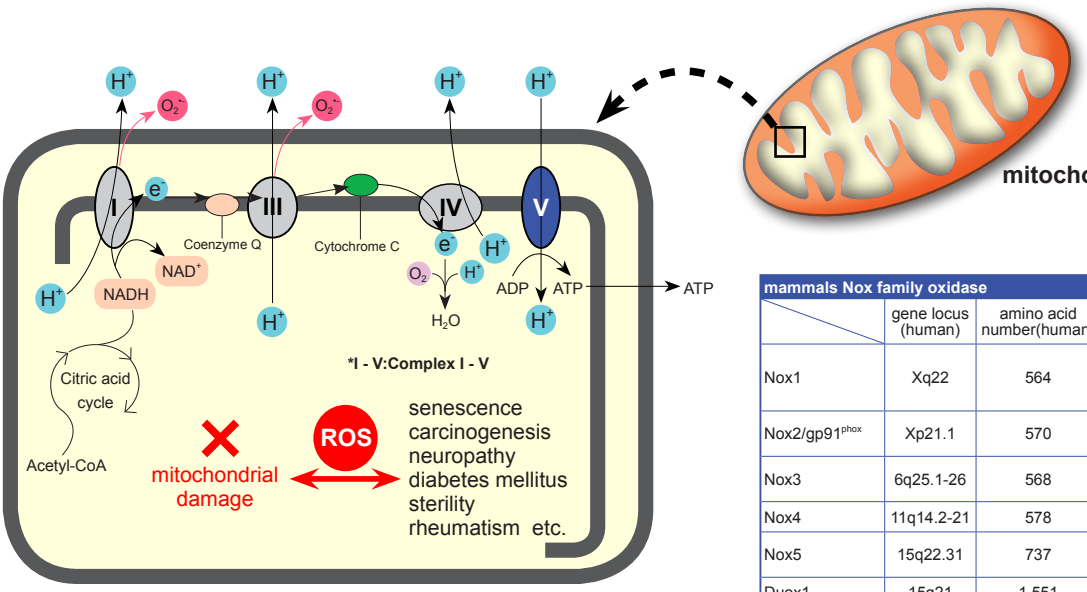
