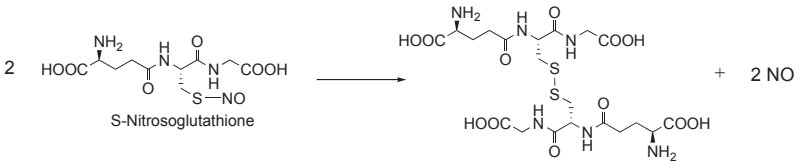
Storage Condition
-20°C, protect from moisture

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
N415-10	25 mg
N415-12	100 mg

Reaction of NO release



Product Description of *S*-Nitrosothiols

Nitrosothiol compounds release NO and become disulfides under specific physiological conditions. While most of the *S*-nitrosothiol compounds are unstable, *S*-Nitrosoglutathione is exceptionally stable. Furthermore, S-Nitrosoglutathione is water-soluble. Although S-nitrosothiol is a good NO donor with no nitrate tolerance, there is evidence that *S*-nitrosothiol itself has NO-like activity during guanylate cyclase activation. Another important reaction of nitrosothiol is NO transfer to other thiol compounds. Since it depends on the pKa of thiols, this transfer reaction proceeds at physiological pH levels. The relaxation efficiency of these nitrosothiol has been compared using rataprta ring samples:SNAP> *S*-Nitrosoglutathione = *S*-Nitroso-*N*-acetylcysteine>*S*-Nitrosocoenzyme A>*S*-Nitroso-L-cysteine. Dr. Kowaluk and others reported that the spontaneous liberation of NO from SNAP could not account for *in vitro* vascular relaxation. The spontaneous release of NO from nitrosothiol compounds may not be a key element of vascular relaxation. Metabolites of nitrosothiol generated at the cell membrane might be the essential element for relaxation.

References

1. A. Gibson, *et al*., An Investigation of Some *S*-Nitrosothiols, and of Hydroxy-arginine, on the Mouse Anococcygeus. *Br J Pharmacol*. 1992;**107**:715-721.
2. M. W. Radomski, *et al*., *S*-Nitroso-glutathione Inhibits Platelet Activation *in Vitro* and *in Vivo*. *Br J Pharmacol*. 1992;**107**:745-749.
3. R. M. Clancy, *et al*., Novel Synthesis of *S*-Nitrosoglutathione and Degradation by Human Neutrophils. *Anal Biochem*. 1992;**204**:365-371.
4. J. W. Park, *et al*., Transnitrosation as a Predominant Mechanism in the Hypotensive Effect of S-Nitrosoglutathione. *Biochem Mol Biol Int*. 1993;**30**:885-891.
5. D. Barrachina, *et al*., Nitric Oxide Donors Preferencially Inhibit Neuronally Mediated Rat Gastric Acid Secretion. *Eur J Pharmacol*. 1994;**262**:181-183.
6. E. A. Konorev, *et al*., *S*-Nitrosoglutathione Improves Functional Recovery in the Isolated Rat Heart After Cardioplegic Ischemic Arrest-evidence for a Cardioprotective Effect of Nitric Oxide. *J Pharmacol Exp Ther*. 1995;**274**:200-2006.
7. S. C. Askew, *et al*., Catalysis by Cu^{2+} of Nitric Oxide Release from *S*-Nitrosothiols (RSNO). *J Chem Soc Perkin Trans 2*. 1995;741-745.
8. D. J. Banett, *et al*., NO-group Transfer(Transnitrosation) between *S*-Nitrosothiols and Thiols. Part 2. *J Chem Soc Perkin Trans 2*. 1995;1279-1282.
9. J. G. De Man, *et al*., Effect of Cu^{2+} on Relaxations to the Nitrergic Neurotransmitter, NO and *S*-Nitrosothiols in the Rat Gastric Fundus. *Br J Pharmacol*. 1996;**119**:990-996.
10. A. P. Dicks, *et al*., Generation of Nitric Oxide from *S*-Nitrosothiols Using Protein-bound Cu^{2+} Sources. *Chem Biol*. 1996;**3**:655-659.
11. J. A. Cook, *et al*., Convenient Colorimetric and Fluorometric Assays for *S*-Nitrosothiols. *Anal Biochem*. 1996;**238**:150-158.
12. S. X. Liu, *et al*., Nitric Oxide Donors: Effects of *S*-Nitrosoglutathione and 4-Phenyl-3-furoxancarbonitrile on Ocular Blood Flow and Retinal Function Recovery. *J Ocul Pharmacol Ther*. 1997;**13**:105-114.
13. C. Alpert, *et al*., Detection of *S*-Nitrosothiols and Other Nitric Oxide Derivatives by Photolysis-chemiluminescence Spectrometry. *Anal Biochem*. 1997;**245**:1-7.
14. T. Akaike, *et al*., Nanomolar Quantification and Identification of Various Nitrosothiols by High Performance Liquid Chromatography Coupled with Flow Reactors of Metals and Griess Reagent. *J Biochem*. 1997;**122**:459-466.

SIN-1 3-(4-Morpholinyl)sydnonimine, hydrochloride [CAS: 16142-27-1]

Application: Spontaneous NO, peroxynitrite donor
Appearance: White or slightly yellowish needles or crystalline powder
MW: 206.63, $C_6H_{11}ClN_4O_2$

Ordering Information

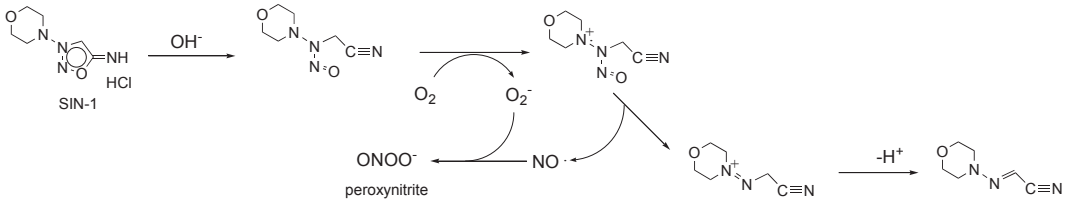
Product code	Unit
S264-10	25 mg

Storage Condition
-20°C, protect from light and moisture

Shipping Condition
with blue ice or dry ice

Product Description
SIN-1, a metabolite of the vasodilator molsidomine, is utilized to separately estimate the effectiveness of NO and peroxynitrite with other NO donors. SIN-1 spontaneously decomposes in the presence of molecular oxygen to generate NO and superoxide. Both products bind very rapidly to form peroxynitrite (rate constant k: $3.7x10^{-7}$ $M^{-1}s^{-1}$). Therefore, SIN-1 is a useful compound that generates peroxynitrite in an efficient manner. Peroxynitrite is a very strong oxidant that generates hydroxyl and nitrosyldioxyl radicals under physiological conditions. Peroxynitrite also decomposes to generate nitrate ion quickly in acidic conditions and slowly in basic conditions. Those species have a different bioactivity from NO.

Reaction of NO release and peroxynitrite production



References

1. M. Feelisch, *et al*., On the Mechanism of NO Release from Sydnonimines. *J Cardiovasc Pharmacol*. 1989;**14**:S13-S22.
2. M. Feelisch, The Biochemical Pathways of Nitric Oxide Formation from Nitrovasodilators: Appropriate Choice of Exogenous NO Donors and Aspects of Preparation and Handling of Aqueous NO Solutions. *J Cardiovasc Pharmacol*. 1991;**17**:S25-S33.
3. N. Hogg, *et al*., Production of Hydroxyl Radicals from the Simultaneous Generation of Superoxide and Nitric Oxide. *Biochem J*. 1992;**281**:419-424.
4. M. E. Murphy, *et al*., Nitric Oxide Hyperpolarizes Rabbit Mesenteric Arteries via ATP-sensitive Potassium Channels. *J Physiol*. 1995;**486**:47-58.
5. H. Kankaanranta, *et al*., 3-Morpholino-sydnonimine-induced Suppression of Human Neutrophil Degranulation in Not Mediated by Cyclic GMP, Nitric Oxide or Peroxynitrite: Inhibition of the Increase in Intracellular Free Calcium Concentration by N-Morpholinoiminoacetoni. *Mol Pharmacol*. 1997;**51**:882-888.
6. S. Yamamoto, *et al*., Subarachnoid Hemorrhage Impairs Cerebral Blood Flow Response to Nitric Oxide but Not to Cyclic GMP in Large Cerebral Arteries. *Brain Res*. 1997;**757**:1-9.
7. S. Pfeiffer, *et al*., Interference of carboxy-PTIO with Nitric-oxide and Peroxynitrite-mediated Reactions. *Free Radic Biol Med*. 1997;**22**:787-794.

8. M. B. Herrero, *et al*., Tyrosine Nitration in Human Spermatozoa: A Physiological Function of Peroxynitrite, the Reaction Product of Nitric Oxide and Superoxide. *Mol Hum Reprod*. 2001;**7**:913-921.
9. P. D. Lu, *et al*., Cytoprotection by Pre-emptive Conditional Phosphorylation of Translation Initiation Factor 2. *EMBO J*. 2004;**23**:169-179.

Carboxy-PTIO

2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, sodium salt [CAS: 148819-93-6]

Application: NO quenching, NO detection by ESR
Appearance: Dark blue or black powder
MW: 299.28, $C_{14}H_{16}N_2NaO_4$

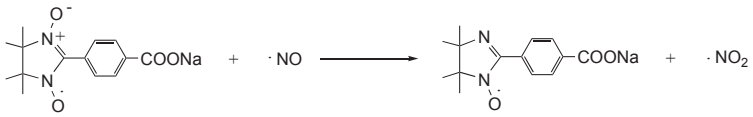
Ordering Information

Product code	Unit
C348-10	10 mg

Storage Condition
-20°C, protect from light, moisture and metal

Shipping Condition
ambient temperature

Reaction of NO quenching



Product Description

Carboxy-PTIO is a stable, water-soluble organic radical that reacts with NO to form NO_2·. This reaction can be monitored by electron spin resonance (ESR). NO is an unstable molecule and has a complex reaction cascade for its metabolism in biological systems. Rapidly generated NO-related metabolites carry out various physiological activities. Commonly used NO scavengers such as hemoglobin trap NO; they also trap NOS inhibitors such as arginine derivatives. These NO scavengers also quench all other NO-related metabolites at the same time. In contrast, Carboxy-PTIO does not dramatically affect other NO-related product systems because it transforms NO to NO_2, which is a metabolite of NO. Thus, Carboxy-PTIO can be used to investigate the effects of NO separately from its downstream metabolites. Dr. Akaike and others showed that Carboxy-PTIO suppresses relaxation of the rat aorta ring, which is induced by acetylcholine, twice as effectively as N^G-nitroarginine. Dr. Yoshida and others reported that downstream metabolites of NO, generated by treatment with Carboxy-PTIO, have an increased antiviral activity compared to NO alone. The NO metabolites play important roles in biological systems; therefore, they should be investigated separately from NO.

References

1. E. F. Ullman, *et al*., Studies of Stable Free Radicals. X. Nitronyl Nitroxide Monoradicals and Biradicals as Possible Small Molecule Spin Labels. *J Am Chem Soc*. 1972;**94**:7049-7059.
2. Y. Miura, *et al*., Polymers Containing Stable Free Raficals, 5. Preparation of a Polymer Containing Imidazoline 3-Oxide 1-Oxyl Groups. *Macromol Chem Phys*. 1973;**172**:233-236.
3. K. Inoue, *et al*., Magnetic Properties of the Crystals of *p*-(1-Oxyl-3-Oxide-4, 4, 5, 5-Tetramethyl-2-Imidazolin-2-Yl)Benzoic acid and Its Alkali Metal Salts. *Chem Phys Lett*. 1993;**207**:551-555.
4. T. Akaike, *et al*., Antagonistic Action of Imidazolineoxyl N-Oxides Against Endothelium-Derived Relaxing Factor/NO Through a Radical Reaction. *Biochemistry*. 1993;**32**:827-832.
5. J. Joseph, *et al*., Trapping of Nitric Oxide by Nitronyl Nitroxides: an Electron Spin Resonance Investigation. *Biochem Biophys Res Commun*. 1993;**192**:926-934.
6. M. Yoshida, *et al*., Therapeutic Effects of Imidazolineoxyl N-Oxide Against Endotoxin Shock Through Its Direct Nitric Oxide-scavenging Activity. *Biochem Biophys Res Commun*. 1994;**202**:923-930.
7. T. Az-Ma, *et al*., Reaction Between Imidazolineoxil N-Oxide(Carboxy-PTIO) and Nitric Oxide Released from Cultured Endothelial Cells:Quantitative Measurement of Nitric Oxide by ESR Spectrometry. *Life Sci*. 1994;**54**:PL185-PL190.
8. H. Maeda, *et al*., Multiple Functions of Nitric Oxide in Pathophysiology and Microbiology: Analysis by a New Nitric Oxide Scavenger. *J Leukoc Biol*. 1994;**56**:588-592.
9. T. Akaike, *et al*., Quantitation of Nitric Oxide Using 2-Phenyl-4, 4, 5, 5-Tetramethylimidazoline-1-Oxyl 3-Oxide(PTIO). *Methods Enzymol*. 1996;**268**:211-221.
10. S. Satoh, *et al*., NO Donors Stimulate Noradrenaline Release from Rat Hippocampus in a Calmodulin-dependent Manner in the Presence of L-Cysteine. *J Cell Physiol*. 1996;**169**:87-96.
11. D. C. Hooper, *et al*., Prevention of Experimental Allergic Encephalomyelitis by Targeting Nitric Oxide and Peroxynitrite: Implications for the Treatment of Multiple Sclerosis. *PNAS*. 1997;**94**:2528-2533.
12. S. Pfeiffer, *et al*., Interference of carboxy-PTIO with Nitric-Oxide and Peroxynitrite-Mediated Reactions. *Free Radic Biol Med*. 1997;**22**:787-794.

2,3-Diaminonaphthalene (for NO detection)

2,3-Diaminonaphthalene [CAS: 771-97-1]

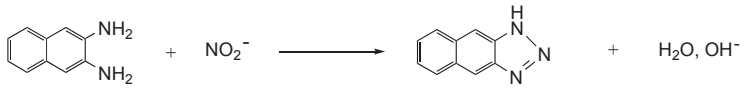
Application: NO_2^- (NO metabolite) detection, fluorometric
Appearance: White or pale yellowish-brown powder
Suitability for NO quantification: pass test
MW: 158.20, $C_{10}H_{10}N_2$

Ordering Information	
Product code	Unit
D418-10	10 mg

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

Reaction of 2,3-Diaminonaphthalene with NO_2^-



Product Description
The Griess assay is a simple and popular method for detecting NO concentration. 2,3-Diaminonaphthalene (DAN) is a highly sensitive alternative to the Griess assay. The DAN method is 50-100 times more sensitive than the Griess assay: While the detection limit of the Griess assay is 1 mM, the limit of the DAN method is 10-50 nM. DAN reacts with NO_2^- in acidic conditions to produce fluorescent naphthalenetriazole. The wavelength of the emission maximum of naphthalenetriazole is 410 nm. However, detection at 450 nm is recommended to avoid fluorescent blanks and increase sensitivity. The fluorescent background of DAN is low for maximum sensitivity. The optimal reaction conditions of DAN with NO_2^- have been determined. The reaction should proceed at pH 2 at room temperature for 5 minutes, and the resulting fluorescence of naphthalenetriazole should be determined at a pH of 10 or more. DAN is a photosensitive reagent and sometimes becomes dark brown colored crystals. Since this brown product cannot be utilized for the fluorescent detection, recrystallization is necessary.

NO_2^- Assay Using 2,3-Diaminonaphthalene (DAN)
1. Dissolve 50 µg DAN in 1 ml 0.62 M HCl to prepare 0.31 mM DAN solution.[a)]
2. Mix 10 µl DAN solution with 100 µl $NaNO_2$ solution (0-10 mM) or sample solution. Incubate the mixture at room temperature for 10-15 minutes.
3. Add 5 µl 2.8 M NaOH solution to the reaction solution.[b)]
4. Dilute 100 µl of this solution with 4 ml water, followed by fluorescent measurement with excitation wavelength at 365 nm and emission wavelength at 450 nm.
5. Prepare a calibration curve using this data where the X-axis is NaNO2 concentration and the Y-axis is fluorescence intensity. Then, use this calibration curve to determine the NO_2^- concentration of the sample solution.

[a)] Acidic conditions are required for a rapid reaction.
[b)] Basic conditions (pH 10 or higher) are required for a high fluorescence signal.

DTCS Na

N-(Dithiocarboxy)sarcosine, disodium salt, dihydrate [CAS: 13442-87-0]

Application: NO detection by ESR
Appearance: White or slightly yellow powder
MW: 245.23, $C_4H_5NNa_2O_2S_2$, $2H_2O$

Ordering Information	
Product code	Unit
D465-10	100 mg
D465-12	500 mg

Storage Condition
0-5 °C, protect from light and moisture

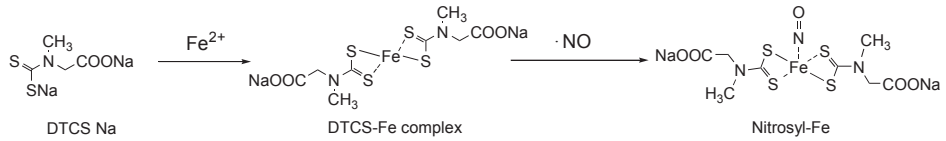
Shipping Condition
ambient temperature

Product Description
Diethyldithiocarbamate (DETC) is a good spin-trapping reagent for nitric oxide *in vivo*. However, DETC has not been widely utilized for NO detection in biological samples due to its poor water solubility. DTCS, an analog of DETC, forms a water-soluble iron(II) complex (Fe-DTCS). The Fe-DTCS complex then forms a complex with NO (NO-Fe-DTCS). Dr. Yoshimura successfully obtained two-dimensional ESR images of NO, induced by lipopolysaccharide in mouse peritoneum. DTCS sodium salt (DTCS Na) was used for this experiment because it is less toxic than ammonium salt (sodium salt LD_{50}: 1942 mg/kg; ammonium salt LD_{50}: 765 mg/kg). Since the Fe-DTCS complex is more stable than the other dithiocarbamate complexes in the air or in aqueous solutions, it could be a useful spintrapping reagent for biochemical research.

Nitric Oxide

The Fe-DTCS complex should be used immediately after preparation. An excessive amount of DTCS Na (usually 5 equivalents DTCS Na to $FeSO_4$) is required to make a more stable solution. Dithiocarbamates tend to decompose under physiological conditions to form toxic carbon disulfide.

Fe Complex formation



Preparation of Fe(II)-DTCS Complex

1. Dissolve 278 mg $FeSO_4$, $7H_2O$ (ferrous sulfate heptahydrate) with 20 ml water[a] to prepare 50 mM $FeSO_4$ solution.[b]
2. Dissolve 123 mg DTCS Na with 10 ml watera) to prepare 50 mM DTCS solution.
3. Mix 1 ml DTCS Na solution with 8.8 ml buffer solutiona) (pH 7 or higher). Add 200 µl $FeSO_4$ solution just prior to use.[c]

[a] *Purge any dissolved oxygen in the water or the buffer by nitrogen gas bubbling for at least 30 minutes prior to dissolving $FeSO_4$.*
[b] *The $FeSO_4$ solution can be stored at -20°C for at least 2 months.*
[c] *Fe(II)-DTCS complex is colorless. If the solution is brown, Fe(III)-DTCS may have formed because of dissolved oxygen in the solution. However, the brown solution can still be used for NO trapping.*

Preparation of NO-Fe(II)-DTCS Complex

1. Under argon gas flow, add 200 µl of $FeSO_4$ solution to 9.4 ml buffer solution (pH 7 or higher). Then introduce NO into the solution by bubbling NO gas through a glass capillary for 15 minutes.
2. Add 400 µl of DTCS Na solution to the $FeSO_4$ solution, and continue to introduce NO by bubbling for another 5 minutes.
3. Remove excess NO with argon gas bubbling for 5 minutes, and store at -20°C. The NO-Fe(II)-DTCS solution can be stored at -20°C for at least 2 months in oxygen-free conditions.

References

1. T. Yoshimura, *et al*., *In vivo* EPR Detection and Imaging of Endogenous Nitric Oxide in Lipopolysaccharide-treated Mice. *Nat Biotechnol*. 1996;**14**:992-994.
2. B. Kalyanaraman, Detectionof Nitric Oxide by Electron Spin Resonance in Chemical, Photochemical, Cellular, Physiological, and Pathophysiological Systems. *Methods Enzymol*. 1996;**268**:168-187.
3. H. Yokoyama, *et al*., In vivo ESR-CT Imaging of the Liver in Mice Receiving Subcutenous Injection of Nitric Oxide-Bound Iron Complex. *Magn Reson Imaging*. 1997;**15**:249-253.

MGD *N*-(Dithiocarbamoyl)-*N*-methyl-D-glucamine, sodium salt [CAS: 94161-07-6 (free acid)]

Application: NO detection with ESR
Appearance: White crystalline powder
Purity: ≥98.0% (HPLC)
MW: 293.34, $C_8H_{16}NNaO_5S_2$

Ordering Information

Product code	Unit
M323-12	500 mg

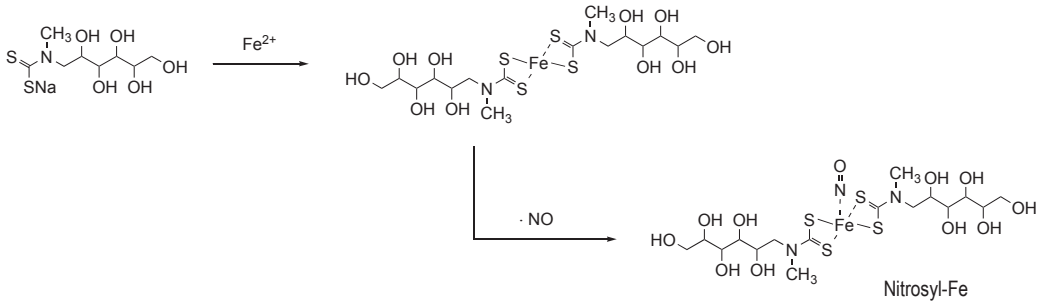
Storage Condition
0-5°C, protect from moisture

Shipping Condition
ambient temperature

Product Description

MGD is a highly water-soluble dithiocarbamate-type chelator that generates with complexes many transitional method such as Fe and Cu. The diethyldithiocarbamate-Fe^{2+} complex has been used for NO detection by electron spin resonance (ESR). However, the poor solubility of this carbamate in an aqueous solution limits its application. Dr. Lai and others improved the technique using a water-soluble dithiocarbamate-Fe^{2+} complex, MGD-Fe^{2+}. They successfully detected *in vivo* NO of a nitroprusside-injected mouse and NO generated by an LPS injection using *in vivo* ESR. The MGD-Fe^{2+} complex is capable of NO detection under physiological conditions, and dissolved oxygen in the solution does not interfere with NO detection.

Fe Complex formation



Preparation of Fe(II)-MGD Complex

1. Dissolve 278 mg $FeSO_4$, $7H_2O$ (ferrous sulfate heptahydrate) with 20 ml water[a] to prepare 50 mM $FeSO_4$ solution.[b]
2. Dissolve 147 mg MGD with 10 ml water[a] to prepare 50 mM MGD solution.
3. Mix 1 ml MGD solution with 8.8 ml buffer solution[a] (pH 7 or higher) and then add 200 µl $FeSO_4$ solution prior to use.[c]

[a] *Purge any dissolved oxygen in the water or buffer by nitrogen gas bubbling for at least 30 minutes prior to dissolving $FeSO_4$.*

[b] *The $FeSO_4$ solution can be stored at -20°C for at least 2 months.*

[c] *Fe(II)-DTCS is colorless. If the solution is brown, Fe(III)-DTCS may have formed because of dissolved oxygen in the solution. However, the brown solution can still be used for NO trapping.*

Preparation of NO-Fe(II)-MGD Complex

1. Under argon gas flow, add 200 µl $FeSO_4$ solution to 9.4 ml buffer solution (pH 7 or higher). Then introduce NO into the solution by bubbling NO gas through a glass capillary for 15 minutes.
2. Add 400 µl MGD solution to the $FeSO_4$ solution and continue to introduce NO by bubbling for another 5 minutes.
3. Remove excess NO with argon gas bubbling for 5 minutes and store at -20°C. The NO-Fe(II)-MGD solution can be stored at -20°C for at least 2 months in oxygen-free conditions.

References

1. L. A. Shinobu, *et al*., Sodium *N*-Methyl-D-glucamine dithiocarbamate and Cadmium in toxication. *Acta Pharmacol Toxicol*. 1984;**54**:189-194.
2. A. Komarov, *et al*., *In vivo* Spin Trapping of Nitric Oxide in Mice. *Biochem Biophys Res Commun*. 1993;**195**:1191-1198.
3. C. S. Lai, *et al*., Spin Trapping of Nitric Oxide Produced *in vivo* in Septic-shock Mice. *FEBS Lett*. 1994;**345**:120-124.
4. P. Giboreau, *et al*., Procedure for the Preparation of Pure Dithiocarbamates. *J Org Chem*. 1994;**59**:1205-1207.
5. B. Kalyanaraman, Detectionof Nitric Oxide by Electron Spin Resonance in Chemical, Photochemical, Cellular, Physiological, and Pathophysiological Systems. *Methods Enzymol*. 1996;**268**:168-187.
6. H. Yokoyama, *et al*., *In vivo* ESR-CT Imaging of the Liver in Mice Receiving Subcutenous Injection of Nitric Oxide-Bound Iron Complex. *Magn Reson Imaging*. 1997;**15**:249-253.
7. V. D. Mikoian, *et al*., Hydrophobic and Hydrophylic Fe^{2+} Complexes with Derivatives of Dithiocarbamates as a Traps of Nitric Oxide in Mouse Organism. *BIOFIZIKA*. 1997;**42**:490-501.

Introduction

Highly stable molecular layers prepared by the self-assembling method have been used for the development of electrochemical, optical, and various other detection systems. Self-assembled monolayers (SAMs) are crystalline, chemisorbed, organic single layers formed on a solid substrate by the spontaneous organization of molecules. Thiol compounds and gold is a well established combination[1]. Carboxylic acids, organosilicon derivatives, and diphosphonates on various metal oxide surfaces have also been explored in recent years in an effort to find a good model for such adhesive processes (Fig. 1). SAMs are easily modified at the single molecular and assembled levels. They are very useful research models to promote our understanding of the self organization mechanism of molecules, molecular structure and property relationships, and phenomena at the interface between different phases. The high flexibility of SAMs has allowed us to study the electron transfer mechanism of proteins, molecular layers, and biosensors.

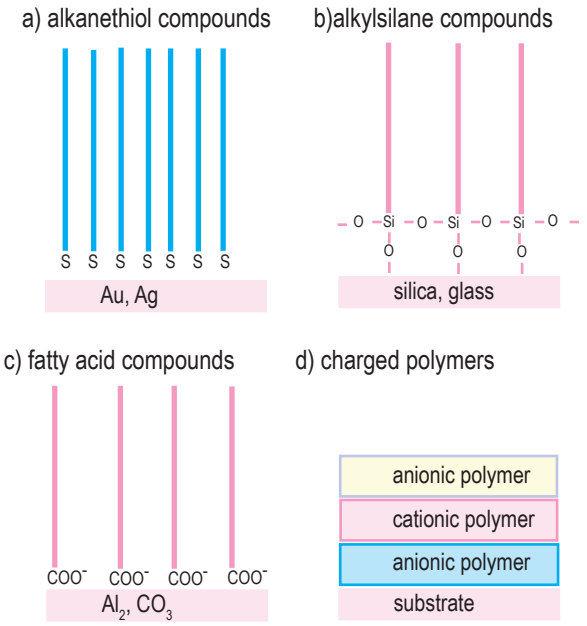


Fig. 1 Model of the Surfaces of Various SAMs

Self-Assembling Process of Alkanelthiols

The self-assembling process of alkanethiols on gold is initiated by the strong chemical interactions between the sulfur and gold surface. This interaction is a result of chemisorption that forces the thiolate molecule to adsorb to a gold lattice. The tail-to-tail interactions of the alkanethiolate molecules (created by lateral, interchain, nonbonded interactions, such as *van der Waals*, steric, repulsive, and electrostatic forces), are strong enough that the molecules align parallel on the gold surface and create a crystalline film.[1),2)] (Fig. 2). Therefore, the packing and ordering of molecules is controlled by a chemisorption mechanism.[3),4)] In practice, the solid surface is simply dipped into a solution containing adsorbing molecules. Organic disulfides, thiols, and sulfides are widely used for the preparation of stable SAMs on gold surfaces. The self-assembling process and the orientation of the molecular layer on a gold surface have been investigated thoroughly using Fourier transform infrared spectroscopy (FT-IR),[5),6)] scanning tunneling microscopy (STM),[1)] atomic force microscopy (AFM),[7)] X-ray photoelectron spectroscopy (XPS),[8),9)] electro-chemistry,[4),10)] Raman spectroscopy,[11)] Ellipsometry,[12),13)] and quartz crystal microbalance (QCM).[14),15)] Please refer to the papers by Dr. Ulman for extensive reviews about the selfassembly process.[3),4)]

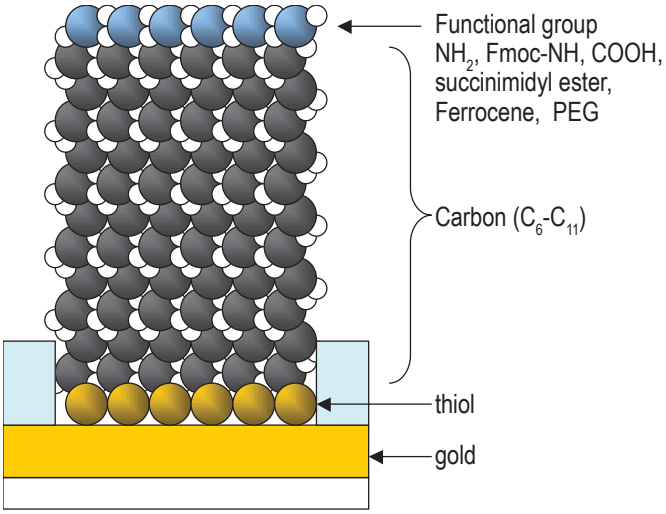


Fig. 2 SAM Structure Prepared by Alkanethiol

Electrochemical Sensing by SAMs-Modified Electrodes

A common application of SAMs is in the creation of sensors with molecular recognition properties. Many groups have studied the electrochemical characteristics of alkylthiols for ion detection and molecular recognition. For example, Turyan and others used SAMs composed of ω-mercapto-carboxylic acids on mercury film and gold electrodes for very sensitive and selective analyses of cadmium(II).[16)] Taniguchi and others created a membrane model with molecular gating by incorporating Meldola's Blue into self-assembled decanethiol monolayer-coated electrodes.[17)] Katayama and Maeda reported the electrochemical detection of cyclic AMP by a 17-mer oligopeptide-coated gold electrode. They showed that this response was dependent on the cyclic AMP concentration, but not ATP, using cyclic voltammograms of ferrocyanide/ferricyanide redox coupled with the electrode.[18)] Wang and others reviewed the behavior, utility, and advantages of an amperometric flow detector coated with unsubstituted *n*-alkylthiols. They indicated that a hydrophilic alkylthiol monolayer has a high selectivity toward lipophilic drugs such as chloropromazine and dipamine.[19)]

Detection of Histidine-Tagged Protein Using NTA-Attached SAMs

The use of a short peptide as an affinity tag is one of the most common methods for the detection and purification of recombinant proteins. These tag proteins are mostly antibody epitopes that are detected with their antibodies.[20)] Sigal and others prepared a self-assembled monolayer that selectively binds protein with a stretch of six histidines (His-tag). They prepared two alkanethiols, one with a nitrilotriacetic acid (NTA) group that forms a tetravalent chelate with Ni(II) ion and the other with a triethyleneglycol group that is capable of avoiding nonspecific adsorption of protein (Fig. 3). This membrane can only recognize a His-tag protein through the nickel(II) ion chelated with NTA on the SAM. This technique is useful for immobilization of His-tag proteins for study using surface plasmon resonance (SPR)[21)].

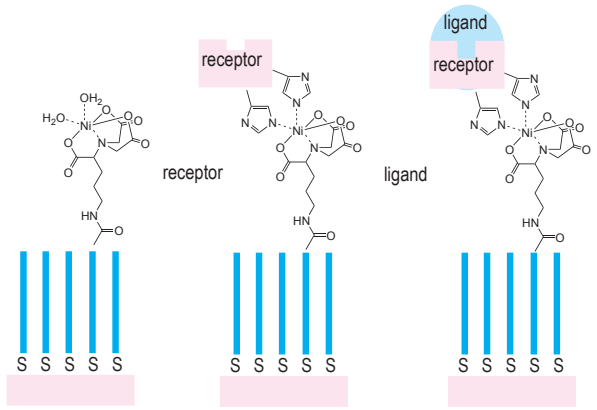


Fig. 3 Procedure for Immobilization of Receptor using His-Tag

Surface Plasmon Resonance Studies for Interactions of SAMs and Proteins

Surface plasmon resonance (SPR) is commonly used to measure the kinetics of association and dissociation of ligands and proteins in aqueous solution. It is particularly powerful for the observation of processes occurring at or near interfaces. The sensing element is a thin (40-50 nm) gold or silver film deposited on a glass surface. The back of the gold-coated glass slide is irradiated with *p*-polarized light, with the reflection angles controlled to minimize the intensity of the reflection. Since the value of this reflection angle linearly depends on the amount of protein adsorbed on the surface, the protein can be monitored by SPR (Fig. 4).[20-22]

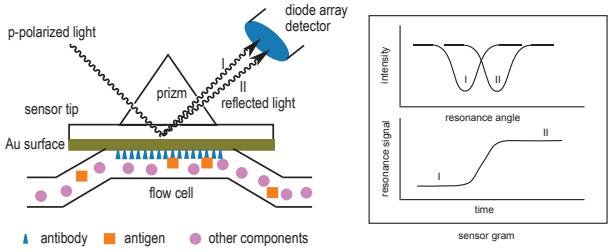


Fig. 4 Schematic Diagram of an Experimental Setup for SPR Measurement

Mrksich and others studied the thermodynamic and kinetic mechanism between benzenesulfonamide-attached SAM and bovine carbonic anhydrase (EC 4.2.1.1) using the SPR technique.[22),23)] Ringsdorf and others prepared protein triple layers on biotin-attached SAMs on gold surface using streptavidin and anti-chorionic gonadotropin-Fab (anti-hCG-Fab) fragments (Fig. 5). They investigated the hinge, or linkage region, of the Fab fragment; the second layer formation (streptavidin, Fab fragment); the third layer formation by antigen hCG; and quantification of these processes. SPR coupled with SAM is an excellent method for determining surface phenomena. The SPR method has been used for the determination of epitopes of monoclonal antibodies, development of immuno-detection systems, research on signal transfer mechanisms, and other studies.

Electrochemical Studies of Proteins on SAMs-Modified Electrodes

Protein-coated electrodes provide suitable systems for the development of devices using protein layer interfaces.[24)] Cytochrome c, ferredoxin, and myoglobin are particularly interesting proteins for interfacial electrochemical studies. For example, Niki's group has been studying diffusionless standard electron transfer rate constants of cytochrome c immobilized on a carboxylic acid-attached SAM with an electroreflectance spectroscope.[25)] Several researchers have used electrochemical techniques for characterizing the absorption of poly(L-lysine)-coated SAMs. Poly(L-lysine) was fixed to carboxylic acid-attached SAMs through an electrostatic binding between a negative charge of carboxylate and a positive charge of amine residue. The poly(L-lysine)-coated SAMs were used to develop a detection system for glucose and for research on the electron transfer mechanism of cytochrome c.[24),26),27),28)] Nakashima's group prepared polyethyleneglycol- attached SAMs and reported that a supramolecular structure was formed by the interaction between poly(ethyleneglycol) and acyclodextrin.[29)]

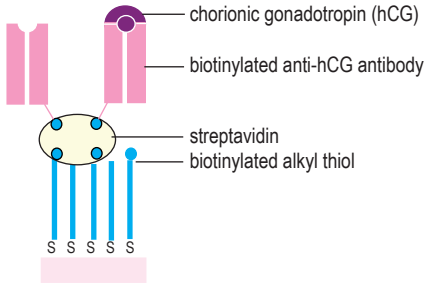


Fig. 5 Protein triple layer on SAM using biotinylated alkyl thiol

DNA Sensor Using Electrochemical and Quartz Crystal Microbalance Method

Electrochemical DNA sensors may be useful devices for the sequence-specific detection and quantification of DNA or RNA in solutions. The amount of DNA or RNA in solution can be measured as an amperometric or voltammetric signal.[24)] Katayama and others prepared a calf thymus DNA-coated gold electrode to detect anti-DNA antibody. They demonstrated that this system could be useful for the development of biosensors for DNA-binding proteins.[30)] Okahata's group used frequency changes in quartz crystal microbalance (QCM) to detect one-to-one hybridization between oligonucleotides immobilized on gold electrodes of the QCM and target M13 phage DNA in aqueous solution. They prepared a 10-mer deoxynucleotide with a mercaptopropyl group at the 5'-phosphate end whose sequence was complementary with the EcoR1 binding site of single-stranded M13 phage DNA.31),[32)] The QCM method is very sensitive because its resonance frequency decreases upon the increase of the mass on the QCM. The QCM method is useful for the study of molecular kinetics of base-pair hybridization in oligonucleotides and to detect various biological materials.

Table 1 Solubility Data

	Methyl alcohol				Ethyl alcohol				Chloroform				Methylene chloride			
	10mM	1mM	100μM	10μM	10mM	1mM	100μM	10μM	10mM	1mM	100μM	10μM	10mM	1mM	100μM	10μM
11-AUT (A423)	S				S				S				I	I	S	
8-AOT (A424)	S				S				S				S			
6-AHT (A425)	S				S				I	S			I	S		
10-CDT (C385)	S				S				S				S			
7-CHT (C386)	S				S				S				S			
5-CPT (C387)	S				S				S				S			
11-HUT (H337)	S				S				S				S			
8-HOT (H338)	S				S				S				S			
6-HHT (H339)	S				S				S				S			
11-FUT (F246)	I	S			L	S			S				S			
8-FOT (F247)	S				L	S			S				S			
6-FHT (F269)	I	S			L	S			S				S			

	Tetrahydrofuran				Acetonitrile				Ethyl acetate				Hexane			
	10mM	1mM	100μM	10μM	10mM	1mM	100μM	10μM	10mM	1mM	100μM	10μM	10mM	1mM	100μM	10μM
11-AUT (A423)	I	I	I	S	I	I	I	I	I	I	I	I	I	I	I	I
8-AOT (A424)	L	L	S		I	L	S		I	I	L	S	I	I	I	I
6-AHT (A425)	I	L	L	S	I	I	I	I	I	I	I	I	I	I	I	I
10-CDT (C385)	S				L	L	L	L	L	L	L	L	S			
7-CHT (C386)	S				L	L	L	L	L	L	L	L	S			
5-CPT (C387)	S				L	L	L	L	L	L	L	L	S			
11-HUT (H337)	S				I	I	L	L	L	L	L	L	I	S		
8-HOT (H338)	S				L	S			L	L	L	L	S			
6-HHT (H339)	S				L	S			L	L	L	L	S			
11-FUT (F246)	S				L	L	L	L	L	L	L	L	S			
8-FOT (F247)	S				L	L	L	L	L	L	L	L	S			
6-FHT (F269)	L	L	S		S				L	L	L	L	I	S		

	Dimethylsulfoxide				N,N-Dimethylformamide				H2O			
	10mM	1mM	100μM	10μM	10mM	1mM	100μM	10μM	10mM	1mM	100μM	10μM
11-AUT (A423)	S				S				I	S		
8-AOT (A424)	S				S				S			
6-AHT (A425)	S				S				I	S		
10-CDT (C385)	S				S				I	I	I	I
7-CHT (C386)	S				S				I	S		
5-CPT (C387)	S				S				S			
11-HUT (H337)	S				S				I	I	I	I
8-HOT (H338)	S				S				I	S		
6-HHT (H339)	S				S				S			
11-FUT (F246)	S				S				I	I	I	I
8-FOT (F247)	S				S				I	I	I	I
6-FHT (F269)	S				S				I	I	I	I

S: Soluble
L: Low Solubility
I: Insoluble

References

1) C. Schonenberger, *et al., J. Phy. Chem.*, **99**, 3259 (1995);2) K. Kajikawa, *et al., Molecular Electronics and Bioelectronics*, **7**, 2 (1996); 3) A. Ulman, "*An Introduction to Ultra Thin Organic Films from Langmuir-Blodgett to Self-Assembly*" Academic Press, San Diego, (1991); 4) A. Ulman, *MRS Bulletin*, **1995(6)**, 46 (1995); 5) Y. Sato, *et al., Langmuir*, **12**, 2726 (1996); 6) Y. Sato, *et al., Bull. Chem. Soc. Jpn.*, **67**, 21 (1994); 7) J. L. Wibur, *Langmuir*, **11**, 825 (1995); 8) C. D. Bain, *et al., J. Am. Chem. Soc.*, **110**, 3665 (1998); 9) P. E. Laibinis, *et al., J. Phy. Chem.*, **95**, 7017 (1991); 10) C. A. Widrig, *et al., J. Electroanal. Chem.*, **310**, 335 (1991); 11) M. A. Bryan, *et al., J. Am. Chem. Soc.*, **113**, 8284 (1991); 12) C. E. D. Chidsey, *et al., Langmuir*, **6**, 682 (1990); 13) T. Ohtsukav, *et al., Langmuir,* **10**, 3658 (1994); 14) K. Shimizu, *et al., Langmuir,* **8**, 1385 (1992); 15) T. W. Schneider, *et al., J. Am. Chem. Soc.*, **115**, 12391 (1993); 16) I. Turyan, *et al., Anal Chem.*, **66**, 58 (1994); 17) I. Taniguchi, *et al., Microchem. J.*, **49**, 340 (1994); 18) Y. Katayama, *et al., Chem. Lett.,* 884 (1997); 19) J. Wang, *et al., Anal. Chem.*, **65**, 1893 (1993); 20) S. Hashimoto, *Bunseki*, **5**, 362 (1997); 21) G. B. Sigal, *et al., Anal. Chem.*, **68**, 490 (1996); 22) M. Mrksich, *et al., J. Am. Chem. Soc.*, **117**, 12009 (1995); 23) M. Mrksich, *et al., Langmuir,* **11**, 4383 (1995); 24) J. L. Anderson, *et al., Anal. Chem.*, **68**, 379R (1996); 25) Z. Q. Feng, *et al., J. Electroanal. Chem.*, **394**, 149 (1995); 26) C. E. Jordan, *et al., Langmuir*, **10**, 3642 (1994); 27) J. D. H. Glenn, *et al., Chem. Lett.*, 399 (1996); 28) F. Mizutani, *et al., Chem. Lett.*, 251 (1996); 29) N. Nakashima, *et al., Chem. Lett.*, 731 (1997); 30) Y. Katayama, *et al., Chem. Lett.*, 1181 (1998); 31) Y. Okahata, *et al., J. Am. Chem. Soc.*, **114**, 8299 (1992); 32) K. Niikura, *et al., Chem. Lett.*, **863** (1996).

Biotin-SAM Formation Reagent

Appearance: White or slightly yellowish solid

Features: Simple preparation of Biotin-SAM on gold surface
Efficient Immobilization of Avidins
Suppress unspecific binding with unknown proteins

Ordering Information

Product code	Unit
B564-10	1 µmol x 3

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Keyword: Self-Assembled Monolayers, SAM, SPR, QCM, biosensors, protein immobilization

Product Description

There are three ways to immobilize a protein on an Surface Plasmon Resonance (SPR) or Quartz Crystal Microbalance (QCM) biosensor consisting of Self-Assembled Monolayer (SAM); 1) Formation of covalent bond of amine functions on proteins and activated carboxy-SAM; 2) immobilize proteins by His-Tag through Ni-NTA-terminated SAM; 3) Iimmobilization of proteins through Biotin and Avidin interaction. Because the Biotin-Avidin formation is known as facile reaction, it is widely utilized for immobilization of protein (Fig 1).

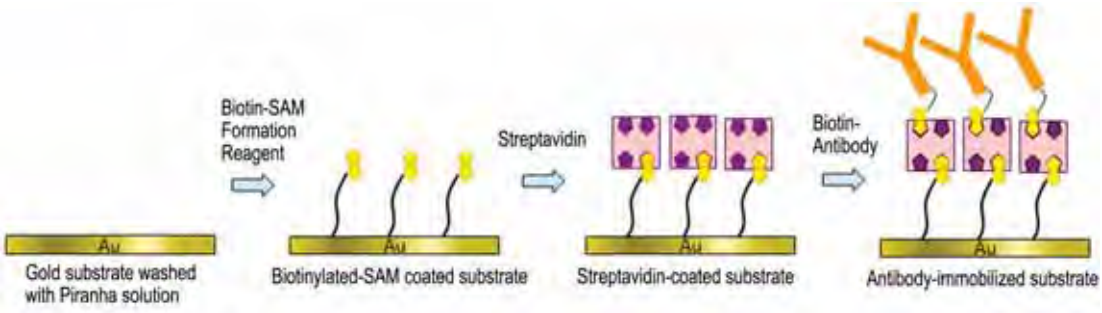


Fig. 1 Illustration of biosensor constructed with Biotin-SAM Formation Reagent

Dojindo's Biotin-SAM Formation Reagent is utilized in a biosensor that is minimized unspecific binding, and it efficiently immobilizes Avidin such as Streptavidin or NeutrAvidin. In fact, a sensor prepared with Dojindo's Biotin-SAM Formation is presented with less unspecific binding than a conventional product (Fig. 2). Therefore, biotinylated proteins are more efficiently immobilized on the sensor for further analysis. The product can also be utilized with biotinylated DNA or peptides.

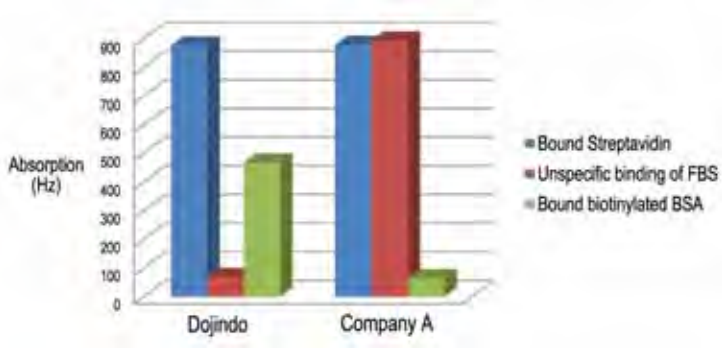


Fig. 2 Comparison of the amount of bound streptavidin, unspecific binding of FBS, and bound biotinylated BSA between Dojindo's Biotin-SAM Formation Reagent and Company A.