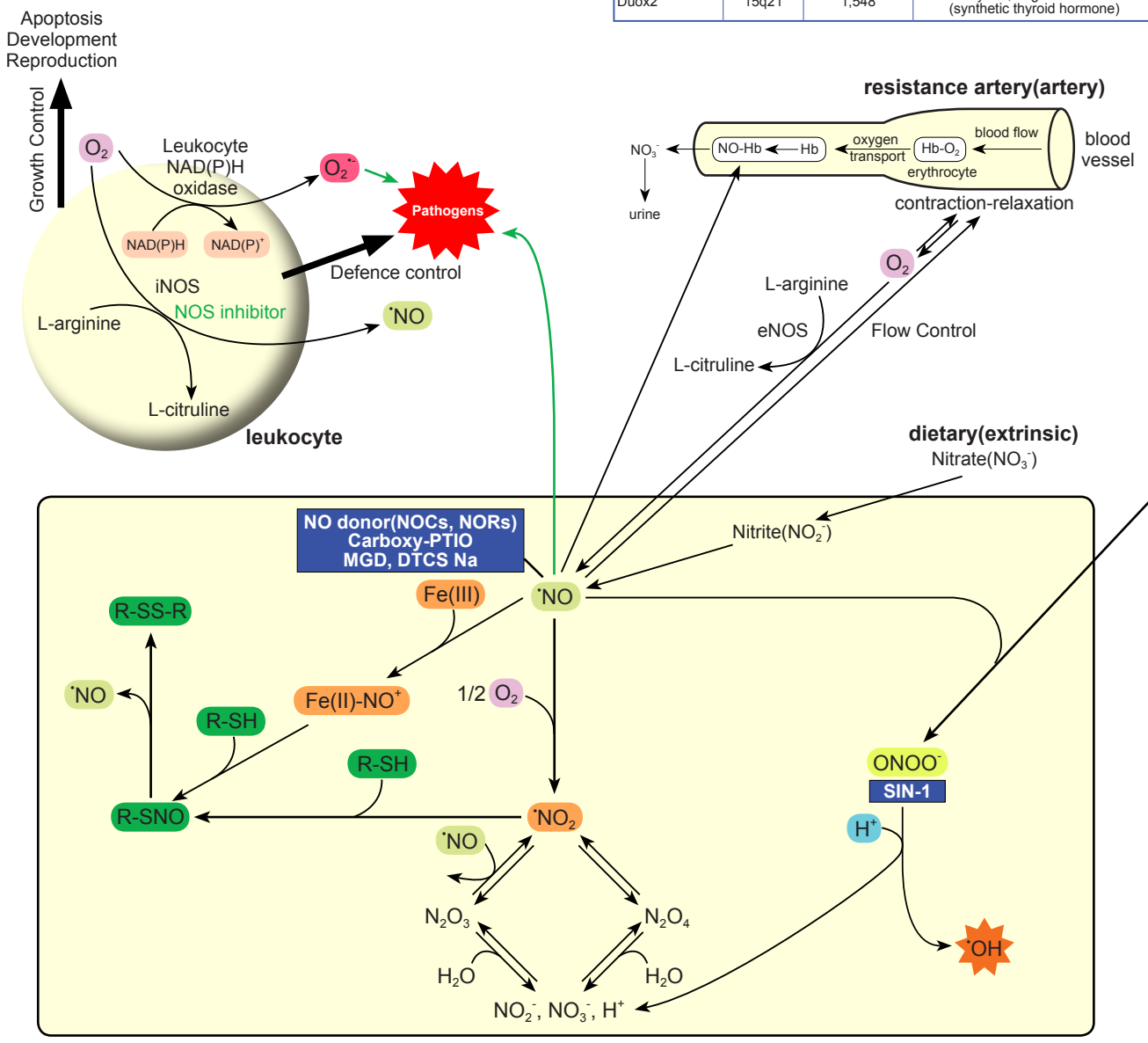
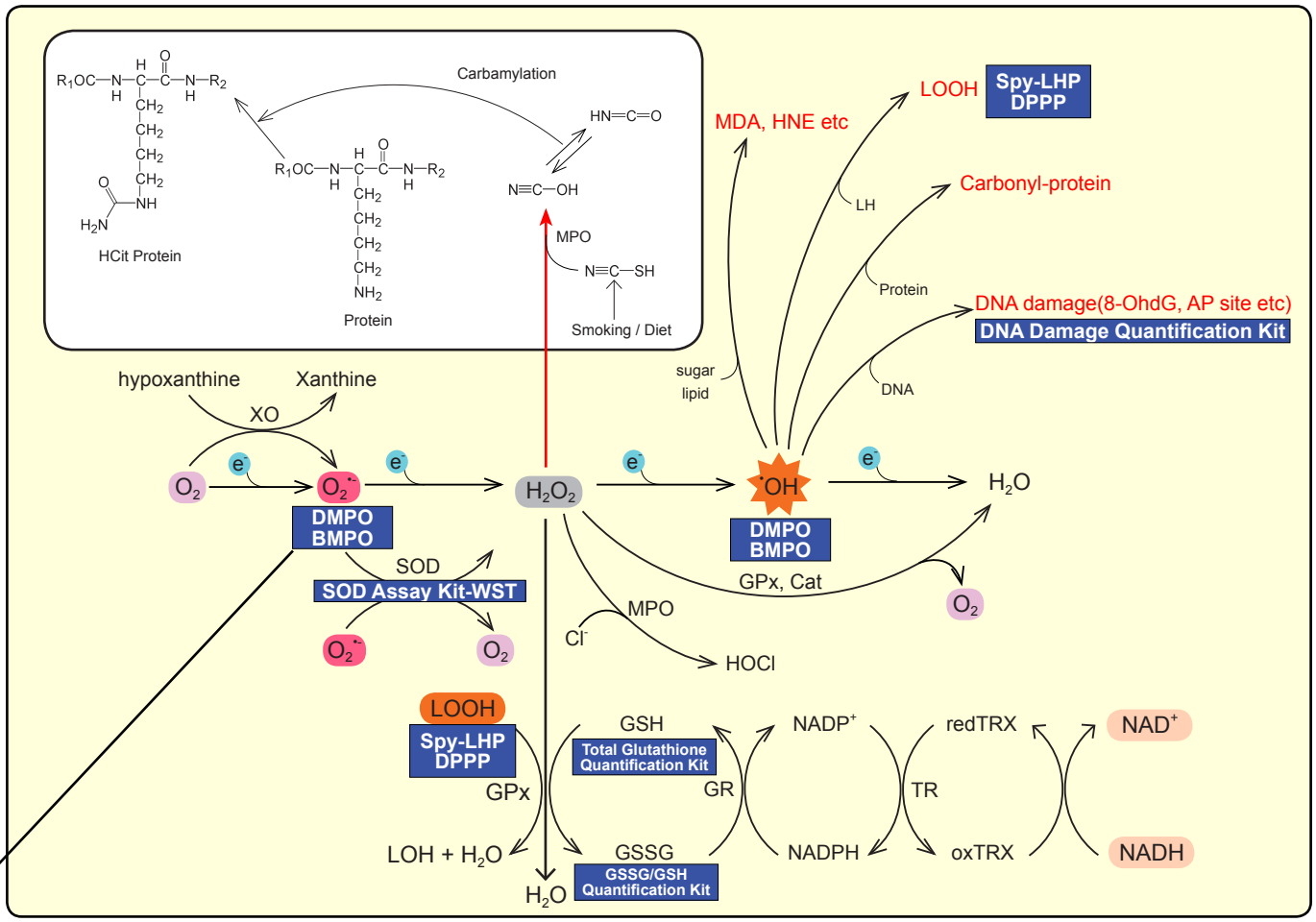
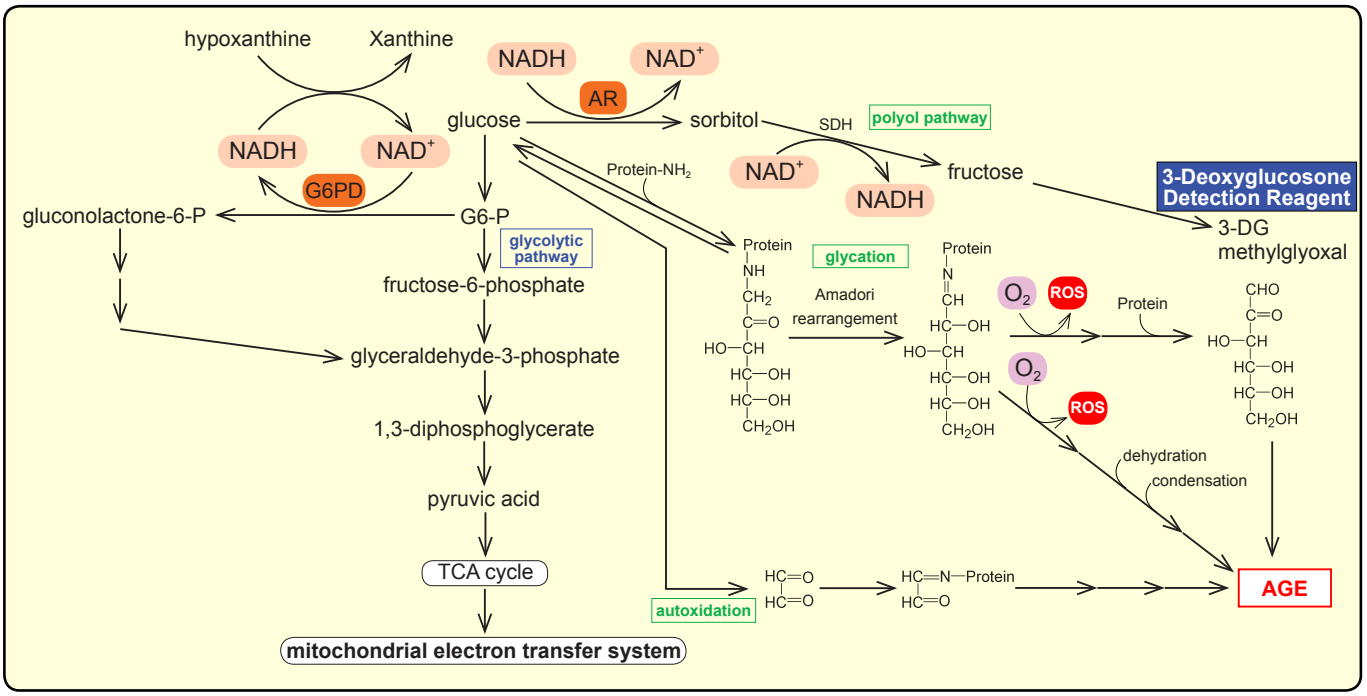