Sulfobetaine3-undecanethiol

N-(11-Mercaptoundecyl)-*N, N*-dimethy 1-3-ammonio-1-propanesulfonate [CAS: 343624-84-0]

Application: SAM preparation

Appearance: White or slightly yellow solid
Purity: 98.0% (HPLC)
NMR Spectrum: Authentic
MW: 353.59, $C_{16}H_{35}NO_3S_2$

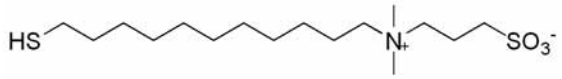
Ordering Information

Product code	Unit
S350-10	10 mg

Storage Condition
0-5°C

Shipping Condition
with blue ice

Chemical Structure



Product Description
It has been reported that sulfobetaine derivative- introduced SAM produces low unspecific binding under ion intensity above 200 mmol/l and mildly alkaline conditions. It is, however, possible to prepare biosensor with higher sensitivity by using the unique characteristics of sulfobetaine derivative-introduced SAM with other thiol and disulfide reagent mixure SAM. In addition, Ostuni ***et al***. used Dojindo's sulfobetaine3-undecanethiol for patterning research of bacteria and mammalian cells on gold substrate. Therefore, sulfobetaine derivatives are applicable in patterning research using biological materials.

Reference
1. R. E. Holmlin, X. Chen, R. G. Chapman, S. Takayama, G. M. Whitesides, *Langmuir,* 2001, **17**, 2841-2850
2. E. Ostuni, R. G. Chapman, M. N. Liang, G. Meluleni, G. Pier, D. E. Ingber, G. M. Whitesides, *Langmuir,* 2001, **17**, 6336-6343

16-Amino-1-hexadecanethiol, hydrochloride

16-Amino-1-hexadecanethiol, hydrochloride

Application: SAM preparation, amine group coating

Appearance: White powder
Purity: ≥95.0% (HPLC)
MW: 309.98, $C_{16}H_{36}ClNS$

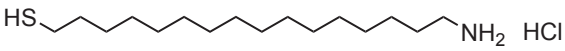
Ordering Information

Product code	Unit
A458-10	10 mg
A458-12	100 mg

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



11-Amino-1-undecanethiol, hydrochloride

11-Amino-1-undecanethiol, hydrochloride [CAS: 143339-58-6]

Application: SAM preparation, amine group coating

Appearance: White or slightly yellowish white crystalline powder
Purity: ≥90.0% (HPLC)
MW: 239.85, $C_{11}H_{26}ClNS$

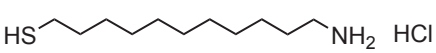
Ordering Information

Product code	Unit
A423-10	10 mg
A423-12	100 mg

Storage Condition
-20°C, protect from metal and moisture

Shipping Condition
ambient temperature

Chemical Structure



8-Amino-1-octanethiol, hydrochloride

8-Amino-1-octanethiol, hydrochloride

Application: SAM preparation, amine group coating

Appearance: White or slightly reddish white crystalline powder
Purity: ≥90.0% (HPLC)
MW: 197.77, $C_8H_{20}ClNS$

Ordering Information

Product code	Unit
A424-10	10 mg
A424-12	100 mg

Storage Condition
-20°C, protect from metal and moisture

Shipping Condition
ambient temperature

Chemical Structure

6-Amino-1-hexanethiol, hydrochloride

6-Amino-1-hexanethiol, hydrochloride [CAS: 31098-40-5]

Application: SAM preparation, amine group coating

Appearance: White or slightly reddish white crystalline powder
Purity: ≥90.0% (HPLC)
MW: 169.72, $C_6H_{16}ClNS$

Ordering Information

Product code	Unit
A425-10	10 mg
A425-12	100 mg

Storage Condition
-20°C, protect from metal and moisture

Shipping Condition
ambient temperature

Chemical Structure

Product Description

Aminoalkanethiols are utilized for the modification of a gold surface to introduce amino groups on the surface. Dojindo's newly developed 16-Amino-1-hexadecanethiol has a 16-carbon chain, which is the longest alkanethiol available in the market. It is expected that 16-Amino-1-hexadecanethiolwill form the most stable SAM on a gold surface among the aminoalkanethiol compounds because of the greater Van-der-Waals force between alkane groups. Five different aminoalkanethiols including Amino-EG6-undecanethiol, hydrochloride are available for gold surface modification. Amino-EG_6-undecanethiol is used for hydrophilic surface preparation. The amino group is usually modified using aminereactivematerials, such as proteins or biomaterials, to functionalize the gold surface. Several researchers have reported SAMs of short alkylchain aminoalkanethiols, and there are an increasing number of reports of long alkyl chain compounds. Takahara and others formed a monolayer of 11-Amino-1-undecanethiol on a gold electrode and studied the effect of the terminal groups on the redox responses of ferrocene derivatives using the voltammetric method. They also reported the relationship between the alkyl chain length of aminoalkanethiols and the redox behavior of 2,3-dichloro-1,4-naphtoquinone attached to the terminal amino group. Tanahashi and coworkers modified a gold surface with SAMs of several kinds of functionalized alkanethiols. They reported the effect of their terminal functional groups on apatite formation in a simulated body fluid using X-ray photoelectron spectroscopic (XPS) measurement and quartz crystal microbalance (QCM) method.

References

A458

1. R. P. Solanki, K. S. Arya, Y. Nishimura, M. Iwamoto, B. D. Malhotra, *Langmuir*, 2007, **23**, 7398-7403.
2. J. M. Brockman, A. G. Fruto and R. M. Corn, *J. Am. Chem. Soc.*, 1999, **121**, 8044-8051.

A423

1. J. M. Brockman, A. G. Frutos and R. M. Corn, A Multistep Chemical Modification Procedure To Create DNA Arrays on Gold Surface for the Study of Protein-DNA Interactions with Surface Plasmon Resonance Imaging, *J. Am. Chem. Soc.,* 1999, **121**, 8044.
2. Y. Yoshimi, T. Matsuda, Y. Itoh, F. Ogata and T. Katsube, Surface Modifications of Functional Electrodes of a Light Addressable Potentiometric Sensor (LAPS): Non-Dependency of pH Sensitivity on the Surface Functional Group, *Mater. Sci. Eng. C*, 1997, ***5***, 131.
3. M. Tanahashi and T. Matsuda, Surface Functional Group Dependence on Apatite Formation on Self-assembled Monolayers in a Simulated Body Fluid, *J. Biomed. Mater. Res.*, 1997, ***34***, 305.
4. J. Tien, A. Terfort and G. M. Whitesides, Microfaburication through Electrostatic Self-Assembly, *Langmuir*, 1997, ***13***, 5349.
5. F. Mukae, H. Takemura and K. Takehara, Electrochemical Behavior of the Naphtoquinone Anchored onto a Gold Electrode through the Self-Assembled monolayers of Aminoalkanethiol, Bull. Chem. *Soc. Jpn.*, 1996, ***69***, 2461.

6. S. Rubin, G. Bar, R. W. Cutts, J. T. Chow, J. P. Ferraris, and T. A. Zawodzinski, Electrical Communication Between Glucose Oxidase and Different Ferrocenylalkanethiol Chain Lengths, Mater. Res. *Soc. Symp. Proc.*, 1996, **413**, 377.
7. K. Takehara and H. Takemura, Electrochemical Behaviors of Ferrocene Derivatives at an Electrode Modified with Terminally Substituted Alkanethiol Monolayer Assemblies, Bull. Chem. *Soc. Jpn.*, 1995, **68**, 1289.
8. K. Takehara, H. Takemura and Y. Ide, Electrochemical Studies of the Terminally Substituted Alkanethiol Monolayers Formed on a Gold Electrode; Effect of the Terminal Group on the Redox Responses of Fe(CN)63-,Ru(NH3)63+ and Ferrocenedimethanol, *Electrochim. Acta*, 1994, **39**, 817.
9. H. J. Lee, A. W. Wark, Y. Li and R. M. Corn, Fabricating RNA Microarrays with RNA-DNA Surface Ligation Chemistry, *Anal. Chem.*, 2005, **77**, 7832.

A424, A425

1. J. M. Brockman, A. G. Frutos and R. M. Corn, A Multistep Chemical Modification Procedure To Create DNA Arrays on Gold Surface for the Study of Protein-DNA Interactions with Surface Plasmon Resonance Imaging, *J. Am. Chem. Soc.*, 1999, **121**, 8044.
2. Y. Yoshimi, T. Matsuda, Y. Itoh, F. Ogata and T. Katsube, Surface Modifications of Functional Electrodes of a Light Addressable Potentiometric Sensor (LAPS): Non-Dependency of pH Sensitivity on the Surface Functional Group, *Mater. Sci. Eng. C*, 1997, **5**, 131.
3. M. Tanahashi and T. Matsuda, Surface Functional Group Dependence on Apatite Formation on Self-assembled Monolayers in a Simulated Body Fluid, *J. Biomed. Mater. Res.*, 1997, **34**, 305.
4. J. Tien, A. Terfort and G. M. Whitesides, Microfaburication through Electrostatic Self-Assembly, *Langmuir*, 1997, **13**, 5349.
5. F. Mukae, H. Takemura and K. Takehara, Electrochemical Behavior of the Naphtoquinone Anchored onto a Gold Electrode through the Self-Assembled monolayers of Aminoalkanethiol, Bull. *Chem. Soc. Jpn.*, 1996, **69**, 2461.
6. S. Rubin, G. Bar, R. W. Cutts, J. T. Chow, J. P. Ferraris, and T. A. Zawodzinski, Electrical Communication Between Glucose Oxidase and Different Ferrocenylalkanethiol Chain Lengths, *Mater. Res. Soc. Symp. Proc.*, 1996, **413**, 377.
7. K. Takehara and H. Takemura, Electrochemical Behaviors of Ferrocene Derivatives at an Electrode Modified with Terminally Substituted Alkanethiol Monolayer Assemblies, *Bull. Chem. Soc. Jpn.*, 1995, **68**, 1289.
8. K. Takehara, H. Takemura and Y. Ide, Electrochemical Studies of the Terminally Substituted Alkanethiol Monolayers Formed on a Gold Electrode; Effect of the Terminal Group on the Redox Responses of Fe(CN)63-,Ru(NH3)63+ and Ferrocenedimethanol, *Electrochim. Acta*, 1994, **39**, 817.

5-Amido-1-pentanethiol

6-Mercaptohexanamide [CAS: 80389-37-3]

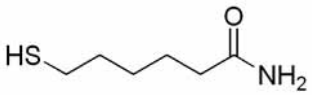
Application: SAM preparation, amine group coating

Appearance: White or slightly yellow solid
Purity: ≥98.0% (HPLC)
MW: 147.24, $C_6H_{13}NOS$

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
A508-10	10 mg
A508-12	100 mg

7-Amido-1-heptanethiol

8-Mercaptooctanamide

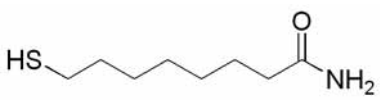
Application: SAM preparation, amine group coating

Appearance: White or slightly yellow solid
Purity: ≥98.0% (HPLC)
MW: 175.29, $C_8H_{17}NOS$

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
A509-10	10 mg
A509-12	100 mg

10-Amido-1-decanethiol

11-Mercaptoundecanamide [CAS: 139041-92-2]

Application: SAM preparation, amine group coating

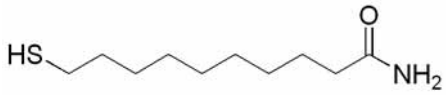
Appearance: White or slightly yellow solid
Purity: ≥98.0% (HPLC)
MW: 217.37, $C_{11}H_{23}NOS$

Ordering Information	
Product code	Unit
A510-10	10 mg
A510-12	100 mg

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Chemical Structure



Product Description

It has been reported that compared to other derivative-introduced SAM, amido derivative- introduced SAM has higher heat stability because of its hydrogen-bonding group. In addition, Mosley ***et al***. used this hydrogen-bonding group to prepare detachable polymer sheet, used the polymer sheet as template, and used amido derivative- introduced SAM. Amido derivative-introduced SAM has been used for the relationship between wettability and protein absorption research, and it has been confirmed that amido derivative-introduced SAM has been known to control protein absorption.

References

A508, A509, A510

1. G. B. Sigal, M. Mrksich, G. M. Whitesides, *J. Am. Chem. Soc.*, 1998, **120**, 3464–3473
2. R. Valiokas, M. Ostblom, S. Svedhem, S. C. T. Svensson, B. Liedberg, *J. Phys. Chem.* B, 2002, **106**, 11550–11556
3. D. W. Mosley, M. A. Sellmyer, E. J. Daida, J. M. Jacobson, *J. Am. Chem. Soc.,* 2003, **125**, 10532–10533
4. A. Sethuraman, M. Han, R. S. Kane, G. Belfort, *Langmuir,* 2004, **20**, 7779–7788

Amino-EG$_6$-undecanethiol, hydrochloride

20-(11-Mercaptoundecanyloxy)-3, 6, 9, 12, 15, 18-hexaoxaeicosane-1-amine, hydrochloride [CAS: 496839-01-1]

Application: SAM preparation, amine group coating, and hydrophilic surface

Appearance: White or pale yellow solid
Purity: ≥90.0% (HPLC)
MW: 504.16, $C_{23}H_{50}ClNO_6S$

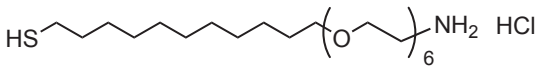
Ordering Information

Product code	Unit
A483-10	10 mg

Storage Condition
-20°C, protect from metal and moisture

Shipping Condition
ambient temperature

Structural Formula



Amino-EG$_6$-hexadecanethiol,hydrochloride

20-(16-Mercaptohexadecanyloxy)-3,6,9,12,15,18-hexaoxaeicosane-1-amine, hydrochloride

Application: SAM preparation, amine group coating

Appearance: White or slightly yellow solid
Purity: ≥95.0% (HPLC)
Solubility:10 mmol/L (Ethyl alcohol, Methyl alcohol, Chloroform)
MW: 574.30, $C_{28}H_{60}ClNO_6S$

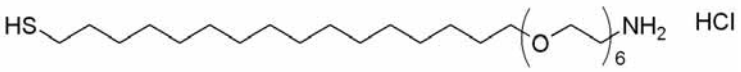
Ordering Information

Product code	Unit
A505-10	10 mg

Storage Condition
-20°C, protect from metal

Shipping Condition
ambient temperature

Structural Formula



Product Description

Polyethyleneglycols (PEGs) are widely used for material modifications to improve hydrophilicity of the surface. PEG-coated materials are usually more stable under physiological conditions. Since Amino-EG$_6$-undecanethiol has 6 ethylene glycol units, 11 carbon atoms, and an SH group at the end, it can be used to prepare a highly oriented and hydrophilic SAM on a gold surface. This is suitable for biomaterial labeling on the surface due to the improved hydrophilicity. The hydrophilic surface can prevent proteins or other biomaterials from non-specific binding. Therefore, the SAM prepared by this reagent will provide a better surface to develop biomaterial sensors or DNA/ protein microarrays. To prepare an Amino-EG$_6$-SAM on a gold surface, hydroxy-EGn-undecanethiols (n=3, 6) are used to dilute the number of amino groups according to the density of the molecules being introduced onto the surface.

How to Prepare SAM

1. Soak a gold-coated glass plate in Piranha solutiona) for 10-15 minutes. Wash the plate with purified water.[a)]
2. Dissolve aminoalkanethiol compound in ethanol to prepare several mM to several ten mM solutions.
3. Soak the plate in the aminoalkanethiol solution for a certain time period.[b)]
4. Wash the SAM-coated plate with ethanol and then water.
5. Dry the plate under nitrogen atmosphere, if necessary.

[a)] Piranha solution: sulfuric acid and 30% hydrogen peroxide, 3:1. Piranha solution is a strong oxidizing agent. Extreme care is necessary when using it. Do not apply Piranha solution to resin-coated plates; it may erode the resin.
[b)] To prepare a SAM-coated plate with the best performance, aminoalkanethiol concentration and soaking time should be individually determined.

Application of SAM-Preparation of DNA Array (Fig. 1)

1. Use SF10 glass slides (Schott Glass Technologies) coated with 5 nm chromium and 45 nm gold thin film.
2. Soak the glass slide in a 1 mM 1-octadecanethiol (ODT)/ethanol solution overnight to prepare ODT SAM-coated slide.
3. Draw 500 µm x 500 µm patterns on the ODT SAM-coated slide by UV irradiation with an Hg-Xe arc lamp.[a)]
4. Soak the slide in a 1 mM 11-amino-1-undecanethiol (AUT)/ethanol solution for 2 hours to form AUT SAM on the 500 µm x 500 µm photopatterned area.
5. Drop 2 mM SPDP solution[b)] onto the slide and leave the slide at room temperature.
6. Wash the slide and dry under nitrogen atmosphere.
7. Apply 1 mM thiol-DNA solution[c)] to each 500 µm x 500 µm pattern and incubate at room temperature overnight.
8. Incubate the slide with a sample solution for 10 minutes and wash with phosphate buffer, followed by SPR imaging.

a) Irradiation time: 1-1.5 hours
b) SPDP: N-succinimidyl 3-(2-pyridyldithio)propionate. Dissolve SPDP in DMSO to prepare 50 mM solution. Dilute it 25 times with 100 mM triethanolamine buffer, pH 7.0.
c) Dissolve thiol-DNA with 100 mM triethanolamine buffer, pH 8.0.

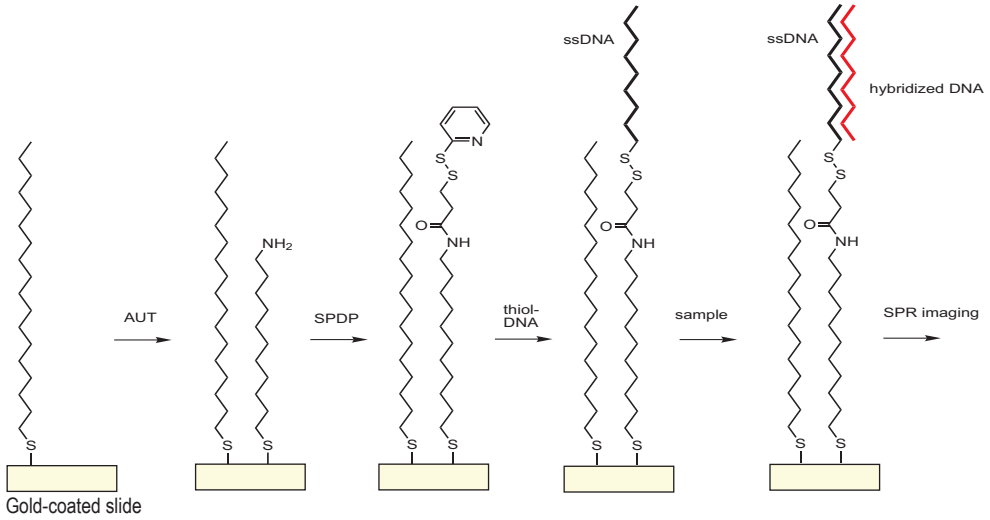


Fig. 1 DNA Array Prepareation Scheme

Reference

A483

1. C. Pale-Grosdemange, E. S. Simon, K. L. Prime, and G. M. Whitesides, *Anal. Chem.*, 1999, ***71***, 777-790.
2. M. Kyo, K.Usui-Aoki, H. Koga, *Anal. Chem*, 2005, ***77***, 7115-7121.
3. G. B. Sigal, M. Mrksich, and G. M. Whitesides, *J. Am. Chem. Soc.*, 1998, ***120***, 3464-3473.
4. Y. Li, H. J. Lee, and R. M. Corn, *Nucleic Acids Research*, 2006, ***34***, 6416-6424.

A505

1. C. Pale-Grosdemange, E. S. Simon, K. L. Prime, and G. M. Whitesides, *Anal. Chem.*, 1999, **71**, 777-790.

N-Fmoc-Aminoundecanethiol

Fluoren-9-ylmethyl *N*-(11-mercaptoundecyl) carbamate

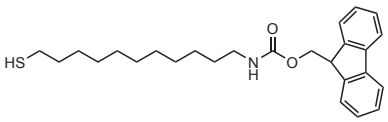
Application: SAM preparation, protected amine group coating

Appearance: White powder or crystalline powder
Purity: ≥90.0% (HPLC)
MW: 425.63, $C_{26}H_{35}NO_2S$

Storage Condition
-20°C, protect from metal

Shipping Condition
with blue ice or dry ice

Structural Formula



Ordering Information

Product code	Unit
F287-10	10 mg
F287-12	50 mg

N-Fmoc-Aminooctanethiol

Fluoren-9-ylmethyl *N*-(8-mercaptooctyl)carbamate

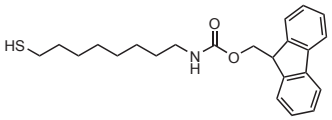
Application: SAM preparation, protected amine group coating

Appearance: White powder or crystalline powder
Purity: ≥90.0%(HPLC)
MW: 383.55, $C_{23}H_{29}NO_2S$

Storage Condition
-20°C, protect from metal

Shipping Condition
with blue ice or dry ice

Structural Formula



Ordering Information

Product code	Unit
F288-10	10 mg
F288-12	50 mg

N-Fmoc-Aminohexanethiol

Fluoren-9-ylmethy*l N*-(6-mercaptohexyl)carbamate

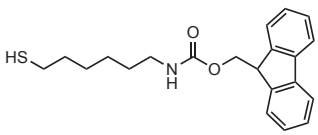
Application: SAM preparation, protected amine group coating

Appearance: White powder or crystalline powder
Purity: ≥90.0% (HPLC)
MW: 355.49, $C_{21}H_{25}NO_2S$

Storage Condition
-20°C, protect from metal

Shipping Condition
with blue ice or dry ice

Structural Formula



Ordering Information

Product code	Unit
F289-10	10 mg
F289-12	50 mg

Product Description

N-Fmoc- Aminoalkanethiols are compounds whose terminal amino group is protected by an Fmoc-group. After adsorption of *N*-Fmoc aminoalkanethiol onto a gold surface, the Fmoc-group can be removed to reproduce the amino group under mild conditions, such as 30 minutes immersion in 20% piperidine/acetonitrile. Brockman and coworkers protected the amino group of 11-Amino-1-undecanethiol with the Fmoc group, after making 11-Amino-1-undecanethiol SAMs, to fabricate DNA arrays with UV photopatterning and a multistep chemical modification procedure. They then used these DNA arrays to study protein-DNA interactions by surface plasmon resonance (SPR) imaging. *N*-Fmoc- Aminoalkanethiols can be used in similar methods with short steps. The modification of a gold substrate with SAMs of NFmoc Aminoalkanethiols may be used to avoid amino group-gold surface interactions and to develop a highly regulated sensor chip by photopatterning (Fig. 1 Preparation of Multi-Element DNA Array on next page).

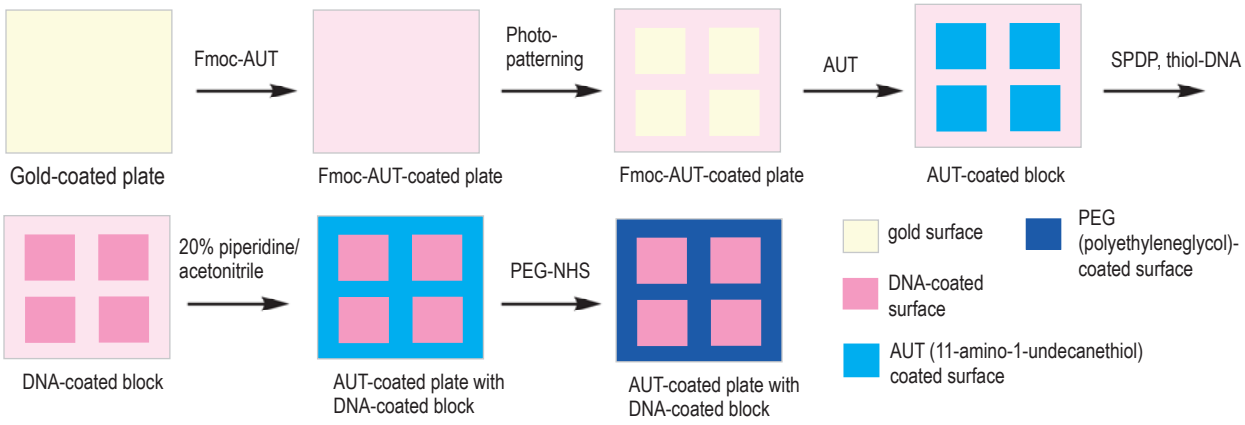


Fig. 1 Preparation of Multi-Element DNA Array

Reference

F287, F288, F289

1. J. M. Brockman, A. G. Frutos and R. M. Corn, A Multistep Chemical Modification Procedure To Create DNA Arrays on Gold Surface for the Study of Protein-DNA Interactions with Surface Plasmon Resonance Imaging, *J. Am. Chem. Soc.*, 1999, ***121***, 8044.

Carboxylic acid-SAM Formation Reagent

Appearance: Colorless or pale yellow liquid
Features: Easily forms carboxy-terminated monolayers on gold
Suppress non-specific binding

Keyword: SAM, Self-Assembled Monolayers
SPR (Surface Plasmon Resonance)
QCM (Quartz Crystal Microbalance)
biosensors, protein immobilization

Ordering Information

Product code	Unit
C488-10	1 µmol x 3

Product Description

Carboxylic acid-SAM Formation Reagent is used to prepare carboxylic acid- terminated self-assembled monolayers (SAMs) on gold surfaces as biosensors detected by QCM (Quartz Crystal Microbalance), SPR (Surface Plasmon Resonance), and electrochemical analysis. Coupled with carboxyl group activation method using NHS/WSC, carboxylic acid-SAM can be used as an interface to immobilize proteins or peptides and other ligands on gold surfaces (Fig. 1). In general, carboxylic acid-SAM provides some non-specific binding because of its negative charge. However, surfaces prepared using -carboxylic acid-SAM Formation Reagent presents less non-specific binding than thiose commonly prepared with carboxylic acid-SAM (Fig. 2).

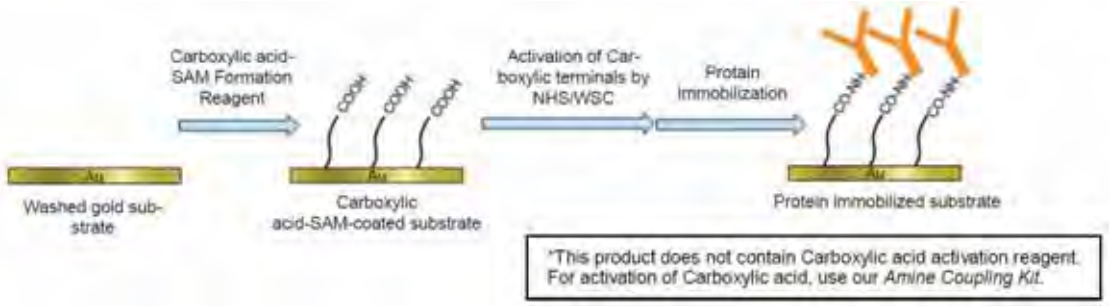


Fig. 1 A biosensor construction by Carboxylic acid-SAM Formation Reagent

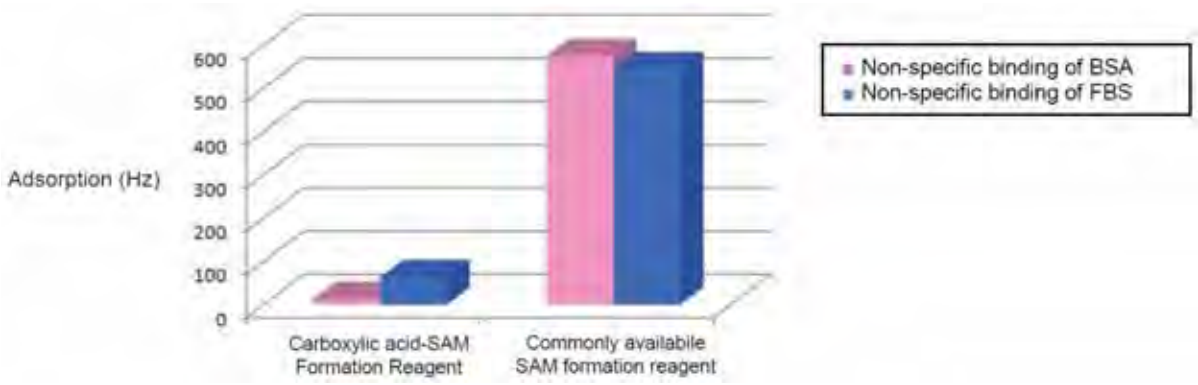


Fig. 2 Comparison of non-specific binding between carboxylic acid-SAM prepared with carboxylic acid-SAM Formation Reagent and commonly prepared SAM

15-Carboxy-1-pentadecanethiol

15-Carboxy-1-pentadecanethiol[CAS: 69839-68-5]

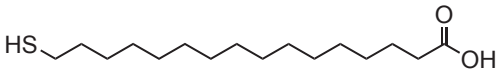
Application: SAM preparation, carboxylate coating

Appearance: White or slightly yellow crystalline powder
Purity: ≥95.0% (HPLC)
MW: 288.49, $C_{16}H_{32}O_2S$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Structural Formula



Ordering Information

Product code	Unit
C429-10	10 mg

10-Carboxy-1-decanethiol

10-Carboxy-1-decanethiol [CAS: 71310-21-9]

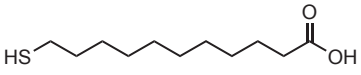
Application: SAM preparation, carboxylate coating

Appearance: White or slightly yellow powder
Purity: ≥97.0% (HPLC)
MW: 218.36, $C_{11}H_{22}O_2S$

Storage Condition
0-5°C, protect from metal

Shipping Condition
ambient temperature

Structural Formula



Ordering Information

Product code	Unit
C385-10	10 mg
C385-12	100 mg

7-Carboxy-1-heptanethiol

7-Carboxy-1-hepanethiol [CAS: 74328-61-3]

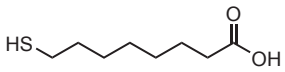
Application: SAM preparation, carboxylate coating

Appearance: Colorless or slightly yellow liquid
Purity: ≥97.0% (HPLC)
MW: 176.28, $C_8H_{16}O_2S$

Storage Condition
0-5°C, protect from metal

Shipping Condition
ambient temperature

Structural Formula



Ordering Information

Product code	Unit
C386-10	10 mg
C386-12	100 mg

5-Carboxy-1-pentanethiol

5-Carboxy-1-pentanethiol [CAS: 17689-17-7]

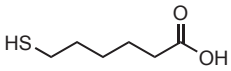
Application: SAM preparation, carboxylate coating

Appearance: Colorless or slightly yellow liquid
Purity: ≥97.0% (HPLC)
MW: 148.22, $C_6H_{12}O_2S$

Storage Condition
0-5°C, protect from metal

Shipping Condition
ambient temperature

Structural Formula



Ordering Information

Product code	Unit
C387-10	10 mg
C387-12	100 mg

Product Description

Carboxyalkanethiols are utilized for the modification of a gold surface to introduce carboxylic groups on it. The carboxylic group is often converted to activated *N*-hydroxysuccinimide ester, which reacts with an amine group of biomaterials. Dojindo's newly developed 15-Carboxy-1-pentadecanethiol has a 15-carbon chain, which is the longest alkanethiol available in the market among carboxyalkanethiols. Five different carboxyalkanethiols including Carboxy-EG_6-undecanethiol are available for gold surface modification. Malone and others fabricated a highly sensitive SPR sensor using 15-Carboxy-1-pentadecanethiol. Glenn and coworkers used carboxyalkanethiol and poly-L-lysine to create an immobilized cytochrome b5 multilayer electrode. Mizutani and others fabricated immobilized glucose oxidase multilayer electrodes in a similar manner. Both groups reported electron transfer from biomaterials to a gold surface. These kinds of multilayer film electrodes are well suited for studies of diffusion electron transfer. Frisbie and others developed a new method, chemical force microscopy, for obtaining the adhesive interactions and the friction image of patterned sample surfaces. They used atomic force microscopy (AFM) to measure the interactions and spatial mapping of chemically distinct functional groups. Frisbie and others formed carboxyalkanethiol monolayers on the gold surfaces of AFM cantilever tips. They used AFM to measure the adhesive and friction forces between molecularly modified probe tips and organic monolayers terminating in a lithographically defined pattern of distinct functional groups.

References

C429

1. M. R. Malone, J-F. Masson, S. Beaudoin, K. S. Booksh, *Proceedings of SPIE-The International Society for Optical Engineering*, 2005, ***6007***.

C385

1. J. D. H. Glenn and E. F. Bowden, Diffusionless Electrochemistry of Cytochrome b5 Adsorbed on a Multilayer Film Electrode, *Chem. Lett.*, 1996, ***399***.
2. F. Mizutani, Y. Sato, S. Yabuki and Y. Hirata, Enzyme Ultra-thin Layer Electrode Prepared by the Co-adsorption of Poly-L-lysine and Glucose Oxidase onto a Mercaptopropionic Acid-Modified Gold Surface, *Chem. Lett.*, 1996, ***251***.
3. C. D. Frisbie, F. Rozsnyai, A. Noy, M. S. Wrighton and C. M. Lieber, Functional Group Imaging by Chemical Force Microscopy, *Science*, 1994, ***265***, 2071.
4. M. Kyo, K. Usui-Aoki and H. Koga, Label-free Detection of Proteins in Crude Cell Lysate with Antibody Arrays by a Surface Plasmon Resonance Imaging Technique, *Anal. Chem.*, 2005, ***77***, 7115.

C386

1. J. D. H. Glenn and E. F. Bowden, Diffusionless Electrochemistry of Cytochrome b5 Adsorbed on a Multilayer Film Electrode, *Chem. Lett.*, 1996, ***399***.
2. F. Mizutani, Y. Sato, S. Yabuki and Y. Hirata, Enzyme Ultra-thin Layer Electrode Prepared by the Co-adsorption of Poly-L-lysine and Glucose Oxidase onto a Mercaptopropionic Acid-Modified Gold Surface, *Chem. Lett.*, 1996, ***251***.

3. C. D. Frisbie, F. Rozsnyai, A. Noy, M. S. Wrighton and C. M. Lieber, Functional Group Imaging by Chemical Force Microscopy, *Science*, 1994, **265**, 2071.

C387

1. J. D. H. Glenn and E. F. Bowden, Diffusionless Electrochemistry of Cytochrome b5 Adsorbed on a Multilayer Film Electrode, *Chem. Lett.*, 1996, **399**.
2. F. Mizutani, Y. Sato, S. Yabuki and Y. Hirata, Enzyme Ultra-thin Layer Electrode Prepared by the Co-adsorption of Poly-L-lysine and Glucose Oxidase onto a Mercaptopropionic Acid-Modified Gold Surface, *Chem. Lett.*, 1996, **251**.
3. C. D. Frisbie, F. Rozsnyai, A. Noy, M. S. Wrighton and C. M. Lieber, Functional Group Imaging by Chemical Force Microscopy, *Science*, 1994, **265**, 2071.

Carboxy-EG$_6$-undecanethiol

20-(11-Mercaptoundecanyloxy)-3,6,9,12,15,18-hexaoxaeicosanoic acid [CAS:221222-49-7]

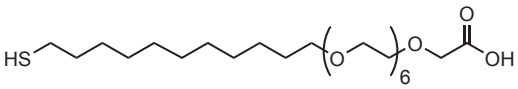
Application: SAM preparation, carboxylate coating, and hydrophilic surface

Appearance: Colorless or slightly yellow liquid
Purity: ≥90.0% (HPLC)
MW: 526.73, $C_{25}H_{50}O_9S$

Storage Condition
-20°C, protect from metal

Shipping Condition
ambient temperature

Structural Formula



Ordering Information

Product code	Unit
C445-10	10 mg
C445-12	100 mg

Carboxy-EG$_6$-hexadecanethiol

20-(16-Mercaptohexadecanyloxy)-3,6,9,12,15,18-hexaoxaeicosanoic acid

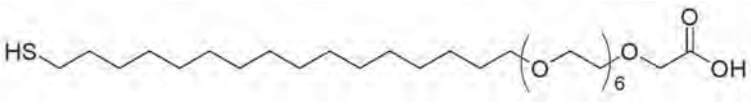
Application: SAM preparation, carboxylate coating, hydrophilic surface

Appearance: White or slightly yellow solid
Purity: ≥95.0% (HPLC)
MW: 526.73, $C_{25}H_{50}O_9S$

Storage Condition
-20°C, protect from metal

Shipping Condition
ambient temperature

Structural Formula



Ordering Information

Product code	Unit
C463-10	10 mg

Product Description

Polyethyleneglycols (PEGs) are widely used for material modifications to improve hydrophilicity of the surface. PEG-coated materials are usually more stable under physiological conditions. Since Carboxy-EG$_6$-undecanethiol has 6 ethylene glycol units, 11 carbon Atoms, and an SH group at the end, it can be used to prepare a highly oriented and hydrophilic SAM on a gold surface suitable for biomaterial-labelings on the surface due to the improved hydrophilicity. The hydrophilic surface can prevent proteins or other biomaterials from non-specific binding. Therefore, the SAM prepared by this reagent will provide a better surface to develop biomaterial sensors or DNA/protein microarrays. To prepare a carboxy- EG$_6$-SAM on a gold surface, hydroxy-EGn-undecanethiols (n=3, 6) are used to dilute the number of carboxyl groups according to the density of the molecules being introduced onto the surface. There are several papers available on labeling proteins such as ovalbumin and cytochrome C.

References
C445
1. C. Pale-Grosdemange, E. S. Simon, K. L. Prime, and G. M. Whitesides, *Anal. Chem*., 1999, ***71***, 777.
2. A. Subramanian, J. Irudayaraj, and T. Ryan, *Biosensors and Bioelectronics*, 2006, ***21***, 998.
3. X. Qian, S. J. Metallo, I. S. Choi, *H. Wu, M. N. Liang and G. M. Whitesides, Anal. Chem.*, 2002, ***74***, 1805.
4. M. Kyo, K.Usui-Aoki, H. Koga, *Anal. Chem*, 2005, ***77***, 7115.

C463
1. C. Pale-Grosdemange, E. S. Simon, K. L. Prime, and G. M. Whitesides, *Anal. Chem*., 1999, **71**, 777-790.
2. M. Kyo, K.Usui-Aoki, H. Koga, *Anal. Chem,* 2005, **77**, 7115-7121.

10-Carboxydecyl disulfide

10-Carboxydecyl disulfide [CAS: 23483-56-9]

Application: SAM preparation, carboxylate coating

Appearance: White powder
Purity: ≥97.0% (HPLC)
MW: 434.70, $C_{22}H_{42}O_4S_2$

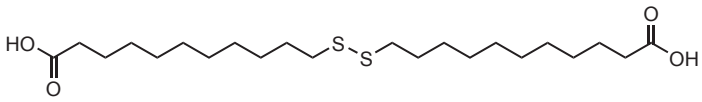
Ordering Information

Product code	Unit
C404-10	10 mg
C404-12	100 mg

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Structural Formula



7-Carboxyheptyl disulfide

7-Carboxyheptyl disulfide [CAS: 107016-79-5]

Application: SAM preparation, carboxylate coating

Appearance: White powder
Purity: ≥97.0% (HPLC)
MW: 350.54, $C_{16}H_{30}O_4S_2$

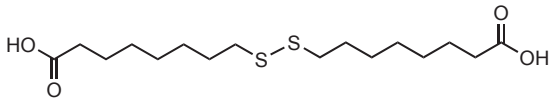
Ordering Information

Product code	Unit
C405-10	10 mg

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Structural Formula



5-Carboxypentyl disulfide

5-Carboxypentyl disulfide [CAS: 92038-67-0]

Application: SAM preparation, carboxylate coating

Appearance: White powder
Purity: ≥97.0% (HPLC)
MW: 294.43, $C_{12}H_{22}O_4S_2$

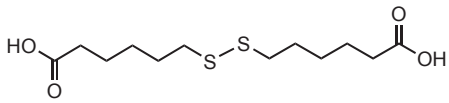
Ordering Information

Product code	Unit
C406-10	10 mg

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Structural Formula



4,4'- Dithiodibutyric acid

4,4'-Dithiodibutyric acid [CAS: 2906-60-7]

Application: SAM preparation, carboxylate coating

Appearance: White crystalline powder
Purity: ≥97.0% (HPLC)
MW: 238.33, $C_8H_{14}O_4S_2$

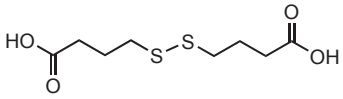
Ordering Information

Product code	Unit
D524-10	500 mg

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Structural Formula



Product Description of Carboxyallanedisulfides Carboxyalkyldisulfides are oxidized carboxyalkanethiols.

They form SAMs similar to the carboxyalkanethiols, but are more stable than thiols. Kanayama and others formed SAMs of 4,4'-dithiodibutyric acid on gold colloids or gold electrodes and introduced phenylboronic acid moieties to its terminus. They succeeded in recognizing various sugars by surface-enhanced Raman spectroscopy (SERS) and cyclic voltammetry (CV) using these SAMs. Takagi and coworkers fixed dinitrophenyl (DNP) groups on carboxyalkyl-disulfide SAMs. They detected an anti-DNP antibody by electrical impedance measurement. This technique could be applied to impedimetric sensing of proteins. Delamarche and his coworkers fabricated 10-carboxydecyl disulfide SAMs on a gold substrate and introduced a photoactivatable benzophenone moiety to the termini. After attaching a protein (IgG), it was tested by a variety of characterization techniques (including ellipsometry, Xray photoelectron spectroscopy and AFM).

References

C405, C406, D524

1. N. Kanayama and H. Kitano, Interfacial Recognition of Sugar by Boronic acid-Carrying self-Assembled Monolayers, *Langmuir*, 2000, **16**, 577.
2. R. D. Vaughan, C. K. O'Sullivan and G. G. Guilbault, Sulfer based Self-assembled Monolayers(SAM's) on Piezoelectric Crystals for Immunosensor development, *Fresenius J. Anal. Chem.*, 1999, **364**, 54.
3. H. Taira, K. Nakano, M. Maeda and M. Takagi, *Electrode Modification by Long-Chain,Dialkyl Disulfide Reagent Having Terminal Dinitrophemyl Group and Its Application to Impedimetric Immunosensors. Anal. Sci.*, 1993, **9**, 199.
4. E. Delamarche, G. Sundarababu, H. Biebuyck, B. Michel, Ch. Gerber, H. Sigrist, H. Wolf, H. Ringsdorf, N. Xanthopoulos and H. J. Mathieu, Immobilization of Antibodies on a Photoactive Self-Assembled Monolater on Gold, *Langmuir*, 1996, **12**, 1997.

Amine Coupling Kit

Application: Immobilization of protein and/or peptide to carboxylic acid groups

Features: Sufficient for 40 immobilizations
All reagents necessary for activation, immobilization, and blocking are included
Blocking buffer enables minimization of non-specific absorption by protein

Ordering Information

Product code	Unit
A515-10	2 ml x 4

Contents of the Kit

WSC.....4 tubes
NHS.....4 tubes
Activation buffer.....20 ml x 1
Reaction buffer.....10 ml x 1
Blocking solution.....20 ml x1

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Required Equipment and Materials
Micropipette, Microtube, Carboxylic acid-SAMs coated substrate

Product Description
Amine and carboxylic acid coupling is one of the most common methods to immobilize a protein or a peptide through a covalent bound onto biosensor surface. Dojindo's Amine Coupling Kit contains all of the reagents and buffers necessary for activation of carboxylic acid, protein immobilization, and blocking. Blocking solution included in the kit minimizes non-specific protein absorption to the surface by capping residual activated esters. Each kit is adequate for approximately 40 immobilizations.

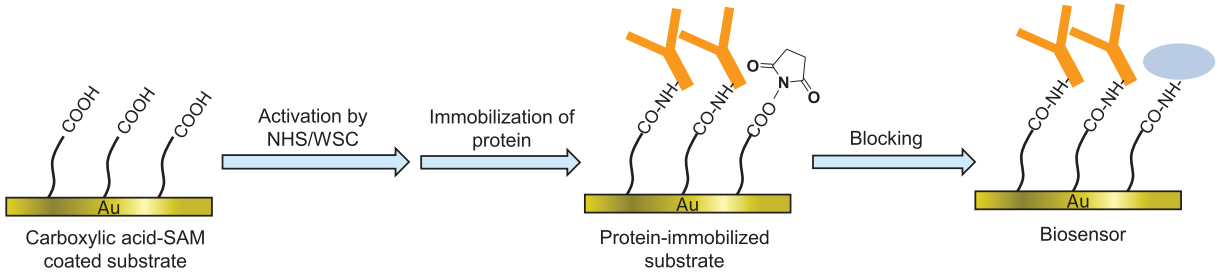


Fig. 1 A biosensor prepared by Amine Coupling Kit

Dithiobis(succinimidyl undecanoate)

Dithiobis(succinimidyl undecanoate)

Application: SAM preparation, amine reactive group coating

Appearance: White powder
Purity: ≥90.0% (HPLC)
MW: 628.84, $C_{30}H_{48}N_2O_8S_2$

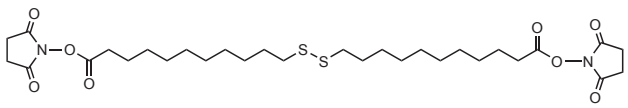
Ordering Information

Product code	Unit
D537-10	10 mg
D537-12	50 mg

Storage Condition
0-5°C, protect from metal and moisture

Shipping Condition
ambient temperature

Structural Formula



Dithiobis(succinimidyl octanoate) Dithiobis(succinimidyl octanoate)

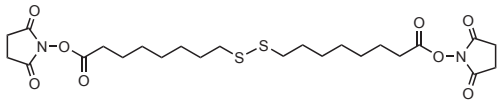
Application: SAM preparation, amine reactive group coating

Appearance: White powder
Purity: ≥90.0% (HPLC)
MW: 544.68, $C_{24}H_{36}N_2O_8S_2$

Storage Condition
0-5°C, protect from metal and moisture

Shipping Condition
ambient temperature

Structural Formula



Ordering Information

Product code	Unit
D538-10	10 mg
D538-12	50 mg

Dithiobis(succinimidyl hexanoate) Dithiobis(succinimidyl hexanoate)

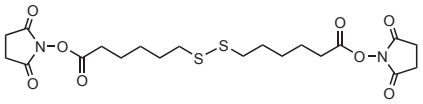
Application: SAM preparation, amine reactive group coating

Appearance: White powder
Purity: ≥90.0% (HPLC)
MW: 488.58, $C_{20}H_{28}N_2O_8S_2$

Storage Condition
0-5°C, protect from metal and moisture

Shipping Condition
ambient temperature

Structural Formula



Ordering Information

Product code	Unit
D539-10	10 mg
D539-12	50 mg

Product Description of Succinimidyl Alkanedisulfides

Succinimidyl ester-terminated alkyldisulfides are amine-reactive analogs of carboxyalkyldisulfide. They are utilized for the modification of a gold surface to introduce amine-reactive sites on the surface. It is possible to use this technique for protein chips and various sensors. There is no need to use coupling agents because these compounds are already activated. Wagner and others characterized dithiobis (succinimidyl undecanoate) SAMs on a gold substrate by scanning tunneling microscopy (STM), radiolabeling, and in situ AFM imaging. The densely packed and highly reactive surfaces allowed them to easily immobilize amino acids and proteins.