mammals Nox family oxidase			
	gene locus (human)	amino acid number(human)	tissue of high expression (function)
Nox1	Xq22	564	colonic epithelium, vascular smooth muscle (blood pressure elevation of Ang II)
Nox2/gp91^{phox}	Xp21.1	570	phagocyte, B lymphocyte (phylaxis)
Nox3	6q25.1-26	568	inner ear, fetal kidneys (otolith formation)
Nox4	11q14.2-21	578	renal tublar, vascular endothelial
Nox5	15q22.31	737	spermatozoon, spleen, lymphocyte
Duox1	15q21	1,551	thyroid, bronchial
Duox2	15q21	1,548	thyroid, large intestine (synthetic thyroid hormone)

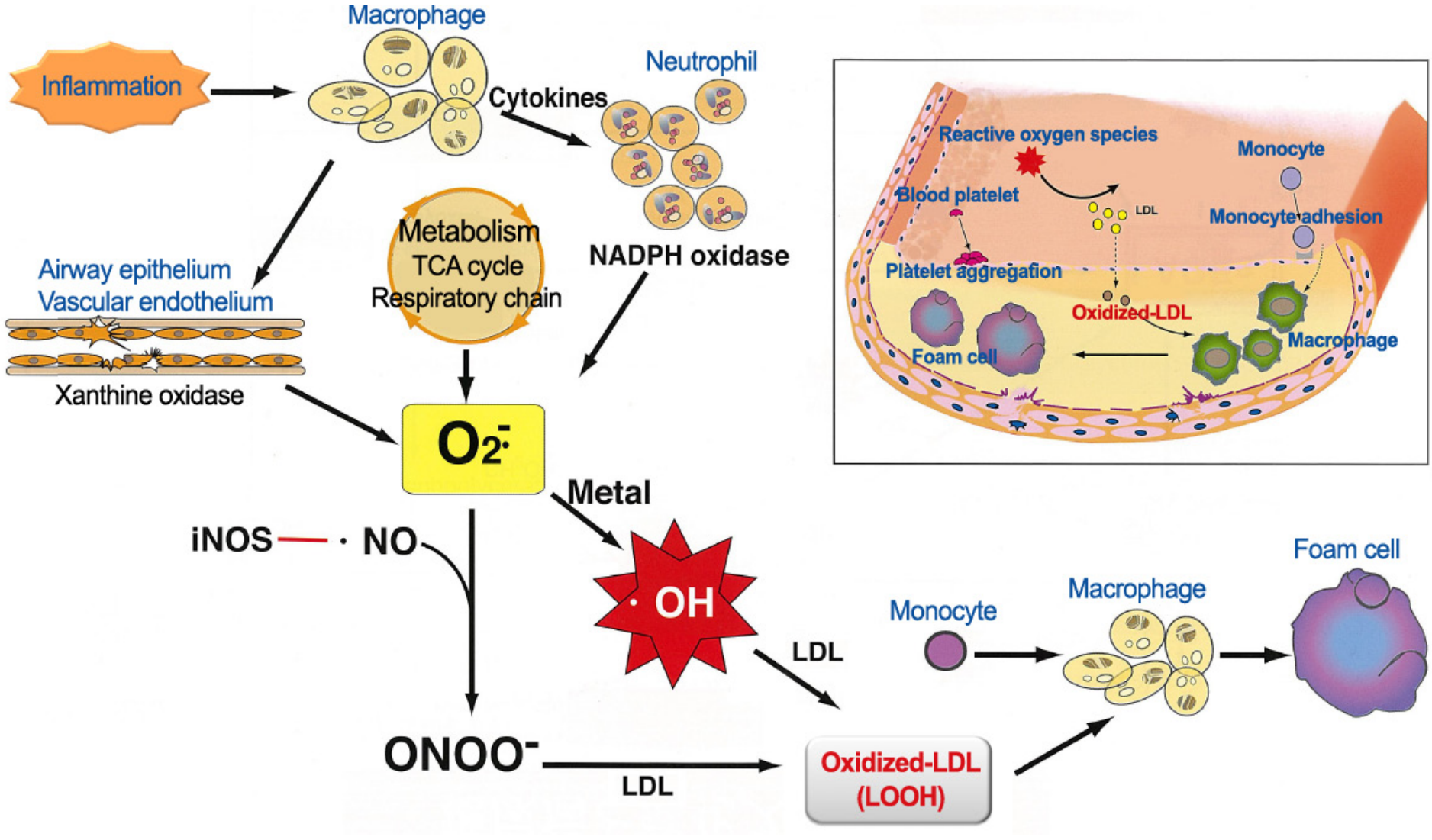






Dojindo Products

GPx: glutathione peroxide
TR: thioredoxin reductase
MPO: myeloperoxide
Cat: catalase
ROS: reactive oxygen species
AR: aldose reductase
GR: glutathione reductase
TRX: thioredoxin
XO: xanthine oxidase
LOOH: lipid peroxide
SDH: sorbetol dehydrogenase
AGE: advanced glycation end product reduct


Inflammation
Macrophage
Cytokines
Neutrophil
NADPH oxidase
Metabolism
TCA cycle
Respiratory chain
Airway epithelium
Vascular endothelium
Xanthine oxidase
$O_2^{-\cdot}$
Metal
iNOS
· NO
· OH
LDL
$ONOO^-$
LDL
Oxidized-LDL
(LOOH)
Monocyte
Macrophage
Foam cell
Reactive oxygen species
Monocyte
Blood platelet
LDL
Monocyte adhesion
Platelet aggregation
Oxidized-LDL
Macrophage
Foam cell

Oxidative Stress Research Products - Index

1. Oxidative Stress Detection

2. Reactive Oxigen Species(ROS) Detection

3. Nitric oxide(NO) Detection

4. NO Donor

* Signaling and metabolic pathways were edited under the supervision of Dr. Keizo Sato/Kyushu University of Health and Welfare.

Introduction

Oxygen is a very important molecule for the synthesis of biologically active materials such as hormones and ATP. The 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. Though 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 oxidative stress. Oxidative stress in cells is caused by ROS 