Dithiobis(C_2-NTA)

3,3'-Dithiobis[*N*-(5-amino-5-carboxypentyl)propionamide-*N,N'*-diacetic acid] dihydrochloride

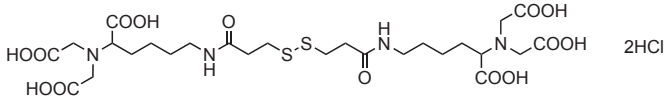
Application: SAM preparation, NTA group coating

Appearance: White or slightly yellow powder or crystal
Purity: ≥95.0% (Titration, as anhydrous)
MW: 771.68, $C_{26}H_{44}Cl_2N_4O_{14}S_2$

Storage Condition
ambient temperature,
protect from metal and moisture

Shipping Condition
ambient temperature

Structural Formula



Ordering Information

Product code	Unit
D550-10	10 mg
D550-12	50 mg

Product Description

Dithiobis(C_2-NTA) is utilized for the modification of a gold surface to introduce NTA groups that can bind most heavy metal ions. It forms SAMs similar to the other alkanethiols. The SAMs prepared using Dithiobis(C_2-NTA) are highly unidirectional due to its alkyl chain interactions. Ni(II)-NTA chelates are commonly used for Histidine-tagged (His-tag) protein purification or separation. Therefore, Ni(II)-NTA-coated gold can be used for His-tag protein analyses. Dithiobis(C_2-NTA) is soluble in water and alcohol. It is also referred to as disulfide-NTA in some papers.

Reference

D537, D538, D539, D550

1. E. Delamarche, G. Sundarabaru, H. Biebuyck, B. Michel, Ch. Gerber, H. Sigrist, H. Wolf, H. Ringsdorf, N. Xanthopoulos and H. J. Mathieu, Immobilization of Antibodies on a Photoactive Self-Assembled Monolater on Gold, *Langmuir*, 1996, ***12***, 1997.
2. P.Wager, F. Zaugg, P. Kernen, M. Hegner and G. Semenza, ω-functionalized self-assembled monolayers chemisorbed on ultraflat Au(111) surfaces for biological scanning probe microscopy in aqueous buffers, *J. Vac. Sci. Technol. B*, 1996, ***14***, 1466.

11-Ferrocenyl-1-undecanethiol

11-Ferrocenyl-1-undecanethiol [CAS: 127087-36-9]

Application: SAM preparation, electrochemical reaction

Appearance: Yellow or yellowish-orange solid
Purity: ≥95.0% (HPLC)
MW: 372.39, $C_{21}H_{32}FeS$

Ordering Information

Product code	Unit
F246-10	10 mg
F246-12	100 mg

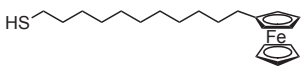
Storage Condition

-20°C, protect from metal

Shipping Condition

with dry ice or blue ice

Structural Formula



8-Ferrocenyl-1-octanethiol

8-Ferrrocenyl-1-octanethiol [CAS: 146056-20-4]

Application: SAM preparation, electrochemical reaction

Appearance: Yellow or yellowish-orange powder
Purity: ≥95.0% (HPLC)
MW: 330.31, $C_{18}H_{26}FeS$

Ordering Information

Product code	Unit
F247-10	10 mg
F247-12	100 mg

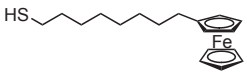
Storage Condition

-20°C, protect from metal

Shipping Condition

with dry ice or blue ice

Structural Formula



6-Ferrocenyl-1-hexanethiol

6-Ferrrocenyl-1-hexanethiol [CAS: 134029-92-8]

Application: SAM preparation, electrochemical reaction

Appearance: Yellow or yellowish-orange solid
Purity: ≥95.0% (HPLC)
MW: 302.26, $C_{16}H_{22}FeS$

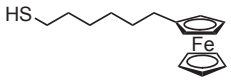
Ordering Information

Product code	Unit
F269-10	10 mg
F269-12	100 mg

Storage Condition
-20°C, protect from metal

Shipping Condition
with dry ice or blue ice

Structural Formula



Product Description of Ferrocenyl Alkanethiols

Ferrocenyl alkanethiols are utilized for the modification of gold surfaces to introduce electrochemically active molecules. The modified gold surface can be utilized for the development of sensitive electrochemical analyses. Rubin and others fabricated mixed SAMs of aminoalkanethiols and ferrocenyl alkanethiols with various chain lengths on a gold electrode surface. They immobilized glucose oxidase on aminoalkanethiol sites and used ferrocenyl-alkanethiol sites as electron mediators. They reported the relationship between electrical response and chain length of mixed SAMs. Uosaki and coworkers reported the results of structural changes and the number of absorbed ferrocenyl alkanethiols during redox reaction of 11-ferrocenyl-1-undecanethiol SAMs on a gold electrode using Fourier transform infrared reflection adsorption spectroscopy (FT-IRRAS) and the electrochemical quartz crystal microbalance (EQCM) method. They suggested the possibility of orientation change of the monolayer during the redox reaction of the ferrocene moiety. They also estimated this change using voltammograms and ellipsometry.

References

F246, F247, F269

1. M. D. Porter, T. B. Bright, D. L. Allara and C. E. D. Chidsey, Spontaneously Organized Molecular Assemblies.4.Structural Characterization of n-Alkyl Thiol Monolayers on Gold by Optical Ellipsometry, Infrared Spectroscopy, and Electrochemistry, *J. Am. Chem. Soc.*, 1987, **109**, 3559.
2. C. E. D. Chidsey, C. R. Bertozzi, T. M. Putvinski and A. M. Mujsce, Coadsorption of Ferrocene-Terminated and Unsubstituted Alkanethiols on Gold: Electroactive Self-Assembled Monolayers, *J. Am. Chem. Soc.*, 1990, **112**, 4301.
3. J. J. Hickman, D. Ofer, P. E. Laibinis, G. M. Whitesides and M. S. Wrighton, Molecular Self-Assembly of Two-Terminal, Voltammetric Microsensors with Internal references, *Science*, 1991, **252**, 688.
4. C. E. D. Chidsey, C. R. Bertozzi, T. M. Putvinski and A. M. Mujsce, Coadsorption of Ferrocene-Terminated and Unsubstituted Alkanethiols on Gold:Electroactive Self-Assembled Monolayers, *Chemtracts*, 1991, **3**, 27.
5. K. Uosaki, Y. Sato and H. Kita, Electrochemical Characteristics of a Gold Electrode Modified with a Self-Assembled Monolayer of Ferrocenylalkane-thiols, *Langmuir*, 1991, **7**, 1510.
6. Y. Kajiya, T. Okamoto and H. Yoneyama, Glucose Sensitivity of Thiol-modified Gold Electrodes Having Immobilized Glucose Oxidase and 2-Amino-ethylferrocene, *Chem. Lett.*, 1993, **2107**.
7. T. Ohtsuka, Y. Sato and K. Uosaki, Dynamic Ellipsometry of a Self-Assembled Monolayer of a Ferrocenylalkanethiol during Oxidation-Reduction Cycles, *Langmuir*, 1994, **10**, 3658.
8. K. Shimazu, I. Yagi, Y. Sato and K. Uosaki, Electrochemical Quartz Crystal Microbalance Studies of Self-Assembled Monolayers of 11-ferrocenyl-1-undecanethiol: Structure-dependent Ion-pairing and Solvent Uptake, *J. Electroanal. Chem.*, 1994, **372**, 117.
9. T. Kondo, M. Takechi, Y. Sato and K. Uosaki, Absorption Behavior of Functionalized Ferrocenylalkane Thiol and Disulfide onto Au and ITO and Electrochemical Properties of Modified Electrodes: Effects of Acyl and Alkyl Groups Attached to the Ferrocene Ring, *J. Electroanal. Chem.*, 1995, **381**, 203.
10. J. I. Anderson, E. F. Bowden and P. G. Pickup, Dynamic Electrochemistry : Methodology and Application", *Anal. Chem.*, 1996, **68**, 379R.
11. K. Chen, F. Xu and C. A. Mirkin, Do Alkanethiols Adsorb onto the Surfaces of Tl-Ba-Ca-Cu-O-Based High-Temperature Superconductors? The Critical Role of H_2O Contact on the Adsorption Process, *Langmuir*, 1996, **12**, 2622.
12. S. Rubin, G. Bar, R. W. Cutts, J. T. Chow, J. P. Ferraris and T. A. Zawodzinski Jr., Electrical Communication Between Glucose Oxidase and Different Ferrocenylalkanethiol Chain Lengths, *Mat. Res. Soc. Symp. Proc.*, 1996, **413**, 377.
13. R. C. Sabapathy, S. Bhattacharyya, W. E. Cleland Jr. and C. L. Hussey, Host-Guest Complexation in Self-Assembled Monolayers: Inclusion of a Monolayer-Anchored Cationic Ferrocene-Based Guest by Cyclodextrin Hosts, *Langmuir*, 1998, **14**, 3797.

16-Hydroxy-1-hexadecanethiol

16-Hydroxy-1-hexadecanethiol [CAS:114896-32-1]

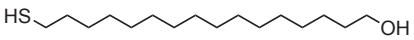
Application: SAM preparation, dilution of functional alkanethiols

Appearance: White or slightly yellowish white crystalline powder
Purity: ≥90% (HPLC)
MW: 274.51, $C_{16}H_{34}OS$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Structural Formula



Ordering Information

Product code	Unit
H394-10	10 mg

11-Hydroxy-1-undecanethiol

11-Hydroxy-1-undecanethiol [CAS: 73768-94-2]

Application: SAM preparation, dilution of functional alkanethiols

Appearance: White or slightly yellowish white crystalline powder
Purity: ≥98.0% (HPLC)
MW: 204.37, $C_{11}H_{24}OS$

Storage Condition
-20°C, protect from metal

Shipping Condition
ambient temperature

Structural Formula

Ordering Information

Product code	Unit
H337-10	10 mg
H337-12	100 mg

8-Hydroxy-1-octanethiol

8-Hydroxy-1-octanethiol [CAS: 33065-54-2]

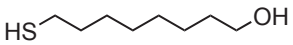
Application: SAM preparation, dilution of functional alkanethiols

Appearance: Colorless or slightly yellow liquid
Purity: ≥98.0% (HPLC)
MW: 162.29, $C_8H_{18}OS$

Storage Condition
-20°C, protect from metal

Shipping Condition
ambient temperature

Structural Formula



Ordering Information

Product code	Unit
H338-10	10 mg
H338-12	100 mg

6-Hydroxy-1-hexanethiol

6-Hydroxy-1-hexanethiol [CAS: 1633-78-9]

Application: SAM preparation, dilution of functional alkanethiols

Appearance: Colorless or slightly yellow liquid
Purity: ≥98.0% (HPLC)
MW: 134.24, $C_6H_{14}OS$

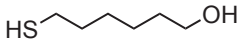
Storage Condition
-20°C protect from metal

Shipping Condition
with dry ice or blue ice

Ordering Information

Product code	Unit
H339-10	10 mg
H339-12	100 mg

Structural Formula



Product Description

Hydroxyalkanethiols are used as dilution reagents on a gold surface to control the density of reactive groups, or as blocking agents to prevent non-specific binding of analytes on the surface. Newly developed 16-Hydroxy-1-hexadecanethiol has a 16-carbon chain, which is the longest alkanethiol available in the market among hydroxyalkanethiols. Six hydroxyalkanethiols, including Hydroxy-EG_6-undecanethiol and Hydroxy-EG_3-undecanethiol, are available for gold surface modification. 16-Hydroxy-1-hexadecanethiol is used to prepare a homogeneous and highly oriented SAM when 16-Amino-1-hexadecanethiol or 15-Carboxy-1-pentadecanethiol is applied. Herne and his coworkers fabricated mixed SAMs of thiol-derivatized single-stranded DNA (HS-ss-DNA) and 6-Hydroxy-1-hexanethiol on a gold surface that prevented non-specific adsorption of HS-ss-DNA. Perez-Luna and others made mixed SAMs of biotin-terminated thiol and 11-hydroxy-1-undecanethiol on a gold surface. They prevented non-specific adsorption of wild type streptavidin and streptavidin mutants. Dubrovsky and coworkers controlled the non-specific adsorption of protein on the surface of gold-coated silicagel using 11-Hydroxy-1-undecanethiol. They mentioned the usefulness of gold-coated silica gel for the preparation of well-defined, surface-functionalized supports for biological assay.

Reference

H394,

1. T. M. Herne and M. J. Tarlov, Characterizathion of DNA Probes Immobilized on Gold Surfaces, *J. Am. Chem. Soc.*, 1997, ***119***, 8916.

H337, H338, H339

1. T. M. Herne and M. J. Tarlov, Characterizathion of DNA Probes Immobilized on Gold Surfaces, *J. Am. Chem. Soc.*, 1997, ***119***, 8916.
2. T. B. Dubrovsky, Z. Hou, P. Stroeve and N. L. Abbott, Self-Assembled Monolayeres Formed on Electroless Gold Deposited on Silica Gel; A Potential Statonary Phase for Biological Assays, *Anal. Chem.*, 1999, ***71***, 327.
3. V. H. Perez-Luna, M. J. O'Brien, K. A. Opperman, P. D. Hampton, G. P. Lopez, L. A. Klumb and P. S. Stayton, Molecular Recognition Between Genetically Engineered Streptavidin and Sueface-Bound Biotin, *J. Am. Chem. Soc.*., 1999, ***121***, 6469.

Hydroxy-EG_6-undecanethiol

11-Mercaptoundecanol hexaethyleneglycol ether [CAS: 130727-44-5]

Application: SAM preparation, dilution of functional alkanethiols

Appearance: Colorless or pale yellow liquid
Purity: ≥90.0% (HPLC)
MW: 468.69, $C_{23}H_{48}O_7S$

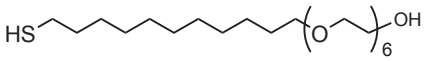
Storage Condition
-20°C, protect from metal

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
H355-10	10 mg
H355-12	100 mg

Structural Formula



Hydroxy-EG_6-hexadecanethiol

16-Mercaptohexadecanol hexathyleneglycol ether

Application: SAM preparation, dilution of functional alkanethiols

Appearance: White or slightly yellow solid
Purity: ≥95.0% (HPLC)
Solubility: 10 mmol/L (Ethyl alcohol, Methyl alcohol, Chloroform)
MW: 538.82, $C_{28}H_{58}O_7S$

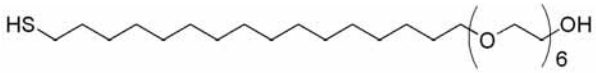
Storage Condition
-20°C, protect from metal

Shipping Condition
with blue ice

Ordering Information

Product code	Unit
H396-10	10 mg

Structural Formula



Hydroxy-EG3-undecanethiol

11-Mercaptoundecanol triethyleneglycol ether [CAS: 130727-41-2]

Application: SAM preparation, dilution of functional alkanethiols

Appearance: Colorless or pale yellow liquid
Purity: ≥90.0% (HPLC)
MW: 336.53, $C_{17}H_{36}O_4S$

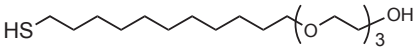
Storage Condition
-20°C, protect from metal

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
H354-10	10 mg
H354-12	100 mg

Structural Formula



Hydroxy-EG3-hexadecanethiol

16-Mercaptohexadecanol triethyleneglycol ether

Application: SAM preparation, dilution of functional alkanethiols

Appearance: White or slightly yellow solid
Solubility:10 mmol/L (Ethyl alcohol, Methyl alcohol, Chloroform)
Purity: ≥95.0% (HPLC)
MW: 406.66, $C_{22}H_{46}O_4S$

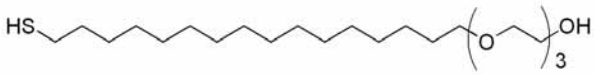
Storage Condition
-20°C, protect from metal

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
H395-10	10 mg

Structural Formula



Product Description

Polyethyleneglycols (PEGs) are widely used for material modifications to improve hydrophilicity of the surface. PEG-coated materials are usually more stable under physiological conditions. Since Hydroxy-EGn-undecanethiol has 3 or 6 ethylene glycol units, 11 carbon atoms, and an SH group at the end, it can be used to prepare a highly oriented and hydrophilic SAM on a gold surface that is suitable for biomaterial-labelings on the surface. This is due to the improvement of hydrophilicity. The hydrophilic surface can prevent proteins or other biomaterials from non-specific binding. Therefore, the SAM prepared by this reagent will provide a better surface to develop biomaterial sensors or DNA/protein microarrays. Hydroxy-EGn-undecanethiols (n=3, 6) are used to dilute Carboxyl -SAM or Amino-SAM according to the density of the molecules be introduced onto the surface. There are several papers available on labeling proteins such as ovalbumin and cytochrome C.

References

H354, H355

4. C. Pale-Grosdemange, E. S. Simon, K. L. Prime, and G. M. Whitesides, *Anal. Chem.*, 1999, **71**, 777.
5. A. Subramanian, J. Irudayaraj, and T. Ryan, *Biosensors and Bioelectronics*, 2006, **21**, 998.
6. X. Qian, S. J. Metallo, I. S. Choi, H. Wu, M. N. Liang and G. M. Whitesides, *Anal. Chem.*, 2002, **74**, 1805.
7. M. Kyo, K.Usui-Aoki, H. Koga, *Anal. Chem*, 2005, **77**, 7115.

ACE Kit-WST

Application: Angiotensin-converting enzyme (ACE) activity detection.
Screening of ACE inhibitors
Features: Colorimetric microplate assay
Simple protocol
No organic solvent required
High reproducibility

Ordering Information

Product code	Unit*
A502-10	100 tests**

* One test corresponds to one well of a 96 well plate.
**100 tests kit contains 2 sets of 50 tests kits

Contents of the Kit:

Enzyme A .....2 tubes
Enzyme B .....2 tubes
Enzyme C ..... 2 tubes
Coenzyme .....2 tubes
Substrate buffer ..... 1 ml x 2
Indicator solution .....5 ml x 2

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Required Equipment and Materials
plate reader with 450 nm filter; 96-well culture plate, 2-20 µl, 20-200 µl, 100-1000 µl and multi-channel pipettes; 37°C incubator, Disposable syringe (1 ml)

Product Description
The kit is used for the determination of ACE inhibition activity. ACE works in the Renin-Angiotensin system, which is one of the mechanisms of blood pressure control, to convert Angiotensin I to the vasopressor Angiotensin II. This enzyme also contributes to elevated blood pressure due to its role in breaking down the antihypertensive peptide Bradykinin. In recent years, food and supplements containing ingredients that block ACE have received attention for their use in preventing high blood pressure. The conventional method of measuring ACE inhibition employs the synthetic substrate Hippuryl-His-Leu. Hippuric acid from the synthetic substrate is extracted with ethyl acetate, condensed, redissolved, and then read at an absorbance of 228 nm. This method is cumbersome and measurement is subjected to error due to residual ethyl acetate. ACE inhibition Assay Kit enzymatically detects 3-Hydroxybutyric acid (3HB), which is made from 3-Hydryoxybutyryl-Gly-Gly-Gly (3HB-GGG). Using a 96-well format, it is possible to test multiple samples at one time. In addition, there is no need to use harmful organic solvents, resulting in a safe, simple, and highly reproducible assay.

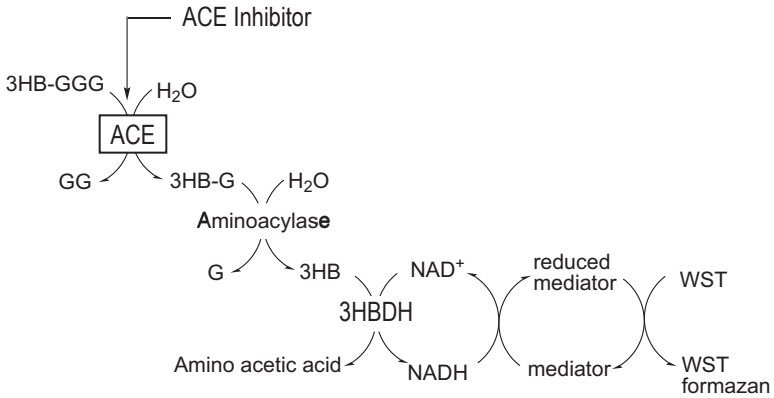


Fig. 1 Principle of the assay system to determine ACE activity or inhibition activity

Preparation of solutions
<Enzyme working solution>
Add 2 ml of purified water to Enzyme B bottle to prepare an Enzyme B solution.[a)] Then, add 1.5 ml of Enzyme B solution to the Enzyme A bottle to prepare a Enzyme working solution.[b)]

[a)] Enzyme A and B are freeze-dried and closed with a rubber cap under vacuum pressure. The contents may fly out of the container if the rubber cap is removed. Add purified water or Enzyme B solutions using a syringe, and then open the bottle after dissolving the contents.
[b)] The Enzyme working solution is stable at -20°C for 2 weeks. If store in a refrigerator, stable for 3 days.

<Indicator working solution>
Add 3 ml of purified water to each of the Enzyme C and Coenzyme bottles and dissolve.[c)] Then, add 2.8 ml each of Enzyme C and Coenzyme to the indicator solution to prepare an indicator working solution.[d)]

[c)] Enzyme C and Coenzyme are freeze-dried and closed with a rubber cap under vacuum pressure. The contents may fly out of the

container if the rubber cap is removed. Add pure water or Enzyme B solution using a syringe, and then open the bottle after dissolving the contents.

[d] *The Indicator working solution is stable at -20°C for 2 weeks. If store in a refrigerator, stable for 3 days.*

Sample solution

Dilute sample solution with purified water.
example: dilution ratio: 1(no dilution), 1/5, $1/5^2$, $1/5^3$, $1/5^4$, $1/5^5$, $1/5^6$

Table 1 Solution and buffer volumes in each well

	sample	blank 1	blank 2
Sample solution	20 µl	-	-
Purified water	-	20 µl	40 µl
Substrate buffer	20 µl	20 µl	20 µl
Enzyme working solution	20 µl	20 µl	-
Indicator working solution	200 µl	200 µl	200 µl

Assay Protocol

1. Add 20 µl of the Sample solution (sample) or purified water (blank 1, blank 2) to each well.
2. Add 20 µl of the Substrate buffer to each well.
3. Add 20 µl of purified water to the blank 2 well.
4. Add 20 µl of the Enzyme working solution to the wells containing Sample solution and blank 1.
   **3-Hydroxybutyric acid (3HB) is produced immediately upon addition of enzyme working solution. To reduce time lag from well to well, use a multichannel pippette.*
5. Incubate the plate at 37°C for 60 minutes.
6. Add 200 µl indicator working solution to each well.
7. Incubate for 10 minutes at room temperature.
8. Measure the absorbance of each well at 450 nm.
9. Use the following equation to calculate the ACE inhibition activity (percent inhibition).

ACE inhibitory activity (inhibition rate %) = $[(A_{blank\ 1} - A_{sample}) / (A_{blank\ 1} - A_{blank\ 2})] \times 100$

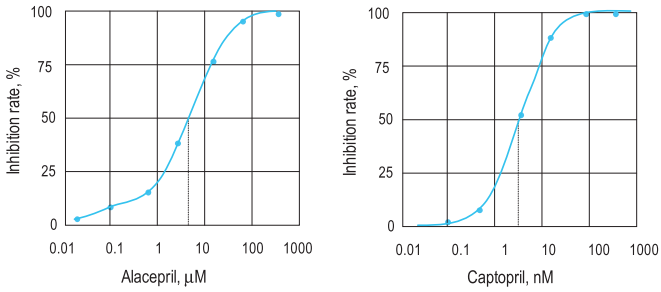


Fig. 2 Inhibition curves prepared by Alacepril and Captopril.

IC_{50} of Alacepril and Captopril are 3.62 µM and 2.14 nM, respectively. Both compounds are ACE inhibitors.

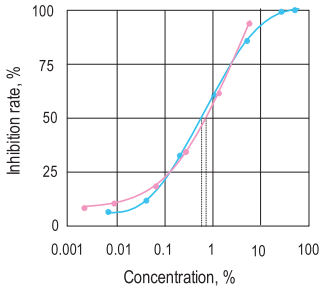


Fig. 3 Inhibition curves prepared by two beverages containing a valyltyrosine (●) or lacto tripeptide (●).

IC_{50} of these beverages are 0.56% and 0.69%, respectively. It is known that these substances have antihypertensive effects.

** Concentration of the beverage in the sample solution.*

References

1. L. H. Lam, *et al*., Assay of angiotensin I-converting enzyme-inhibiting activity based on the detection of 3-hydroxybutyric acid. *Anal Biochem*. 2007;**364**:104-111.
2. L. H. Lam, *et al*., Assay of angiotensin I-converting enzyme-inhibiting activity based on the detection of 3-hydroxybutyrate with water-soluble tetrazolium salt. *Anal Sci*. 2008;**24**:1057-1060.
3. L. H. Lam, *et al*., Flow injection analysis of angiotensin I-converting enzyme inhibitory activity with enzymatic reactors. *Talanta*. 2009;**79**:1130-1134.

Get *pure*DNA Kit - Cell, Tissue

Application: Genomic DNA isolation from cell and tissue
Features: No phenol or chloroform required
Short DNA isolation time
No need for spin columns or Filtration tubes
DNA recovery from a wide range of sample volumes:
up to 6 g tissue or 2x10^9 cells for 200 samples kit

Ordering Information

Product code	Unit*
GK03-20	200 samples

* 1 x 10^7 cells/sample, 25-30 mg tissue/sample

Contents of the Kit

Lysis buffer ..... 100 ml x 1 bottle
Precipitation solution I .....20 ml x 1 bottle
Precipitation solution II ..... 22 ml x 1 bottle
Proteinase K solution .....1 ml x 2 tubes
RNase solution ..... 0.5 ml x 1 tube

Storage Condition

0-5 °C

Shipping Condition

ambient temperature

Required Equipment and Materials

microcentrifuge, vortex mixer, 100 µl and 100-1000 µl adjustable pipettes, ethanol, homogenizer (for tissue), 65°C water bath (for tissue)

Product Description

Get *pure*DNA Kit-Cell, Tissue enables isolation and purification of genomic DNA from cell cultures and tissue samples in three easy steps: 1) sample lysis, 2) removal of RNA and protein, and 3) DNA recovery using ethanol precipitation. To isolate genomic DNA from 1 x 10^8 cells or a 1 g tissue sample, simply increase the volume of each solution (protocols for large samples are available at www.dojindo.com). This kit does not require phenol, chloroform, centrifugal columns, or Filtration tubes. The isolated genomic DNA can be utilized for downstream experiments such as restriction enzyme digestion, ligation, PCR, and other enzymatic reactions.

Protocol for 3 x 10^6 to 1 x 10^7 cells

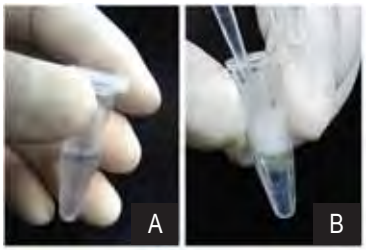


Photo A and B

1. Transfer cell suspension into a 1.5 ml tube and centrifuge at 1,500 rpm for 5 minutes.
2. Discard the supernatant and add 500 µl PBS. Vortex for 5 seconds and centrifuge at 1,500 rpm for 5 minutes.
3. Discard the supernatant and add 250 µl Lysis buffer and 10 µl Proteinase K solution.Dissolve the cells completely using a pipette. Incubate the cell lysate at 65°C for 10 minutes. Be sure that all clumps of cells dissolve before proceeding. *This step is essential for high yield isolation of genomic DNA.
4. Add 2 µl RNase solution and vortex for 5 seconds. Leave the solution at room temperature for 2 minutes.

Photo C

5. Add 50 µl Precipitation solution I and vortex for 5 seconds. White precipitate should appear immediately.

Photo D

6. Add 50 µl Precipitation solution II and vortex for 5 seconds. More white precipitate should appear.

Photo E, F and G

7. Centrifuge at 12,000-14,000 rpm for 5 minutes. Transfer the supernatant to a new 1.5 ml tube. To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If there are white precipitates remaining in the transferred supernatant, repeat this step.

Photo H

8. Add an equal volume of ethanol to the supernatant and mix by inverting the tube several times, then vortex for 5 seconds.

Photo I

9. Centrifuge at 6,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate. A white pellet should be visible at the bottom of the tube. To avoid contamination, do not disturb the white precipitate during the removal of supernatant.

Photo J, K

10. Add 1 ml 70% ethanol and vortex for 5 seconds. Centrifuge at 6,000 rpm for 2 minutes and discard the supernatant. A white pellet should be visible at the bottom of the tube. Carefully remove as much of the supernatant as possible.

Photo L

11. Dry the DNA pellet using a vacuum desiccator for 10 minutes. Dissolve the pellet in TE buffer and use for downstream experiments. The DNA solution can be stored at 4°C for more than one year without degradation.

Protocol for 25 to 30 mg Tissue Sample

1. Transfer 25-30 mg tissue sample into 1.5 ml tube. Add 400 µl Lysis buffer and 10 µl Proteinase K solution.
2. Homogenize the tissue sample using a homogenizer and incubate at 65°C for 10 minutes.
   - *or* -Incubate at 55°C for 2-3 hours with occasional vortexing or pipetting (no need for homogenizing).
3. Leave the tube at room temperature for 2 minutes. Add 2 µl RNase solution and vortex for 5 seconds. Leave the tube at room temperature for 2 minutes.
4. Add 80 µl Precipitation solution I and vortex for 5 seconds. White precipitate should appear immediately.
5. Add 80 µl Precipitation solution II and vortex for 5 seconds. More white precipitate should appear. Leave the tube at room temperature for 2 minutes.
6. Centrifuge at 12,000-14,000 rpm for 5 minutes. Transfer the supernatant to a new 1.5 ml tube. To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If white precipitates remain in the transferred supernatant, repeat this step.
7. Add an equal volume of ethanol to the supernatant and mix by inverting the tube several times, then vortex for 5 seconds.
8. Centrifuge at 6,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate. A white pellet should be visible at the bottom of the tube. Carefully remove as much of the supernatant as possible.
9. Add 1 ml 70% ethanol and vortex for 5 seconds. Centrifuge at 6,000 rpm for 2 minutes, and discard the supernatant. A white pellet should be visible on the bottom of the tube. Carefully remove the supermatant as much as possible.
10. Dry the DNA pellet using a vacuum desiccator for 10 minutes. Dissolve the pellet in TE buffer and use for downstream experiments. The DNA solution can be stored at 4°C over one year without degradation.

Table 1 Typical Amount of DNA Recovered from Given Sample Types

Sample	DNA Recovery	Sample	DNA Recovery
HeLa (1 x 10^7 cells)	80-120 µg	Mouse heart (25-30 mg)	20-25 µg
HeLa (1 x 10^8 cells)	1-1.5 µg	Mouse tail (0.5 -1 cm)	40-60 µg
HL60 (1 x 10^7 cells)	40-60 µg	Mouse liver (1 g)	2-2.5 mg
HL60 (1 x 10^8 cells)	500-900 µg	Mouse brain (1 g)	600-800 µg
Mouse liver (25-30 mg)	40-100 µg	Mouse kidney (1 g)	1.8-2.3 mg
Mouse brain (25-30 mg)	20-40 µg	Rat heart (0.8-0.9g)	600-800 µg
Mouse kidney (25-30 mg)	50-60 µg	Rat tail (10 cm)	2.5-3.5 mg

The $A_{280\ nm}/A_{260\ nm}$ ratio of the recovered DNA is between 1.7 and 1.9.

Troubleshooting

♦No or low DNA recovery

a) Completely dissolve the tissue sample in step 2.
b) Make sure there is a DNA pellet at the bottom of the tube before discarding the supernatant in steps 8 and 9.

♦Difficulty dissolving the tissue sample in step 2

a) Mince or cut the tissue sample into small pieces before transferring it into a tube.
b) Vortex or pipette every 30 minutes during the incubation. If pipetting, use a pipette tip with the point cut off, and pipette gently.

♦Too much precipitate after centrifugation in step 6

a) Completely dissolve the tissue before adding Precipitation solution I.
b) Thoroughly mix the solution by inverting the tube several times after adding precipitation solutions I and II.
c) Increase the centrifugation time if 12,000-14,000 rpm (10,000 g) centrifugation is difficult to achieve.

♦Low DNA purity

a) Be sure to incubate at room temperature for 2 minutes after adding RNase solution in step 3.

Get *pure*DNA Kit – Agarose

Application: DNA fragment extraction from agarose gel
Features: No phenol or chloroform required
DNA isolation in 30 minutes
No need for columns or filtration tubes

Ordering Information

Product code	Unit*
GK01-20	200 samples

* 200 mg agarose/sample

Contents of the Kit

Gel lysis buffer ..... 65 ml x 1 bottle
Co-precipitation solution .....0.5 ml x 1 tube
Precipitation solution ..... 65 ml x 1 bottle

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Required Equipment and Materials
microcentrifuge, transiluminator or UV lamp, vortex mixer, 100 µl and 1000 µl adjustable pipettes, ethanol

Product Description
Get *pure*DNA Kit-Agarose enables isolation and purification of double-stranded DNA from agarose gel in three easy steps: 1) lysis of agarose gel, 2) removal of agarose gel, and 3) DNA recovery using ethanol precipitation. Double-stranded DNA can be isolated from up to 200 mg of agarose gel using 1.5 ml tubes. To isolate DNA from a larger quantity of agarose gel, simply increase the volume of each solution. This kit does not require phenol, chloroform, centrifugal columns, or filtration tubes. The extracted double-stranded DNA can be utilized for restriction enzyme digestion, ligation, PCR, and other enzymatic reactions.

Protocol for 100 - 200 mg Agarose Gel Slice

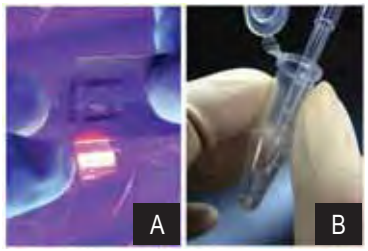


Photo A

1. Cut and weigh the agarose gel slice that contains double-stranded DNA fragment. Trim the edge using a razor if the weight is over 200 mg.

Photo B

2. Put the agarose gel slice into a 1.5 ml tube, and break it into several pieces using a pipette tip.

Photo C, D

3. Add 300 µl Gel lysis buffer. Incubate at 60°C for 10 minutes, vortexing every 2 minutes. Continue incubation if agarose gel is not completely dissolved after 10 minutes.

Photo E

4. Allow the tube to cool at room temperature for 2 minutes, and then add 300 µl Precipitation Solution. White precipitate should appear immediately. Mix the solution by inverting the tube 5 times.

Photo F

5. Centrifuge at 12,000-14,000 rpm for 5 minutes.

Photo G, H

6. Transfer the supernatant to a new 1.5 ml tube. To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If white precipitates remain in the transferred supernatant, repeat step 5 and 6.

Photo I

7. Add 2 µl Co-precipitation solution to the supernatant and vortex for 5 seconds. Add 800 µl ethanol to the supernatant and vortex for 5 seconds. Co-precipitation solution is a DNase-free glycogen solution (20 mg/ml). If you prefer not to use this solution, you can simply omit this step. Please note that the DNA pellet may not be visible without Co-precipitation solution. Be careful not to disturb the solution during the removal of the supernatant, and do not leave the tube in the centrifuge for more than 1 minute after centrifugation. Avoid using a clear tube since the DNA pellet easily peels off the wall of the tube.

Photo J

8. Centrifuge at 12,000-14,000 rpm for 3 minutes and discard the supernatant. A fairly large white pellet should be visible at the bottom of the tube if you use Co-precipitation solution. Be careful not to disturb the white pellet during the removal of supernatant.

Photo K

9. Add 1 ml 70% ethanol and vortex for 5 seconds. Centrifuge at 12,000-14,000 rpm for 3 minutes and discard the supernatant. Repeat this step once. A white pellet should be at the bottom of the tube. Carefully remove as much of the supernatant as possible.

Photo L

10. Dry the DNA pellet using a vacuum desiccator for 10 minutes or leave it at room temperature for 20-30 minutes. Dissolve the pellet in TE buffer and use for downstream experiments. The DNA solution can be stored at 0-5°C.

Troubleshooting

♦No or low DNA recovery

a) Completely dissolve the agarose gel slice by vortexing in step 2. It may be difficult to see undissolved agarose gel pieces in Gel lysis buffer.

b) Make sure there is a DNA pellet at the bottom of the tube before discarding the supernatant in steps 8 and 9.

♦Difficulty dissolving the agarose gel slice in step 3

a) Crush the gel slice in a 1.5 ml tube with a pipette tip.

b) Vortex the tube every 2 minutes during solubilization of the gel slice at 60°C.

♦Too much precipitate after centrifugation in step 5

a) Completely dissolve the agarose gel slice before adding precipitation solution.

b) Thoroughly mix the solution by vortexing after adding Precipitation solution.

c) Increase the centrifugation time if 12,000-14,000 rpm (10,000 g) is difficult to achieve.

♦Low DNA purity

a) Do not disturb the white precipitate in step 6.

b) Remove as much of the supernatant as possible in steps 8 and 9.

♦Degradation of isolated DNA

a) Minimize the UV irradiation time during preparation of agarose gel slice in step 1.

b) Avoid using short wavelength UV light.

Get *pure*RNA Kit

Application: Total RNA isolation from cell, tissue and blood
Features: No phenol or chloroform required
Short RNA isolation time
No need for spin columns or filtration tubes
RNA recovery from a wide range of sample volumes:
up to 5x10^8 cells, 1.5 g tissue or 1 ml whole blood for GK04-05

Ordering Information

Product code	Unit*
GK04-05	50 samples

* 1x10^7 cells/sample, 25-30 mg tissue/sample, 200 µl whole blood/sample

Contents of the Kit

Lysis buffer ..... 13 ml x 2 bottles
DNase .....0.55 ml x 1 tube
DNase dilution buffer .....12 ml x 1 bottle
Precipitation solution I ..... 13 ml x 1 bottle
Precipitation solution II ..... 8 ml x 1 bottle

Storage Condition

0-5°C

Shipping Condition

ambient temperature

Required Equipment and Materials

microcentrifuge, vortex mixer, 100 µl and 1000 µl adjustable pipettes, ethanol, ß-mercaptoethanol, homogenizer (for tissue)

Product Description

Get *pure*RNA Kit enables quick isolation and purification of total RNA from animal cells, tissue samples, and whole blood. This kit does not use harmful organic solvents such as phenol or chloroform. Unlike the CsCl method, this kit does not require ultracentrifugation. Get *pure*RNA Kit can be used for a wider range of sample volumes than silica-based spin column kits. The isolated RNA can be used for downstream experiments such as RT-PCR, Northern blotting, and cDNA synthesis

Protocol for 3 x 10^6 to 1 x 10^7 Cells

1. Transfer cell suspension into a 1.5 ml tube and centrifuge at 1,500 rpm for 5 minutes. Discard the supernatant and add 500 µl PBS. Vortex for 5 seconds and centrifuge at 1,500 rpm for 5 minutes.
2. Discard the supernatant and add 500 µl Lysis buffer and 5 µl ß-mercaptoethanol (not included in this kit). Completely dissolve the cells by pipetting (for 2-3 minutes). Make sure that all clumps of cells dissolve before proceeding. This step is essential for high yield RNA isolation.
3. Add 100 µl Precipitation solution I and vortex for 5 seconds. White precipitate should appear immediately.
4. Centrifuge at 12,000-14,000 rpm for 5 minutes. Transfer the supernatant to a new 1.5 ml tube. To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If white precipitate remains in the transferred supernatant, repeat this step.
5. Add an equal volume of ethanol to the supernatant and vortex for 5 seconds.
6. Centrifuge at 12,000-14,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate. A white pellet should be visible on the side or bottom of the tube. To avoid contamination, do not disturb the white precipitate during the removal of supernatant.
7. Add 1.2 ml 70% ethanol and vortex for 5 seconds.
8. Centrifuge at 12,000-14,000 rpm for 2 minutes and discard the supernatant. A white pellet should be visible at the side or bottom of the tube. Carefully remove as much of the supernatant as possible.
9. Prepare 20X diluted DNase working solution in a separate tube using DNase dilution buffer (e.g., 10 µl DNase + 190 µl DNase dilution buffer).
10. Add 200 µl DNase working solution to the tube from step 8 and dissolve the pellet.
11. Incubate at 37°C for 15 minutes.
12. Add 50 µl Precipitation solution I and vortex for 5 seconds. White precipitate should appear immediately.

13. Add 50 µl Precipitation solution II and vortex for 5 seconds. More white precipitate should appear.
14. Centrifuge at 12,000-14,000 rpm for 5 minutes, then transfer the supernatant to a new 1.5 ml tube. To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If white precipitates remain in the transferred supernatant, repeat this step.
15. Add an equal volume of ethanol to the supernatant and vortex for 5 seconds.
16. Centrifuge at 12,000-14,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate. A white pellet should be visible on the side or bottom of the tube. To avoid contamination, do not disturb the white precipitate during the removal of supernatant.
17. Add 1.2 ml 70% ethanol and vortex for 5 seconds.
18. Centrifuge at 12,000-14,000 rpm for 2 minutes and discard the supernatant. A white pellet should be visible on the side or bottom of the tube. Carefully remove as much of the supernatant as possible.
19. Dissolve the pellet in RNase-free water (DEPC-treated water) or RNase-free buffer.

Protocol for 25 to 30 mg Tissue Sample

1. Transfer 25-30 mg of chopped tissue sample pieces into 1.5 ml tube. Add 500 µl Lysis buffer and 5 µl ß-Mercaptoethanol (not included in this kit). Cool the sample in an ice bucket.
2. Homogenize the sample in the ice bucket using a homogenizer for 30-60 seconds.
3. Add 100 µl Precipitation solution I and vortex for 5 seconds.
4. Centrifuge at 12,000-14,000 rpm for 5 minutes. Transfer the supernatant to a new 1.5 ml tube. To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If white precipitate remains in the transferred supernatant, repeat this step.
5. Add an equal volume of ethanol to the supernatant and vortex for 5 seconds.
6. Centrifuge at 12,000-14,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate. A white pellet should be visible on the side or bottom of the tube. Carefully remove as much of the supernatant as possible.
7. Add 1.2 ml 70% ethanol and vortex for 5 seconds.
8. Centrifuge at 12,000-14,000 rpm for 2 minutes and discard the supernatant. A white pellet should be on the side or bottom of the tube. Carefully remove as much of the supernatant as possible.
9. Prepare 20X diluted DNase working solution in a separate tube using DNase dilution buffer. (e.g., 10 µl DNase + 190 µl DNase dilution buffer).
10. Add 200 µl DNase working solution to the tube from step 8, and dissolve the pellet.
11. Incubate at 37°C for 15 minutes.
12. Add 50 µl Precipitation solution I and vortex for 5 seconds. White precipitate should appear immediately.
13. Add 50 µl Precipitation solution II and vortex for 5 seconds. More white precipitate should appear.
14. Centrifuge at 12,000-14,000 rpm for 5 minutes. Transfer the supernatant to a new 1.5 ml tube. To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If white precipitate remains in the transferred supernatant, repeat this step.
15. Add an equal volume of ethanol to the supernatant and vortex for 5 seconds.
16. Centrifuge at 12,000-14,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate. A white pellet should be visible on the side or bottom of the tube. To avoid contamination, do not disturb the white precipitate during the removal of supernatant.
17. Add 1.2 ml 70% ethanol and vortex for 5 seconds.
18. Centrifuge at 12,000-14,000 rpm for 2 minutes and discard the supernatant. A white pellet should be visible on the side or bottom of the tube. Carefully remove as much of the supernatant as possible.
19. Dissolve the pellet in RNase-free water (DEPC-treated water) or RNase-free buffer.

DNA RNA Isolation

Protocol for 200 µl of Whole Blood

1. Transfer 200 µl whole blood into a 1.5 ml tube. Add 300 µl Lysis Buffer and 5 µl ß-Mercaptoethanol (not included in this kit). Completely dissolve the cells by pipetting for 2-3 minutes. Make sure that all clumps of cells dissolve before proceeding. This step is essential for high-yield RNA isolation.
2. Add 100 µl Precipitation solution I and vortex for 5 seconds. Precipitate should appear immediately.
3. Add 100 µl Precipitation solution II and vortex for 5 seconds. More precipitate should appear.
4. Centrifuge at 12,000-14,000 rpm for 5 minutes. Transfer the supernatant to a new 1.5 ml tube. To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If white precipitates remaining in the transferred supernatant, repeat this step.
5. Add 100 µl Precipitation solution I and vortex for 5 seconds. White precipitate should appear immediately.
6. Centrifuge at 12,000-14,000 rpm for 5 minutes, and transfer the supernatant to a new 1.5 ml tube. To avoid contamination, do not disturb the white precipitate.
7. Add an equal volume of ethanol* to the supernatant and vortex for 5 seconds.
   * *Optionally, 2 µl glycogen solution (20 mg/ml, not provided) can be added as a co-precipitation solution before adding the ethanol.*
8. Add 1.2 ml 70% ethanol and vortex for 5 seconds.
9. Centrifuge at 12,000-14,000 rpm for 2 minutes and discard the supernatant.
10. Prepare 20X diluted DNase working solution in a separate tube using DNase dilution buffer (e.g., 10 µl DNase + 190 µl DNase dilution buffer).
11. Add 200 µl DNase working solution to the tube from step 9 and dissolve the pellet.
12. Incubate at 37°C for 15 minutes.
13. Add 50 µl Precipitation solution I and vortex for 5 seconds. White precipitate should appear immediately.
14. Add 50 µl Precipitation solution II and vortex for 5 seconds. More white precipitate should appear.
15. Centrifuge at 12,000-14,000 rpm for 5 minutes. Transfer the supernatant to a new 1.5 ml tube. To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If white precipitate remains in the transferred supernatant repeat this step.
16. Add an equal volume of ethanol* to the supernatant and vortex for 5 seconds.
    * *Optionally, 2 µl glycogen solution (20 mg/ml, not provided) can be added as a co-precipitation solution before adding the ethanol.*
17. Centrifuge at 12,000-14,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate. A white pellet should be visible on the side or bottom of the tube. To avoid contamination, do not disturb the white precipitate during the removal of supernatant.
18. Add 1.2 ml 70% ethanol and vortex for 5 seconds.
19. Centrifuge at 12,000-14,000 rpm for 2 minutes and discard the supernatant. A white pellet should be visible on the side or bottom of the tube. Carefully remove as much of the supernatant as possible.
20. Dissolve the pellet in RNase-free water (DEPC-treated water) or RNase-free buffer.

Table 1 RNA Recovery

Sample	RNA Recovery	Sample	RNA Recovery
HeLa (1 x 10^7 cells)	90-150 µg	Mouse kidney (30 mg)	40-55 µg
Balb3T3 (1 x 10^7 cells)	90-150 µg	Mouse heart (30 mg)	7-15 µg
HL60 (1 x 10^7 cells)	30-60 µg	Mouse liver (1 g)	2.8-4.4 mg
Mouse liver (20 mg)	60-75 µg	Mouse brain (1 g)	700-1000 µg
Mouse brain (30 mg)	15-25 µg	Mouse blood (1 ml)	14-24 µg

The $A_{260\ nm}$ / $A_{280\ nm}$ ratio of the recovered RNA is between 2.0 -2.2. The A_{260}/A_{280} values are based on recovered RNA dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). The use of DEPC-treated water may lower the A_{260}/A_{280} values.

Troubleshooting

♦No or low RNA recovery

Dissolve the samples compeletely with pipetting. Make sure that the RNA pellet is on the side or bottom of the tube before discarding the supermatant.

♦Difficulty dissolving the tissue sample

Mince or chop the tissue sample into very small pieces before transferring it to a tube.

♦The precipitates are not packed tightly after adding Precipitation solution II.

Completely dissolve the sample before adding Precipitation solution I. Mix the solution thoroughly by vortexing after adding Precipitation solutions I and II. Increase the centrifugation time if 12,000-14,000 rpm (10,000 g) centrifugation is difficult.

♦Low RNA purity

Do not disturb the white precipitate during the supernatant transfers. Use a small volume micropipette (<20 µl) when removing the supernatants after the ethanol precipitation.

♦Degradation of isolated RNA

Use freshly prepared samples. Make sure that all materials and equipment are RNase-free (e.g., ethanol, microtubes, pipette tips).

Introduction

Enzymatic oxidation reactions, based on the hydrogen peroxide and peroxidase reactions, have been utilized over several decades for diagnostic analyses because of their high selectivity and sensitivity. To determine the concentration of a specific element in a biological sample, the specificity of the reaction is necessary. A large number of oxidases have been found and used in detection systems. In most cases, the enzymatic reaction of an oxidase generates hydrogen peroxide, and the concentration of hydrogen peroxide indicates the concentration of a substrate in a reaction solution. Therefore, the concentration of substrate can be determined by measuring the amount of hydrogen peroxide. The amount of hydrogen peroxide is determined by spectrophotometer using an oxidative chromogenic dye and peroxidase. Since peroxidase is one of the most commonly used enzymes for enzyme immunoassays (EIA), several oxidative chromogenic dyes are available for staining tissue sections and membrane filters. It can also be utilized for microplate assays. The stability of these oxidative chromogenic dyes, however, is sometimes low due to the oxidation by oxygen. Dojindo offers stable and water-soluble oxidative chromogenic dyes for the hydrogen peroxide (H_2O_2)-peroxidase assay. The table on page 197 indicates the characteristics of water-soluble aniline derivatives (New Trinder's Reagents) that are used for hydrogen peroxide detection.

Peroxidase Detection

Tetramethylbenzidine (TMBZ) is a commonly utilized oxidative chromogenic dye for peroxidase detection on a membrane filter or an EIA plate. Peroxidase catalyses the reduction of hydrogen peroxide to generate two water molecules. TMBZ is the electron source for this reduction. Since the oxidized forms of TMBZ have intense colors, peroxidase can be determined colorimetrically. Horseradish peroxidase (HRP) is widely applied in EIA and diagnostic analyses. HRP (brown color) reacts with hydrogen peroxide to form Compound I (yellowishgreen color). Then Compound I turns back to HRP by one electron oxidoreduction via Compound II (red color). Most of the chromogenic dyes are easily oxidized with activated oxygen by metals or light. TMBZ is a stable oxidative chromogenic dye utilized for microplate assays. Colorless TMBZ solution is turned blue or greenish-blue by the hydrogen peroxide and peroxidase oxidation reaction. Sulfuric acid (1-2 M) is sometimes added to the assay solution to enhance the color development of oxidized TMBZ. Oxidized TMBZ solution turns from blue to yellow when sulfuric acid is added, and its molar absorptivity is 2 to 3 times that of the blue oxidized TMBZ.

Hydrogen Peroxide Detection

Enzymatic oxidation reactions are highly selective and sensitive enough to measure a specific substrate in complicated mixtures such as biological samples. Due to their selectivity, oxidases have been used in various assay systems established for diagnostic analyses. There are two steps to determining the amount of substrate: 1) the oxidation reaction of the substrate with oxidase, and 2) the detection of hydrogen peroxide generated by the oxidation reaction. Aniline and phenol compounds have been used as oxidative chromogenic reagents for hydrogen peroxide detection. However, they are not stable enough to prepare assay solutions or test papers. New Trinder's Reagents, which are aniline analogs, have propylsulfonic acid (PS) or hydroxypropylsulfonic acid (OS) attached to an amino group of their aniline structure. These PS or OS groups give aniline water solubility and markedly improved its stability. Trinder's reagent requires a coupling reagent such as 4-aminoantipyrin or methylbenzothiazolinonehydrazone (MBTH), to produce a stable dye. Dojindo offers various New Trinder's Reagents for the best combination with oxidase-substrate reactions.

TMBZ 3,3',5,5'-Tetramethylbenzidine [CAS: 54827-17-7]

Application: Peroxidase detection, colorimetric
Appearance: White to pale grayish-brown crystalline powder
Purity: ≥99.0 % (HPLC)
MW: 240.34, $C_{16}H_{20}N_2$

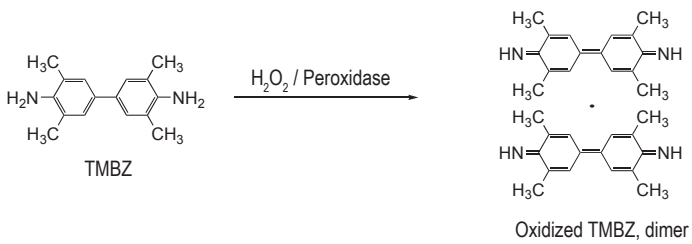
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
T022-10	1 g
T022-12	5 g

Oxidation Reaction



TMBZ

Oxidized TMBZ, dimer

TMBZ·HCl

3,3',5,5'-Tetramethylbenzidine, dihydrochloride, dihydrate [CAS: 64285-73-0]

Applicaton: Peroxidase detection, colorimetric
Appearance: White to slightly pink crystalline powder
Purity: ≥98.0% (Titration)
MW: 349.30, $C_{16}H_{22}Cl_2N_2 \cdot 2H_2O$

Ordering Information

Product code	Unit
T039-10	1 g

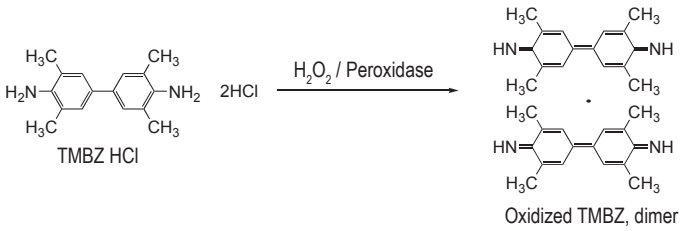
Storage Condition

ambient temperature, protect from light metal, and moisture

Shipping Condition

ambient temperature

Oxidation Reaction



TMBZ HCl

Product Description of TMBZ Compounds

TMBZ is a chromogenic reagent utilized for peroxidase detection. It has been developed as an alternative to benzidine, which is a carcinogenic chemical. Because of the ortho methyl groups on its benzene ring, TMBZ is not metabolized into highly carcinogenic o-hydroxybenzidines or o,o'- dihydroxybenzidines. Therefore, TMBZ compounds are much less carcinogenic than benzidine. Although the TMBZ solution is colorless, it turns bluish-green (λmax: 655 nm) in the presence of hydrogen peroxide and peroxidase. The structure of this bluish-green complex is thought to be a radical form of two oxidized TMBZ molecules. TMBZ HCl is a hydrochloride form of TMBZ that is readily soluble in water (100 mg TMBZ HCl per 10 ml water).

Assay Protocol

1. Dissolve 6 mg of TMBZ with 1 ml DMSO to prepare 100X TMBZ solution.
2. Mix 5 µl of 30% hydrogen peroxide solution with 1 ml PBS to prepare 200X H_2O_2 solution.
3. Add 10 µl of 100X TMBZ solution and 5 µl of 200X H_2O_2 solution to 1 ml PBS to prepare staining solution.*
4. Add 100-200 µl of the staining solution to each well of a 96-well microplate. Incubate the plate at room temperature or at 37°C for 5 minutes to 1 hour.
5. Wash the sample with PBS several times to stop the staining reaction.

* For the best results, modification of the final concentration of TMBZ and hydrogen peroxide may be necessary. The staining solution is not stable. Please prepare fresh solution prior to use.

References

1. V. R. Holland, *et al*., A Safer Substitute for Benzidine in the Detection of Blood. *Tetrahedron*. 1974;**30**:3299-3302.
2. W. Levin, *et al*., Preparation of Partially Purified, Lipid-depleted Cytochrome P-450 and Reduced Nicotinamide Adenine Dinucleotide Phosphate-cytochrome c Reductase from Rat Liver Microsomes. *J Biol Chem*. 1974;**249**:1747-1754.
3. P. E. Thomas, *et al*., An Improved Staining Procedure for the Detection of the Peroxidase Activity of Cytochrome p-450 on Sodium Dodecyl Sulfate Polyacrylamide Gels. *Anal Biochem*. 1976;**75**:168-176.
4. H. H. Liem, *et al*., Quantitative Determination of Hemoglobin and Cytochemical Staining for Peroxidase Using 3, 3', 5, 5'-Tetramethylbenzidine dihydrochloride, a Safe Substitute for Benzidine. *Anal Biochem*. 1979;**98**:388-393.
5. R. C. Lijana, *et al*., Tetramethylbenzidine- A substitute for Benzidine in Hemoglobin Analysis. *J Lab Clin Med*. 1979;**94**:266-276.
6. R. M. Jaffe, *et al*., A New Occult Blood Test Not Subject to False-Negative Results from Reducing Substances. *J Lab Clin Med*. 1979;**93**:879-886.
7. K. Suzuki, *et al*., Assay Method for Myeloperoxidase in Human Polymorphonuclear Leukocytes. *Anal Biochem*. 1983;**132**:345-352.
8. F. H. Pujol, *et al*., A Double Sandwich Monoclonal Enzyme Immunoassay for Detection of Hepatitis B Surface Antigen. *J Immunoassay*. 1993;**14**:21-31.
9. F. B. Serrat, Colorimetric Method for Determiantion of Chlorine with 3, 3', 5, 5'-Tetramethylbenzidine. *Talanta*. 1994;**41**:2091-2094.

DAB 3,3'-Diaminobenzidine, tetrahydrochloride [CAS: 7411-49-6]

Application: Peroxidase detection, colorimetric
Appearance: White or slightly pinkish-gray powder
Purity: ≥97% (Titration)
MW: 360.11, $C_{12}H_{18}Cl_4N_4$

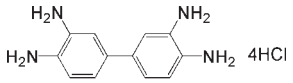
Ordering Information

Product code	Unit
D006-10	1 g
D006-12	5 g

Storage Condition
0-5 °C, protect from light

Shipping Condition
Ambient temperature

Chemical Structure



Product Description:
DAB is one of the most commonly used oxidative chromogenic dyes for the detection of peroxidase in immunohistochemistry. In the presence of H_2O_2 and peroxidase, DAB is oxidized into a brown pigment. These brown pigments are aniline black type compounds and are firmly deposited around peroxidase on cell membranes or tissues. Most DAB available on the market appears brown as a result of oxidation during processing or storage. On the other hand, Dojindo offers a high quality DAB with a white or slightly reddish-gray powder appearance that is good for sharp staining.

Preparation of Sample Staining Solution
1. Dissolve 9 mg DAB with 1 ml PBS to prepare 100X DAB solution.
2. Mix 5 µl of 30% hydrogen peroxidase solution with 1 ml PBS to prepare 200X H_2O_2 solution.
3. Add 10 ul of 100X DAB solution and 5 ul of 200X H_2O_2 solution to 1 ml PBS to prepare staining solution[a].
4. Add the staining solution to a sample in a staining chamber, and incubate the sample at room temperature for 5 minutes to 1 hour [b].
5. Wash the sample with PBS several times to stop the staining reaction.

[a] *The staining solution is not stable. Prepare fresh solution prior to use.*
[b] *For better staining, incubate the sample with 0.09 mg DAB/PBS solution for 10 minutes before adding the staining solution.*

References

1. A. B. Novikoff, *et al*., Studies on Microperoxisomes V. Are Microperoxisomes Ubiquitous in Mammalian Cells. *J Histochem Cytochem*. 1973;**21:**737.
2. V. Herzog, *et al*., A New Sensitive Colorimetric Assay for Peroxidaase Using 3, 3'-Diaminobenzidine as Hydrogen Donor. *Anal Biochem*. 1973;**55**:554.
3. H. Yamada, *et al*., Improvement of Technique of Immunohistochemical Demonstraction of Bioactive Substances in the Central Nervous System. *Acta Histochem Cytochem*. 1987;**20**:629.
4. K. L. Cheng, Determination of Traces of Selenium 3,3'-Diaminobenzidine as Selenium(IV) Organic Reagent. *Anal Chem*. 1956;**28**:1738.

SAT-3 *N,N'*-Bis(2-hydroxy-3-sulfopropyl)tolidine, disodium salt, tetrahydrate

Application: Peroxidase detection
Appearance: White or pale-brown powder
Purity: ≥95.0% (HPLC)
MW: 604.62, $C_{20}H_{26}N_2Na_2O_8S_2 \cdot 4H_2O$

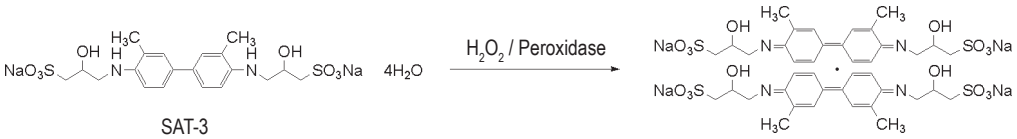
Ordering Information

Product code	Unit
S302-10	1 g

Storage Condition
0-5°C, protect from light

Shipping Condition
ambient temperature

Oxidation Reaction



SAT-3 Oxidized SAT-3, dimer

Product Description
SAT-3 is a stable, highly water-soluble o-Tolidine analog. It is easily oxidized by peroxidase and hydrogen peroxide into a green dye at pH 4-6. While TMBZ requires an organic solvent or detergent to solubilize, SAT-3 can be solubilized with just buffer solution. The SAT-3 solution can be stored at room temperature with protection from light. The maximum wavelength of the dye is 675 nm. Using the Ames test, Nno mutagenicity of SAT-3 was detected. using the Ames test.

Assay Protocol
1. Mix 1 ml of 100 mM SAT-3 solution and 1 ml of 30 mM hydrogen peroxide solution with 8 ml of 50 mM citrate buffer (pH 4) to prepare an assay solution.
2. Add 100-200 µl of the assay solution to each well of a 96-well plate and incubate at 37°C for 5-30 minutes.
3. Read the O.D. at 670 nm or add 50 µl of 4 M sulfuric acid to each well and read the O.D. at 490 nm instead.

Reference
M. Mizoguchi, *et al*., Sensitive Chromogenic Substrate for Detecting Peroxidase Activity. *Anal Commun*. 1998;**35**:179.

New Trinder's Reagents

Application: Hydrogen peroxide detection, colorimetric
Features: Highly water-soluble
Stable aniline analogs
Wide pH range in color development and oxidization reactions

Product Description
New Trinder's Reagents, which are highly water-soluble aniline derivatives, are widely used in diagnostic assays and biochemical examinations. They have several advantages over conventional chromogenic reagents in the colorimetric determination of hydrogen peroxide activity. New Trinder's Reagents are stable enough to use in both solution and test strip detection systems. New Trinder's reagents form highly stable purple or blue dyes through an oxidative coupling reaction with 4-Aminoantipyrine (4-AA) or 3-Methylbenzothiazolinone hydrazone (MBTH) in the presence of hydrogen peroxide and peroxidase (Table 1).The molar absorptivity of the coupled dye with MBTH is 1.5 to 2 times higher than that with 4-AA; however, 4-AA solution is more stable than MBTH solution. Enzymatic oxidization of a substrate by its oxidase produces hydrogen peroxide. The hydrogen peroxide concentration corresponds to the substrate concentration.
Therefore, the amount of the substrate can be determined by the color development of an oxidative coupling reaction. Glucose, alcohol, acyl-CoA, and cholesterol are utilized for the detection of those substrates coupled with New Trinder's Reagents and 4-AA. There are 10 kinds of New Trinder's reagents available. The table below shows the maximum wavelength and molar absorptivity of each oxidized New Trinder's Reagent complex with 4-AA. Among the New Trinder's reagents, TOOS is the most frequently used to develop assay systems. However, experimentation with the different New Trinder's reagents will be necessary to develop the best detection system for a given substrate.

Table 1 Characteristics of Dyes Derived from New Trinder's Reagent

New Trinder's Reagant	λmax (nm), pH	Molar Absorptivity
ADOS	542, 5.5-9.5	2.72×10^4
ADPS	540, 5.9-9.5	2.79×10^4
ALPS	561, 6.5-7.5	4.13×10^4
DAOS	593, 5.5-9.5	1.75×10^4
HDAOS	583, 5.5-9.5	1.73×10^4

New Trinder's Reagant	λmax (nm), pH	Molar Absorptivity
MADB	630, 5.5-9.5	1.65×10^4
MAOS	630, 5.5-9.5	2.25×10^4
TODB	550, 5.5-9.5	3.80×10^4
TOOS	555, 5.5-9.5	3.92×10^4
TOPS	550, 5.5-9.5	3.74×10^4

ADOS *N*-Ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3-methoxyaniline, sodium salt, dihydrate [CAS: 82692-96-4]

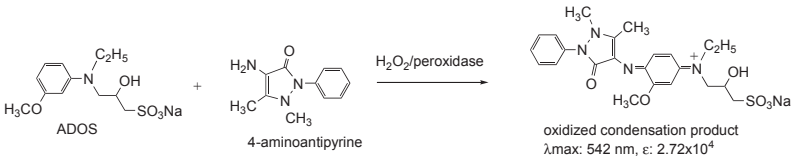
Application: Hydrogen peroxide detection, colorimetric
Appearance: White or slightly brown powder
Molar absorptivity(pH10): ≥8,200(around 255 nm)
MW: 347.36, $C_{12}H_{18}NNaO_5S$, $2H_2O$

Ordering Information
Product code	Unit
OC01-10	1 g

Storage Condition
0-5 °C, protect from light

Shipping Condition
ambient temperature

Oxidation Reaction



ADPS *N*-Ethyl-*N*-(3-sulfopropyl)-3-methoxyaniline, sodium salt, monohydrate [CAS: 82611-88-9]

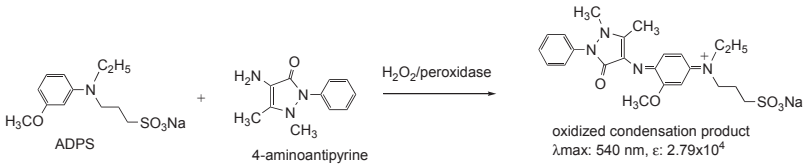
Application: Hydrogen peroxide detection, colorimetric
Appearance: White or slightly brown powder
Molar absorptivity (pH10): ≥8,200 (around 255 nm)
MW: 313.35, $C_{12}H_{18}NNaO_4S$, H_2O

Storage Condition	Shipping Condition
0-5 °C, protect from light	ambient temperature

Ordering Information

Product code	Unit
OC02-10	1 g

Oxidation Reaction



ALPS *N*-Ethyl-*N*-(3-sulfopropyl)aniline, sodium salt [CAS: 82611-85-6]

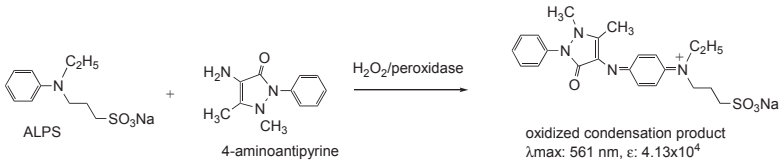
Application: Hydrogen peroxide detection, colorimetric
Appearance: White or slightly grayish-yellow powder
Molar absorptivity: ≥9,500 (around 255 nm)
MW: 265.31, $C_{11}H_{16}NNaO_3S$

Storage Condition	Shipping Condition
0-5 °C, protect from light	ambient temperature

Ordering Information

Product code	Unit
OC04-10	1 g

Oxidation Reaction



DAOS *N*-Ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, sodium salt [CAS: 83777-30-4]

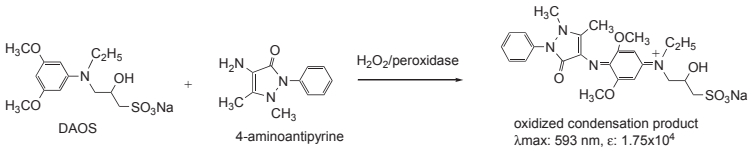
Application: Hydrogen peroxide detection, colorimetric
Appearance: White or pale blueish white powder
Purity: ≥97.0% (Absorbance)
MW: 341.36, $C_{13}H_{20}NNaO_6S$

Storage Condition	Shipping Condition
0-5 °C, protect from light	ambient temperature

Ordering Information

Product code	Unit
OC06-10	1 g

Oxidation Reaction



HDAOS *N*-(2-Hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline, sodium salt [CAS: 82692-88-4]

Application: Hydrogen peroxide detection, colorimetric
Appearance: White or almost white powder
Purity: ≥98.0% (Absorbance)
MW: 313.30, $C_{11}H_{16}NNaO_6S$

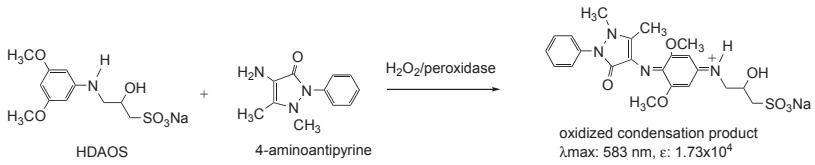
Storage Condition
0-5 °C, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
OC08-10	1 g

Oxidation Reaction



MADB *N*,*N*-Bis(4-sulfobutyl)-3,5-dimethylaniline, disodium salt

Application: Hydrogen peroxide detection, colorimetric
Appearance: White or slightly brown powder
Purity: ≥97.0% (HPLC)
MW: 437.48, $C_{16}H_{25}NNa_2O_6S_2$

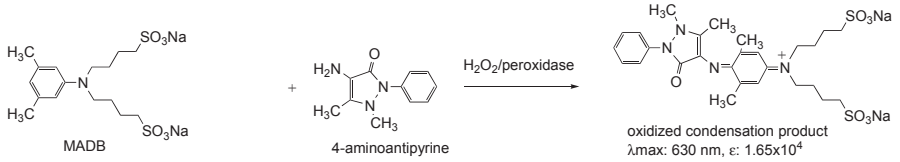
Storage Condition
0-5 °C, protect from moisture and light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
OC21-10	1 g

Oxidation Reaction



MAOS *N*-Ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3,5-dimethylaniline, sodium salt, monohydrate [CAS: 82692-97-5]

Application: Hydrogen peroxide detection, colorimetric
Appearance: White or slightly yellowish-pink powder
Molar absorptivity (pH10): ≥10,000 (around 257 nm)
MW: 327.37, $C_{13}H_{20}NNaO_4S$, H_2O

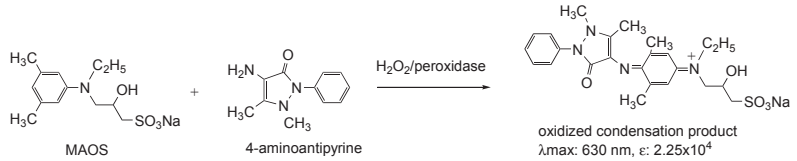
Storage Condition
0-5 °C, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
OC11-10	1 g

Oxidation Reaction



TODB *N*,*N*-Bis(4-sulfobutyl)-3-methylaniline, disodium salt

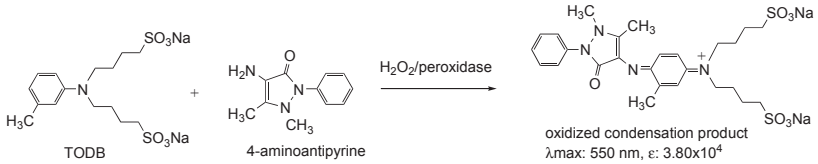
Application: Hydrogen peroxide detection, colorimetric
Appearance: White or slightly yellowish white powder
Purity: ≥98.0% (HPLC)
Molar absorptivity (pH10): ≥9,900 (around 257 nm)
MW: 423.46, $C_{15}H_{23}NNa_2O_6S_2$

Ordering Information	
Product code	Unit
OC22-10	1 g

Storage Condition
0-5 °C, protect from moisture and light

Shipping Condition
ambient temperature

Oxidation Reaction



TOOS *N*-Ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3-methylaniline, sodium salt, dihydrate [CAS: 82692-93-1]

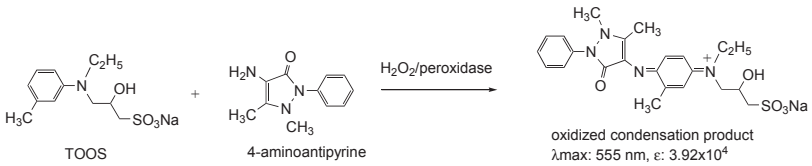
Application: Hydrogen peroxide detection, colorimetric
Appearance: White or slightly blue powder
Purity: ≥98.0% (Absorbance)
MW: 331.36, $C_{12}H_{18}NNaO_4S$, $2H_2O$

Ordering Information	
Product code	Unit
OC13-10	1 g

Storage Condition
ambient temperature, protect from light

Shipping Condition
ambient temperature

Oxidation Reaction



TOPS *N*-Ethyl-*N*-(3-sulfopropyl)-3-methylaniline, sodium salt, monohydrate [CAS: 40567-80-4]

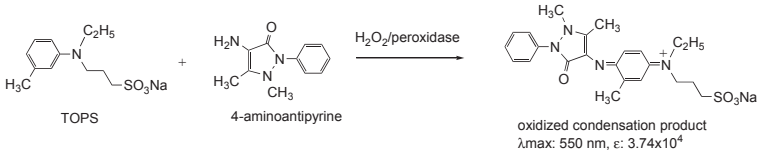
Application: Hydrogen peroxide detection, colorimetric
Appearance: White or slightly blue powder
Purity: ≥97.0% (Absorbance)
MW: 297.34, $C_{12}H_{18}NNaO_3S$, H_2O

Ordering Information	
Product code	Unit
OC14-10	1 g

Storage Condition
0-5 °C, protect from light

Shipping Condition
ambient temperature

Oxidation Reaction



Preparation of Assay Solution - TOOS

1. Dissolve 20 mg TOOS with 10 ml PBS to prepare 6.6 mM TOOS solution.
2. Dissolve 14 mg 4-aminoantipyrin (AA) with 10 ml PBS to prepare 6.6 mM 4-AA solution.
3. Prepare 2 U/ml horseradish peroxidase solution with PBS.
4. Mix the same volume of each solution together to prepare assay solution. Store the assay solution at 4 °C with protection from light.

Assay Protocol

1. Prepare sample solutions for the enzymatic oxidation reaction. The pH range of the buffer solution should be from 5.5-9.5.
2. Prepare standard solutions containing known amounts of substrate using the same buffer.
3. Add the appropriate units of oxidase to the sample solution; then add the same volume of the assay solution.
4. Incubate the mixture at room temperature or at 37 °C for 30 minutes to 1 hour.
5. Measure the O.D. at 555 nm.
6. Prepare a standard curve, and determine the substrate concentration in the sample solution.

References

1. K. Tamaoku, *et al*., New water-soluble Hydrogen Donors for the Enzymatic Photometric Determination of Hydrogen Peroxide. II. N-Ethyl-N-(2-hydroxy-3-sulfopropyl)aniline derivatives. *Chem Pharm Bull*. 1982;**30**:2492-2497.
2. K. Tamaoku, *et al*., New water-soluble Hydrogen Donors for the Enzymatic Spectrophotometric Determination of Hydrogen Peroxide. *Anal Chim Acta*. 1982;**136**:121-127.
3. B. C. Madsen, *et al*., Flow Injection and Photometric Determination of Hydrogen Peroxide in Rainwater with N-Ethyl-N-(sulfopropyl)aniline sodium salt. *Anal Chem*. 1984;**56**:2849-2850.

Tetrazolium Salts

Application: Dehydrogenase detection

Introduction

Several tetrazolium salts have been developed to determine dehydrogenase activity. Tetrazolium salts are unique compounds with a slightly yellow color that generate intense yellow to purple formazan dyes when reduced. Nitro blue tetrazolium salt (NTB or NitroTB) has been widely used for dehydrogenase detection on agarose gels due to its extremely low water solubility. Tetrazolium salts have been used in a variety of quantitative assays, such as blood glucose monitoring, lactate dehydrogenase detection, and glucose-6-phosphate dehydrogenase detection. The solubility of these tetrazolium salts, however, is so poor that detergents or organic solvents are always required to solubilize their formazan dyes. Dojindo has developed several water-soluble tetrazolium salts (WSTs) that are utilized for cell viability assays, dehydrogenase detection, superoxide detection, and reduced sugar detection. For example, WST-8 is used in Cell Counting Kit-8 and WST-1 is used in SOD Assay Kit-WST. The maximum wavelengths of formazan dyes are shown in the table. The water-solubility of WSTs ranges from 10 mM to 100 mM. The spectra of formazan dyes, especially bis-type dyes, are broad and shift according to the progress of the reaction. The spectra of formazan dyes also drastically change in alkaline conditions such as at pH 11 to 12. Most of the water-soluble formazan dyes turn blue at higher pH levels. Since tetrazolium salts are not stable in light, it is necessary to protect them from light during storage. The mechanism of the dehydrogenase detection is shown in Fig. 1. Dehydrogenase oxidizes its substrate and reduces its co-enzyme, β-nicotinamidoadenine-dinucleotide (NAD) or R. -nicotinamidoadenine dinucleotide-phosphate (NADP).Then an electron from NADH or NADPH is transferred to the tetrazolium salt via an electron mediator, such as 1-Methoxyphenazine methosulfate (1-Methoxy PMS) or diaphorase. 1-Methoxy PMS is one of the most stable electron mediators and has no selectivity when reacting with NADH and NADPH. On the other hand, diaphorase has more specificity with NAD(P) reaction and with certain types of tetrazolium salt.

Table 1 Molar Absorptivity(ε) of Formazan Dyes

Tetrazolium Salt	Formazan Dye	
water-soluble type	ε	λ max
WST-1	3.7 x 10^4	438 nm
WST-3	3.1 x 10^4	433 nm
WST-4	1.0 x 10^4	530 nm
WST-5	2.7 x 10^4	550 nm
WST-9	1.6 x 10^4	490 nm
water-insoluble type	ε	λ max
INT	1.5 x 10^4	490 nm
NTB	3.6 x 10^4	530 nm
TB	2.6 x 10^4	525 nm

Product Description of WSTs

Water-soluble tetrazolium salts (WSTs) were developed by introducing positive or negative charges and hydroxy groups to the phenyl ring of the tetrazolium salt. Positive charges, such as trialkylammonio groups, improve the water-solubility of the formazan dye. However, a large cation is easily precipitated out with organic anions such as carboxylate or phosphate. Although a hydroxy group also improved the water solubility of the tetrazolium salt, the corresponding formazan dye was not sufficiently water-soluble. Dojindo's WSTs have sulfonate groups added directly or indirectly to the phenyl ring to improve water-solubility. Dojindo also offers several newly developed phenylazo-type tetrazolium salts, which are easily reduced with NADH or other reducing agents to give orange or purple formazan dyes. Due to the phenylazo group, the color changes with heavy metal ion. Since the water solubility of Dojindo's WSTs is high, 10 mM to 100 mM solutions can be prepared.

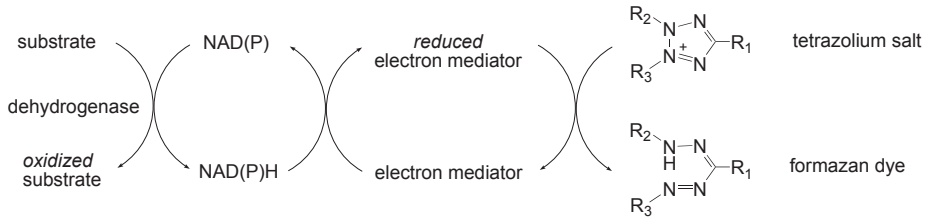


Fig. 1 Mechanism of Electron Transfer to Tetrazolium salt

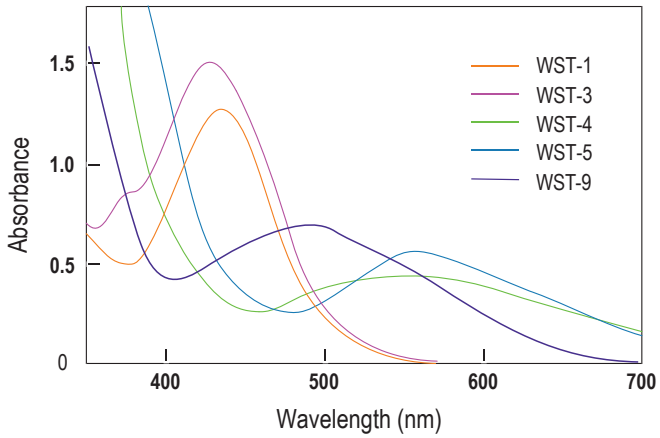


Fig. 2 Absorption Spectra of WST Formazan Dyes

WST-1

2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt [CAS: 150849-52-8]

Application: NADH and NADPH detection, superoxide detection
Appearance: Pale yellow or yellowish brown powder
Molar absorptivity (pH8): ≥21,600 (around 244 nm)
MW: 651.35, $C_{19}H_{11}IN_5NaO_8S_2$

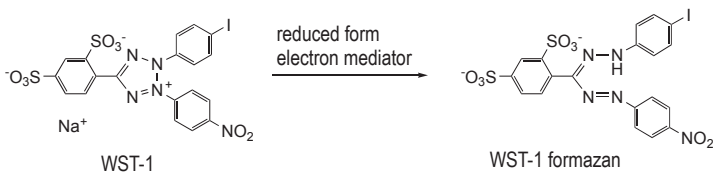
Storage Condition
0-5°C

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
W201-10	100 mg
W201-12	500 mg

Reaction Scheme



WST-3

2-(4-Iodophenyl)-3-(2,4-dinitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt [CAS: 515111-36-1]

Application: NADH and NADPH detection
Appearance: Pale yellow to pale brown powder

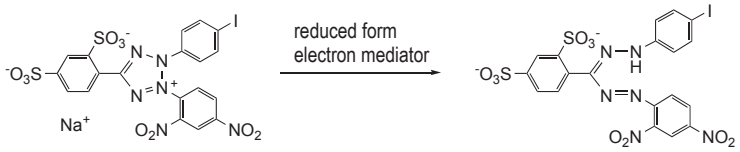
Molar absorptivity (pH8): ≥36,000 (around 235 nm)
MW: 696.34, $C_{19}H_{10}IN_6NaO_{10}S_2$

Storage Condition	Shipping Condition
0-5°C	ambient temperature

Ordering Information

Product code	Unit
W202-10	100 mg

Reaction Scheme



WST-3 | WST-3 formazan

WST-4

2-Benzothiazolyl-3-(4-carboxy-2-methoxyphenyl)-5-[4-(2-sulfoethylcarbamoyl)phenyl]-2*H*-tetrazolium [CAS: 178925-54-7]

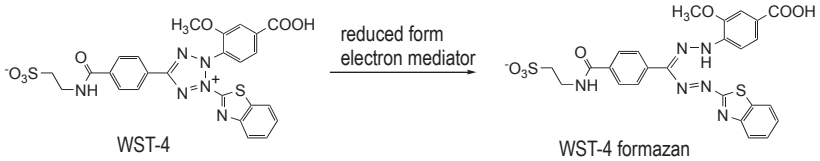
Application: NADH and NADPH detection
Appearance: Yellow or brown powder
Molar absorptivity (pH8): ≥28,000 (around 265 nm)
MW: 580.59, $C_{25}H_{20}N_6O_7S_2$

Storage Condition	Shipping Condition
0-5°C	ambient temperature

Ordering Information

Product code	Unit
W203-10	100 mg

Reaction Scheme



WST-4 | WST-4 formazan

WST-5

2,2'-Dibenzothiazolyl-5,5'-bis[4-di(2-sulfoethyl)carbamoylphenyl]-3,3'-(3,3'-dimethoxy-4,4'-biphenylene)ditetrazolium, disodium salt [CAS: 178925-55-8]

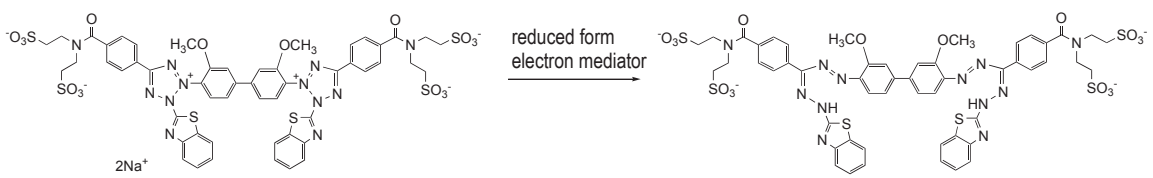
Application: NADH and NADPH detection
Appearance: Yellow or brown powder
Molar absorptivity (pH8): ≥50,000 (around 262 nm)
MW: 1331.35, $C_{52}H_{44}N_{12}Na_2O_{16}S_6$

Storage Condition	Shipping Condition
0-5°C	ambient temperature

Ordering Information

Product code	Unit
W204-10	100 mg

Reaction Scheme



WST-5 | WST-5 formazan

WST-9 2-(4-Nitrophenyl)-5-phenyl-3-[4-(4-sulfophenylazo)-2-sulfophenyl]-2*H*-tetrazolium, monosodium salt

Application: NADH and NADPH detection
Appearance: Reddish orange or darkly reddish orange powder
Molar absorptivity (pH8): ≥24,000 (around 320 nm)
MW: 629.56, $C_{25}H_{16}N_7NaO_8S_2$

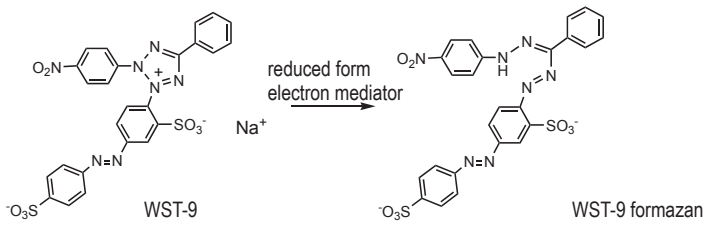
Ordering Information

Product code	Unit
W217-10	100 mg

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Reaction Scheme



References

W201

1. M. Ishiyama, *et al*., A New Sulfonated Tetrazolium Salt That produces a Highly Water-soluble Formazan Dye. *Chem Pharm Bull*. 1993;**41**:1118-1122.
2. T. Yano, *et al*., Ras Oncogene Enhances the Production of a Recombinant Protein Regulated by the Cytomegalovirus Promoter in BHK-21 Cells. *Cytotechnology*. 1994;**16**:167-178.
3. K. Teruya, *et al*., Ras Amplification in BHK-21 Cells Produces a Host Cell Line for Further Rapid Establishment of Recombiant Protein Hyper-producing Cell Lines. *Biosci Biotech Biochem*. 1995;**59**:341-344.
4. M. Ishiyama, *et al*., Novel Disulfonated Tetrazolium Salt That can be Reduced to a Water-soluble Formazan and Its Application to the Assay of Lactate Dehydrogenase. *Analyst*. 1995;**120**:113-116.
5. M. Ishiyama, *et al*., Novel Cell Proliferation and Cytotoxicity Assays Using a Tetrazolium Salt That Produces a Water-soluble Formazan Dye. *In Vitro Toxicol*. 1995;**8**:187-190.
6. S. Q. Liu, *et al*., Induction of Human Autologous Cytotoxic T Lymphocytes on Formalin-Fixed and Paraffin-Embedded Tumour Sections. *Nat Med*. 1995;**1**:267-271.
7. T. Takenouchi, *et al*., Trophic Effects of Substance P and β-Amyloid Peptide on Dibutyryl Cyclic AMP-Differentiated Human Leukemic (HL-60) Cells. *Life Sci*. 1995;**56**:PL479-PL484.
8. T. Iwaki, *et al*., β-Crystallin in C6 Glioma Cells Supports their Survival in Elevated Extracellular K^+: the Implication of a Protective Role of β-Crystallin Accumulation in Reactive Glia. *Brain Res*. 1995;**673**:47-52.
9. M. Ishiyama, *et al*., A Combined Assay of Cell Viability and vitro Cytotoxycity with a Highly Water-soluble Tetrazolium Salt, Neutral Red and Crystal Violet. *Biol Pharm Bul*. 1996;**19**:1518-1520.
10. T. Mosmann, *et al*., Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J Immunol Methods*. 1983;**65**:55-63.
11. A. H. Cory, *et al*., Use of an Aqueous Soluble Tetrazolium/Formazan Assay for Cell Growth Assays in Culture. *Cancer Commun*. 1991;**3**:207-212.

W202

1. M. Ishiyama, *et al*., Novel Disulfonated Tetrazolium Salt That can be Reduced to a Water-soluble Formazan and Its Application to the Assay of Lactate Dehydrogenase. *Analyst*. 1995;**120**:113-116.
2. I. Sakurabayashi, *et al*., New Enzymatic Assay for Glycohemoglobin. *Clin Chem*. 2003;**49**:269-274.

W203, W205

1. M. Ishiyama, *et al*., Benzothiazole-Containing Tetrazolium Salts That Produce Water-Soluble Formazan Dyes Absorbing at a Long Wavelength upon NADH Reduction. *Anal Chem*. 1996;**12**:515-51.

INT 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride [CAS: 146-68-9]

Application: Dehydrogenase detection, staining
Appearance: Pale yellow or pale yelowish-orange powder
Purity: ≥95.0% (Titration)
Molar absorptivity: ≥33,000 (around 249 nm)
MW: 505.70, $C_{19}H_{13}ClIN_5O_2$

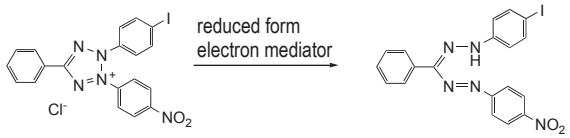
Ordering Information

Product code	Unit
I003-10	1 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Reaction Scheme



INT INT formazan

References

1. A. Johannsson, *et al*., A Fast Highly Sensitive Colorimetric Enzyme Immunoassay Sysytem Demonstrating Benefits of Enzyme Amplification in Clinical Chemistry. *Clin Chim Acta*. 1985;**148**:119-124.

MTT 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide [CAS: 2348-71-2]

Application: Dehydrogenase detection, staining
Appearance: Yellow or yellowish-orange powder
Purity: ≥97.0% (absorbance)
Molar absorptivity: ≥8,250 (around 375 nm)
MW: 414.32, $C_{18}H_{16}BrN_5S$

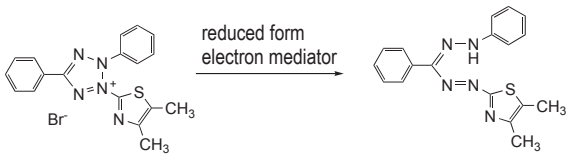
Ordering Information

Product code	Unit
M009-08	100 mg
M009-10	1 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Reaction Scheme



MTT MTT formazan

Product Description
MTT is slightly soluble in methanol, but has very low solubility in ethanol. It barely dissolves in ether, acetone, or ethyl acetate. MTT is utilized for cell viability detection. Since MTT has a positive charge, it can pass through a cell membrane and is reduced by mitochondria to form a purple formazan dye. Organic solvent is required to dissolve MTT formazan dyes.

References

1. T. F. Slater, *et al*., Studies on Succinate-tetrazolium Reductase Systems III. Points of Coupling of Four Different Tetrazolium Salts. *Biochim Biophys Acta*. 1963;**77**:383-393.
2. T. Mosmann, *et al*., Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J Immunol Methods*. 1983;**65**:55-63.
3. H. Tada, *et al*., An Improved Colorimetric Assay for Interleukin 2. *J Immunol Methods*. 1986;**93**:157-165.
4. M. C. Alley, *et al*., Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Res*. 1988;**48**:589-601.
5. E. Aoyama, *et al*., Determination of Selenium by Flow Injection Analysis Based on the Selenium(III)-Catalyzed Reduction of 3-(4, 5-Dimethyl-2-Thiazolyl)-2, 5-Diphenyl-2*H* Tetrazolium Bromide. *Anal Sci*. 1991;**7**:103-107.
6. H. Yamaue, *et al*., Chemosensitivity Testing with Highly Purified Fresh Human Tumor Cells with the MTT Colorimetric Assay. *Eur J Cancer*. 1991;**27**:1258-1263.

7. H. Yamaue, *et al*., Chemosensitivity Testing of Fresh Human Gastric Cancer with Highly Purified Tumour Cells Using the MTT Assay. *Br J Cancer*. 1992;**66**:794-799.
8. M. G. Stevens, *et al*., Comparative Analysis of Using MTT and XTT in Colorimetric Assays for Quantitating Bovine Neutrophil Bactericidal Activity. *J Immunol Methods*. 1993;**157**:225-231.

Nitro-TB

3,3'-[3,3'-Dimethoxy-(1,1'-biphenyl)-4,4'-diyl]-bis[2-(4-nitrophenyl)-5-phenyl-2*H*-tetrazolium chloride] [CAS: 298-83-9]

Application: Dehydrogenase detection, staining
Appearance: Yellow crystalline powder
Purity: ≥98.0% (absorbance)
Molar absorptivity: ≥63,000 (around 257 nm)
MW: 817.64, $C_{40}H_{30}Cl_2N_{10}O_6$

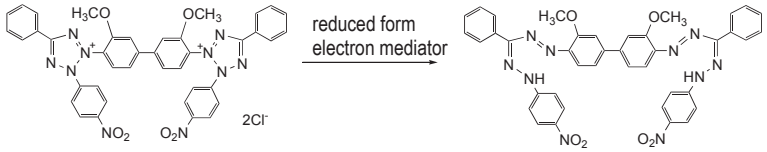
Ordering Information

Product code	Unit
N011-10	100 mg
N011-12	1 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Reaction Scheme



Nitro-TB　　Nitro-TB formazan

Product Description
Nitro-TB is dissolved by hot water or hot methanol. Other organic solvents such as acetone and ether cannot solubilize Nitro-TB. Nitro-TB is easily reduced by dehydrogenase to purple formazan dye aggregates. It is used for dehydrogenase detection on agarose gels.

References
1. K. Tsou, *et al*., Synthesis of Some p-Nitrophenyl Substituted Tetrazolium Salts Electron Acceptors for the Demonstration of Dehydrogenases. *J Am Chem Soc*. 1956;**78**:6139-6144.
2. J. R. Baker, *et al*., Mechanism of Fructosamine Assay: Evidence against Role of Superoxide as Intermediate in Nitroblue Tetrazolium Reduction. *Clin Chem*. 1993;**39**:2460-2465.

TB

3,3'-[3,3'-Dimethoxy-(1,1'-biphenyl)-4,4'-diyl]bis(2,5-diphenyl-2*H*-tetrazolium chloride) [CAS: 1871-22-3]

Application: Dehydrogenase detection, staining
Appearance: Pale yellow or pale yellowish orange powder
Molar absorptivity: ≥52,500 (around 254 nm)
MW: 727.64, $C_{40}H_{32}Cl_2N_8O_2$

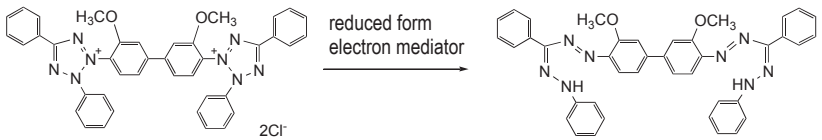
Ordering Information

Product code	Unit
T012-10	1 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Reaction Scheme



TB　　TB formazan

Product Description
TB is slightly soluble in water, but does not dissolve easily in acetone, ethyl acetate, and ether. Other organic solvents such as methanol, ethanol, and tetrachloromethane can solubilize TB pretty well. Since TB is easily reduced with dehydrogenase to generate reddish-purple formazan dye aggregates, it is used for dehydrogenase detection in tissue samples.

References
1. K. Tsou, *et al*., Synthesis of Some p-Nitrophenyl Substituted Tetrazolium Salts Electron Acceptors for the Demonstration of Dehydrogenases. *J Am Chem Soc*. 1956;**78**:6139-6144.
2. S. S. Karmarkar, *et al*., Preparation of Nitrotetrazolium Salts Containing Benzothiazole. *J Org Chem*. 1960;**25**:575-578.
3. J. E. Sinsheimer, *et al*., Reactivity of Blue Tetrazolium with Nonketol Compounds. *Anal Chem*. 1965;**37**:566-569.

1-Methoxy PMS

1-Methoxy-5-methylphenazinium methylsulfate [CAS: 65162-13-2]

Application: Electron mediator for NAD(P)H-tetrazolium salt
Appearance: Dark red or reddish-purple powder
Purity (Absorbance): ≥95.0%
Molar absorptivity: ≥2,700 (around 505 nm)
MW: 336.36, $C_{15}H_{16}N_2O_5S$

Ordering Information

Product code	Unit
M003-10	100 mg

Storage Condition
ambient temperature, protect from moisture

Shipping Condition
ambient temperature

Product Description
1-Methoxy PMS is easily dissolved by water and alcohol. Its redox potential is +63 mV. Though phenazinium methylsulfate (PMS) is commonly used as an electron carrier for NADH-tetrazolium, the stability of PMS is very poor. However, 1-Methoxy PMS solution can be stored at room temperature for over 3 months without protection from light. An electron of NAD(P)H is transferred by 1-Methoxy PMS to tetrazolium salt (Fig. 1). Therefore, it is a useful reagent for NAD(P)H-tetrazolium-based assay systems.

Reaction Scheme

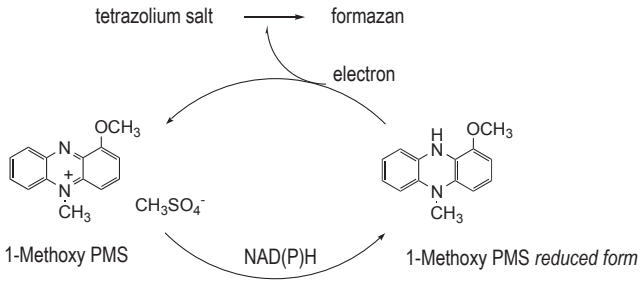


Fig. 1 Electron transfer system

References
1. R. Hisada, *et al*., 1-methoxy-5-methylphenazinium Methyl Sulfate. *J Biochem*. 1977;**82**:1469-1473.
2. S. Nakamura, *et al*., Use of 1-methoxy-5-methylphenazinium Methyl Sulfate(1-methoxyPMS) in the Assay of Some Enzymes of Diagnosticimportance. *Clin Chim Acta*. 1980;**101**:321-326.
3. C. J. Van Noorden, *et al*., The Role of Exogenous Electron Carriers in NAD(P)-dependent Dehydrogenase Cytochemistry Studied in vitro and with a Model System of Polyacrylamide Films. *J Histochem Cytochem*. 1982;**30**:12-20.
4. H. Tsuge, *et al*., Partial Purification and Property of Pyridoxine (Pyridoxamine)-5'- phosphate Oxidase Isozymes from Wheat Seedlings. *Arch Biochem Biophys*. 1982;**217**:479-484.
5. M. Rabinovitch, *et al*., Destruction of Leishmania Mexicana Amazonensis Amastigotes within Macrophages in Culture by Phenazine Methosulfate and Other Electron Carriers. *J Exp Med*. 1982;**155**:415-431.

Derivatization for HPLC Analysis

Introduction

A large number of labeling reagents have been developed to modify, detect, or monitor certain materials. Fluorometric and colorimetric derivatizing compounds are popular labeling reagents for the analysis of biological samples such as proteins, nucleotides, peptides, and amino acids. Detection and monitoring of fluorescent-labeling materials by fluorescent microscopy or flow cytometry have become the most useful methods for analyzing the functions of materials in cells or tissues. With the development of various kinds of columns and labeling reagents, high performance liquid chromatography (HPLC) has become one of the most important tools for the analysis of non-volatile or unstable compounds in complicated matrixes such as biological samples. Several HPLC, UV-visible, and fluorescence detection systems are the most common methods for the determination of purity or content of a specific compound that has UV-visible absorption or fluorescence. The detection limits of certain compounds depend on their molar absorptivity or fluorescent intensity. These detection limits can be lowered by either of two derivatization Methods, one pre-column and the other post-column. Compounds to be derivatized should have functional groups such as amino, carboxy, sulfhydryl, hydroxyl, and aldehyde groups. Most analytes of biological samples are water-soluble and some are unstable, so labeling reactions should be carried out in mild aqueous conditions. Labeling reagents that derivatize target materials in mild conditions are necessary, especially for post-column labeling. Dojindo offers a wide variety of labeling reagents for HPLC analysis.

Amine-Reactive Labeling Reagents

Amino compounds readily react with isothiocyanates, succinimidyl esters, acid chlorides, sulfonyl chlorides, and activated aryl halides under mild conditions (Fig. 1). The most commonly used fluorescent reagents for protein labeling and DNA sequencing are fluoresceins and rhodamines, such as FITC, carboxyfluorescein succinimidyl ester, TRITC, and sulforhodamine acid chloride. Amine labeling reagents are also used for protein sequencing. Biotinyl-succinimide reacts with an amino group, and the biotin-labeled compound can be determined with high sensitivity by enzyme immunoassay (EIA) coupled with peroxidase or alkalinephosphatase-labeled avidin. Meares' reagents are used to attach chelating functions to protein.

Sulfhydryl-Reactive Labeling Reagents

Maleimido, bromoacetamido, chlorobenzofurazan, and fluorobenzofurazan compounds react with sulfhydryl groups. Maleimido compounds, such as NAM, are sulfhydryl-specific labeling reagents. The quenched fluorescence is recovered upon the addition of sulfhydryl groups to maleimide moieties. Thus, the fluorescent intensities of maleimide compounds are greatly increased upon labeling. Bromoacetamido compounds, such as BABE or FeBABE, are readily conjugated with SH compounds to form a highly stable thioester bond. Chlorobenzofurazan and fluorobenzofurazan compounds also react with amino groups. Biotin-labeling reagents and Meares' reagents for sulfhydryl group labeling are also available.

Carboxylate-Reactive Labeling Reagents

Hydrazides and alkyl halides react with carboxylic acids in the presence of a base and a condensing agent, such as carbodiimide. Carboxylic acid labeling reagents are useful for the determination of free fatty acids, bile acids, or prostaglandins in biological samples.

Hydroxy-Reactive Labeling Reagents

The reactivity of hydroxyl groups is low, so highly reactive acid chloride is usually used as a labeling reagent. DMEQ-COCl reacts with primary, secondary, and even tertiary alcohols. These reactive reagents are easily hydrolyzed, so it is necessary to keep them from moisture and store them in a refrigerator or a freezer.

Aldehyde-Reactive Labeling Reagents

Aromatic diamines, such as DDB, react with aldehydes to produce highly fluorescent imidazole derivatives that can be used as labeling reagents for aldehyde. They also react with alpha-keto acid to produce fluorescent quinoxaline derivatives. Aldehyde Reactive Probe (ARP) contains an aminooxy group that has highly specific aldehyde and ketone reactivity.

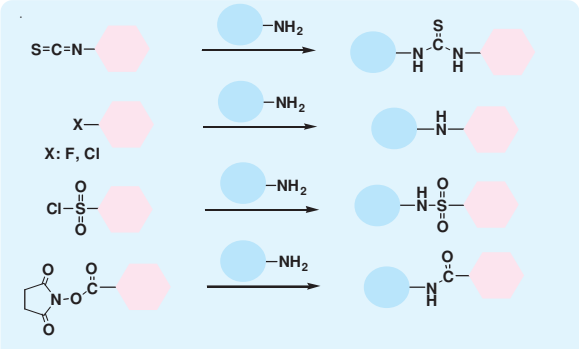


Fig. 1 Amine-Reactive Groups

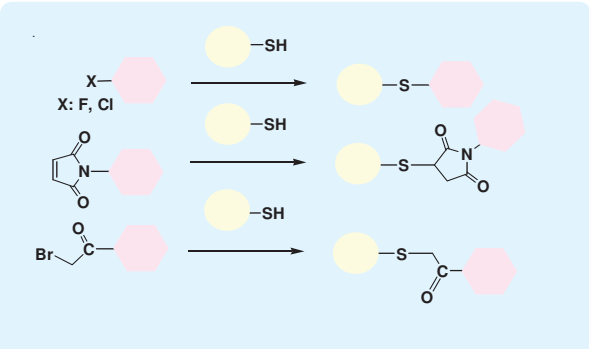


Fig. 2 Sulfhydryl-Reactive Groups

NBD-F 4-Fluoro-7-nitrobenzofurazan [CAS: 29270-56-2]

Application: Amine compound derivatization for HPLC analysis
Appearance: Pale yellow powder
Purity: ≥99.0% (HPLC)
MW: 183.10, $C_6H_2FN_3O_3$

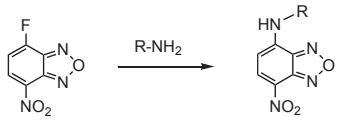
Ordering Information

Product code	Unit
N020-10	50 mg
N020-12	100 mg

Storage Condition
-20°C, protect from light and moisture

Shipping Condition
ambient temperature

Derivatization Reaction



Product Description
NBD-F is highly reactive and can label primary and secondary amines under mild conditions (1 minute reaction at 60°C in a weak basic solution). NBD-F is a pre-labeling compound for HPLC analysis of small molecules. NBD-labeled compounds are orange with a maximum wavelength at 470 nm. The excitation and emission of the derivatized compound are 470 nm and 530 nm, respectively.

NBD Labeling Protocol
1. To prepare sample solution, mix or dissolve a sample with 50 mM borate buffer (pH 8.0) containing 20 mM EDTA.
2. Mix 300 µl of the sample solution and 100 µl of 100 mM NBD-F/acetonitrile solution in a reaction vial.
3. Heat the vial at 60°C for 1 minute and then cool it on an ice bath.
4. Add 400 µl of 50 mM HCl aqueous solution to the reaction mixture.
5. Use this mixture for HPLC analysis to determine NBD-labeled compounds.

References

1. K. Imai, *et al*., Fluorimetric Determination of Secondary Amino Acids by 7-fluoro-4-nitrobenzo-2-oxa-1, 3-diazole. *Anal Chim Acta*. 1981;**130**:377-383.
2. Y. Watanabe, *et al*., High-Performance Liquid Chromatography and Sensitive Detection of Amino Acids Derivatized with 7-fluoro-4-nitrobenzo-2-oxa-1, 3-diazole. *Anal Biochem*. 1981;**116**:471-472.
3. Y. Watanabe, *et al*., Pre-column Labelling for High-performance Liquid Chromatography of Amino Acids with 7-fluoro-4-nitrobenzo-2-oxa-1, 3-diazole and Its Application to Protein Hydrolysates. *J Chromatogr A*. 1982;**239**:723-732.
4. T. Toyo'oka, *et al*., Reaction of Amines of Biological Importance with 4-fluoro-7-nitrobenzo-2-oxa-1, 3-diazole. *Anal Chim Acta*. 1983;**149**:305-312.
5. Y. Watanabe, *et al*., Liquid Chromatographic Determination of Amino and Imino Acids and Thiols by Postcolumn Derivatization with 4-Fluoro-7-nitrobenzo-2, 1, 3-oxadiazole. *Anal Chem*. 1983;**55**:1786-1791.
6. Y. Watanabe, *et al*., Sensitive Detection of Amino Acids in Human Serum and Dried Blood Disc of 3mm Diameter for Diagnosis of Inbornerrors of Metabolism. *J Chromatogr*. 1984;**309**:279-286.
7. H. Miyano, *et al*., Further Studies on the Reaction of Amines and Proteins with 4-fluoro-7-nitrobenzo-2-oxa-1, 3-diazole. *Anal Chim Acta*. 1985;**170**:81-87.
8. H. Kotaniguchi, *et al*., Automatic Amino Acid Analysis Utilizing 4-fluoro-7-nitrobenzo-2-oxa-1, 3-diazole. *J Chromatogr*. 1987;**420**:141-145.
9. K. Imai, *et al*., High-Performance Liquid Chromatography with Photochemical Fluorimetric Detection of Tryptophan Based on 4-fluoro-7-nitrobenzo-2-oxa-1, 3-diazole Total Protein Amino Acid Analysis. *Anal Chim Acta*. 1988;**205**:7-14.
10. K. Imai, *et al*., Dynamic Analytical Chemistry: A Trial Study of the Interaction of Fluorogenic Reagents with Living Chinese Hamster Ovary Cells. *Anal Chim Acta*. 1989;**223**:299-308.
11. C. Nakamura, *et al*., Dioxin-binding Pentapeptide for Use in a High-sensitivity On-bead Detection Assay. *Anal Chem*. 2005;**77**:7750-7757.

ABD-F 4-Fluoro-7-sulfamoylbenzofurazan [CAS: 91366-65-3]

Application: Thiol compound derivatization for HPLC analysis
Appearance: White or slightly yellow crystalline powder
Purity: ≥99.0% (HPLC)
MW: 217.18, $C_6H_4FN_3O_3S$

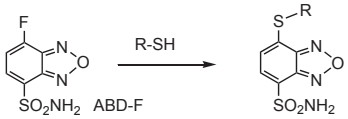
Ordering Information

Product code	Unit
A016-10	50 mg
A016-12	100 mg

Storage Condition
-20°C, protect from light and moisture

Shipping Condition
ambient temperature

Derivatization Reaction



Product Description

ABD-F has a benzofurazan moiety that produces a highly fluorescent compound through reaction with a sulfhydryl group. The excitation and emission of the derivatized compound are 389 nm and 513 nm, respectively. The reaction rate of ABD-F is 30 times faster than that of SBD-F. ABD-F reactions with thiol compounds are completed within 5 minutes in aqueous conditions at 50°C, pH 8. However, ABD-F does not react with alanine, proline, or cysteine under these conditions. Its maximum fluorescence intensity can be observed at pH 2. In reverse phase HPLC analysis, pre-labeled ABD-thiol compounds can be detected separately. The detection limits (S/N=3) are 0.6 pmol per injection for cysteine, 0.4 pmol per injection for glutathione, 1.9 pmol per injection for N-acetylcysteine, and 0.5 pmol per injection for cysteamine.

ABD Labeling Protocol

1. To prepare sample solution, mix or dissolve a sample with 100 mM borate buffer, pH 8.0 containing 2 mM EDTA.
2. Mix 500 µl of the sample solution and 500 µl of 1 mM ABD-F/100 mM borate buffer in a reaction vial.
3. Heat the vial at 50°C for 5 minutes and cool it on an ice bath.
4. Add 300 µl of 100 mM HCl aqueous solution to the reaction mixture.
5. Use this mixture for HPLC analysis to determine ABD-labeled compounds; excitation: 389 nm, emission: 513 nm.

References

1. T. Toyo'oka, *et al*., New Fluorogenic Reagent Having Halogenobenzofurazan Structure for Thiols: 4-(Aminosulfonyl)-7-fluoro-2, 1, 3-benzoxadiazole. *Anal Chem*. 1984;**56**:2461-2464.
2. T. Toyo'oka, *et al*., Isolation and Characterization of Cysteine-Containing Regions of Proteins Using 4-(Aminosulfonyl)-7-fluoro-2, 1, 3-benzoxadiazole and High-Performance Liquid Chromatography. *Anal Chem*. 1985;**57**:1931-1937.
3. T. Toyo'oka, *et al*., Amino Acid Composition Analysis of Minute Amounts of Cysteine-containing Proteins Using 4-(Aminosulfonyl)-7-fluoro-2, 1, 3-benzoxadiazole and 4-fluoro-7-nitro-2, 1, 3-benzoxadiazole in Combination with HPLC. *Biomed Chromatogr*. 1986;**1**:15-20.
4. T. Toyo'oka, *et al*., Simultaneous Determination of Thiols and Disulfides by High-performance Liquid Chromatography with Fluorescence Detection. *Anal Chim Acta*. 1988;**205**:29-41.
5. Y. Luo, *et al*., Antichymotrypsin interaction with chymotrypsin. Intermediates on the way to inhibited complex formation. *J Biol Chem*. 1999;**274**:17733-17741.

NAM *N*-(9-Acridinyl)maleimide [CAS: 49759-20-8]

Application: Thiol compound derivatization for HPLC analysis
Appearance: Yellow crystalline powder
Purity: ≥96.0% (HPLC)
MW: 274.27, $C_{17}H_{10}N_2O_2$

Ordering Information

Product code	Unit
N018-08	10 mg

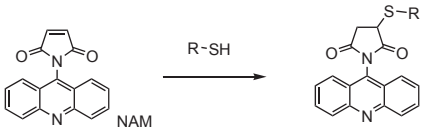
Storage Condition

0-5°C

Shipping Condition

ambient temperature

Derivatization Reaction



Product Description

NAM is a labeling reagent for sulfhydryl groups that forms highly fluorescent compounds. It reacts with thiol compounds in aqueous solutions at pH 3-10 and can be used to detect picomolar levels of cysteine per injection by HPLC. The sensitivity of NAM is approximately 100 times that of DTNB. The excitation and emission wavelength of the derivatized compound are 365 nm and 435-440 nm, respectively.

References

1. Y. Nara, *et al*., Synthesis of N-(9-Acridinyl)maleimide, a Fluorometrical Reagent for Thiol Compounds. *Agric Biol Chem*. 1978;**42**:793-798.
2. T. Yagi, *et al*., A Sensitive Method for the Detection of Aspartate: 2-Oxoglutarate Aminotransferase Activity on Polyacrylmide Gels. *Anal Biochem*. 1981;**110**:146-149.

3. H. Meguro, *et al*., A Novel Fluorometry of Sulfite with High Specificity. *Anal Lett*. 1983;**16**:1625-1632.
4. H. Tanno, *et al*., Assay of L-Methionineγ-Lyase with N-(9-Acridinyl)Maleimide. *Agric Biol Chem*. 1985;**49**:1517-1518.
5. K. Akasaka, *et al*., A Fluorometric Assay of L-Aspartate:2-Oxo-Glutarate Aminotransferase in Human Serum with N-(9-Acridinyl)Maleimide. *Anal Lett*. 1985;**18**:357-368.
6. K. Akasaka, *et al*., Fluorometric Determination of Sulfite with N-(9-Acridinyl)-Maleimide for High Performance Liquid Chromatography. *Agric Biol Chem*. 1986;**50**:1139-1144.
7. K. Akasaka, *et al*., Fluorometric Determination of Sulfites in Rainwater. *Anal Sci*. 1986;**2**:443-446.
8. K. Akasaka, *et al*., 9-Bromomethylacridine a Novel Fluorescent Labeling Reagent of Carboxylic Group for HPLC. *Anal Lett*. 1987;**20**:1581-1594.
9. K. Akasaka, *et al*., Fluorometric Determination of Sulfite in Wine by *N*-(9-Acridinyl)Maleimide. *Agric Biol Chem*. 1990;**54**:501-504.
10. T. Kamata, *et al*., A Sensitive Fluorometric Assay of Glutathione Reductase Activity with N-(9-Acridinyl)Maleimide. *Anal Sci*. 1993;**9**:867-870.

SBD-F 4-Fluoro-7-sulfobenzofurazan, ammonium salt [CAS: 84806-27-9]

Application: Thiol compound derivatization for HPLC analysis
Appearance: White or pale yellow crystalline powder
Purity: ≥98.0% (HPLC)
MW: 235.19, $C_6H_6FN_3O_4S$

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
S013-10	50 mg
S013-12	100 mg

Derivatization Reaction

Product Description
SBD-F is a water-soluble reagent that reacts with sulfhydryl groups to produce highly fluorescent compounds. The detection limit of thiol compounds such as glutathione, cysteine, N-acetylcysteine, CoA, and BSA by HPLC analysis is in the range of 100-500 pmol per injection. The excitation and emission of the derivatized compound are 385 nm and 515 nm, respectively.

References
1. K. Imai, *et al*., A Novel Fluorogenic Reagent for Thiols: Ammonium 7-Fluorobenzo-2-Oxa-1, 3-Diazole-4-Sulfonate. *Anal Biochem*. 1983;**128**:471-473.
2. T. Toyo'oka, *et al*., High-Performance Liquid Chromatography and Fluorometric Detection of Biologically Important Thiols, Derivatized with Ammonium 7-Fluorobenzo-2-Oxa-1, 3-Diazole-4-Sulphonate(SBD-F). *J Chromatogr*. 1983;**282**:495-500.
3. T. Toyo'oka, *et al*., Fluorescence Analysis of Thiols with Ammonium 7-Fluorobenzo-2-Oxa-1, 3-Diazole-4-Sulphonate. *Analyst*. 1984;**109**:1003-1007.
4. T. Toyo'oka, *et al*., Determinaiton of Total Captopril in Dog Plasma by HPLC After Prelabelling with Ammonium 7-Fluorobenzo-2-Oxa-1, 3-Diazole-4-Sulphonate(SBD-F). *J Pharm Biomed Anal*. 1984;**2**:473-479.
5. T. Sueyoshi, *et al*., Application of a Fluorogenic Reagent, Ammonium 7-Fluorobenzo-2-Oxa-1, 3-Diazole-4-Sulphonate for Detection of Cystine-Containing Peptides. *J Biochem*. 1985;**97**:1811-1813.
6. T. Toyo'oka, *et al*., Simultaneous Determination of Thiols and Disulfides by High-performance Liquid Chromatography with Fluorescence Detection. *Anal Chim Acta*. 1988;**205**:29-41.
7. T. Araki, *et al*., The Position of the Disulfide Bonds in Human Plasmaα2HS-glycoprotein and the Repeating Double Disulfide Bonds in the Domain Structure. *Biochim Biophys Acta*. 1989;**994**:195-199.
8. B. A. McMullen, *et al*., Location of the Disulfide Bonds in Human Plasma Prekallikrein; The Presence of Four Novel Apple Domains in the Amino-Terminal Portion of the Molecule. *Biochemistry*. 1991;**30**:2050-2056.
9. D. Hess, *et al*., Identification of the Disulfide Bonds of Human Complement C1s. *Biochemistry*. 1991;**30**:2827-2833.

Br-DMEQ 3-Bromomethyl-6,7-dimethoxy-1-methyl-1,2-dihydroquinoxaline-2-one [CAS: 100595-07-1]

Application: Carboxylic acid derivatization for HPLC analysis
Appearance: Yellow needles
Purity: ≥96.0% (HPLC)
MW: 313.15, $C_{12}H_{13}BrN_2O_3$

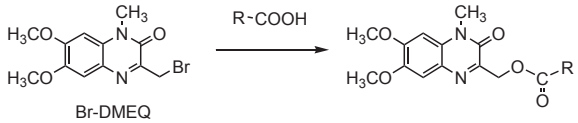
Ordering Information

Product code	Unit
B036-10	10 mg

Storage Condition
-20°C

Shipping Condition
ambient temperature

Derivatization Reaction



Br-DMEQ

Product Description
Br-DMEQ is a highly fluorescent labeling reagent for carboxylates, such as fatty acids and prostaglandins. Quinoxalinone compounds are formed by a reaction between 1,2-diamino-4,5-dimethoxybenzene and pyruvic acid. Br-DMEQ also reacts with pyrimidine bases of nucleic acids. Potassium carbonate and 18-crown-6 are necessary for carboxylate labeling with Br-DMEQ. The HPLC detection limit of saturated fatty acids with 3 to 20 carbons is 0.3 to 1 femtomoles per injection. The excitation and emission wavelength of labeled materials are 370 nm and 450 nm, respectively.

DMEQ Labeling Protocol
1. Dissolve a sample with acetonitrile to prepare sample solution.
2. Mix 500 µl of the sample solution, 250 µl of 3.8 mM Br-DMEQ/acetonitrile, and 250 µl of 0.8 mM 18-crown-6/acetonitrile in a reaction vial.
3. Heat the vial at 80°C for 20 minutes and then cool it in water bath.
4. Use the mixture for HPLC analysis to determine DMEQ-labeled compounds; excitation: 370 nm, emission: 450 nm.

References

1. M. Yamaguchi, *et al*., 3-Bromomethyl-6, 7-dimethoxy-1-methyl-2(1*H*)-quinoxalinone as a New Fluorescence Derivatization Reagent for Carboxylic Acids in High-performance Liquid Chromatography. *J Chromatogr A*. 1985;**346**:227-236.
2. M. Yamaguchi, *et al*., Highly Sensitive Fluorogenic Reagent for Carboxylic Acid, 3-Bromomethyl-6, 7-Dimethoxy-1-Methyl-2(1H)-Quinoxalinone, in High Performance Liquid Chromatography. *Anal Sci*. 1985;**1**:295-296.
3. M. Yamaguchi, *et al*., Fluorimetric High-Performance Liquid Chromatography of Prostaglandins and Its Application to Their Determination in Human Seminal Fluid. *J Chromatogr*. 1986;**380**:257-265.
4. M. Yamaguchi, *et al*., Highly Sensitive Determination of Free Polyunsaturated, Long-Chaine Fatty Acids in Human Serum by High-Performance Liquid Chromatography with Fluorescence Detection. *Anal Biochem*. 1986;**155**:256-261.
5. M. Yamaguchi, *et al*., Highly Sensitive Determination of Free Fatty Acids in Human Serum by High-Performance Liquid Chromatography with Fluorescence Detection. *J Chromatogr*. 1986;**375**:27-35.
6. M. Yamaguchi, *et al*., Determination of 5-Fluorouracil and 5-Fluoro-2'-Deoxyuridine in Human Serum by High Performance Liquid Chromatography with Fluorescence Detection. *Anal Sci*. 1987;**3**:75-79.
7. M. Yamaguchi, *et al*., 3-Bromomethyl-6, 7-methylenedioxy-1-methyl-2(1H)-quinoxalinone as a Highly Sensitive Fluorescence Derivatization Reagent for Carboxylic Acids in High-performance Liquid Chromatography. *Chem Pharm Bull*. 1988;**36**:2263-2266.
8. Y. Ohkura, High Performance Liquid Chromatography of Bioactive Substances Using Bifunctional Fluorogenic Reagents for Derivatization a Review", *Anal Sci*. 1989;**5**:371-388.

Br-Mmc 4-Bromomethyl-7-methoxycoumarin [CAS: 35231-44-8]

Application: Carboxylic acid derivatization for HPLC analysis
Appearance: Pale yellow or pale yellowish gray crystals
Molar absorptivity: ≥11,000 (around 332 nm)
MW: 269.09, $C_{11}H_9BrO_3$

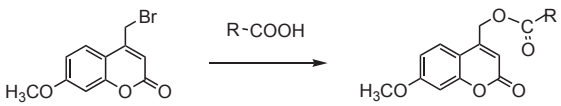
Ordering Information

Product code	Unit
B023-10	100 mg

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Derivatization Reaction



Br-Mmc

Product Description
Br-Mmc, a coumarin derivative, is a blue fluorescent labeling reagent for carboxylates. This reagent is used for the determination of trace fatty acids in medicines or agricultural chemicals by TLC or HPLC. Potassium carbon is necessary for the labeling. The reaction is completed

within 40 to 60 minutes in acetone reflux. Br-Mmc does not react with pyruvic acid, trichloroacetic acid, fumaric acid, or acetylenedicarboxylic acid. The excitation and emission wavelength of labeled materials are 360 nm and 410 nm, respectively.

References

1. W. Dunges, 4-Bromomethyl-7-methoxycoumarin as a New Fluorescence Label for Fatty Acids. *Anal Chem*. 1977;**49**:442-445.
2. E. Grushka, *et al*., Fluorescence Labeling of Dicarboxylic Acids for High Performance Liquid Chromatoguraphic Separation. *Anal Chem*. 1978;**50**:1398-1399.
3. S. Okuyama, *et al*., The Improved Method of High Performance Liquid Chromatographic Separation of Individual Bile Acids: Free and Glycine-conjugated Bile Acid. *Chem Lett*. 1979;**8**:461-462.

DMEQ-COCl 3-Chlorocarbonyl-6,7-dimethoxy-1-methyl-2(1*H*)-quinoxalinone [CAS: 104077-15-8]

Application: Alcohol derivatization for HPLC analysis
Appearance: Reddish-orange or orange powder
Purity: ≥95.0% (HPLC, derivatization)
MW: 282.68, $C_{12}H_{11}ClN_2O_4$

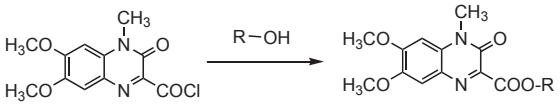
Ordering Information

Product code	Unit
D049-10	10 mg

Storage Condition
-20°C, protect from light anf moisture

Shipping Condition
with blue ice or dry ice

Derivatization Reaction



DMEQ-COCl

Product Description
DMEQ-COCl is a labeling reagent for primary and secondary alcohols. In organic solvents such as benzene and acetonitrile, this reagent readily reacts with alcohols to yield highly fluorescent derivatives. The HPLC detection limits of benzylalcohol, n-hexanol, and cyclohexanol are 2-3 femtomoles per injection. Steroids that have primary and secondary alcohols can be detected as fluorescent DMEQ derivatives. However, tert-alcohols, hydroxycarboxylic acids, and phenols do not react under the same labeling conditions. DMEQ-COCl also reacts with amines; as little as 0.3 pmol per ml of β-phenylethylamine has been detected in human serum. The excitation and emission wavelengths of the labeled materials are 400 nm and 500 nm, respectively.

References

1. S. Inoue, *et al*., Cypridina Bioluminescence VI a New Route for the Synthesis of Cypridina Luciferin and Its Analogs. *Tetrahedron Lett*. 1969;**10**:1609-1610.
2. T. Iwata, *et al*., 3, 4-Dihydro-6, 7-dimethoxy-4-methyl-3-oxo-quinoxaline-2-carbonyl chloride as a Highly Sensitive Fluorescence Derivatization Reagent for Alcohols in High-performance Liquid Chromatography. *J Chromatogr A*. 1986;**362**:209-216.
3. A. Nishida, *et al*., A Sensitive and Specific Chemiluminescence Method for Estimating the Ability of Human Granulocytes and Monocytes to Generate O^{2-}. *Clin Chim Acta*. 1989;**179**:177-181.
4. H. Nagaoka, *et al*., 2-(5-Chlorocarbonyl-2-oxazolyl)-5, 6-methylenedioxybenzofuran as Fluorescence Derivatization Reagent for Alcohols in High Performance Liquid Chromatography. *Anal Sci*. 1989;**5**:525-530.
5. J. Ishida, *et al*., 3,4-dihydro-6, 7-dimethoxy-4-methyl-3-oxoquinoxaline-2-carbonyl chloride as a Sensitive Fluorescence Derivatization Reagent for Amines in Liquid Chromatography. *Anal Chim Acta*. 1989;**223**:319-326.
6. Y. Ohkura, High Performance Liquid Chromatography of Bioactive Substances Using Bifunctional Fluorogenic Reagents for Derivatization a Review, *Anal Sci*. 1989;**5**:371-388.
7. J. Ishida, *et al*., High-Performance Liquid Chromatographic Determination of β-Phenylethylamine in Human Plasma with Fluorescence Detection. *Anal Biochem*. 1990;**184**:86-89.
8. K. J. Dave, *et al*., Improved Preparation and Structural Conformation of the Fluorescence Labelling Reagents 1, 2-Diamino-4, 5-dimethoxybenzene and 3, 4-dihydro-6, 7-dimethoxy-4-methyl-3-oxo-quinoxaline-2-carbonyl chloride. *J Pharm Biomed Anal*. 1990;**8**:307-312.
9. J. Ishida, *et al*., High-Performance Liquid Chromatographic Determination of 1, 2, 3, 4-Tetrahydroisoquinoline in Rat Brain with Fluorescence Detection. *Anal Biochem*. 1991;**195**:168-171.

DDB 1,2-Diamino-4,5-dimethoxybenzene, dihydrochloride [CAS: 131076-14-7]

Application: Aromatic aldehyde derivatization
Appearance: White or slightly pinkish gray crystalline powder
Purity: ≥98.0% (Titration)
MW: 241.11, $C_8H_{14}Cl_2N_2O_2$

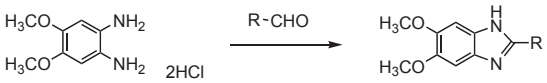
Ordering Information

Product code	Unit
D034-10	50 mg

Storage Condition
-20°C, protect from light

Shipping Condition
with blue ice or dry ice

Derivatization Reaction



Product Description
DDB reacts with aromatic aldehydes in acidic conditions to give strong fluorescent benzimidazole. This reagent can be used for the detection of aromatic aldehydes , and is soluble in aqueous solutions. The excitation and emission wavelength of the labeled materials are 338 nm and 402 nm, respectively.

References

1. M. Nakamura, *et al*., Fluorimetric Determination of Aromatic Aldehydes with 4, 5-Dimethoxy-1, 2-Diaminobenzene. *Anal Chim Acta*. 1982;**134**:39-45.
2. M. Nakamura, *et al*., Fluorescent Product in the Determination of Aromatic Aldehydes with 4,5-Dimethoxy-1,2-Diaminobenzene. *Chem Pharm Bull*. 1983;**31**:2910-2912.
3. S. Hara, *et al*., Highly Sensitive Determination of N-Acetyl- and N-Glycolylneuraminic Acids in Human Serum and Urine and Rat Serum by Reversed-Phase Liquid Chromatography with Fluorescence Detection. *J Chromatogr*. 1986;**377**:111-119.

MDB 1,2-Diamino-4,5-methylenedioxybenzene, dihydrochloride [CAS: 38608-07-0]

Application: Alpha-keto acid derivatization
Appearance: White or slightly pink crystalline powder
Purity: ≥98.0% (Titration)
MW: 225.07, $C_7H_{10}Cl_2N_2O_2$

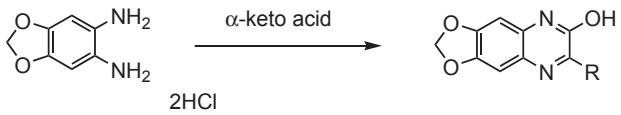
Ordering Information

Product code	Unit
M021-10	50 mg
M021-12	100 mg

Storage Condition
-20°C, protect from light and moisture

Shipping Condition
with dry ice or blue ice

Derivatization Reaction



Product Description
Phenylenediamine (OPD) reacts with α-keto acids to form highly fluorescent quinoxaline derivatives. MDB is an o-phenylenediamine analogue. It is the best labeling reagent for α-keto acids because of its reactivity and sensitivity. In HPLC analysis, more than ten different α-keto acids, such as α-keto glutaric acid, pyruvic acid, and phydroxyphenyl-pyruvic acid, can be separately detected at a time, even at concentrations of only a few femtomoles per injection. The sensitivity of MDB is almost 150 times as high as OPD. The excitation and emission wavelength of the labeled materials are 367 nm and 445 nm, respectively.

References

1. M. Nakamura, *et al*., 1, 2-Diamino-4, 5-methylenedioxybenzene as a Highly Sensitive Fluorogenic Reagent for α-Keto Acids. *Chem Pharm Bull*. 1987;**35**:687-692.
2. S. Hara, *et al*., Fluorometric High-Performance Liquid Chromatography of N-Acetyl-and N-Glycolylneuraminic Acids and Its Application to Their Microdetermination in Human and Animal Sera, Glycoproteins, and Glycolipids. *Anal Biochem*. 1987;**164**:138-145.
3. S. Hara, *et al*., 1, 2-Diamino-4, 5-methylenedioxybenzene as a Highly Sensitive Fluorogenic Reagent for α-Dicarbonyl Compounds. *Anal Chim Acta*. 1988;**215**:267-276.

Good's Buffers

Introduction

In biological experiments, it is important to maintain the pH of the solutions used. Buffers, mixtures of appropriate weak acids, and their conjugate bases, are usually used. Most biological reactions occur at a neutral pH, from 6 to 8; the buffer needs to be effective in this range. Furthermore, the acids and bases used in the buffer should not produce chelates with metal ions, which are essential in biological systems. For these reasons, Dr. Good developed several aminoethane and aminopropane sulfonic acids that are now widely used for biological research and analysis. Good's buffers have the following characteristics:

1) High water-solubility
2) Low cell membrane permeability
3) Consistent acid-base dissociation constants
4) Low metal chelating capability
5) High chemical stability
6) Low absorption spectra in UV and visible regions.

Table 1 Active pH Range of Good's Buffers

Good's Buffer	pKa (20 °C)	pH
MES	6.15	5.5-7.0
Bis-Tris	6.46	5.7-7.3
ADA	6.60	5.8-7.4
PIPES	6.80	6.1-7.5
ACES	6.90	6.0-7.5
MOPSO	6.95	6.2-7.4
BES	7.15	6.6-8.0
MOPS	7.20	6.5-7.9
TES	7.50	6.8-8.2
HEPES	7.55	6.8-8.2
TAPSO	7.70	7.0-8.2
POPSO	7.85	7.2-8.5
HEPPSO	7.90	7.4-8.6
EPPS	8.00	7.5-8.5
Tricine	8.15	7.8-8.8
Bicine	8.35	7.7-9.1
TAPS	8.40	7.7-9.1
CHES	9.50	8.6-10.0
CAPS	10.40	9.7-11.1

Preparation of Good's Buffer Solution

ADA, PIPES, POPSO

Solution A: 0.1 M solution

ADA	19.02 g + NaOH 4.0 g / 1 L
PIPES	30.24 g + NaOH 4.0 g / 1 L
POPSO	39.85 g + NaOH 4.0 g / 1 L

Solution B: 0.1 M NaOH solution

NaOH	4.0 g / 1 L

Solution A	Solution B				
25 ml	0 ml	5 ml	10 ml	15 ml	20 ml
ADA	5.8	6.6	6.9	7.3	7.8
PIPES	5.6	6.4	6.8	7.2	7.7
POPSO	6.4	7.3	7.7	8.1	8.5

Bis-Tris

Solution A: 0.1 M Bis-Tris
Solution B: 0.1 M HCl solution

Solution A	Solution B			
25 ml	0 ml	5 ml	10 ml	15 ml
Bis-Tris	9.5	7.1	6.6	6.1

Other Good's Buffers

Solution A: 0.1 M Good's Buffer solution
Solution B: 0.1 M HCl solution

Solution A	Solution B				
25 ml	0 ml	5 ml	10 ml	15 ml	20 ml
ACES	4.6	6.6	7.0	7.3	7.7
BES	3.8	6.6	7.0	7.4	8.0
Bicine	5.1	7.8	8.2	8.6	10.4
CAPS	6.8	10.0	10.5	10.8	11.2
CHES	5.9	9.0	9.4	9.7	10.1
EPPS	5.2	7.3	7.8	8.2	8.8
HEPES	5.3	7.0	7.4	7.7	8.1
HEPPSO	5.7	7.6	7.8	8.2	8.6
MES	3.7	5.6	6.0	6.4	8.4
MOPS	3.8	6.6	7.0	7.4	8.8
MOPSO	3.9	6.2	6.6	7.0	7.4
TAPS	4.6	7.8	8.3	8.6	9.0
TAPSO	4.7	7.0	7.4	7.8	8.2
TES	4.4	6.7	7.4	7.7	8.2
Tricine	4.9	7.5	7.9	8.3	8.6

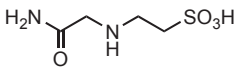
ACES *N*-(2-Acetamido)-2-aminoethanesulfonic acid [CAS: 7365-82-4]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 182.20, $C_4H_{10}N_2O_4S$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB01-10	25 g
GB01-12	100 g

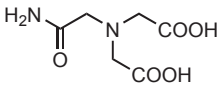
ADA *N*-(2-Acetamido)iminodiacetic acid [CAS: 26239-55-4]

Appearance: white crystalline powder
Purity: ≥99.0% (Titration)
MW: 190.15, $C_6H_{10}N_2O_5$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB02-10	25 g
GB02-12	100 g

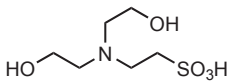
BES *N*,*N*-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid [CAS: 10191-18-1]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 213.25, $C_6H_{15}NO_5S$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB03-10	25 g
GB03-12	100 g

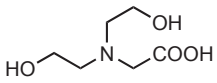
Bicine *N*,*N*-Bis(2-hydroxyethyl)glycine [CAS: 150-25-4]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 163.17, $C_6H_{13}NO_4$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB04-10	25 g
GB04-12	100 g

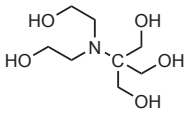
Bis-Tris Bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane [CAS: 6976-37-0]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 209.24, $C_8H_{19}NO_5$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB05-10	25 g
GB05-12	100 g

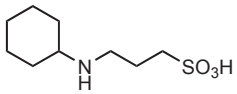
CAPS *N*-Cyclohexyl-3-aminopropanesulfonic acid [CAS: 1135-40-6]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 221.32, $C_9H_{19}NO_3S$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB06-10	25 g
GB06-12	100 g

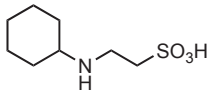
CHES *N*-Cyclohexyl-2-aminoethanesulfonic acid [CAS: 103-47-9]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 207.29, $C_8H_{17}NO_3S$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB07-10	25 g

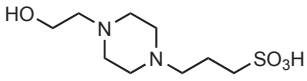
EPPS 3-[4-(2-Hydroxyethyl)-1-piperazinyl]propanesulfonic acid [CAS: 16052-06-5]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 252.33, $C_9H_{20}N_2O_4S$

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB09-10	25 g

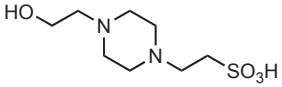
HEPES 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid [CAS: 7365-45-9]

Appearance: White crystalline powder
Purity: >99.0% (Titration)
MW: 238.31, $C_8H_{18}N_2O_4S$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB10-10	25 g
GB10-12	100 g

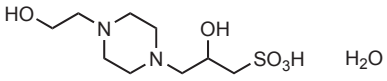
HEPPSO 2-Hydroxy-3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid, monohydrate [CAS: 68399-78-0]

Appearance: White crystalline power
Purity: ≥99.0% (Titration)
MW: 286.35, $C_9H_{20}N_2O_5S$, H_2O

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB11-10	25 g

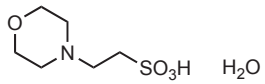
MES 2-Morpholinoethanesulfonic acid, monohydrate [CAS: 145224-94-8]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 213.25, $C_6H_{13}NO_4S$, H_2O

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB12-10	25 g
GB12-12	100 g

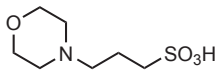
MOPS 3-Morpholinopropanesulfonic acid [CAS: 1132-61-2]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 209.26, $C_7H_{15}NO_4S$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB13-10	25 g
GB13-12	100 g

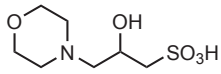
MOPSO 2-Hydroxy-3-morpholinopropanesulfonic acid [CAS: 68399-77-9]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 225.26, $C_7H_{15}NO_5S$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB14-10	25 g

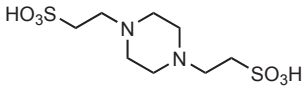
PIPES Piperazine-1,4-bis(2-ethanesulfonic acid) [CAS: 5625-37-6]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 302.37, $C_8H_{18}N_2O_6S_2$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB15-10	25 g
GB15-12	100 g

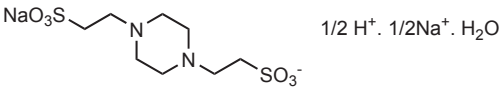
PIPES sesquisodium Piperazine-1,4-bis(2-ethanesulfonic acid), sesquisodium salt, monohydrate

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 353.36, $C_8H_{16.5}N_2Na_{1.5}O_6S_2$, H_2O

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB25-10	25 g

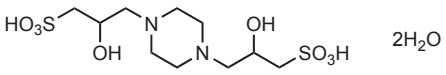
POPSO Piperazine-1,4-bis(2-hydroxy-3-propanesulfonic acid), dihydrate [CAS: 68189-43-5]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 398.45, $C_{10}H_{22}N_2O_8S_2$, $2H_2O$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB16-10	25 g

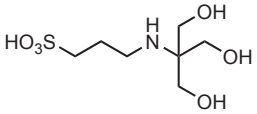
TAPS *N*-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid [CAS: 29915-38-6]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 243.28, $C_7H_{17}NO_6S$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB17-10	25 g
GB17-12	100 g

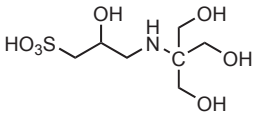
TAPSO 2-Hydroxy-*N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid [CAS: 68399-81-5]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 259.28, $C_7H_{17}NO_7S$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB20-10	25 g

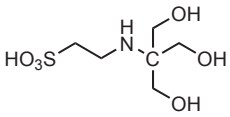
TES *N*-Tris(hydroxymethyl)methyl-2-aminoethenesulfonic acid [CAS: 7365-44-8]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 229.25, $C_6H_{15}NO_6S$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB18-10	25 g
GB18-12	100 g

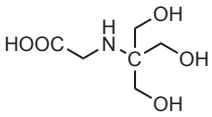
Tricine *N*-[Tris(hydroxymethyl)methyl]glycine [CAS: 5704-04-1]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 179.17, $C_6H_{13}NO_5$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
GB19-10	25 g
GB19-12	100 g

References

1. N. E. Good, Uncoupling of the Hill reaction from photophosphorylation by anions. *Arch Biochem Biophys*. 1962;**96**:653-661.
2. N. E. Good, *et al*., Hydrogen ion buffers for biological research. *Biochemistry*. 1966;**5**:467-477.
3. C. Ceccarini, *et al*., Induction and reversal of contact inhibition of growth by pH modification. *Nat New Biol*. 1971;**233**:271-273.
4. E. L. Medzon, *et al*., Substitution of 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) for bicarbonate in protein-free animal cell culture medium: application to vaccinia virus quantitation and fluorogenic acetylesterase assay in living LM cells. *Can J Microbiol*. 1971;**17**:651-653.
5. A. Itagaki, *et al*., TES and HEPES buffers in mammalian cell cultures and viral studies: problem of carbon dioxide requirement. *Exp Cell Res*. 1974;**83**:351-361.
6. W. J. Ferguson, *et al*., Hydrogen ion buffers for biological research. *Anal Biochem*. 1980;**104**:300-310.
7. J. K. Grady, *et al*., Radicals from "Good's" buffers. *Anal Biochem*. 1988;**173**:111-115.
8. J. W. Hanrahan, *et al*., Inhibition of an outwardly rectifying anion channel by HEPES and related buffers. *J Membr Biol*. 1990;**116**:65-77.
9. T. Kudo, *et al*., A simple and improved method to generate human hybridomas. *J Immunol Methods*. 1991;**145**:119-125.

Introduction

Metal indicators, colorimetric metal chelators, and fluorometric metal chelators are chromogenic chelating agents. They can be used to determine the metal ion concentration in solutions. There are several methods for measuring a particular metal ion in solution, including chelate titration, colorimetric detection, fluorometric detection, colorimetric detection coupled with solvent extraction, and precipitation titration. In general, metal indicators are utilized for chelate titration, and colorimetric and fluorometric chelating agents are utilized for the determination of the metal concentration by spectrophotometry.

Table 1 Application of Metal Indicators

Indicators	Metals	pH	Color Change
BT	alkaline earth metals Zn, Cd, Hg, Pb	10 7-10	reddish purple → blue
XO	In, Th, Bi Zn, Cd, rare earth metals, Hg, Pb	1-3 5-6	pink → yellow
NN	Ca	12-13	pink → blue
PAN	Cu	3-10	pink → yellow
Cu-PAN	Al Zn, Cd, Co, Hg, Fe, Pb, Ni	3-3.5 4-5	pink → yellow
PC	alkaline earth metals	11	pink → colorless
MX	Ca Ni	8 10	yellow → purple
Calcein	Ca	12-13	green fluorescence → no flourescence
Calcein Blue	Ca	12-13	blue fluorescence → no fluorescence

Chelate Titration

Metal indicators and chelating agents are used to determine the concentration of specific metal ions in solution by chelate titration. The endpoint of the titration can be determined by the color of the solution. The color of the metal indicator varies sharply with the association and dissociation of metal ions, so the total amount of a metal ion can be estimated by the amount of titration reagent used. EDTA-metal complexes are very stable and have high dissociation constants. EDTA forms a 1:1 complex with most metal ions that are divalent or more. For these reasons, EDTA is a widely used titration reagent. Fluorescent metal indicators are useful for determining the endpoint of titration of metal ions in stained samples.

Spectrophotometry

Colorimetric chelating reagents form colored complexes with metal ions in pH-controlled solutions. Their selectivity depends on the dissociation constants of metal ions and their sensitivity depends on the molar absorptivity of the complex. However, few colorimetric chelating reagents are highly selective. To increase selectivity, the choice of masking reagents or solvents for the extraction procedures is important. The maximum wavelength of the complex is also an important factor for selectivity and sensitivity. For example, Nitroso-PSAP forms complexes with several heavy metal ions, but the maximum wavelength of the Nitroso-PSAP-Fe complex is considerably longer than that of the other metal complexes. Thus, iron can be determined without interference from other metal ions. Water-soluble colorimetric chelating agents enable the determination of metal ions in aqueous solution without solvent extraction. Therefore, these reagents are useful for automatic detection systems. Calcium is one of the most important metal ions for signal transduction in living cells. Several unique reagents are available for monitoring the calcium concentration in living cells.

Masking Reagent

EDTA forms stable complexes with various metal ions in chelate titration. The total consumption of EDTA solution indicates the total amount of mixed metal ions contained in the sample solution. To determine the amount of one specific metal ion in the sample solution, masking reagents should be added to remove other metal ions.Table 2 shows popular masking reagents for chelatometry and colorimetry.

Table 2 Application of Masking Reagents

Masking Reagent	Metal	pH
Acetylacetone	Al, Fe(III), Pd, Be, UO_2	acidic pH
Citric acid	Zr, Th	5-6
KCN	Co, Hg, Fe, Cu, Ni, Zu, Cd	> 7
Diethyldithiocarbamate	Cu, Mn	alkaline pH
Dimercaptopropanol	Zn, Sb, Cd, Hg, Sn, Pb, Bi	alkaline pH / NH_4OH
Dimercaptopropane sulfonate	Zn, Cd, Hg, Sn	weak acidic pH
Oxalic acid Dithioozialic acid	Sn	weak acidic pH
Tartaric acid	Ti	alkaline pH
	Sb, UO_2	5-6
Tiron	Al, Ti, Fe	acidic pH
Thiocarbazide	Cu	acidic pH
Thioglucolic acid	Zn, Cd, Hg, Cu, Pb	alkaline pH
Thiourea	Cu	2.5-6
	Pb, Pt	5-6
Thiosulfate	Cu, Bi	acidic pH
Triethanolamine	Al, Fe(III), Mn(III)	10-12 / KOH, NaOH
o-Phenanthroline 2,2'-Dipyridine	Zn, Cd, Co, Hg, Cu, Ni	acidic pH
NH_4F	Al, Sn, Ti, Fe	acidic pH
	Ca, Sr, Ba, Mg	alkaline pH
KI	Hg(III)	>7
H_2S	Zn, Cd, Co, Hg	acidic pH
Na_2S	Cu, Ni	alkaline pH

Reagents for Ion Selective Electrodes

Real-time monitoring of electrolytes is increasing in importance for clinical use. For example, monitoring sodium and potassium ion concentrations in the blood flow is indispensable during cardiac surgery. Although lithium ion is used in the treatment of manic symptoms, its serum level must be strictly monitored due to its toxicity. For simple and quick determination of these alkaline and alkaline earth metal ions, polyvinyl chloride (PVC) membrane electrodes have been widely used. The concentration of neutral carriers, plasticizers, and counteranions used to prepare the PVC electrode determines its ion selectivity. A large number of crown ether compounds have been developed. Some of them are superior to naturally existing neutral carriers such as valinomycin, which is highly selective for potassium ions.

Table 3 Reagent Index

Ion	Reagent
Ag	*o*-Phenanthroline
Al	Calcein Cu-PAN PR (Pyrogallol sulfonphthalein) XO (Xylenol Orange)
As	Arsemate Bismuthiol-II
B	Azomethine H
Ba Bi	Calcein Calcein Blue HNB PC (Phthalein Complexone) Bismuthiol-II PAR XO (Xylenol Orange)
Ca	BT Calcein Calcein Blue Chlorophosphonazo-III Cu-PAN HDOPP-Ca t-HDOPP-Ca HNB Murexide MX NN PC (Phthalein Complexone) Zincon
Cd	BT Zincon
Cl^-	Bisthiourea-1
CN^-	Cyanoline Blue
Co	BT Calcein Blue Cu-PAN 5-Br-PSAA PAN 5-Br-PAPS Nitro-PAPS PAR PR (Pyrogallol sulfonphthalein) TPPS
Cr	Calcein Blue
Cs	Cesibor
Cu	Bathocuproine Bathocuproindisulfonic acid Calcein Calcein Blue Calcichrome Cu-PAN 5-Br-PSAA PAN PR (Pyrogallol sulfonphthalein) SATP
Cu	Sodium bicinchoninate 5-Br-PAPS Nitro-PAPS TMPyP TPPS Zincon
F^-	ALC Calcein Blue
Fe	Bathophenanthroline Bathophenanthrolinedisulfonic acid Calcein Blue 5-Br-PAPS Nitro-PAPS Cu-PAN Diantipyrylmethane
Ga	Cu-PAN PAN PAR
	PAR
Ge	PR (Pyrogallol sulfonphthalein)
H^+ Hg	BT Cu-PAN PC (Phthalein Complexone) Zincon
In K	Cu-PAN Bis(benzo-15-crown-5)
La	Kalibor Chlorophosphonazo-III PC (Phthalein Complexone)
Li	Dibenzyl-14-crown-4
Mg	TTD-14-crown-4 BT C14-K22B5 Calcein XB-I Chlorophosphonazo-III Cu-PAN
	PC (Phthalein Complexone)
Mo	Diantipyrylmethane
Na	Bis(12-crown-4)
Nb	XO (Xylenol Orange)
Nd	Chlorophosphonazo-III
NH_4^+	Cyanoline Blue
Ni	TD19C6 Calcein Blue Calcichrome Cu-PAN 5-Br-PSAA Nitro-PAPS PAN PAR SATP
NO_2^-	DAN
Np	Chlorophosphonazo-III
OCN^-	Cyanoline Blue
Os	PAN
Pa	Chlorophosphonazo-III
Pb	BT TPPS Zincon
Pd	5-Br-PAPS
Pt	5-Br-PAPS
Pu	Chlorophosphonazo-III
Rare earth metals	Chlorophosphonazo-III HNB Murexide MX PAN
Rh	5-Br-PAPS
Ru	5-Br-PAPS
Sb	Arsemate
Sc	Chlorophosphonazo-III
SCN^-	Cyanoline Blue
Se	DAB DAN
Sn	SATP
Sr	Calcein Blue Chlorophosphonazo-III PC (Phthalein Complexone)
Th	Chlorophosphonazo-III
Ti	Chlorophosphonazo-III Diantipyrylmethane PR (Pyrogallol sulfonphthalein)
U	Chlorophosphonazo-III Diantipyrylmethane
UO_2	HNB NN
V	Cu-PAN DAB PAR
Zn	BT Calcein Cu-PAN 5-Br-PAPS Nitro-PAPS PAN PAR XO (Xylenol Orange) Zincon
Zr	Calcein Blue Chlorophosphonazo-III

ALC

3-[*N*,*N*-Bis(carboxymethyl)aminomethyl]-1,2-dihydroxyanthraquinone [CAS: 3952-78-1]

Application: F^- detection, colorimetric
Appearance: Yellowish orange or Yellowish brown powder
Absorbance (0.10 mmol/l, pH5.1): ≥0.420 (around 430 nm)
MW: 385.32, $C_{19}H_{15}NO_8$

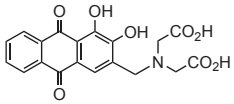
Ordering Information

Product code	Unit
A006-10	100 mg
A006-12	1 g

Storage Condition
ambient temperature, protect from light

Shipping Condition
ambient temperature

Chemical Structure



Product Description
ALC is utilized for colorimetric detection of fluoride ion. A red solution of the La(III) or Ce(III) complex turns blue in the presence of fluoride ions. The maximum wavelength of the fluoride complex is 620 nm at pH 4-5. As little as 0.1 to 1 ppm of fluoride ion can be determined using this method. The structure of this complex has been reported as $La_2L_2F_2$, $(La_5L_4F_2)n$ or $Ce_5L_4F_4$ (L: ALC). ALC is insoluble in alcohol and ether, slightly soluble in water, and easily soluble in alkaline water. The fluoride complex can be extracted with iso-amylalcohol. Since ALC is less toxic than Alizarin red, it is used to mark and trace young fish. The aqueous solution of ALC is yellow at pH < 6, red at pH 6 - 10, and blue violet at pH > 11. ALC can also be used to detect aluminum ions. pKa_1(COOH)=2.40, pKa_2(OH)=5.54, $pKa_3(NH^+)$=10.07, pKa_4(OH)=11.98 (m=0.1).

References

1. R. Belcher, *et al*., A Study of the Cerium-Alizarin Complexan Fluoride Reaction. *Talanta*. 1961;**8**:853-862.
2. F. Ingman, Annotation the Acid Stability Constants of Alizarin Fluorine Blue. *Talanta*. 1973;**20**:135-138.
3. F. Ingman, Photometric Determination of Aluminium with Alizarin Fluorine Blue. *Talanta*. 1973;**20**:999-1007.
4. Y. Yamashita, *et al*., Effects of Release Size on Survival and Growth of Japanese Flounder Paralichthys Olivaceus in Coastal Waters of Iwate Prefecture, Northeastern Japan. *Mar Ecol Prog Ser*. 1994;**105**:269-276.

Arsemate

Diethyldithiocarbamic acid, silver salt [CAS: 1470-61-7]

Application: As, Sb detection, colorimetric
Appearance: Yellow powder
Purity: ≥98.0% (Titration)
MW: 256.14, $C_5H_{10}AgNS_2$

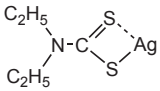
Ordering Information

Product code	Unit
A012-10	5 g
A012-12	25 g

Storage Condition
ambient temperature, protect from light

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Arsemate is soluble in pyridine and chloroform, but insoluble in water and other organic solvents. For arsenic detection, a 0.5% Arsemate/pyridine solution is used. The sample should be treated with sulfuric acid, 15% potassium iodide, and 40% $SnCl_2$/HCl solution to convert arsenic to AsH_3. Arsemate reacts with AsH_3 in pyridine and forms red colloidal silver with a maximum wavelength at 520-540 nm. The detection range of arsenic using the Arsemate/pyridine solution is 4-12 ppm. Alternatively, 0.5% Arsemate/chloroform solution with 30 mM triethylamine can be used for the assay. For the colorimetric detection of arsenic, an AsH_3 gas generator is required.

References

1. A. G. Howard, *et al*., Sequential Spectrophotometric Determination of Inorganic Arsenic(III) and Arcenic(V) Species. *Analyst*. 1980;**105**:338-343.

Azomethine H

8-Hydroxy-1-(salicylideneamino)-3,6-naphthalenedisulfonic acid, disodium salt [CAS: 32266-60-7]

Application: B detection, colorimetric
Appearance: Yellowish-orange or yellowish-brown crystalline powder
Absorbance (B complex): ≥0.900 (around 413 nm)
MW: 467.38, $C_{17}H_{11}NNa_2O_8S_2$

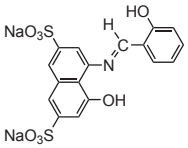
Ordering Information

Product code	Unit
A015-10	5 g
A015-12	25 g

Storage Condition
ambient temperature, protect from light

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Azomethine H is a colorimetric reagent for boron detection; it forms an orange complex with boron in aqueous solution. The molar absorptivity of this complex is 1.01×10^4 at 415 nm. The detection range of boron in sample solutions is 1-6 µg per ml. To detect boron in plant samples, EDTA is used to mask copper, iron, and aluminum ions. Azomethine H is used to detect microgram levels of boron in glass and steel samples.

Working Solution
1. Mix 9 g of Azomethine H and 2 g of ascorbic acid with 700 ml of deionized or double-distilled water.
2. Heat the mixture at 70°C or lower to dissolve Azomethine H.
3. Add water to prepare 1,000 ml of assay solution.
4. Measure the absorbance at 410 nm to detect bron(1-6 µg / ml)

References

1. R. Capelle, Microdosage Colorimetrique Du Bore En Milieu Aqueux, Au Moyen De Reactifs a Groupement Azoique Ou Imine Derives Des Acides H Et K. *Anal Chim Acta*. 1961;**24**:555-572.
2. W. D. Basson, *et al*., An Automated Procedure for the Determination of Boron in Plant Tissue. *Analyst*. 1969;**94**:1135-1141.
3. R. A. Edwards, Automatic Determination of Boron(0.10-10.0 mg/l)in Raw and Waste Waters. *Analyst*. 1980;**105**:139-146.

Bathocuproine

2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline [CAS: 4733-39-5]

Application: Cu(I) detection with solvent extraction, colorimetric
Appearance: White or yellow crystalline powder
Absorbance: ≥0.810 (around 280 nm)
Absorbance (Cu complex): ≥0.580 (around 475 nm)
MW: 360.45, $C_{26}H_{20}N_2$

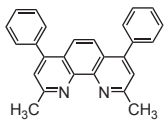
Ordering Information

Product code	Unit
B001-10	100 mg

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Bathocuproine is a Cu(I)-specific colorimetric reagent. An orange Bathocuproine-Cu(I) complex can be extracted with n-hexylalcohol or iso-amylalcohol. The maximum wavelength of the complex extract is 479 nm. The Cu(I) detection range is 0.1-10 ppm. Bathocuproine has been used to determine trace amounts of Cu(I) in wine and purified tellurium samples. Bathocuproine is soluble in organic solvents such as nitrobenzene and insoluble in water.

References

1. W. M. Banick, *et al*., The in situ Determination of Iron and Copper in Wine. *Anal Chim Acta*. 1957;**16**:464-472.

Bathocuproinedisulfonic acid, disodium salt

2,9-Dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonic acid, disodium salt [CAS:52698-84-7]

Application: Cu(I) detection, colorimetric
Appearance: White or pale yellowish-brown powder
Absorbance (0.02 mmol/l): ≥0.740 (around 285 nm)
Absorbance (0.05 mmol/l, Cu complex): ≥0.210 (around 480 nm)
MW: 564.54, $C_{26}H_{18}N_2Na_2O_6S_2$

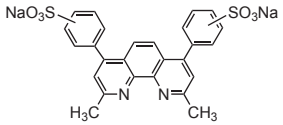
Ordering Information

Product code	Unit
B002-10	1 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Bathocuproinedisulfonic acid is a water-soluble analog of bathocuproine that emits fluorescence under UV light. Since the Bathocuproine-Cu(I) complex formed by this analog is water-soluble, Cu(I) can be determined by direct measurement of the assay solution at 483-522 nm without solvent extraction. The molar absorptivity of the bathocuproinedisulfonic acid-Cu(I) complex is $1.2x10^4$. Dr. Faizullah developed a system to quickly determine Cu(I) at around 30 ppm by using Bathocuproine-disulfonic acid in flow injection analysis. Dr. Sawada developed a highly sensitive Cu(I) detection system using the long flow cell. Dr. Sawada's system can detect as little as 0-15 ppb Cu(I) in water samples without pretreatment.

References
1. T. Ishii, *et al*., The Synergistic Action of the Copper Chelator Bathocuproine Sulphonate and Cysteine in Enhancing Growth of L1210 Cells in Vitro. *J Cell Physiol*. 1985;**125**:151-155.

Bathophenanthroline

4,7-Diphenyl-1,10-phenanthroline [CAS:1662-01-7]

Application: Fe(II), Cu(I) and Ru(II) detection with solvent extraction, colorimetric
Appearance: White or pale yellow powder
Molar absorptivity (Fe complex): ≥21,000 (around 535 nm)
Absorbance (Fe complex): ≥0.600 (around 535 nm)
MW: 332.40, $C_{24}H_{16}N_2$

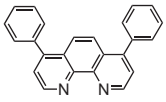
Ordering Information

Product code	Unit
B003-10	1 g
B003-12	5 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Bathophenanthroline is a highly selective colorimetric reagent for Fe(II) detection. Bathophenanthroline is soluble in organic solvents and slightly soluble in acidic aqueous solutions, but insoluble in neutral or alkaline solutions. It chelates with Fe(II) at pH 2-9, and the red color complex can be extracted with iso-amylalcohol ($\log\beta_3$=21.8, λmax=533 nm, ε=22,400). The detection range of Fe(II) by Bathophenanthroline is 10 ppb to 4 ppm. The pKa_2 of Bathophenanthroline is reported to be 4.30 in a 50% dioxane solution (m=0.3) at 25°C, and the redox potential of FeL_3^{2+}-FeL_3^{3+} in the 1 M H_2SO_4 solution is 1.24 V. Cu(I) and Ru(II) can also be detected using Bathophenanthroline. The maximum wavelength of the Cu(I) and Ru(II) complexes are 457 nm and 460 nm, respectively.

References
1. G. F. Smith, *et al*., The Colorimetric Determination of Iron in Raw and Treated Municipal Water Supplies by Use of 4,7-Diphenyl-1,10-phenanthroline. *Analyst*. 1952;**77**:418-422.
2. F. R. Short, *et al*., Spectrophotometric Determination of Parts-per-billion Iron in High-temperature Hydrocarbon Jet Fuels. *Anal Chem*. 1967;**39**:251-253.

Bathophenanthrolinedisulfonic acid, disodium salt

4,7-Diphenyl-1,10-phenanthrolinedisulfonic acid, disodium salt [CAS:52746-49-3]

Application: Fe(II) and Cu(I) detection, colorimetric
Appearance: White, pale pink, or pale brown powder
Absorptivity (Fe^{2+} free): ≥ 0.010 at 535 nm
Absorptivity (Fe^{2+} complex): ≥0.500 (around 535 nm)
MW: 536.49, $C_{24}H_{14}N_2Na_2O_6S_2$

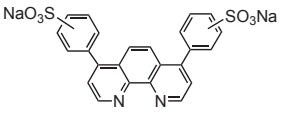
Ordering Information

Product code	Unit
B004-10	1 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Bathophenanthrolinedisulfonic acid, disodium salt is a water-soluble derivative of Bathophenanthroline. It is insoluble in organic solvents. Its pKa_1 and pKa_2 are 2.83 and 5.20, respectively. Due to its high water solubility, Bathophenanthrolinedisulfonic acid can be used to detect Fe(II) in aqueous samples such as blood or biological fluids. The detection range of Fe^{2+} using Bathophenanthrolinedisulfonic acid is 0.25-4 ppm at 535 nm. The molar absorptivity of the complex is $2.24x10^4$. The reduction and oxidation potential of FeL_3^{2+}-FeL_3^{3+} in 1 M H_2SO_4 solution is reported to be 1.09 V. Cu^+ can also be detected using Bathophenanthrolinedisulfonic acid. The maximum wavelength and molar absorptivity of the Cu^+ complex is 483 nm and $1.23x10^4$, respectively.

References
1. D. Blair, *et al*., Bathophenanthrolinedisulphonic Acid and Bathocuproinedisulphonic Acid, Water Soluble Reagents for Iron and Copper. *Talanta*. 1961;**7**:163-174.

Bis(benzo-15-crown-5)

Bis[(benzo-15-crown-5)-4-methyl]pimelate [CAS:69271-98-3]

Application: Ionophore for K^+ selective electrode
Appearance: White powder
Purity: ≥98.0% (HPLC)
MW: 720.80, $C_{37}H_{52}O_{14}$

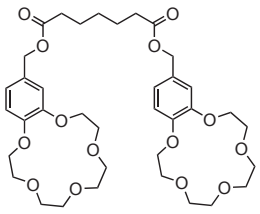
Ordering Information

Product code	Unit
B020-10	100 mg

Storage Condition
ambient temperature, protect from light

Shipping Condition
ambient temperature

Chemical Structure



Product Description

Bis(benzo-15-crown-5) is a potassium-selective ionophore. Its potassium ion selectivity is almost equal to that of valinomycin, which is a well known neutral carrier for the potassium ion in nature. This reagent, combined with NPOE as a plasticizer, is the most recommended ionophore for potassium-selective PVC membrane electrodes. This ionophore also forms a thallium complex, so it can be used for thallium sensors.

Ion selectivity: $LogK^{pot}(K/Na)$:-3.6, $LogK^{pot}(K/Rb)$:-0.7, $LogK^{pot}(K/NH_4)$:-2.0, $LogK^{pot}(K/Cs)$:-2.0
Formulation: Ionophore: 2.7%, NPOE: 70%, TFPB: 50 mol% of ionophore/PVC, THF

References

1. H. Tamura, *et al*., Thallium(I)-selective PVC Membrane Electrodes Based on Bis(crown ether)s. *J Electroanal Chem*. 1980;**115**:115-121.
2. H. Tsukube, *et al*., Biomimetic Application of Natural Valinomycin and Nonactine Ionophores to Artificial Transport of Amino Acid Salts. *Bull Chem Soc Jpn*. 1986;**59**:2021-2022.
3. Y. Yamashoji, *et al*., Polymer Membrane Thallium(I)-selective Electrodes Based on Dibenzo-crown-6 Ethers. *Anal Sci*. 1991;**7**:485-486.

Bis(12-crown-4) Bis[(12-crown-4)methyl]-2-dodecyl-2-methylmalonate [CAS: 80403-59-4]

Application: Ionophore for Na^+ selective electrode
Appearance: Colorless or slightly yellow liquid
Purity: ≥95.0% (HPLC; RI)
MW: 662.85, $C_{34}H_{62}O_{12}$

Ordering Information

Product code	Unit
B021-10	100 mg
B021-12	500 mg

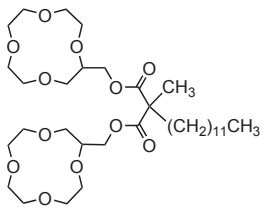
Storage Condition

-20°C, protect from light

Shipping Condition

ambient temperature

Chemical Structure



Product Description

The NAS_{11-8} glass electrode is utilized as a selective sodium ion sensor. However, it is easily spoiled by biomaterials in sample solutions. Bis(12-crown-4), a sodium ionophore, is 100 times more selective for sodium than potassium in the PVC electrode using NPOE as a plasticizer. The same selectivity for sodium over potassium is reported when Bis(12-crown-4) is used on a coated wire electrode. It can be used in either a liquid membrane or a PVC membrane. TFPB, a lipophilic anion, can help improve detection sensitivity.

Ion selectivity: $LogK^{pot}(Na/Li)$:-3.0, $LogK^{pot}(Na/K)$:-2.0, $LogK^{pot}(Na/Cs)$:-2.0, $LogK^{pot}(Na/Rb)$:-2.4, $LogK^{pot}(Na/NH4)$:-3.0, $LogK^{pot}(Na/Mg)$:-4.0, $LogK^{pot}(Na/Ca)$:-4.0, $LogK^{pot}(Na/Ba)$:-3.7, $LogK^{pot}(Na/Sr)$:-3.7
Formulation: Ionophore: 6%, NPOE: 70%, TFPB: 50 mol% of ionophore

References

1. I. Ikeda, *et al*., Syntheses of Ester-type Bis-12-crown-4 Ethers and Their Complexing Abilities toward Sodium Cation. *Tetrahedron Lett*. 1981;**22**:3615-3616.
2. H. Tamura, *et al*., Coated Wire Sodium- and Potassium-selective Electrodes Based on Bis(crown ether) Compounds. *Anal Chem*. 1982;**54**:1224-1227.
3. T. Shono, *et al*., Sodium-selective PVC Membrane Electrodes Based on Bis(12-crown-4)s. *J Electroanal Chem*. 1982;**132**:99-105.
4. H. Tamura, *et al*., Simultaneous Determination of Sodium and Potassium in Human Urine or Serum Using Coated-wire Ion-selective Electrodes Based on Bis(crown ether)s. *Mikrochim Acta*. 1983;**80**:287-296.
5. H. Sakamoto, *et al*., Lipophilic Bis(monoaza crown ether) Derivatives: Synthesis and Cation-complexing Properties. *J Org Chem*. 1986;**51**:4974-4979.
6. T. Maruizumi, *et al*., Neutral Carrier-based Na^+ -selective Electrode for Application in Blood Serum. *Mikrochim Acta*. 1986;**1**:331-336.
7. N. Nakashima, *et al*., Design of a Novel Bilayer System Responsive to Chemical Signals; Selective Discrimination of Na^+ by a Spectroscopic Method. *J Chem Soc Chem Commun*. 1987:617-619.
8. H. Widmer, *et al*., The Increase of Oxygen Consumption after a Flash of Light Is Tighly Coupled to Sodium Pumping in the Lateral Ocellus of Barnacle. *J Gen Physiol*. 1990;**96**:83-108.
9. M. Tanaka, *et al*., Evaluation of Complex Formation of Bis(12-crown-4)s with Sodium Picrate in Solution by ^{23}Na NMR Spectroscopy. *Chem Lett*. 1990;**19**:1419-1422.
10. Y. Shibutani, *et al*., Chiral Bis(12-crown-4)-based Electrodes for Sodium Ion. *Chem Lett*. 1997;**26**:49-50.

Bisthiourea-1

2,7-Di-*tert*-butyl-9,9-dimethyl-4,5-bis(*N'*-*n*-butyl-thioureido)xanthene [CAS:187404-67-7]

Application: Ionophore for Cl^- selective electrode
Appearance: White or slightly yellow powder
Purity: ≥98.0% (HPLC)
MW: 582.91, $C_{33}H_{50}N_4OS_2$

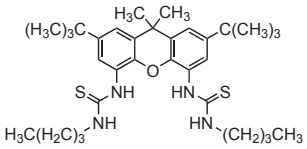
Ordering Information

Product code	Unit
B432-10	25 mg

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Bisthiourea-1 is a chloride-selective neutral ionophore, and it is utilized for preparation of chloride electrodes. Bisthiourea-1 forms a 1:1 complex with chloride ion. The detection limit of chloride ion using Bisthiourea-1 is (6.5+3.0) x 10^{-6} M.

References

1. K. P. Xiao, *et al*., A Chloride Ion-Selective Solvent Polymeric Membrane Electrode Based Forming Ionophore. *Anal Chem*. 1997;**69**:1038-1044.
2. P. Buhlmann, *et al*., Strong Hydrogen Bond-Mediated Complexation of $H_2PO_4^-$ by Neutral Bis-Thiourea Hosts. *Tetrahedron*. 1997;**53**:1647-1654.

5-Br-PAPS

2-(5-Bromo-2-pyridiylazo)-5-[*N*-*n*-propyl-*N*-(3-sulfopropyl)amino]phenol, disodium salt, dehydrate [CAS:91599-24-5]

Application: Zn^{2+} detection, colorimetric
Appearance: Orange or reddish-brown crystalline powder
Absorbance (0.01 mmol/l): ≥0.420 (around 449 nm)
Absorbance (0.01 mmol/l): ≤0.040 (at 552 nm)
MW: 537.34, $C_{17}H_{19}BrN_4Na_2O_4S,2H_2O$

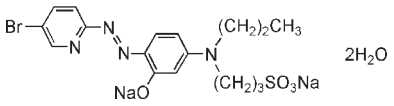
Ordering Information

Product code	Unit
B026-10	100 mg

Storage Condition
ambient temperature, protect from metal and moisture

Shipping Condition
ambient temperature

Chemical Structure



Product Description
5-Br-PAPS, a water-soluble derivative of 5-Br-PADAP, is a highly sensitive colorimetric reagent for zinc detection. This reagent forms a red chelate with zinc at pH 7.5-9.5 (λmax=552 nm, λ=13.3 x 10^4). 5-Br- PAPS is also utilized for the determination of Cu(II), Fe(II), Ni(II), Co(II), Pt(II), Ru(II), and Rh(II). 5-Br-PAPS is utilized to determine serum levels of Zn, Cu, Fe, Ni, and Co. For serum Zn levels, Fe ions can be masked with citric acid or metaphosphoric acid, and fluoride and Cu ions can be masked with 2-mercaptobenzothiazole or dithiocarboxysarcosine. 5-Br-PAPS-Ti complex can be used for hydrogen peroxide detection.

References

1. T. Makino, *et al*., A Highly Sensitive Colorimetric Determination of Serum Zinc Using Water-soluble Pyridylazo Dye. *Clin Chim Acta*. 1982;**120**:127-135.
2. D. Horiguchi, *et al*., Water Soluble Pyridylazoaminophenols and Pyridylazoaminobenzoic Acids as Highly Sensitive Photometric Reagents for Zinc, Uranium, Cobalt and Nickel. *Anal Sci*. 1985;**1**:461-465.
3. C. Matsubara, *et al*., Spectrophotometric Determination of Hydrogen Peroxide with Titanium 2-((5-bromopyridyl)azo)-5-(N-proryl-N-sulfopropylamino) phenol Reagent and Its Application to the Determination of Serum Glucose Using Glucose Oxidase. *Anal Chem*. 1985;**57**:1107-1109.
4. Y. Shijo, *et al*., Spectrophotometric and Analogue Derivative Spectrophotometric Determination of Chromium(III) with 2-(5-Bromo-2-pyridylazo)-5-(N-

propyl-N-sulfopropylamino)phenol. *Bull Chem Soc Jpn*. 1986;**59**:1455-1458.
5. Y. Shijo, *et al*., Spectrophotometric and Analogue Derivative Spectrophotometric Determination of Rhodium(III) with 2-(5-Bromo-2-pyridylazo-5-propyl-N-sulphopropylamino)phenol. *Analyst*. 1988;**113**:519-521.
6. Y. Shijo, *et al*., High Performance Liquid Chromatographic Separation of Iron, Bismuth, Indium and Thallium by Pre-column Chelation with 2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)phenol. *Analyst*. 1988;**113**:1201-1203.
7. C. Ohtsuka, *et al*., Reversed-phase Ion-pair Partition Liquid Chromatoguraphy of Chelates with 2-(3,5-Dibromo-2-pyridylazo)-5-[N-ethyl-N-(3-sulphopropyl)amino]phenol and Analogues. *Anal Chim Acta*. 1989;**223**:339-347.
8. I. Kasahara, *et al*., Bis[2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)phenolato]cobaltate(III) as a Counter Ion for the Extraction and Spectrophotometric Determination of Long-chain Quaternary Ammonium Salts and Tertiary Alkylamines in the Presence of Each Other. *Anal Chim Acta*. 1989;**219**:239-245.
9. N. Uehara, *et al*., Simultaneous Determination of Platinum(II), Rhodium(III) and Palladium(II) with 2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)phenol by High Performance Liquid Chromatography. *Anal Sci*. 1989;**5**:111-112.
10. Y. Hayashibe, *et al*., Direct Determination of Zinc in Human Serum by Flow-injection Spectrophotometric Analysis. *Anal Sci*. 1994;**10**:795-799.
11. N. Uehara, *et al*., Determination of Cobalt in Natural Water as a 2-(5-Bromo-2-pyridylazo)-5-[N-propyl-N-(3-sulphopropyl)amino]phenol Chelate by On-Line Preconcentration HPLC with Column-Switching Technique. *Anal Sci*. 1998;**14**:343-348.

5-Br-PSAA 2-(5-Bromo-2-pyridylazo)-5-[*N*-*n*-propyl-*N*-(3-sulfopropyl)amino]aniline, sodium salt [CAS:86035-60-7]

Application: Fe(II) and Co(III) detection, colorimetric
Appearance: Reddish-orange or orange powder
Molar absorptivity (Fe complex): ≥86,000 (around 558 nm)
Absorbance: ≥0.400 (around 458 nm)
MW: 478.34, $C_{17}H_{21}BrN_5NaO_3S$

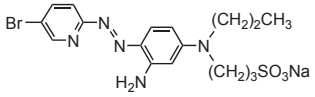
Ordering Information

Product code	Unit
B027-10	100 mg

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description
5-Br-PSAA is a water-soluble colorimetric reagent for Fe(II) and Co(III) detection. This reagent forms colored complexes with Fe, Ni, Cu, and Co, but not with Zn. The Fe(II) and Co(III) complexes are very stable, and even EDTA cannot remove metals from these complexes. However, Cu(II) and Ni(II) complexes are decomposed by EDTA, so 5-Br-PSAA can be used to determine Fe(II) in the presence of Cu(II) or Ni(II). The molar absorptivity of Fe(II) is reported to be $8.9x10^4$ (λmax=558 nm). The 5-Br-PSAA assay is 4 times as sensitive as Bathophenanthroline assay. Dr. Sakai and others determined ppb levels of Pb (λ=98,400) using 5-Br-PSAA by flow injection analysis. Dr. Makino and others determined Cu(II) in 0.2 ml of serum by direct colorimetric detection (λmax=580 nm,ε =64,000).

References
1. D. Horiguchi, *et al*., Water-soluble Pyridylazo Reagents for the Spectrophotometric Determination of Metals Determination of Iron(II) with 2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulfopropylzmino)aniline. *Anal Chim Acta*. 1983;**151**:457-463.
2. T. Sakai, *et al*., Flow-injection Spectrophotometric Determination of Palladium in Catalysts and Dental Alloys with 2-(5-Bromo-2-Pyridylazo)-5-(N-Propyl-N-Sulfopropylamino)Aniline. *Anal Chim Acta*. 1988;**214**:271-277.
3. T. Makino, A Sensitive, Direct Colorimetric Assay of Serum Copper Using 5-Br-PSAA. *Clin Chim Acta*. 1989;**185**:7-16.
4. S. W. Kang, *et al*., Simultaneous Spectrophotometric Determination of Iron and Copper in Serum with 2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)aniline by Flow-injection Analysis. *Anal Chim Acta*. 1992;**261**:197-203.

BT 2-Hydroxy-1-(1-hydroxy-2-naphthylazo)-6-nitro-4-naphthalenesulfonic acid, sodium salt [CAS:1787-61-7]

Application: Metal indicator, Ca^{2+} and Mg^{2+} detection, colorimetric
Appearance: Black or blackish brown powder
Absorbance in borate buffer(1 mg / 100 ml, pH9)
: ≥0.400(around 620 nm)
MW: 461.38, $C_{20}H_{12}N_3NaO_7S$

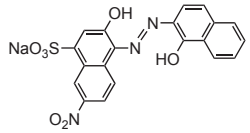
Ordering Information

Product code	Unit
B015-10	25 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description
BT in aqueous solution changes color according to pH: red at pH<6, blue at pH 7-11, and orange at pH>12. Its proton dissociation constants are reported to be pKa_2=6.3 and pKa_3=11.55 (m=0.08, 20°C). BT is one of the most commonly used indicators of Ca, Mg, Zn, Cd, Pb, Hg, and rare earth metals for EDTA chelate titration. BT solution turns red in the presence of the metals at pH 10, and then changes to blue at the endpoint of the titration. Cu and Fe(III), as well as Al, Co, and Mn(III), should be masked with KCN because they interfere with the color change. Triethanolamine is also a suitable masking reagent for Al, Fe(III), and Mn(III). Since metal ions such as Mn(III) and Fe(III) oxidize BT, hydroxylamine and hydrochloride should be added to prevent oxidation.

References
1. P. F. Lott, *et al*., Spectrophotometric Determination of Thorium with Eriochrome Black T. *Anal Chem*. 1960;**32**:1702-1704.

C14-K22B5

4,13-Bis[*N*-(1-adamantyl)carbamoylacetyl]-8-tetradecyl-1,7,10,16-tetraoxa-4,13-diazacyclooctadecane

Application: Ionophore for Mg selective electrode
Appearance: White powder or solid
Purity: ≥95.0% (HPLC)
MW: 897.28, $C_{52}H_{88}N_4O_8$

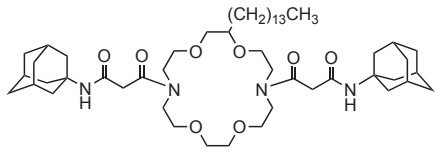
Ordering Information

Product code	Unit
C391-10	10 mg

Storage Condition
-20°C, protect from moisture

Shipping Condition
ambient temperature

Chemical Structure



Product Description
C14-K22B5 is the best magnesium selective ionophore. The PVC membrane stability is improved by this ionophore's high lipophilicity due to tetraoctadecane and adamantyl groups. C14-K22B5 has a linear response between $1x10^{-5}$ M and 0.1 M magnesium ion.

Reference
1. K. Suzuki, *et al*., Design and Synthesis of Calcium and Magnesium Ionophores Based on Double-Armed Diazacrown Ether Compounds and Their Application to an Ion-Sensing Component for an Ion-Selective Electrode. *Anal Chem*. 1995;**67**:324-334.

Calcein

Bis[*N*,*N*-bis(carboxymethyl)aminomethyl]fluorescein [CAS:1461-15-0]

Application: Metal indicator for Ca^{2+}, fluorometric
Appearance: Yellowish orange powder
Free fluorescein: To pass test
MW: 622.53, $C_{30}H_{26}N_2O_{13}$

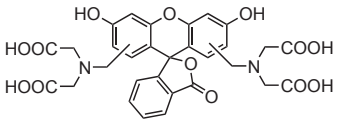
Ordering Information

Product code	Unit
C001-10	1 g
C001-12	5 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description

Calcein is a fluorescent metal indicator for calcium detection with EDTA titration. It is slightly soluble in water. The proton dissociation constants are reported to be pKa_1=2.1, pKa_2=2.9, pKa_3=4.2, pKa_4=5.5, pKa_5=10.8, and pKa_6=11.7. Calcein solution emits a yellowish-green fluorescence in acidic conditions and no fluorescence in basic conditions. However, Calcein will emit fluorescence in the presence of metal ions such as Al, Ba, Ca, Cu, Mg, and Zn in basic conditions. Therefore, Calcein is also used for direct fluorometric EDTA titration of these metal ions. Since Calcein has a clear endpoint even if the sample has color or contains phosphate, it is suitable for calcium detection in biological samples or fertilizers. TPC, murexide, or PPC are added to get a clear endpoint. For example, by mixing equal amounts of TPC with Calcein, intense green fluorescence will turn reddish-purple with no fluorescence at the endpoint.

References

1. J. Korbl, *et al*., Metallochromic Indicators. IV. A Note on the Preparation and Properties of "Calceine". *Collect Czech Chem Commun*. 1958;**23**:622-627.
2. R. O. Ashby, *et al*., A Microdetermination of Calcium in Blood Serum. *J Lab Clin Med*. 1957;**49**:958-961.
3. M. A. Demertzis, Fluorimetric Determination of Calcium in Serum with Calcein Complexation of Calcein with Calcium and Alkali Metals. *Anal Chim Acta*. 1988;**209**:303-308.

Calcein Blue

8-[*N*,*N*-Bis(carboxymethyl)aminomethyl]-4-methylunbelliferone [CAS: 54375-47-2]

Application: Metal indicator, fluorometric
Appearance: White or pale yellow powder
Sensitivity: To pass test
MW: 321.28, $C_{15}H_{15}NO_7$

Ordering Information

Product code	Unit
C002-08	1 g

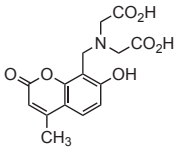
Storage Condition

ambient temperature, protect from metal

Shipping Condition

ambient temperature

Chemical Structure



Product Description

Calcein Blue is a fluorescent metal indicator in EDTA titration. It is highly soluble in alkaline solutions, but slightly soluble in water. Its proton dissociation constants are reported to be pKa_1=2.45, pKa_2=7.24, and pKa_3=11.3. Calcein Blue solution emits bright blue fluorescence (λem=445 nm) at pH 4-11, but no fluorescence at pH 12 or higher. The blue fluorescence of the Calcein Blue solution at pH 4-11 is quenched by metal ions such as Co, Cu, Mn, Ni, and Pb. However, Calcein Blue forms a highly fluorescent complex with alkaline earth metals at pH 12 or higher. Since the endpoint of the chelate titration is clear by UV irradiation at 200-370 nm in a dark room, highly stained samples can be used. Calcein Blue is also suitable to determine Fe and Zr ions. Calcein Blue-Zn chelate can detect 10 ppb of F^-. Calcein Blue aqueous solution emits laser at 449-490 nm.

References

1. D. H. Willkins, Calcein Blue - A New Metalfluorechromic Indicator for Chelatometric Titrations. *Talanta*. 1960;**4**:182-184.
2. J. H. Eggers, Umbellikomplexon Und Xanthokomplexonein Beitrag zur Kenntnnis Komplexometrischer Fluoreszensindikatoren. *Talanta*. 1960;**4**:38-43.
3. H. N. Elsheimer, Complexometric Determination of Gallium with Calcein Blue as Indicator. *Talanta*. 1967;**14**:97-102.
4. M. A. Salam Khan, *et al*., A Structural Study of the Methyleneiminodiacetic Acid Derivatives of Some 7-Hydroxycoumarins. *Anal Chim Acta*. 1968;**43**:153-156.
5. R. V. Hems, *et al*., Spectrofluorimetric Determination of Submicrogram Amounts of Zirconium with Calcein Blue. *Anal Chem*. 1970;**42**:784-787.
6. T. L. Har, *et al*., Spectrofluorimetric Determination of Traces of Fluoride Ion by Ternary Complex Formation with Zirconium and Calcein Blue. *Anal Chem*. 1971;**43**:136-139.
7. T. Imasaka, *et al*., Characteristics of Coordination Compounds of Calcein Blue for a Tunable Oraganic Liquid Laser. *Bull Chem Soc Jpn*. 1976;**49**:2687-2695.
8. K. A. Matsoukas, *et al*., Spectrofluorimetric Determination of Magnesium in Blood Serum with Calcein Blue. *Anal Chim Acta*. 1989;**227**:211-218.

Cesibor

Tetrakis(4-fluorophenyl)borate, sodium salt, dihydrate [CAS: 25776-12-9]

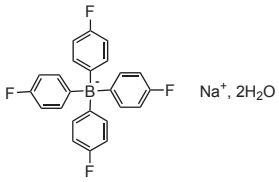
Application: Cs^+ detection, precipitation titration
Appearance: White powder
Purity: ≥98.0% (Grav.)
MW: 450.21, $C_{24}H_{16}BF_4Na$, $2H_2O$

Ordering Information	
Product code	Unit
C007-10	1 g

Storage Condition
0-5°C

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Cesibor is a cesium-ion-selective tetraphenylborate compound utilized as a precipitation reagent for cesium ion. Cesibor can precipitate only cesium ion from a mixture of ammonium, potassium, and cesium ions.

References

1. C. E. Moore, *et al*., Studies in the Tetraarylborates Part II. The Preparation and Reagent Properties of Sodium tetrakis(p-fluorophenyl)borate, a Selective Reagent for Cesium. *Anal Chim Acta*. 1966;**35**:1-5.
2. M. Tsubouchi, *et al*., Two-Phase Titration of Poly(oxyethylene) Nonionic Surfactantswith Tetrakis(4-fluorophenyl)borate. *Anal Chem*. 1985;**57**:783-784.

Chlorophosphonazo-III

2,7-Bis(4-chloro-2-phosphonophenylazo)-1,8-dihydroxy-3,6-naphthalenedisulfonic acid [CAS: 1914-99-4]

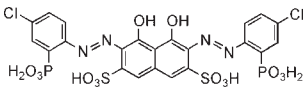
Application: Alkaline earth metals detection, colorimetric
Appearance: Reddish brown or black purple crystalline powder
Absorbance (0.018 mmol/l): ≥0.530 (around 550 – 570 nm)
MW:757.37, $C_{22}H_{16}Cl_2N_4O_{14}P_2S_2$

Ordering Information	
Product code	Unit
C010-10	100 mg
C010-12	1 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Chlorophosphonazo-III is a colorimetric reagent for thorium, uranium, lanthanoids, actinoids, and alkaline earth metals. This reagent is readily soluble in water and is violet at pH 4 or lower, blue in NaOH solution, and brilliant green in acids such as H_2SO_4, HCl, and diluted HNO_3 (it decomposes in concentrated HNO_3). Its proton dissociation constants are reported to be -1.1, -1.1, 0.6, 0.8, 1.5, 2.5, 5.47, 7.20, 12.15 and 15.13 (μ=0.1, 20°C). Chlorophosphonazo-III is used as a selective colorimetric reagent for calcium in a mixed Ca/Mg sample in the pH range of 2-3. It is also a useful indicator for the precipitation titration of SO_4^{2-} using Ba^{2+} as a standard solution.

References

1. L. R. Snyder, *et al*., Characterization and Routine Determination of Nonbasic Nitrogen Types in Cracked Gas Oils by Linear Elution Adsorption Chromatography. *Anal Chem*. 1964;**36**:767-773.
2. J. W. Ferguson, *et al*., Simultaneous Spectrophotometric Determination of Calcium and Magnesium with Chlorophosphonazo-III. *Anal Chem*. 1964;**36**:796-799.
3. D. S. Howell, *et al*., Ultramicro Spectrophotometric Determination of Calcium in Biologic Fluids. *Anal Chem*. 1966;**38**:434-438.
4. T. Yamamoto, Extraction-photometric Determination of Uranium(IV) with Chlorophosphonazo-III. *Anal Chim Acta*. 1973;**65**:329-334.
5. M. Zenki, *et al*., Repetitive Determination of Calcium Ion and Regeneration of a Chromogenic Reagent Using Chlorophosphonazo III and an Ion Exchanger in a Circulatory Flow Injection System. *Anal Sci*. 2002;**18**:1137-1140.

Co(III)-5-Cl-PADAP

Bis[2-(5-chloro-2-pyridylazo)-5-diethylaminophenolato]cobalt(III) chloride [CAS: 81342-98-5]

Application: Anionic detergent detection with solvent extraction, colorimetric
Appearance: Dark purple crystalline
Molar absorptivity: ≥84,000 (around 585 nm)
MW: 701.92, $C_{30}H_{32}Cl_3CoN_8O_2$

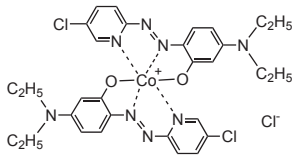
Ordering Information

Product code	Unit
C021-10	100 mg

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Since Co(III)-5-Cl-PADAP is not influenced by chloride or nitrate ions,it is suitable for the colorimetric determination of anionic detergents in seawater. Anionic surfactant can be determined at the ppb level.

Protocol
1. Add 1 ml of HCl (5 M), 1 ml of Co(III)-5-Cl-PADAP solution (0.1% w/v), and 5 ml of toluene to 50 ml of sample solution.
2. Shake the mixture for 5 minutes.
3. Discard the aqueous phase and wash the toluene solution with 25 ml of Co(III)-5-Cl-PADAP (0.002% w/v, pH 1 HCl).
4. Measure the absorbance of the toluene phase at 560 nm.

References

1. S. Taguchi, *et al*., Silaned Glass Beads for the Preconcentration and Spectrophotometric Determination of Cobalt with 2-(2-Pyridylazo)-5-diethylaminophenol. *Talanta*. 1980;**27**:819-821.
2. S. Taguchi, *et al*., Bis[2-(2-Pyridylazo)-5-Diethylaminophenolato]Cobalt(III) Chloride as a New Extraction and Spectrophotometric Reagent for Trace Anions. *Talanta*. 1980;**27**:289-291.
3. S. Taguchi, *et al*., An Application of Bis[2-(5-Chloro-2-Pyridylazo)-5-Diethylaminophenolato]Cobalt(III) Chloride to the Extraction and Spectrophotometric Determination of Sulphonated and Sulphate Surfactants. *Talanta*. 1981;**28**:616-618.
4. K. Seno, *et al*., Extraction-spectrophotometric Determination of Higher Carboxylic Acids with Bis[2-(5-Chloro-2-Pyridylazo)-5-Diethylaminophenol] Cobalt(III) Chloride. *Chem Lett*. 1982;**11**:645-646.
5. M. Taga, *et al*., Flotation-spectrophotometric Determination of Phosphate with Bis[2-(5-Chloro-2-Pyridylazo)-5-Diethylaminophenolato]Cobalt(III) Chloride. *Anal Sci*. 1988;**4**:181-184.
6. M. Taga, *et al*., Capillary Isotachophoretic Determination of Phosphate After Enrichment on Membrane Filter as Ion Pair Molybdophosphate with Bis[2-(5-Chloro-2-Pyridylazo)-5-Diethylaminophenolato]Cobalt(III). *Anal Sci*. 1989;**5**:219-220.
7. M. Taga, *et al*., The Spectrophotometric Determination of Phosphate Following the Collection of Ion Pair of Molybdophosphate with Bis-[2-(5-chloro-2-pyridylazo)-5-diethylaminophenolato]cobalt(III) on an Organic Solvent-soluble Membrane Filter. *Bull Chem Soc Jpn*. 1989;**62**:1482-1485.

Cu-PAN

Composite preparation of Cu-EDTA and PAN (11.1:1)

Application: Metal indicator
Appearance: Grayish red brown or yellowish brown powder
Absorbance: ≥0.500 (around 470 nm)

Ordering Information

Product code	Unit
C016-10	10 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Product Description
Cu-PAN, a mixture of Cu-EDTA and pyridylazo-naphthol PAN, is a very popular metal indicator for chelatometry. Usually, 20-50% aqueous dioxane (or isopropyl alcohol) or 50-70% aqueous methanol (or ethanol) is used as a solvent (1 g Cu-PAN per 100 ml solvent). The solution should be heated during the chelating titration.

Applications
Chelate titration (acidic condition): Al, C, Co, Fe(III), Ga, Hg, In, Ni, V, Zn, rare earth metal ions Chelate titration (alkaline condition): Ca, Cu, Mg, Mn(II)

References
1. H. Flaschka, *et al*., EDTA Titrations Using Copper-PAN Complex as Indicator. *Chem Anal*. 1956;**45**:58-61.

Cyanoline Blue Composite preparation of monopyrazolone and bisbyrazolone (12.5:1)

Application: CN^- detection, colorimetric
Appearance: White or pale yellow powder
Sensitivity: To pass test

Ordering Information

Product code	Unit
C017-10	25 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Product Description
Cyanoline Blue is a mixture of monopyrazolone and bispyrazolone. It is used as a colorimetric reagent for CN^- detection. The detection range of CN^- by this method is 5 ppb to 10 ppm. The maximum wavelength is 620 nm. A ready-to-use solution can be prepared by dissolving 0.27 g Cyanoline Blue in 20 ml pyridine, followed by dilution with 100 ml of water. The solution is stable for one week at 4°C. Cyanoline Blue is also used to detect SCN^-, OCN^-, NH_3, and vitamin B_{12}.

References
1. J. M. Kruse, *et al*., Colorimetric Determination of Cyanide and Thiocyanate. *Anal Chem*. 1953;**25**:446-450.
2. L. Prochazkova, Spectrophotometric Determination of Ammonia as Rubazoic Acid with Bispyrazolone Reagent. *Anal Chem*. 1964;**36**:865-871.
3. S. Baar, The Micro Determination of Cyanide: Its Application to the Analysis of Whole Blood. *Analyst*. 1966;**91**:268-272.

DAN 2,3-Diaminonaphthalene [CAS 771-97-1]

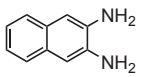
Application: Se detection
colorimetric and fluorometric Nitrite detection fluorometric
Appearance: White to pale yellowish brown powder
MW: 158.20, $C_{10}H_{10}N_2$

Ordering Information

Product code	Unit
D027-10	1 g

Storage Condition
-20°C, protect from light

Shipping Condition
ambient temperature

Chemical Structure


Product Description
DAN is a highly selective colorimetric and fluorometric reagent for Se detection. This reagent is almost insoluble in cold water and alcohol, but it is highly soluble in water at 50°C. DAN forms 4,5-benzopiaselenol by the complex with Se, and this complex is extracted with chloroform, toluene, or cyclohexane. Since the Se complex is volatile, Se can be determined by gas chromatography. Further, DAN generates fluorescent naphthalenetriazole by the reaction with nitrite ion, so it is also used for the fluorometric determination of nitrite.

Applications
Solvent extraction, colorimetric: Se(IV) (in toluene at pH 1.2-2.2, λmax=380 nm, λ=11,800, detection range 0-4 ppm)
Solvent extraction, fluorometric: Se(IV) (λem=520 nm in toluene, λem=538 nm in cyclohexane, detection range 1-100 ppb)

References
1. J. H. Wiersma, 2,3-Diaminonaphthalene as a Spectrophotometric and Fluorometric Reagent for the Determination of Nitrite Ion. *Anal Lett*. 1970;**3**:123-132.
2. G. L. Wheeler, *et al*., Rapid Determination of Trace Amounts of Selenium(IV), Nitrate, and Nitrate by High-pressure Liquid Chromatography Using 2,3-Diaminonaphthalene. *Microchem J*. 1974;**19**:390-405.
3. E. M. Rodriguez, *et al*., Critical Study of Fluorimetric Determination of Selenium in Urine. *Talanta*. 1994;**41**:2025-2031.
4. A. M. Miles, *et al*., Fluorometric Determination of Nitric Oxide. *Methods*. 1995;**7**:40-47.

Diantipyrylmethane

Di(4-antipyryl)methane, monohydrate [CAS 1251-85-0]

Application: Ti detection, colorimetric
Appearance: White crystalline powder
Purity: ≥99.0% (HPLC)
Absorbance (Ti complex): ≥0.280 (around 390 nm)
MW: 406.48, $C_{23}H_{24}N_4O_2$, H_2O

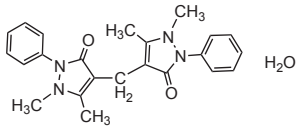
Ordering Information

Product code	Unit
D008-10	25 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Diantipyrylmethane is a colorimetric reagent for Ti detection, and is also used as a gravimetric and solvent extraction reagent for various kinds of metal ions. This reagent is readily soluble in mineral acids and organic solvents, and slightly soluble in benzene (1.7%) and carbon tetrachloride (0.28%). Diantipyrylmethane forms a yellow complex with Ti in 0.5-4.0 M HCl solution (λmax=385-390 nm, λ=15,000). The resulting Ti complex can be extracted by organic solvents in the presence of I^-, SCN^-, tartaric acid, or pyrocatechol. The detection range is 0.2-3.0 ppm. Diantipyrylmethane also makes a brown water-soluble complex with Fe in a weak acidic solution.

Application
Solvent extraction, colorimetric detection: Fe (λmax=450 nm, λ=5,400), Mo, Ti, U
Gravimetric detection: Be, Ca, Cd, Co, Cu, Hg, Ir, Os, Pb, Sr, Tl, Zn

References

1. C. H. Chung, Simultaneous Determination of Iron and Titanium in Silicate Rocks by Using Diantipyrinylmethane with Dual-wavelength Spectrophotometry. *Anal Chim Acta*. 1983;**154**:259-265.
2. N. Uehara, *et al*., Determination of Titanium(III) in River Water by Ion-pair Reversed-phase High-performance Liquid Chromatography with 4,4'-Diantipyrylmethane. *Analyst*. 1991;**116**:27-29.

Dibenzyl-14-crown-4

6,6-Dibenzyl-1,4,8,11-tetraoxacyclotetradecane [CAS: 106868-21-7]

Application: Li^+ selective electrode
Appearance: White crystalline powder
Purity: ≥98.0% (HPLC)
MW: 384.51, $C_{24}H_{32}O_4$

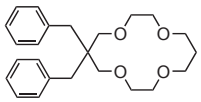
Ordering Information

Product code	Unit
D043-10	50 mg

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Dibenzyl-14-crown-4 is 200 times more selective for lithium than for sodium or potassium. Though Dibutyl-phenanthroline is 1,000 times more selective for lithium than sodium at a controlled pH, Dibenzyl-14-crown-4 is less pH-sensitive.

Selectivity: $LogK^{pot}$(Li/Na): -2.5, $LogK^{pot}$(Li/K): -2.3, $LogK^{pot}$(Li/Cs): -1.6, $LogK^{pot}$(Li/Rb): -2.2, $LogK^{pot}$(Li/NH4): -3.1, $LogK^{pot}$(Li/H): -3.2, $LogK^{pot}$(Li/Mg): -4.3, $LogK^{pot}$(Li/Ca): -4.9, $LogK^{pot}$(Li/Ba): -4.7, $LogK^{pot}$(Li/Sr): -4.3
Formulation: Ionophore: 1%, NPOE: 70%, K-TCPB: 50 mol% of ionophore/PVC, TH

References
1. K. Kimura, *et al*., Lithium Ion Selective Electrodes Based on Crown Ethers for Serum Lithium Assay. *Anal Chem*. 1987;**59**:2331-2334.
2. K. Kimura, *et al*., Synthesis and Selectivity for Lithium of Lipophilic 14-Crown-4 Derivatives Bearing Bulky Substituents or an Additional Binding Site in the Side Arm. *J Chem Soc Perkin Trans 2*. 1986;**12**:1945-1951.

HDOPP-Ca Bis(4-*n*-octylphenyl)phosphate, calcium salt

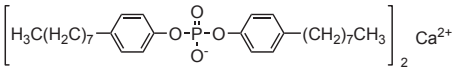
Application: Ion exchanger for Ca^{2+} selective electrode
Appearance: White powder
Molar absorptivity (in THF): ≥3,600 (around 270 nm)
MW: 987.29, $C_{56}H_{84}CaO_8P_2$

Ordering Information	
Product code	Unit
H003-10	1 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
HDOPP-Ca is a liquid cation exchanger for use in the calcium ion selective electrode. HDOPP is the most suitable compound among the dialkyl phosphate derivatives and is used in either a liquid membrane or a PVC membrane using DOPP (dioctylphenylphosphate calcium salt) as a plasticizer.

References
1. J. W. Ross, Calcium-Selective Electrode with Liquid Ion Exchanger. *Science*. 1967;**156**:1378-1379.
2. J. Ruzicka, *et al*., Selectrode - The Universal Ion-selective Electrode Part VI. The Calcium(II) Selectrode Employing a New Ionexchanger in a Nonporous Membrane and a Solid-state Reference System. *Anal Chim Acta*. 1973;**67**:155-178.
3. H. M. Brown, *et al*., A Calcium-sensitive Microelectrode Suitable for Intracellular Measurement of Calcium(II) Activity. *Anal Chim Acta*. 1976;**85**:261-276.

HFPB Tetrakis[3,5-bis(1,1,1,3,3,3-hexafluoro-2-methoxy-2-propyl)phenyl]borate, sodium salt, trihydrate [CAS: 120945-63-3]

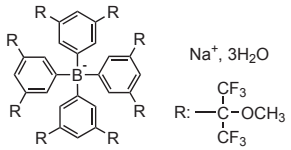
Application: Lipophilic anion, counter anion for ion selective electrode
Appearance: White crystalline powder
Purity: ≥95.0% (Titration)
MW: 1836.65, $C_{56}H_{36}BF_{48}NaO_8$, $3H_2O$

Ordering Information	
Product code	Unit
H209-10	100 mg

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Highly lipophilic tetraphenylborate (HFPB) is a very stable anion in a wide pH range. It is used as a cation phase transfer reagent. A vitamin B_{12} electrode using HFPB as an ion site detects 10^{-7} M level of vitamin B_{12} with linear response.

References
1. N. Ishibashi, *et al*., Vitamin B1-Sensitive Poly(vinyl chloride) Membrane Electrode Based on Hydrophobic Tetraphenylborate Cation Exchangers. *Anal Sci*. 1988;**4**:527-528.
2. G. H. Zhang, *et al*., Vitamin B1 sensitive poly(vinyl chloride) membrane electrode based on hydrophobic tetraphenylborate derivatives and their application. *Anal Chem*. 1990;**62**:1644-1648.
3. T. Katsu, *et al*., A New Bretylium-Selective Electrode for Monitoring the Drug in Blood Serum. *Anal Lett*. 1996;**29**:1281-1292.
4. T. Katsu, *et al*., Potentiometric determination of alkaline phosphatase in blood serum using a hordenine-sensitive membrane electrode. *Electroanalysis*. 1996;**8**:1101-1104.

HNB 2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3,6-naphthalendisulfonic acid, trisodium salt [CAS: 63451-35-4]

Application: Alkaline earth metal ions, rare earth metal ions detection
Appearance: Black purple powder
Absorbance (Ca complex): ≥0.310 (around 562 nm)
Sensitivity: To pass test
MW: 620.48, $C_{20}H_{11}N_2Na_3O_{11}S_3$

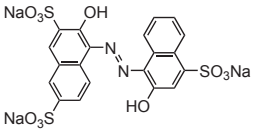
Ordering Information

Product code	Unit
H007-10	1 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description
HNB is a metal indicator for calcium, and a colorimetric reagent for alkaline earth metal ions, rare earth metal ions, and uranium ions. HNB is readily soluble in water and alcohol. Aqueous solution of HNB is blue at pH 7-12, red above pH 13, and pink in the presence of calcium at pH 10. The detection range of alkaline earth metal ions and rare earth metal ions are 1-600 ppm and 1-300 ppm, respectively. The maximum wavelength of the complex is 650 nm. HNB is also used for UO_2(II) detection. The proton dissociation constants of HNB are reported to be pKa_4=6.44 and pKa_5=12.91(μ=0.1, 24°C).

References

1. A. Itoh, *et al*., Evaluation of 2-Hydroxy-1-(2-Hydroxy-4-Sulpho-1-Naphthylazo)-3-Naphthoic Acid and Hydroxynaphthol Blue as Metallochromic Indicators in the EDTA Titration of Calcium. *Analyst*. 1970;**95**:583-589.
2. M. Sugawara, *et al*., Catalytic Decomposition of the Excess Reagent in the Spectrophotometric Determination of Copper(II) with O,O'-Dihydroxyazo Compounds. *Bull Chem Soc Jpn*. 1977;**50**:3206-3208.
3. M. M. Ferris, *et al*., Examination of Metallochromic Indicators and Water-soluble Reagents for Metals by Planar Electrophoresis. *Analyst*. 1986;**111**:351-354.

Kalibor Tetraphenylborate, sodium salt [CAS: 143-66-8]

Application: Lipophilic anion, counter anion for ion selective electrode
Appearance: White crystal
Purity: ≥99.5% (Grav.)
MW: 342.22, $C_{24}H_{20}BNa$

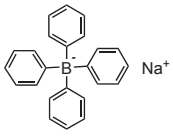
Ordering Information

Product code	Unit
K003-12	25 g
K003-14	100 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Kalibor is utilized for gravimetric analyses of K^+, Rb^+, Cs^+, NH_4^+, amine compounds, alkaloids, onium compounds, and other monovalent ions, such as Ag(I), Cu(I) and Tl(I).

References

1. R. E. Jensen, *et al*., Non-aqueous Acids-base Properties of Sodium Tetraphenylboron. *Anal Chem*. 1972;**44**:846-848.
2. N. M. Hanken, The Use of Sodium Tetraphenylborate and Sodium Chloride in the Extraction of Fossils from Shales. *J Paleontology*. 1979;**53**:738-741.
3. M. Tsubouchi, *et al*., Determination of Cationic Surfactants by Two-phase Titration. *Anal Chem*. 1981;**53**:1957-1959.
4. S. S. Badawy, *et al*., Potentiometric and Thermal Studies of a Coated-wire Antazoline-selective Electrode. *Anal Chem*. 1988;**60**:758-761.
5. K. Vytras, *et al*., Titrations of Non-ionic Surfactans with Sodium Tetraphenylborate Using Simple Potentiometric Sensors. *Analyst*. 1989;**114**:1435-1441.
6. A. Yoo, *et al*., The Synthesis of Some Lipophilictetra-arylborates for Use in Membrane Electrode Preparation. *Talanta*. 1991;**38**:493-496.

Murexide Purpuric acid, ammonium salt [CAS 3051-09-0]

Application: Ca^{2+} and rare earth metal ion detection, colorimetric
Appearance: Reddish or dark reddish purple powder
Absorbance (0.018 mmol/l): ≥0.200 (around 525 nm)
Sensitivity: To pass test
MW: 284.19, $C_8H_8N_6O_6$

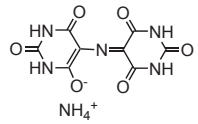
Ordering Information

Product code	Unit
M011-10	5 g
M011-12	25 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



References

1. H. Gordon, *et al*., Spectrophotometric Determination of Calcium in Zirconium Powder by Use of Murexide. *Talanta*. 1972;**19**:1-6.
2. K. S. Balaji, *et al*., Stability Constants of Calcium and Lanthanide Ions with Murexide. *Anal Chem*. 1978;**50**:1972-1975.

MX Composite preparation of ammonium purpurate and potassium sulfate (Ratio 1:250)

Application: Ca^{2+} and rare earth metal ions detection, colorimetric
Appearance: Pink or reddish purple powder
Absorbance: 0.150 – 0.200 (at 525 nm)
Sensitivity: To pass test

Ordering Information

Product code	Unit
M012-08	100 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Product Description of Murexide and MX

MX is a 1:250 mixture of Murexide and potassium sulfate. Murexide is a metal indicator for Ca, Co, Cu, Ni, Th, and rare earth metals; it is also a colorimetric reagent for calcium and rare earth metals. Murexide is slightly soluble in water, alcohol, and ether. Murexide is unstable in aqueous solution. Its proton dissociation constants are reported to be pKa_1=0, pKa_2=9.2 and pKa_3=10.5. The solution is yellow in strong acidic conditions, reddish-purple in weak acidic conditions, and bluepurple in alkaline conditions. Detection conditions for calcium are pH 11.3, maximum wavelength 506 nm and detection range 0.2-1.2 ppm.

Nitro-PAPS 2-(5-Nitro-2-pyridylazo)-5-[*N*-*n*-propyl-*N*-(3-sulfopropyl)amino]phenol, disodium salt, dihydrate [CAS: 143205-66-7]

Application: Heavy metal ion detection, colorimetric
Appearance: Dark green or dark greenish brown powder
Purity: ≥90.0% (Absorbance, as anhydride)
MW: 503.45, $C_{17}H_{19}N_5Na_2O_6S$, $2H_2O$

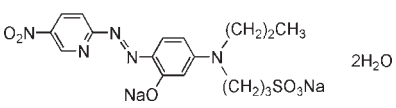
Ordering Information

Product code	Unit
N031-10	100 mg

Storage Condition
ambient temperature, protect from light and moisture

Shipping Condition
ambient temperature

Chemical Structure



Product Description

Nitro-PAPS is a highly sensitive colorimetric reagent for Fe(II) detection that forms a water-soluble complex at pH 3.0-8.0 (λmax=582 nm, λ=107,000). This reagent is suitable for the determination of Fe(II) in serum and can also be used to determine micromolar levels of Cu, Zn, Ni, and Co. Cu and Zn in serum are masked by a mixture of thioglycolic acid and SDS. Zn in serum can be determined by Nitro-PAPS using CN^- as a masking reagent of Fe and Cu.

Metal	λmax	Molar Absorptivity
Co(II)	590 nm	113,000
Cu(II)	566 nm	71,000
Fe(II)	582 nm	107,000

Metal	λmax	Molar Absorptivity
Ni(II)	568 nm	150,000
Zn(V)	566 nm	150,000

References

1. T. Makino, *et al*., A Sensitive, Direct Colorimetric Assay of Serum Iron Using the Chromogen, Nitro-PAPS. *Clin Chim Acta*. 1988;**171**:19-27.
2. T. Makino, A Sensitive, Direct Colorimetric Assay of Serum Zinc Using Nitro-PAPS and Microwell Plates. *Clin Chim Acta*. 1991;**197**:209-220.
3. S. Yamashita, *et al*., Sensitive, Direct Procedures for Simultaneous Determination of Iron and Copper in Serum, with Use of 2-(5-Nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)phenol(Nitro-PAPS) as Ligand. *Clin Chem*. 1992;**38**:1373-1375.
4. T. Yamane, *et al*., Flow-injection Spectrophotometric Determination of Trace Iron in Various Salts. Elimination of Blank Peak Effect and Use of 2-(5-Nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)phenol as Chromogenic Agent. *Anal Chim Acta*. 1995;**308**:433-438.
5. T. Yamane, *et al*., Complex Formation of 2-(5-Nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)phenol with Lead, Cadmium and Manganese for Their Sensitive Spectrophotometric Detection in Flow Injection and Ion Chromatography Systems. *Anal Chim Acta*. 1997;**345**:139-146.

Nitroso-PSAP

2-Nitroso-5-[*N*-*n*-propyl-*N*-(3-sulfopropyl)amino]phenol [CAS 80459-15-0]

Application: Fe(II) detection, colorimetric
Appearance: Yellow or yellowish brown powder
Purity: ≥97.0% (Absorbance)
Molar absorptivity (Fe^{2+} complex): ≥44,000 (around 756 nm)
Absorbance (Fe^{2+} free, Blank): ≤0.050 (around 756 nm)
MW: 302.35 $C_{12}H_{18}N_2O_5S$

Ordering Information

Product code	Unit
N010-10	100 mg
N010-12	1 g

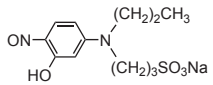
Storage Condition

0-5°C, protect from metal

Shipping Condition

ambient temperature

Chemical Structure



Product Description

Nitroso-PSAP is a highly sensitive water-soluble colorimetric reagent for Fe(II) detection that forms a green complex with Fe(II) (λmax=756 nm, ε=4.5x10^4). This reagent is suitable for the determination of blood serum levels of Fe, Co, Ni, and Cu. The detection range is from 5 ppb to 5 ppm under the same detection condition. Nitroso-PSAP is useful in flow injection analyses. The workable pH range of the nitroso compounds is 5.6-10.1. The molar absorptivities and maximum wavelengths of the Co, Cu, and Ni complexes are 4.0x10^4 (490 nm), 2.8x10^4 (429 nm), and 2.6x10^4 (394 nm), respectively.

Metal	λmax	Molar Absorptivity
Co(II)	490 nm	40,000
Cu(II)	429 nm	28,000

Metal	λmax	Molar Absorptivity
Fe (II)	756 nm	45,000
Ni(II)	394 nm	26,000

References

1. K. Toei, *et al*., Nitrosophenol and Nitrosonaphthol Derivatives as Reagents for the Spectrophotometric Determination of Iron and Determination of Micro-amounts in Waters with 2-Nitroso-5-dimethylaminophenol. *Analyst*. 1975;**100**:629-636.
2. N. Ohno, *et al*., Spectrophotometric Determination of Iron in Boiler and Well Waters by Flow Injection Analysis Using 2-Nitroso-5-(N-propyl-N-sulpho-propylamino)phenol. *Analyst*. 1987;**112**:1127-1130.
3. I. Yoshida, *et al*., Studies of the Stabilities of Scandium, Yttrium, and Lanthanoid-ion Complexes of 2-Nitroso-5-(N-propyl-3-sulfopropylamino)phenol. *Bull Chem Soc Jpn*. 1988;**61**:2639-2640.
4. I. Yoshida, *et al*., Potentiometric Studies on the Binding Properties of Protons, Some Divalent and Tervalent Metal Ions with 2-Nitroso-5-(N-propyl-3-sulfopropylamino)phenol. *Anal Sci*. 1988;**4**:69-72.

NN 2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid [CAS: 3737-95-9]

Application: Ca detection, colorimetric
Appearance: Black purple powder
Absorbance (0.046 mmol/l, in MeOH): 0.600 – 0.800 (around 555 nm)
MW: 438.41, $C_{21}H_{14}N_2O_7S$

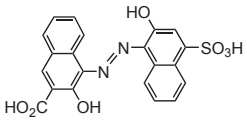
Ordering Information

Product code	Unit
N013-08	1 g

Storage Condition
ambient temperature, protect from light

Shipping Condition
ambient temperature

Chemical Structure



Product Description
NN is a colorimetric and chelatometric reagent for calcium (pH 12, λmax=470 nm) and UO_2^{2+} (λmax=570 nm, ε=1.36x10^5). Although NN is soluble in water, the solution is unstable and decomposes in a few minutes, especially in the presence of oxidative ions. Its proton dissociation constants are reported to be pKa_2=9.26 and pKa_3=13.67 (μ=0.1, 24°C). An NN solution of 0.1% w/v in 50% aqueous methanol is stable for one week if stored in a cool and dark place. Ascorbic acid is used as a stabilizer of the NN stock solution. Al, Cu, Fe(III), and Ni interfere with the determination of calcium by NN and should be masked by KCN or triethanolamine.

Reference
1. J. Patton, *et al*., New Indicator for Titration of Calcium with (Ethylenedinitrilo) Tetraacetate. *Anal Chem*. 1956;**28**:1026-1028.
2. A. Itoh, *et al*., Evaluation of 2-Hydroxy-1-(2-Hydroxy-4-Sulpho-1-Naphthylazo)-3-Naphthoic Acid and Hydroxynaphthol Blue as Metallochromic Indicators in the EDTA Titration of Calcium. *Analyst*. 1970;**95**:583-589.

NN diluted with potassium sulfate

Composite preparation of NN and potassium sulfate (Ratio 1:200)

Application: Ca detection, colorimetric
Appearance: Purple powder
Absorbance (1 g / 1000 ml, 550 nm): ≥0.150
Sensitivity: pass test

Ordering Information

Product code	Unit
N012-10	25 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

PAN 1-(2-Pyridylazo)-2-naphthol [CAS: 85-85-8]

Application: Metal indicator, heavy metal ion detection
Appearance: Yellowish or reddish orange crystalline powder
Absorbance (0.040 mmol/l): ≥0.600 (around 470 nm)
MW: 249.27, $C_{15}H_{11}N_3O$

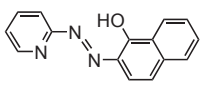
Ordering Information

Product code	Unit
P002-10	1 g
P002-12	10 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description

PAN is a metal indicator and colorimetric reagent for heavy metal and rare earth metal ions. This reagent is slightly soluble in acidic solutions and soluble in alkaline solutions. Its proton dissociation constants are reported to be pKa_1=2.9 and pKa_2=11.6 (μ=0.1, $NaClO_4$, at room temperature). The aqueous solution is yellow at pH<12 and red at pH>12. The distribution constants, $logK_D$, of chloroform/water and carbon tetrachloride/water are reported to be 5.4 and 4.0, respectively.

Application

Chelate titration: Bi, Cd, Ce, Cu, Ga, In, Tl, UO_2(II), Zn
Solvent extraction colorimetru: Ag, Bi, Cd, Co, Cr, Cu, Fe, Ga, Hf, Hg, In, Ir, Mn, Nb, Ni, Os, Pd, Pt, Rh, Ru, Sb, Ti, Tl, UO_2(II), VO(II), Zn, Zr, rare earth metals

Table 1 Characteristics of PAN Metal Complex

Metal	$LogK_{ML}$	$LogK_{ML2}$	Conditions
Co(II)	>12 -	50 %	dioxane
Cu(II)	15.6	8.4	50 % dioxane
Mn(II)	8.5	7.9	50 % dioxane
Ni	12.7	12.6	50 % dioxane
Zn	11.2 10.2	50 %	dioxane

Metal	$LogK_{ML}$	$LogK_{ML2}$	Conditions
Al	12.9	-	50 % ethanol
Ga	15.1	-	50 % ethanol
In	13.1	-	50 % ethanol
Eu	12.4	11.4	μ=0.05 ClO_4^-

*M=metal Ion, L=PAN

Table 2 Photometric Detection Conditions Using PAN

Metal	Conditions	λMax (nm)	ε ($x10^{-4}$)	Extraction Solvent	Range (ppm)
Cd	pH 8.7-19	555	4.9	Chloroform	0-2.5
Hf	pH 4, 40% methanol	545	3.9	Water	0.2-3.6
Mn(II)	pH 8.8-9.6, NH_3, KCN	562	4.8	Chloroform	0.2-1.2
Ni	pH 4-10, NH_3, KCN	570	5.0	Benzene	0-1.5
Os(VIII)	pH 3.5-5.2	550	2.8	Chloroform	0-9.2
Zn	pH 8.0-9.5, Triton x 100	555	5.6	Water	0.2-2
rare earth metals	pH 8.0-9.5	530	6-7	Chloroform	0-2

Reference

1. S. Shibata, Solvent Extraction Behavior of Some Metal-1-(2-Pyridylazo)-2-naphthol Chelates. *Anal Chim Acta*. 1960;**23**:367-369.

PAR 4-(2-Pyridylazo)resorcinol [CAS: 1141-59-9]

Application: Heavy metal ions detection, colorimetric
Appearance: Yellowish orange or orange-red powder
Absorbance: ≥0.450 (around 385 nm)
Sensitivity: To pass test
MW: 215.21, $C_{11}H_9N_3O_2$

Ordering Information

Product code	Unit
P003-10	1 g
P003-12	5 g

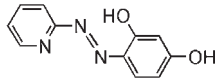
Storage Condition

ambient temperature, protect from metal

Shipping Condition

ambient temperature

Chemical Structure



Product Description
PAR is a metal indicator and colorimetric reagent for heavy metal ions. The solubility of this reagent in water (5 mg per 100 ml at 10°C) is higher than that of PAN. PAR is slightly soluble in alcohol. The aqueous solution is red at pH 5.5 or less, orange at pH 6-12.5, and red at pH 13 or higher. Its proton dissociation constants are reported to be $pKa_1(NH^+)$=3.1, pKa_2(*p*-OH)=5.6 and pKa_3(*o*-OH)=11.9 (*m*=0.2). PAR forms complexes with various metal ions that can be extracted with chloroform.

Application
Chelate titration: Al, Bi, Cd, Cu, Ga, Hg, In, Mn, Ni, Pb, Zn
Precipitation titration: MoO_4(II), WO_4(II)
Colorimetry: Al, Au, Bi, Co, Cr, Cu, Fe, Hf, Ga, In, Nb, Ni, Pb, Pd, Sb, Sn, Ti, Tl, U, V, Zn

Table 1 Photometric Detection Conditions Using PAR

Metal	Conditions	Complex	λMax (nm)	ε (x10^{-4})	Solvent
Bi	pH 3.0-6.0, ClO_4^-, antipyrine	$M(HL)_2X_2ClO_4$	520	2.9	chloroform-isobutanol (1:1)
Co(II)	pH 4-10, TPAC	ML_2X	518	6.0	chloroform
V(V)	pH 4.6-5.1, Crystal violet	MLX	585	11.0	benzene-MIBK (3:2) + 15% ethanol
Zn	pH 6-7, Dipenylguanidine	ML_2X_3	515	6.7	chloroform

M=metal ion, L=PAR, X=antipyrine, TPAC, Crystalviolet, or Diphenylguanidine

Reference

1. W. J. Geary, *et al*., The Metal Complexes of Some and Azomethine Dyestuffs Part I. Spectra in Water, and in Dioxan/Water in the Wavelength Range 320-600 mμ. *Anal Chim Acta*. 1962;**26**:575-582.
2. R. M. Dagnall, *et al*., Determination of Lead with 4-(2-Pyridylazo)-resorcinol-I Spectrophotometry and Solvent Extraction. *Talanta*. 1965;**12**:583-588.
3. G. Pilloni, *et al*., Spectrophotometric Determination of Diethyllead and Diethyltin Ions with 4-(2-Pyridylazo)-resorcinol. *Anal Chim Acta*. 1966;**35**:325-329.
4. L. Sommer, *et al*., Complexation of Aluminium, Yttrium, Lanthanum and Lanthanides with 4-(2-Pyridylazo)Resorcinol (PAR). *Talanta*. 1967;14:457-471.
5. R. Sawyer, Determination of Dialkyltin Stabilisers in Aqueous Extracts from PVC and Other Plastics. *Analyst*. 1967;**92**:569-574.
6. Z. Kleckova, *et al*., Spectrophotometric Study of Complex Equilibria and Determination of Lead(II) with 4-(2-Pyridylazo)resorcinol. *Collect Czech Chem Commun*. 1978;**43**:3163-3178.
7. A. Rios, *et al*., New Configuration for Construction of pH Grandients in Flow Injection Analysis. *Anal Chem*. 1986;**58**:663-664.

PC 3,3'-Bis[*N*,*N*-bis(carboxymethyl)aminomethyl]-*o*-cresolphthalein [CAS: 2411-89-4]

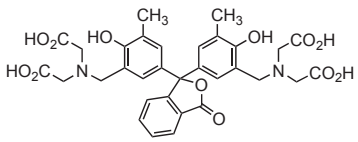
Application: Alkaline earth metal detection, colorimetric
Appearance: White or slightly yellowish pink powder
Absorbance (Ba chelate): ≥0.800 (around 578 nm)
Absorbance (Blank): ≤0.090 (around 575 nm)
MW: 636.60, $C_{32}H_{32}N_2O_{12}$

Ordering Information

Product code	Unit
P004-10	1 g
P004-12	5 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure


Product Description
PC is a colorimetric reagent and metal indicator for alkaline earth metal ions. This reagent is slightly soluble in water and readily soluble in aqueous ammonia or organic solvents. The aqueous solution is colorless at pH 11, but turns a strong pink in the presence of alkaline earth metals. PC is widely utilized for the determination of serum calcium by an autoanalyzer.

Applications
Chelate titration: Ca, Ba, Mg, Sr, CN^-, SO_4^{2-}
Colorimetry: Ca, Ba, Hg(II), La, Mg, Sr

Conditions
Ba: pH 11.3, 575 nm, detection range 0-5 ppm Ca: pH 10.5, 575 nm, detection range 0-1 ppm
Hg: pH 10, 585 nm, detection range 0.1-4 ppm La: cetylpyridiniumbromate, 617 nm, detection range 0-2 ppm
Mg: pH 10, 570 nm, detection range 5-30 ppm Sr: pH 11.2, 575 nm, detection range 0-3 ppm

Reference

1. G. Anderegg, *et al*., Metallindikatoren VII. Ein Auf Erdalkaliionen Ansprechendes Phtalein und Seine Analytische Verwendung. *Helv Chim Acta*. 1954;**38**:113-120.
2. J. Bosholm, Spektrofotometrische Bestimmung von Calciumspuren Nachihrer Abstrennung aus Konzentrierten Lithiumchlorid-losungen Mittels Kationenaustausch. *Anal Chim Acta*. 1966;*34*:71-77.

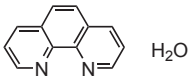
o-Phenanthroline 1,10-Phenanthroline, monohydrate [CAS: 5144-89-8]

Application: Ag and Fe detection, colorimetric
Appearance: White crystalline powder
Purity: 99.0-101.0% (Titration)
Sensitivity: To pass test
MW: 198.22, $C_{12}H_8N_2$, H_2O

Ordering Information
Product code	Unit
P007-10	5 g
P007-12	25 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure


Product Description
o-Phenanthroline is a colorimetric reagent for iron detection. It is also used as a reagent for the solvent extraction of anions. This reagent is slightly soluble in water (3.3 g/l at room temperature) and benzene (14 g/l at room temperature), and fairly soluble in alcohol (540 g/l), acetone, and diluted mineral acids. Its proton dissociation constants are reported to be pKa_1=0.70 and pKa_2=4.98 (μ=0.1, 25°C). *o*-Phenanthroline forms a red chelate with Fe(II) [$\log\beta_3$=21.1(μ=0.1, 20°C), λmax=510 nm, ε =1.1x10^5]. This complex is not easily extracted by organic solvents. The redox potential of FeL_3^{3+} -FeL_3^{2+} (L: o-phenanthroline) in 1 M H_2SO_4 is reported to be 1.06 V, and the color of the solution changes from red to pale blue. This reagent also forms colored complexes with Cu, Ni, and Co, and colorless complexes with Cd and Zn. Therefore, *o*-Phenanthroline can be used as a colorimetric reagent for Cu, Ni, and Co, and as a masking reagent of Cd and Zn. FeL_3^{2+} is also utilized for precipitation detection and ion-pair extraction.

Applications
Masking: Cd, Zn
Reduction and oxidation indicator: Ce
Colorimetry: Ag, Fe(II) :510 nm, detection limit 0.8 ppm
Fluorometry: Ru(II), Eu
Extraction colorimetry: halogen, ClO_4^-, $PtCl_6^{2-}$, ReO_4^-, $HCrO_4^-$, SCN^-, $AuCl_4$, $Ag(CN)_2^-$, $Sn(C_2O_4)_3^{2-}$, phosphomolybdate and organic compounds (Trichloroacetic acid, dehydroacetic acid, syclamic acid, saccharin, salicylic acid, alkylbenzenesulfonic acid, maleic acid, pentachlorophenol, tetraphenylborate, and chloranilic acid)

PR Pyrogallol sulfonphthalein [CAS: 32638-88-3]

Application: Heavy metal ions and rare earth metal ions detection colorimetric
Appearance: Dark reddish brown or dark purple powder
Absorbance (5 mg / 500 ml, pH5): ≥0.330 (around 505 nm)
Sensitivity: To pass test
MW: 400.36, $C_{19}H_{12}O_8S$

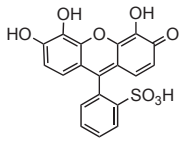
Ordering Information

Product code	Unit
P012-10	1 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description
PR is a colorimetric reagent and indicator for heavy metal ions and rare earth metals. This reagent is hardly soluble in water and alcohol, and insoluble in organic solvents. The color of PR in aqueous solution is orange-red in weak acidic pH, yellow in neutral pH, and violet in alkaline pH. The aqueous PR solution turns red in the presence of Bi or Pb at acidic pH, and blue-violet in the presence of Co, Ni, and rare earth metal ions at neutral or alkaline pH. Its proton dissociation constants are reported to be pKa_1=2.56, pKa_2=6.28, pKa_3=9.75 and pKa_4=11.94.

Applications
Chelate titration: Bi, Co, Ni, Pb, rare earth metals
Precipitation titration: Br^-, Cl^-, I^-, SCN^-
Colorimetry: Ag, Al, Cu, Fe, Ge, In, Mo, Sb, Ti, U, V, W, rare earth metals

References
1. J. Medina-Escriche, *et al*., Spectrophotometric Study of the Cerium(IV) - Pyrogallol Red System. *Analyst*. 1985;**110**:807-810.

SATP Salicylideneamino-2-thiophenol [CAS: 3449-05-6]

Application: Heavy metal detection with solvent extraction, colorimetric
Appearance: White crystalline powder
Absorbance (Sn complex): ≥0.530 (around 415 nm)
MW: 229.30, $C_{13}H_{11}NOS$

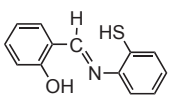
Ordering Information

Product code	Unit
S003-10	1 g
S003-12	5 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
SATP is a colorimetric solvent extraction reagent for Cu, In, Ni, and Sn. It is insoluble in water, but soluble in organic solvents. SATP forms yellow or brown complexes with various metal ions. The Sn(II) complex can be extracted with benzene (maximum wavelength: 415 nm, molar absorptivity: $1.61x10^5$), xylene and toluene.

Reference
1. E. Uhleman, *et al*., Extraktion Und Photometrische Bestimmung Von Zinn Und Blei Mit 2-(o-Hydroxyphenyl)Benzthiazolin. *Anal Chim Acta*. 1973;65:319-328.

Sodium bicinchoninate

4,4'-Dicarboxy-2,2'-biquinoline, disodium salt [CAS: 979-88-4]

Application: Cu(I) determination, colorimetric
Appearance: Slightly yellow or yellow powder
Molar absorptivity: ≥15,500 (around 332 nm)
MW: 388.28, $C_{20}H_{10}N_2Na_2O_4$

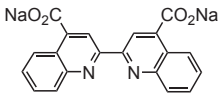
Ordering Information

Product code	Unit
B037-10	5 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Strcuture



Product Description
Sodium bicinchoninate is a Cu(I)- specific colorimetric reagent that is more sensitive than neocuproine. Its absorption maximum is at 562 nm (molar absorptivity: 7,900). In alkaline solution, Cu(II) is reduced by proteins to Cu(I), and then forms a purple complex with Sodium bicinchoninate. The protein detection range of this assay is 1-2,000 mg per ml.

References

1. K. Ueno, *et al*., Colloid Titration - A Rapid Method for the Determination of Charged Colloid. *J Chem Educ.* 1985;**62**:627-629.
2. P. K. Smith, *et al*., Measurement of Protein Using Bicinchoninic Acid. *Anal Biochem*. 1985;**150**:76-85.
3. M. G. Redinbaugh, *et al*., Adaptation of the Bicinchoninic Acid Protein Assay for Use with Microtiter Plates and Sucrose Gradient Fractions. *Anal Biochem*. 1986;**153**:267-271.

TD19C6

2,6,13,16,23,26-Hexaoxaheptacyclo[25.4.4.4^{7,12}.4^{17,22}.0^{1,17}.0^{7,12}.0^{17,22}]tritetracontane

Application: Ammonium selective electrode
Appearance: White powder
MW: 602.88, $C_{37}H_{62}O_6$

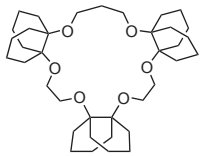
Ordering Information

Product code	Unit
T402-10	10 mg

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description
TD19C6 is an ammonium ion selective ionophore. It is difficult to separate ammonium ion and potassium ion because they are close in ion size. The selectivity of TD19C6 for ammonium ion is about 10 times that of potassium ion, which is nearly the same selectivity of nonactin.

References

1. K. Suzuki, *et al*., Design and Synthesis of a More Highly Selective Ammonium Ionophore Than Nonactin and Its Application as an Ion-Sensing Component for an Ion-Selective Electrode. *Anal Chem*. 2000;**72**:2200-2205.

TFPB Tetrakis[3,5-bis(trifluoromethyl)phenyl]borate, sodium salt [CAS: 79060-88-1]

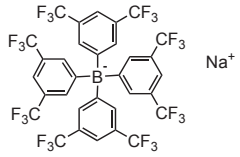
Application: Lipophilic anion, phase transfer reagent
Appearance: White crystalline powder
Purity: ≥99.0% (Titration, as anhydrous))
MW: 886.20, $C_{32}H_{12}BF_{24}Na$

Ordering Information	
Product code	Unit
T037-10	100 mg

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
Tetraphenylborate is unstable in acidic conditions because protons attack the ipso-carbon of the phenyl group and benzene is released. However, the electron density of the ipso-carbon of TFPB is low due to a trifluoromethyl group. Thus TFPB is stable in acidic conditions (such as 50 mM sulfuric acid solution). TFPB is used in Friedel-Craft alkylation or diazo coupling reactions as a phase transfer reagent. It is also used for ion selective electrodes. TFPB is known as Kobayashi's reagent.

References

1. H. Kobayashi, *et al*., Tetrakis[3, 5-di(*F*-methyl) phenyl] borate as the First Efficient Negatively Charged Phase Transfer Catalyst. Kinetic Evidences. *Chem Lett*. 1981;**10**:579-580.
2. H. Kobayashi, *et al*., The First Application of Anion-catalyzed Phase-transfer Catalysis to Friedel-Crafts Alkylation. *Chem Lett*. 1982;**11**:1185-1186.
3. Y. Takahashi, *et al*., Facile and Highly Selective Synthesis of 2, 2-Dialkyl-1, 5-Lactones by the Calboxylation of Primary, Tertiary-1, 4-Diols with Formic Acid or Copper(I)Carbonyls in the Presence of Concentrated Sulfuric Acid. *Chem Lett*. 1982;**11**:1187-1188.
4. H. Iwamoto, *et al*., Anion-Catalyzed Phase-Transfer Catalysis. I. Application to Diazo-Coupling Reactions. *Bull Chem Soc Jpn*. 1983;**56**:796-801.
5. H. Iwamoto, *et al*., Diazotization of Pentafluoroaniline by Means of Anion-Catalyzed Phase Transfer Catalysis in a Hydrophobic Organic Solvent. *J Fluor Chem*. 1984;**24**:535-537.
6. H. Nishida, *et al*., Tetrakis[3, 5-bis(trifluoromethyl)phenyl]borate. Highly Lipophilic Stable Anionic Agent for Solvent-extraction of Cations. *Bull Chem Soc Jpn*. 1984;**57**:2600-2604.
7. Y. Shiraki, *et al*., Anion-Catalyzed Phase-Transfer Catalysis. II. Effects of Anionic Tetrakis[3, 5-Bis(Trifluoromethyl)Phenyl]Borate Catalyst in Phase-Transfer-Catalyzed Sulfonium Ylide Formation. *Bull Chem Soc Jpn*. 1985;**58**:3041-3042.
8. N. Ishibashi, *et al*., Vitamin B1-Sensitive Poly(vinyl chloride) Membrane Electrode Based on Hydrophobic Tetraphenylborate Cation Exchangers. *Anal Sci*. 1988;**4**:527-528.
9. G. H. Zhang, *et al*., Vitamin B1 sensitive poly(vinyl chloride) membrane electrode based on hydrophobic tetraphenylborate derivatives and their application. *Anal Chem*. 1990;**62**:1644-1648.
10. T. Nagamura, *et al*., Control of Molecular Orientation of 4, 4'-Bipyridinium Cation Radicals in Novel Photochromic Monolayer Assemblies. *J Chem Soc Chem Commun*. 1990;**9**:703-704.
11. T. Nagamura, *et al*., Novel Photochromic Polymer Films Containing Ion-pair Charge-transfer Complexes of 4, 4'-Bipyridinium Ions for Optical Recording. *J Chem Soc Chem Commun*. 1991;**2**:72-74.
12. M. Kira, *et al*., An NMR Study of the Formation of Silyloxonium Ions by Using Tetrakis[3, 5-Bis(Trifluoromethyl)Phenyl]Borate as Counteranion. *J Am Chem Soc*. 1992;**114**:6697-6700.

Tiron 1,2-Dihydroxy-3,5-benzenedisulfonic acid, disodium salt, monohydrate [CAS: 149-45-1]

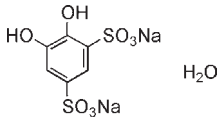
Application: Fe, Al, Ti detection, colorimetric
Appearance: White crystal or powder
Purity: ≥98.0% (Titration)
Absorbance (Ti complex): ≥0.280 (around 380 nm)
Sensitivity: To pass test
MW: 332.22, $C_6H_4Na_2O_8S_2 \cdot H_2O$

Ordering Information	
Product code	Unit
T021-10	5 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description

Tiron is used as a metal indictor of Fe, and as a colorimetric reagent of Fe, Al, Ti, and other metal ions. Tiron is readily soluble in water as a colorless solution. The pKa_2 is reported to be 12.6 (μ=0.1, 20°C). Tiron forms colored chelates with Fe, Ti, and other metal ions. The color of the Fe complex is blue at pH 1-4 (1:1, logK=20.4), purple at pH 5-7 (1:2, logK=15.1), and red at pH 7 or over (1:3, logK=10.8).

Applications

Chelate titration: Fe
Colorimetry: Al, B, Co, Cu, Fe, Ga, Mo, Nb, Os, Sr, Ti, UO_2(II), V
Fluorometry: rare earth metals
Masking reagent: Al, Cr, Fe, Ti

References

1. G. F. Atkinson, *et al.*, Oxidation of the Analytical Reagent Tiron (Disodium-4,5-Dihydroxybenzene-1,3-Disulphonate). *Can J Chem*. 1957;**35**:477-487.
2. L. J. Clark, Titanium Determination with Disodium-1,2-Dihydroxybenzene-3,5-Disulfonate (Tiron) in Oxalic Acid Solution. *Anal Chem*. 1970;**42**:694-698.

TMPyP

5,10,15,20-Tetrakis(*N*-methylpyridinium-4-yl)-21*H*, 23*H*-porphine, tetrakis(*p*-toluenesulfonate) [CAS: 36951-72-1]

Application: Cu detection, colorimetric
Appearance: Purple powder
Molar absorptivity (Cu complex): ≥140,000 (around 423 nm)
Molar absorptivity (in acidic solution): ≥280,000 (around 445 nm)
MW: 1363.61, $C_{72}H_{66}N_8O_{12}S_4$

Ordering Information

Product code	Unit
T001-12	1 g

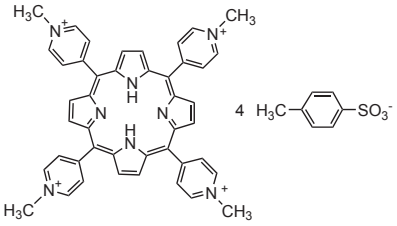
Storage Condition

ambient temperature, protect from metal

Shipping Condition

ambient temperature

Chemical Structure



Product Description

TMPyP is a highly sensitive colorimetric reagent for Cu. Its proton dissociation constants are reported to be pKa_1=0.8 and pKa_2=2.06. The maximum wavelength of the Soret band is 422 nm (molar absorptivity:149,000) at pH 4-7. In 1 M HCl solution, the maximum wavelength of the Soret band is 446 nm (molar absorptivity: 193,000).

TOPO Tri-*n*-octylphosphine oxide [CAS: 78-50-2]

Application: Solvent extraction
Appearance: White or slightly yellow crystalline powder
Purity: ≥98.0% (GC)
MW: 386.63, $C_{24}H_{51}OP$

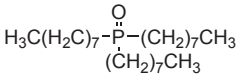
Ordering Information

Product code	Unit
T024-10	5 g
T024-12	25 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description
TOPO dissolves in cyclohexane. TOPO forms adducts with metal chelates to enhance solvent extraction efficiency (synergistic effect). It can be used for rare earth metals, actinoids, and other metal ions indicated below.

Applications
Solvent extraction: Al, Au, Co, Cr, Fe, Hf, Re, Ti, UO_2^{2+}, V, Zr, rare earth metals, actinoids

References

1. R. Y. Xie, *et al*., Lithium Ion-selective Electrodes Containing TOPO: Determination of Serum Lithium by Flow Injection Analysis. *Analyst*. 1987;**112**:61-64.
2. S. Tsurubou, *et al*., Liquid-liquid Extraction of Cinchona Alkaloids by Using Some Metal Complexes of Optically Pure Usnic Acids. *Anal Chim Acta*. 1991;**248**:501-506.

TPPS 5,10,15,20-Tetraphenyl-21*H*, 23*H*-porphinetetrasulfonic acid, disulfuric acid, tetrahydrate [CAS: 35218-75-8]

Application: Heavy metal ion detection, colorimetric
Appearance: Deep green or deep greenish blue powder
Molar absorptivity (pH 6.5): ≥510,000 (around 413 nm)
MW: 1203.21, $C_{44}H_{34}N_4O_{20}S_6 \cdot 4H_2O$

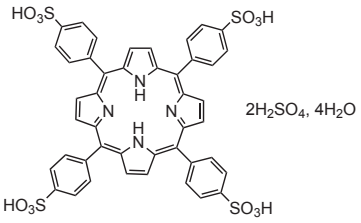
Ordering Information

Product code	Unit
T003-10	100 mg

Storage Condition
ambient temperature,
protect from metal and moisture

Shipping Condition
ambient temperature

Chemical Structure



Product Description
TPPS is a water-soluble porphyrin that is reddish-purple in aqueous solution at pH 6.5 (maximum wavelength: 413 nm, molar absorptivity: 5.1×10^5) and green at pH 4 (maximum wavelength: 434 nm, molar absorptivity: 5.0×10^5). Its proton dissociation constants are reported to be pKa_1=4.86 and pKa_2=4.96. TPPS selectively forms a complex with Cu^{2+} in acidic conditions, and with Cd, Cu, Fe, Pb, and Pd in alkaline conditions.

Table 1 Colorimetric Determination of Metal Ions Using TPPS

Metal	Condition	λmax (nm)	ε (x10^{-5})	Range (ppb)	Interference
Cu(II)	pH 4	434	4.8	6-60	Zn
Cd(II)	pH 12, bipyridine	432	4.5	(0)-100	-
Fe(II)	pH 3.9-4.2	395	1.4	20-180	Co, Cu, Mn, Pd, Sn, Zn
Pb(II)	pH 10.2, KCN	464	2.8	50-500	Cr, In, Mn, Sn
Pd(II)	pH 3	413	2.2	(0)-250	Cu, Hg

References

1. A. D. Adler, *et al*., A Simplified Synthesis for Meso-tetraphenylporphin. *J Org Chem*. 1967;**32**:476-476.
2. J. Itoh, *et al*., Spectrophotometric Determinaton of Copper with α, β, γ, δ-Tetraphenylporphine Trisulfonate. *Anal Chim Acta*. 1975;**74**:53-60.

XB-I

3-[3-(2,4-Dimethylphenylcarbamoyl)-2-hydroxynaphthalen-1-yl-azo]-4-hydroxybenzenesulfonic acid, sodium salt [CAS: 14936-97-1]

Application: Mg detection, colorimetric
Appearance: Dark red or dark reddish brown powder
Absorbance in 55% EtOH (0.027 mmol/l):
≥0.500 (around 605 nm)
Absorbance in 60% EtOH (0.055 mmol/l, Mg complex):
≥0.200 (around 505 nm)
MW: 513.50, $C_{25}H_{20}N_3NaO_6S$

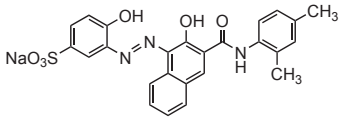
Ordering Information

Product code	Unit
X001-10	1 g
X001-12	5 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
XB-I is a colorimetric reagent for Mg detection. It is slightly soluble in water and alcohol, and readily soluble in aqueous alkaline solution. The aqueous solution of XB-1 is red and turns reddish-violet in the presence of Mg at pH 9 (maximum wavelength: 510 nm, molar absorptivity: 49,000, detection range 0.02-0.4 ppm).

Reference
C. K. Mann, *et al*., Spectrophotometric Determination of Magnesium with 1-Azo-2-hydroxy-3-(2,4-dimethylcarboxanilido)naphthalene-1'-(2-hydroxybenzene). *Anal Chim Acta*. 1957;**16**:155-160.

XO 3,3'-Bis[*N*,*N*-bis(carboxymethyl)aminomethyl]-*o*-cresolsulfonphthalein, disodium salt [CAS: 1611-35-4]

Application: Metal indicator
Appearance: Reddish orange or reddish purple powder
Absorbance (0.014 mmol/l): ≥0.210 (around 440 nm)
Sensitivity: To pass test
MW: 716.62, $C_{31}H_{30}N_2Na_2O_{13}S$

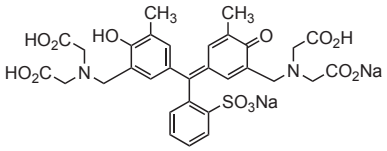
Ordering Information

Product code	Unit
X003-10	1 g
X003-12	5 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description
XO is a colorimetric reagent and metal indicator of various metal ions. It is readily soluble in water. Its proton dissociation constants are reported to be -1.74, -1.09, 0.76, 1.15, 2.58, 3.23, 6.40, 10.5, and 12.6 (ion strength: 0.2). The color of the aqueous XO solution is yellow at pH < 6.3 and pink at pH > 6.5, and turns reddish-violet in the presence of metal ions. XO forms complexes with various metal ions in acidic conditions. Therefore, XO can be used to determine Ca, Cd, Mg, Pb, and Zn in the presence of Zephiramine in acidic conditions. XO is also a very versatile indicator for EDTA titration in acidic solutions.

Table 1 Colorimetric Determination of Metal Ions Using XO

Metal	Condition	λmax (nm)	Range (ppm)	Interference
Al	pH 3.4, 100 °C	536	0.2-1	Fe(III), Th
Bi	80-120 mM HNO_3, ascorbic acid	530	0.4-3.2	-
Fe(III)	60-100 mM $HClO_4$	535	0.12-1.8	Zr
Np(IV)	4 M HNO_3, trimethylbenzene	535	(0)-5.5	Cr(VI), Th
Pu(IV)	0.1 M HNO_3	560	0.5-4.0	> 6000x UO_2^{2+}
Th	pH 3, diphenylguanidine, butanol	578	0.14-0.83	U, F^-
UO_2^{2+}	pH 3.5-3.7, ascorbic acid, 100 °C	568	0.8-4.0	Cu, Fe, Pd
Zr	acetic acid, TOA	550	0.03-2	Mo, Pd, Ti: + error; Cd, Cu, Hg: - error

Reference

1. D. C. Olson, *et al*., Semixylenol Orange: A Sensitive Reagent for Zirconium. *Anal Chem*. 1962;**34**:1299-1302.
2. M. Murakami, *et al*., Separation and Acid Equilibria of Xylenol Orange and Semi-xylenol Orange. *Talanta*. 1967;**14**:1293-1307.
3. Z. Nan, *et al*., Spectrophotometric Determination of Bismuth with Semi-xylenol Orange and Its Application in Metal Analysis. *Talanta*. 1989;**36**:733-737.

Zincon 1-(2-Hydroxycarbonyl-phenyl)-5-(2-Hydroxy-5-sulfophenyl)-3-phenylformazan, sodium salt [CAS: 62625-22-3]

Application: Cu and Zn detection, colorimetric
Appearance: Dark reddish purple powder
Sensitivity: To pass test
Absorbance (0.02 mmol/l, pH9): ≥0.375 (around 490 nm)
MW: 462.41, $C_{20}H_{15}N_4NaO_6S$

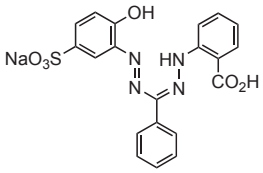
Ordering Information

Product code	Unit
Z002-10	1 g
Z002-12	5 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



Product Description

Zincon is a colorimetric reagent for Zn and Cu detection, and a metal indicator in EDTA titration. This reagent is insoluble in organic solvents, but slightly soluble in water and alcohol. Its proton dissociation constants are reported to be pKa_1=7.9-8.3, pKa_2=7.9-8.3 and pKa_3=13-14. The aqueous solution of Zincon is yellow or orange-yellow in weak alkaline conditions, and turns blue in the presence of Cu (maximum wavelength: 600 nm, molar absorptivity: $1.9x10^4$) and Zn (pH 8.5-9.5, maximum wavelength: 620 nm, molar absorptivity: 23,000). Dr. Koupparis and others used Zincon for the automated flow injection spectrometric determination of 1-10 mg per ml levels of Zn at 80 samples per hour. The detection limit was reported to be 0.05 µg per ml.

Applications

Chelate titration: Ca, Cd, Hg, Pb, Zn
Colorimetry: Cu, Zn

References

1. D. W. Margerum, *et al*., Evaluation of Methods for Trace Zinc Determination. *Anal Chem*. 1960;**32**:1157-1161.
2. G. Ackermann, *et al*., Vergleichende Untersuchung an Reagentienzur Spektralphotometrischem Bestimmungvon Zink. *Talanta*. 1979;**26**:693-703.
3. K. Yoshimura, *et al*., Ion-exchanger Phase Absorptiometry for Trace Analysis. *Talanta*. 1985;**32**:345-352.
4. M. A. Koupparis, *et al*., Automated Flow Injection Spectrophotometric Determination of Zinc Using Zincon: Applications Analysis of Waters, Alloys and Insulin Formulations. *Analyst*. 1989;**111**:1311-1315.

Introduction

Organic compounds that coordinate metal ions into circular structures (chelate circles) are called chelating reagents. Most chelating reagents include oxygen, nitrogen, or sulfur atoms in their molecules. Chelate structures with five or six member rings form the most stable chelate circle. In chelating reactions of typical chelating reagents, such as ethylenediamine, acetylacetone, and oxine, several molecules are coordinated with one metal ion. Ethylenediamine tetraacetic acid (EDTA), which has many coordinated atoms, forms a very stable chelate between one molecule of EDTA and metal ion. Chelating reagents are used for chelate titration, isolation, and separation of metal ions. They are also used to mask certain ions, to solubilize metals in organic solvents, and for gas chromatography of metal ions. Metal indicators are chelating reagents that can be used to colorimetrically or fluorometrically determine metal ion concentration in solutions. Acetylacetone (AA) and its analogs are used for solvent extractions of metal ions from aqueous solutions. These metal complexes are used as catalysts of polymerization, dryness, and combustion. Chelating reagents, including EDTA, EDTA analogs, metal salts, and others, are available in bulk quantities from Dojindo. The following table indicates the stability constants of chelators with various metal ions and chelate reagents.

Table 1 Stability Constants

Metal	EDTA	NTA	CyDTA	DTPA	EDTA-OH	GEDTA	TTHA*	DHEG	IDA	DTPA-OH	NTP	Me-EDTA	HIDA	EDDP	EDTPO	NTPO	BAPTA
Ag(I)	7.32	5.16	8.15	8.70	6.71	6.88	8.67 (13.89)	-	-	-	-	-	-	-	-	-	-
Al(III)	16.13	9.50	18.63	18.40	12.43	13.90	19.7 (28.90)	-	8.16	14.40	-	-	-	-	-	-	-
Am(III)	18.16	-	18.79	22.92	-	-	-	-	6.93	-	-	-	9.75	-	22.47	-	-
Ba(II)	7.76	4.83	8.64	8.63	5.54	8.41	8.22 (11.63)	-	1.67	4.91	-	8.10	3.42	-	11.14	-	5.77
Be(II)	9.27	7.11	10.81	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bi(III)	27.90	-	31.20	29.70	21.80	23.80	-	-	-	-	-	-	-	-	-	-	-
Bk(III)	-	-	19.60	22.79	-	-	-	-	-	-	-	-	-	-	-	-	-
Ca(II)	10.96	6.41	12.50	10.74	8.14	11.00	10.06 (14.16)	-	2.59	6.69	-	10.40	5.30	1.00	6.93	6.68	6.97
Cd(II)	16.46	9.54	19.23	19.31	13.60	16.70	18.65 (26.85)	6.30	5.73	12.10	3.40	16.00	7.41	5.60	16.53	-	12.20
Ce(III)	15.98	10.83	16.76	20.50	14.11	15.70	19.20	7.50	6.18	-	-	16.79	8.50	-	21.10	-	-
Ce(IV)	24.20	10.97	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cf(III)	19.09	-	19.42	22.57	-	-	-	-	-	-	-	-	9.61	-	-	-	-
Cm(III)	18.45	-	18.81	22.99	-	-	-	-	-	-	-	-	9.27	-	21.89	-	-
Co(II)	16.31	10.38	18.92	18.40	14.40	12.50	17.1 (28.80)	6.10	6.95	13.92	4.80	14.40	9.00	7.30	15.49	-	8.70
Co(III)	40.60	-	-	-	-	-	-	-	29.60	-	-	-	-	-	-	-	-
Cr(II)	13.61	-	-	-	-	-	-	-	-	-	-	-	7.73	-	-	-	-
Cr(III)	23.40	>10	-	-	-	2.54	-	-	-	-	-	-	-	-	-	-	-
Cs(I)	0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cu(II)	18.80	12.96	21.30	21.53	17.35	17.80	19.2 (32.60)	8.60	10.63	17.21	9.10	17.70	13.38	15.10	18.95	-	11.70
Dy(III)	18.30	11.74	19.69	22.82	15.30	17.42	23.29	7.60	6.88	-	-	19.09	8.88	-	-	-	-
Er(III)	18.38	12.03	20.20	22.74	15.42	17.40	13.19 (26.92)	7.70	7.09	-	-	19.61	9.24	-	-	-	-
Eu(III)	17.35	11.52	18.77	22.39	15.35	17.10	23.85	8.00	6.73	-	-	18.26	8.99	-	-	-	-
Fe(II)	14.33	8.84	16.27	16.55	12.20	11.92	17.10	4.31	5.80	12.90	-	14.30	6.78	6.30	-	-	-
Fe(III)	25.10	15.87	28.05	28.60	19.80	20.50	26.8 (40.50)	-	10.42	-	-	-	11.64	13.10	19.60	-	-
Fm(III)	-	-	-	22.70	-	-	-	-	-	-	-	-	-	-	-	-	-
Ga(III)	20.27	13.60	22.91	23.00	16.90	-	-	-	-	-	-	-	9.01	-	-	-	-
Gd(III)	17.00	11.54	18.80	22.46	15.22	16.94	23.83	7.70	6.68	-	-	18.21	-	-	21.80	-	-
Hf(IV)	29.50	20.34	-	35.40	-	-	19.08	-	-	-	-	-	14.63	-	-	-	-
Hg(II)	21.80	14.60	24.30	27.00	20.10	23.12	26.8 (39.10)	14.17	11.76	18.40	-	21.70	5.48	-	-	-	-
Ho(III)	18.05	11.90	19.89	22.78	15.32	17.38	23.59	7.60	6.97	-	-	19.30	9.14	-	21.85	-	-
In(III)	24.95	16.90	28.74	29.00	17.16	-	-	-	9.54	-	-	-	11.00	-	-	-	-
La(III)	15.50	10.36	16.75	19.48	13.46	15.79	22.22 (25.62)	7.30	5.88	-	-	16.42	8.00	5.80	21.15	-	-
Li(I)	2.79	2.51	4.13	-	-	1.17	-	-	-	-	-	-	-	-	-	-	-
Lu(III)	19.65	12.49	20.91	22.44	15.88	17.81	-	7.50	7.61	-	-	20.56	9.50	-	-	-	-
Mg(II)	8.69	5.46	10.32	9.30	7.00	5.21	8.10 (14.38)	1.15	2.94	5.30	<1	8.80	3.50	1.60	5.69	6.49	1.77
Mn(II)	14.04	7.44	16.78	15.60	10.70	12.30	14.68 (21.19)	3.90	-	9.06	-	14.50	6.40	3.40	12.70	-	8.73
Mn(III)	24.80	-	28.90	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* The data in parentheses are the stability constants of the 1:2 (metal:chelate) complexes

Table 1 Stability Constants (continued)

Metal	EDTA	NTA	CyDTA	DTPA	EDTA-OH	GEDTA	TTHA*	DHEG	IDA	DTPA-OH	NTP	Me-EDTA	HIDA	EDDP	EDTPO	NTPO	BAPTA
Mo(V)	6.36	-	17.68	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mo(VI)	19.50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Na(I)	1.66	2.15	2.70	-	-	1.38	-	-	-	-	-	-	-	-	-	-	-
Nb(V)	40.78	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Nd(III)	16.61	11.26	17.69	21.60	14.86	16.28	22.82 (26.75)	7.60	6.50	-	-	17.54	8.80	-	21.47	-	-
Ni(II)	18.62	11.54	19.40	20.32	17.00	13.60	18.1 (32.40)	7.70	8.90	16.63	5.80	14.20	10.20	9.30	15.30	-	-
NpO2(II)	9.70	-	-	-	-	-	-	-	6.27	-	-	-	6.08	-	-	-	-
Pa(V)	8.19-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pb(II)	18.04	11.39	19.68	18.80	15.50	14.71	17.1 (28.10)	7.50	7.45	14.40	-	17.30	9.41	-	-	-	11.30
Pd(II)	26.40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pm(III)	16.75	-	18.71	-	-	-	-	-	-	-	-	-	8.97	-	-	-	-
Pr(III)	16.40	11.07	17.23	21.07	14.61	16.50	23.45	7.70	6.44	-	-	17.17	8.64	-	-	-	-
Pu(III)	18.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pu(IV)	17.66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pu(VI)	16.39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sb(III)	24.80	-	-	-	20.20	-	-	-	-	-	-	-	-	-	-	-	-
Sc(III)	23.10	12.70	25.40	-	-	25.40	-	8.00	-	-	-	-	-	-	-	-	-
Sm(III)	16.70	11.53	18.63	22.34	15.28	23.85	23.81	7.80	6.64	-	-	17.97	9.10	-	22.39	-	-
Sn(II)	18.30	-	-	-	-	23.85	-	-	-	15.20	-	15.10	-	-	-	-	-
Sr(II)	8.63	4.98	10.54	9.68	6.92	8.50	9.26 (12.70)	-	2.23	5.33	-	10.70	3.77	-	5.41	-	5.13
Tb(III)	17.81	11.59	19.30	22.71	15.32	17.27	23.61	8.00	6.78	-	-	18.64	9.08	-	-	-	-
Th(IV)	23.20	12.40	29.25	28.78	18.50	-	31.90	7.80	9.32	-	-	-	10.70	-	-	-	-
Ti(III)	17.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TiO(II)	17.30	12.30	19.90	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tl(I)	6.53	4.75	5.33	5.97	5.45	-	-	-	1.32	4.40	-	-	-	-	-	-	-
Tl(III)	22.50	18.00	38.30	48.00	-	-	-	-	-	-	-	-	-	-	-	-	-
Tm(III)	18.62	12.20	20.46	22.72	15.59	17.48	-	-	7.22	-	-	20.08	9.35	-	21.41	-	-
U(IV)	25.80	-	26.90	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UO2(II)	10.40	9.56	-	-	-	-	-	-	8.93	-	-	-	8.32	-	-	-	-
V(II)	12.70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VO(II)	18.77	-	19.40	-	-	-	-	-	9.01	-	-	-	-	-	-	-	-
V(V)	18.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Y(III)	18.09	11.48	19.41	22.05	14.65	16.82	-	7.20	6.78	-	-	18.78	9.22	-	15.06	-	-
Yb(III)	18.88	12.40	20.80	22.62	15.88	17.78	23.58	7.70	7.42	-	-	20.25	9.38	-	-	-	-
Zn(II)	16.50	10.66	18.67	18.75	14.50	14.50	16.65 (28.70)	6.50	7.27	13.70	5.30	16.20	8.33	7.60	17.05	-	9.38
Zr(IV)	29.90	20.80	20.74	36.90	-	-	19.74	-	-	-	-	-	-	-	-	-	-

* The data in parentheses are the stability constants of the 1:2 (metal:chelate) complexes

Table 2 Acid Dissociation Constants

pKa	EDTA	NTA	CyDTA	DTPA	EDTA-OH	GEDTA	TTHA*	DHEG	IDA	DTPA-OH	NTP	Me-EDTA	HIDA	EDDP	EDTPO	NTPO	BAPTA
a1	1.99	1.89	2.43	2.14	2.51	2.00	2.42	8.14	2.58	1.60	3.62	2.60	2.25	6.87	1.46	0.30	-
a2	2.67	2.49	3.52	2.38	5.31	2.68	2.95	-	9.33	2.60	4.24	3.03	8.75	9.60	2.72	1.50	-
a3	6.16	9.73	6.12	4.26	9.86	8.85	4.16	-	-	6.96	9.30	6.20	-	-	5.05	4.64	5.47
a4	0.26	-	11.70	8.60	-	1.38	6.16	-	-	9.49	-	10.84	-	-	6.18	5.86	6.36
a5	-	-	-	10.53	-	9.46	9.40	-	-	-	-	-	-	-	6.63	7.30	-
a6	-	-	-	-	-	-	10.19	-	-	-	-	-	-	-	7.43	12.10	-
a7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.22	-	-
a8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.60	-	-

4H(EDTA free·acid)

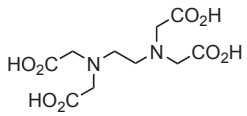
Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid [CAS: 60-00-4]

Appearance: White powder
Purity: ≥99.0% (Titration)
MW: 292.24, $C_{10}H_{16}N_2O_8$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
H001-10	500 g

2NA(EDTA·2Na)

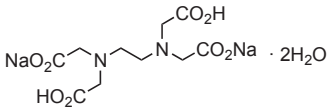
Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid, disodium salt, dihydrate [CAS: 6381-92-6]

Appearance: White powder
Purity: ≥99.5%
MW: 372.24, $C_{10}H_{14}N_2Na_2O_8$, $2H_2O$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
N001-10	500 g

3NA(EDTA·3Na)

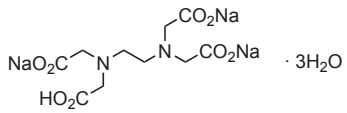
Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid, trisodium salt, trihydrate [CAS: 85715-60-2]

Appearance: White powder
Purity: ≥98.0% (Titration)
MW: 412.23, $C_{10}H_{13}N_2Na_3O_8$, $3H_2O$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
N002-10	500 g

4NA(EDTA·4Na)

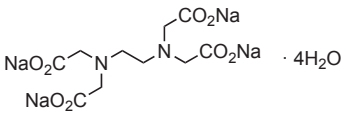
Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid, tetrasodium salt, tetrahydrate [CAS: 13235-36-4]

Appearance: White crystalline powder
Purity: ≥98.0% (Titration)
MW: 452.23, $C_{10}H_{12}N_2Na_4O_8$, $4H_2O$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
N003-10	500 g

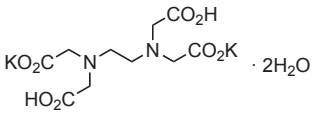
2K(EDTA·2K) Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid, dipotassium salt, dihydrate [CAS: 25102-12-9]

Appearance: White powder or white crystalline powder
Purity: ≥99.0% (Titration)
MW: 404.45, $C_{10}H_{14}K_2N_2O_8$, $2H_2O$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
K001-10	50 g
K001-12	500 g

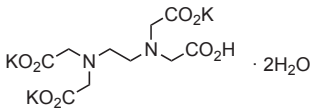
3K(EDTA·3K) Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid, tripotassium salt, dihydrate [CAS: 65501-24-8]

Appearance: White crystalline powder
Purity: ≥99.0% (,Titration)
MW: 442.54, $C_{10}H_{13}K_3N_2O_8$, $2H_2O$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
K002-10	50 g
K002-12	500 g

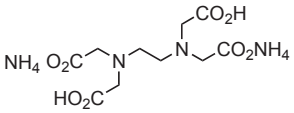
$2NH_4$(EDTA·$2NH_4$) Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid, diammonium salt [CAS: 20824-56-0]

Appearance: White powder
Purity: ≥99.0% (Titration)
MW: 326.30, $C_{10}H_{22}N_4O_8$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
N008-10	500 g

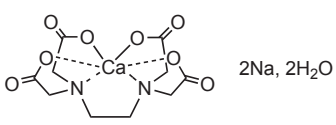
Ca(II)-EDTA Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid, calcium(II), disodium salt, dihydrate [CAS: 62-33-9]

Appearance: White powder
Purity: ≥99.0% (Titration)
MW: 410.30, $C_{10}H_{12}CaN_2Na_2O_8$, $2H_2O$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
E008-10	50 g

Cu(II)-EDTA

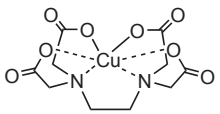
Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid, copper(II), disodium salt, tetrahydrate [CAS: 39208-15-6

Appearance: Blue powder
Purity: ≥98.0% (Titration)
MW: 469.80, $C_{10}H_{12}CuN_2Na_2O_8$, $4H_2O$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
E010-08	10 g

Fe(III)-EDTA

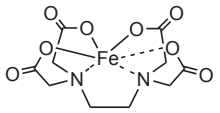
Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid, iron(III), sodium salt, trihydrate [CAS: 15708-41-5]

Appearance: Yellowish brown powder
Purity: ≥98.0% (Titration)
MW: 421.09, $C_{10}H_{12}FeN_2NaO_8$, $3H_2O$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
E011-10	50 g
E011-12	500 g

Mg(II)-EDTA

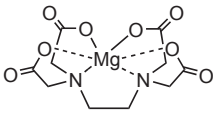
Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid, magnesium(II), disodium salt, tetrahydrate [CAS: 14402-88-1]

Appearance: White powder
Purity: ≥99.0% (Titration)
MW: 430.56, $C_{10}H_{12}MgN_2Na_2O_8$, $4H_2O$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
E013-10	25 g

Zn(II)-EDTA

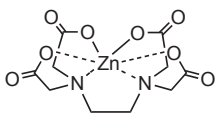
Ethylenediamine-*N*,*N*,*N*',*N*'-tetraacetic acid, zinc(II), disodium salt, tetrahydrate [CAS: 39208-16-7]

Appearance: White powder
Purity: ≥99.0% (Titration)
MW: 471.64 $C_{10}H_{12}N_2Na_2O_8Zn$, $4H_2O$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
E017-10	25 g
E017-12	500 g

CyDTA *trans*-1,2-Diaminocyclohexane-*N*,*N*,*N*',*N*'-tetraacetic acid, monohydrate [CAS: 125572-95-4]

Appearance: White powder
Purity: ≥99.0% (Titration)
MW: 364.35, $C_{14}H_{22}N_2O_8$, H_2O

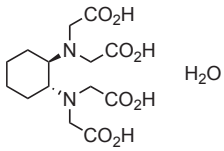
Ordering Information

Product code	Unit
C018-10	25 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
CyDTA is used for masking various metal ions. The two amino groups of CyDTA are in the trans formation, and its free acid is water soluble. CyDTA chelates at a slower rate than EDTA with metal ions, especially Al, C, Ni, and Zr. However, the stability constants of CyDTA metal complexes are higher than that of EDTA. Dojindo offers highly purified CyDTA for use as a masking reagent on automatic amino acid analyzers. It does not interfere with the ninhydrin reaction.

DTPA Diethylenetriamine-*N*,*N*,*N*',*N*'',*N*''-pentaacetic acid [CAS: 67-43-6]

Appearance: White powder
Purity: ≥99.0% (Titration)
MW: 393.35, $C_{14}H_{23}N_3O_{10}$

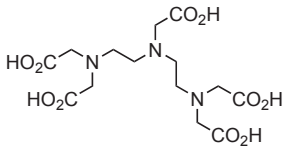
Ordering Information

Product code	Unit
D022-10	5 g
D022-12	25 g

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Product Description
The stability constant of the DTPA-metal complex is the second highest, next only to the CyDTA-metal complex. DTPA is a free acid that has low solubility in water. DTPA is useful as a strong masking reagent. It is also used for the extraction of metal ions, such as Al, Fe, Ma, Zn, Cu, Cd, and Ni from soil at pH 5.3.

References

1. R. Pribil, *et al*., Determination of Rare Earths in the Presence of Phosphate. *Chem Anal*. 1967;**56**:23-24.
2. J. Kinnunen, *et al*., Rapid Determination of the Rare Earths in Phosphate Rock. *Chem Anal*. 1967;**56**:24-25.
3. J. Kinnunen, *et al*., Determination of Rare Earths in Phosphate Rock by Atomic Absorption Flame Photometry. *Chem Anal*. 1967;**56**:25-27.
4. W. A. Norvell, Comparison of Chelating Agents as Extractants for Metals in Diverse Soil Materials. *Soil Sci Soc Am J*. 1984;**48**:1285-1292.

EDTA-OH

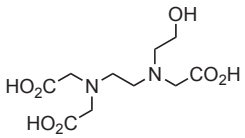
N-(2-Hydroxyethyl)ethylenediamine-*N*,*N*',*N*'-triacetic acid [CAS: 150-39-0]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 278.26, $C_{10}H_{18}N_2O_7$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
E005-10	5 g
E005-12	25 g

GEDTA (EGTA)

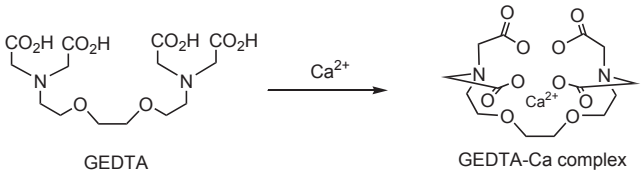
O,*O*'-Bis(2-aminoethyl)ethyleneglycol-*N*,*N*,*N*',*N*'-tetraacetic acid [CAS: 67-42-5]

Appearance: White crystalline powder
Purity: ≥97.0% (Titration)
MW: 380.35, $C_{14}H_{24}N_2O_{10}$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



GEDTA

GEDTA-Ca complex

Ordering Information

Product code	Unit
G002-12	100 g

References

1. C. L. Luke, *et al*., Photometric Determination of Magnesium in Electronic Nickel. *Anal Chem*. 1954;**26**:1778-1780.
2. F. S. Sadek, *et al*., Visual EGTA Titration of Calcium in Thepresence of Magnesium. *Talanta*. 1959;**2**:38-51.
3. R. Pribil, *et al*., Contributions to the Basic Problems of Complexometry-XX Determination of Calcium and Magnesium. *Talanta*. 1966;**13**:233-236.
4. T. Tatsumi, *et al*., Hypochlorous Acid Mobilizes Intracellular Zinc in Isolated Rat Heart Myocytes. *J Mol Cell Cardiol*. 1994;**26**:471-479.
5. H. Ohata, *et al*., Confocal Imaging Analysis of ATP-Induced Ca^{2+} Response in Individual Endothelial Cells of the Artery in Situ. *Am J Physiol*. 1997;**272**:C1980-C1987.
6. I. Sakabe, *et al*., Induction of Apoptosis in Neuro-2A Cells by Zn^{2+} Chelating. *Cell Struct Funct*. 1998;**23**:95-99.

HIDA

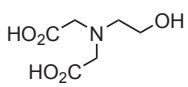
N-(2-Hydroxyethyl)iminodiacetic acid [CAS: 93-62-9]

Appearance: White crystalline powder
Purity: ≥98.0% (Titration)
MW: 177.16, $C_6H_{11}NO_5$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
H006-10	5 g

IDA Iminodiacetic acid [CAS: 142-73-4]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 133.10, $C_4H_7NO_4$

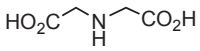
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
I001-10	25 g
I001-12	500 g

Chemical Structure



References

1. R. J. Motekaitis, *et al*., New Multidenate Ligands. XIII. Ethylenediaminetetra(methylenephosphonic) acid. *Inorg Nucl Chem Lett*. 1971;**7**:1103-1107.
2. E. N. Rizkalla, *et al*., Metal Chelates of Phosphonate-containing Ligands-I Stability of Some N, N, N', N' -Ethylenediaminetetra(methylenephosphonic) acid Metal Chelates. *Talanta*. 1979;**26**:507-510.

NTA Nitrilotriacetic acid [CAS: 139-13-9]

Appearance: White crystalline powder
Purity: ≥99.0% (Titration)
MW: 191.14, $C_6H_9NO_6$

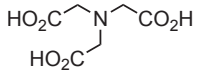
Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
N016-10	500 g

Chemical Structure



NTPO Nitrilotris(methylphosphonic acid), trisodium salt [CAS: 7611-50-9]

Appearance: White powder
Purity: ≥75.0% (Titration)
MW: 365.00, $C_3H_9NNa_3O_9P_3$

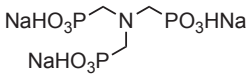
Storage Condition
ambient temperature, protect from moisture

Shipping Condition
ambient temperature

Ordering Information

Product code	Unit
N030-10	5 g

Chemical Structure



References

1. R. J. Motekaitis, *et al*., New Multidenate Ligands. XIII. Ethylenediaminetetra(methylenephosphonic) acid. *Inorg Nucl Chem Lett*. 1971;**7**:1103-1107.
2. E. N. Rizkalla, *et al*., Metal Chelates of Phosphonate-containing Ligands-I Stability of Some N, N, N', N' -Ethylenediaminetetra(methylenephosphonic) acid Metal Chelates. *Talanta*. 1979;**26**:507-510.
3. E. N. Rizkalla, *et al*., Metal Chelates of Phosphonate-containing Ligands-VI Complexes of Ethylenediaminetetra(methylenephosphonic) acid with Cd, Mg, Ca and Ba. *Talanta*. 1980;**27**:769-770.

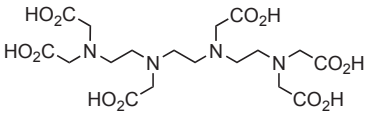
TTHA Triethylenetetramine-*N*,*N*,*N*',*N*",*N*"',*N*"'-hexaacetic acid [CAS: 869-52-3]

Appearance: White crystalline powder
Purity: ≥98.0% (Titration)
MW: 494.45, $C_{18}H_{30}N_4O_{12}$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
T031-10	5 g

D-Luciferin K salt

(*S*)-4,5-Dihydro-2-(6-hydroxy-2-benzothiazolyl)-4-thiazolecarboxylic acid, monopotassium salt [CAS: 115144-35-9]

Application: Luciferase substrate, bioluminescence
Appearance: Pale yellow or yellow powder
Purity: ≥98.0% (HPLC)
MW: 318.42, $C_{11}H_7N_2O_3S_2K$

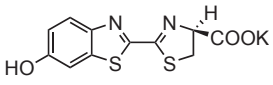
Ordering Information

Product code	Unit
L226-10	25 mg

Storage Condition
-20°C, protect from light and moisture, under nitrogen gas

Shipping Condition
ambient temperature

Chemical Structure



Product Description
D-Luciferin is a firefly luciferase substrate. Its quantum efficiency is 0.88, which is 20 times that of Luminol. The reaction mechanism is shown below. First, Luciferin reacts with ATP by luciferase in the presence of magnesium ion, then it is oxidized to form dioxetane structure and emits yellow-green light. Luciferin-luciferase luminescence is used for ATP monitoring to determine cell viability and bacteria counting. It is also used for reporter gene assays.

References
1. V. T. Nguyen, *et al*., Firefly Luciferase Luminescence Assays Using Scintillation Counters for Quantitation in Transfected Mammalian Cells. *Anal Biochem*. 1988;**171**:404-408.
2. S. P. Crouch, *et al*., The Use of ATP Bioluminescence as a Measure of Cell Proliferation and Cytotoxicity. *J Immunol Methods*. 1993;**160**:81-88.
3. S. R. Ford, *et al*., Improvements in the Application of Firefly Luciferase Assays. *Methods Mol Biol*. 1998;**102**:3-20.

Ferrocenyl PEG

11-Ferrocenylundecyl polyoxyethylene ether [CAS: 126879-04-7]

Application: Surfactant for organic thin layer preparation
Appearance: Yellow or orange brown waxy solid
Purity: ≥98.0% (HPLC)
MW: 929.01, $C_{47}H_{84}FeO_{14}$

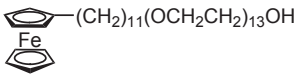
Ordering Information

Product code	Unit
F017-12	1 g

Storage Condition
0-5°C, protect from light

Shipping Condition
ambient temperature

Chemical Structure



Ferrocenyl TMA

11-Ferrocenyltrimethylundecylammonium bromide

Application: Surfactant for organic thin layer preparation
Appearance: Yellowish orange powder
Purity: ≥95.0% (Titration)
MW: 478.33, $C_{24}H_{40}BrFeN$

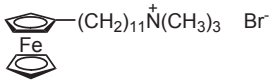
Ordering Information

Product code	Unit
F018-12	1 g

Storage Condition
0-5°C, protect from light and moisture

Shipping Condition
ambient temperature

Chemical Structure



Product Description of Ferrocenyl Surfactants

Ferrocenyl PEG and Ferrocenyl TMA are used to prepare a thin layer of a hardly dissolved organic compound on a solid surface. For example, these surfactants can solubilize phtharocyanine in aqueous solution. After preparing a homogeneous phtharocyanine solution, a thin layer of phtharocyanine is formed on a solid surface by electrolysis of the ferrocenyl surfactant.

References

1. T. Saji, *et al*., Reversible Formation and Disruption of Micelles by Controlof the Redox State of the Head Group. *J Am Chem Soc*. 1985;**107**:6865-6868.
2. J. S. Facci, *et al*., Characterization of Electroactive Langmuir-Blodgett Monolayers of (Ferrocenylmethyl)Dimethyloctadecylammonium Sulfate at Gold and Air/Water Interfaces. *Langmuir*. 1986;**2**:732-738.
3. K. Hoshino, *et al*., Electrolyte Effects on the Electrochemical Activity of Micelle-Solubilized Substance. *Chem Lett*. 1986;**29**:979-982.
4. K. Hoshino, *et al*., Electrochemical Formation of an Organic Thin Film by Disruption of Micelles. *J Am Chem Soc*. 1987;**109**:5881-5883.
5. T. Saji, *et al*., Formation of Copper Phthalocyanine Thin Films by Electrolysis of Surfactans with Ferrocenyl Moiety. *J Electrochem Soc*. 1989;**136**:2953-2956.
6. T. Saji, *et al*., Formation of Organic Thin Films by Electrolysis of Surfactants with the Ferrocenyl Moiety. *J Am Chem Soc*. 1991;**113**:450-456.

WSC 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, hydrochloride [CAS: 25952-53-8]

Application: Condensing agent
Appearance: White powder
Purity: ≥98.0% (Titration)
MW: 191.70, $C_8H_{18}ClN_3$

Ordering Information

Product code	Unit
W001-10	5 g
W001-12	25 g

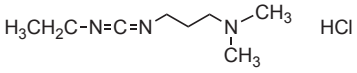
Storage Condition

ambient temperature, protect from moisture

Shipping Condition

ambient temperature

Chemical Structure



Product Description of Calixarenes

WSC is a water-soluble carbodiimide. It is also soluble in alcohol, acetone, chloroform, dioxane, and DMF. WSC is used as a condensing agent for peptide syntheses. WSC and its urea form can be easily removed after the reaction due to their high water solubility. WSC, EDC, EDAC, EDCI, and ethyl-CDI are the same reagent.

References

1. J. H. Saunders, *et al*., The Chemistry of the Organic Isocyanates. *Chem Rev*. 1948;**43**:203-218.
2. R. J. Slocombe, *et al*., Phosgene Derivatives. The Preparation of Isocyanates, Carbamyl Chlorides, and Cyanuric Acid. *J Am Chem Soc*. 1950;**72**:1888-1891.
3. H. Ozawa, Reaction of Insulin with Ethyl Glycinate and 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide. *Biochemistry*. 1970;**9**:2158-2163.
4. T. Y. Lin, Lactose Synthetase. Modification of Carboxyl Groups in α-Lactalbumin. *Biochemistry*. 1970;**9**:984-995.
5. J. C. Sheehan, *et al*., Total Synthesis of a Monocyclic Peptide LactoneAntibiotic, Etamycin", *J Am Chem Soc*. 1973;**95**:875-879.
6. M. E. Addy, *et al*., A Problem in the Mechanism of Carbodiimide-Mediated Synthesis of Peptides in Aqueous Medium. *Biochem Biophys Res Commun*. 1973;**52**:1034-1038.
7. A. Williams, *et al*., A New Mechanism Involving Cyclic Tautomers for the Reaction with Nucleophiles of the Water-Soluble Peptide Coupling Reagent 1-Ethyl-3-(3-(Dimethylamino)Propyl)Carbodiimide(EDC). *J Am Chem Soc*. 1981;**103**:7090-7095.
8. H. Yamada, *et al*., Selective Modification of Aspartic Acid-101 in Lysozyme by Carbodiimide Reaction. *Biochemistry*. 1981;**20**:4836-4842.
9. J. W. Chase, *et al*., F Sex Factor Encodes a Single-Stranded DNA Binding Protein (SSB) with Extensive Sequence Homology to Escherichia Coli SSB. *PNAS*. 1983;**80**:5480-5484.
10. J. V. Staros, *et al*., Enhancement by N-Hydroxysulfosuccinimide of Water-Soluble Carbodiimide-Mediated Coupling Reactions. *Anal Biochem*. 1986;**156**:220-222.
11. M. Taniuchi, *et al*., Induction of Never Growth Factor Receptor in Schwann Cells After Axotomy. *PNAS*. 1986;**83**:4094-4098.
12. M. Taniuchi, *et al*., Never Growth Factor Receptor Molecules in Rat Brain. *PNAS*. 1986;**83**:1950-1954.

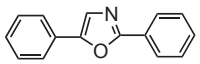
DPO 2,5-Diphenyloxazole [CAS: 92-71-7]

Application: Scintillator reagent
Appearance: White crystalline powder
Purity: ≥99.0% (HPLC)
MW: 221.25, $C_{15}H_{11}NO$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
D018-10	25 g
D018-12	100 g

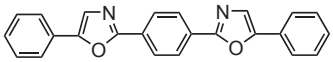
POPOP 1,4-Bis(5-phenyl-2-oxazolyl)benzene [CAS: 1806-34-4]

Application: Scintillation reagent
Appearance: Pale yellow needles
Purity: ≥99.0% (HPLC)
MW: 364.40, $C_{24}H_{16}N_2O_2$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
P009-10	5 g
P009-12	25 g

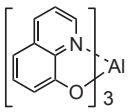
Alq3, sublimed Tris(8-hydroxyquinolinato)aluminum(III) [CAS: 2085-33-8]

Application: Electron carrier
Appearance: Yellow or yellowish green powder
Purity: ≥98.5% (Titration)
MW: 459.43, $C_{27}H_{18}AlN_3O_3$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

Product code	Unit
T203-10	1 g

Bathocuproine, sublimed

2,9-Dimethyl-4,7-diphenyl-1,10-phenanthroline [CAS: 4733-39-5]

Application: Electron carrier
Appearance: White or pale yellow powder
Purity: ≥98.0% (HPLC)
MW: 360.45, $C_{26}H_{20}N_2$

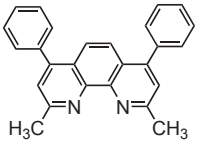
Ordering Information

Product code	Unit
B446-10	1 g

Storage Condition
ambient temperature, protect from metal

Shipping Condition
ambient temperature

Chemical Structure



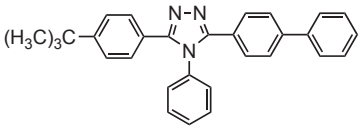
TAZ-01 3-(4-Biphenylyl)-4-phenyl-5-(4-*tert*-butylphenyl)-1,2,4-triazole [CAS: 150405-69-9]

Application: Electron carrier
Appearance: White crystals or white crystalline powder
Purity: ≥98.0% (HPLC)
MW: 429.56, $C_{30}H_{27}N_3$

Storage Condition
ambient temperature

Shipping Condition
ambient temperature

Chemical Structure



Ordering Information

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B363-10	1 g

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A423	11-Amino-1-undecanethiol, hydrochloride	164
A424	8-Amino-1-octanethiol, hydrochloride	165
A425	6-Amino-1-hexanethiol, hydrochloride	165
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