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 in water 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 the biological systems. In response, aerobic organisms created defense mechanisms to avoid oxidative stress. Oxidative stress has recently become the focus of many studies conducted for the understanding of defense mechanisms and 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.

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, there have been many contradictory reports published in NO research. These contradicting results are due to NO's unique chemical properties. Since NO is a free radical, it is highly 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 that reason, it is vital to separately investigate each function of the NO-related metabolites.

SOD Activity Detection

SOD Assay Kit-WST

Product Code: S311

Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion ($O_2^{\cdot-}$) into hydrogen peroxide and molecular oxygen, is one of the most important antioxidative enzymes. In order to determine the SOD activity, several direct and indirect methods have been developed. Among these methods, an indirect method using nitroblue tetrazolium (NBT) is commonly used due to its convenience and ease of use. However, there are several disadvantages to the NBT method, such as poor water solubility of the formazan dye, and the interaction with the reduced form of xanthine oxidase.

SOD Assay Kit-WST allows very convenient SOD assay by utilizing Dojindo's highly water-soluble tetrazolium salt, WST-1 (2-(4-Iodophenyl)- 3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) that produces a water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with $O_2^{\cdot-}$ are linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD, as shown below. Therefore, the IC_{50} (50% inhibition activity of SOD or SOD-like materials) can be determined by a colorimetric method.

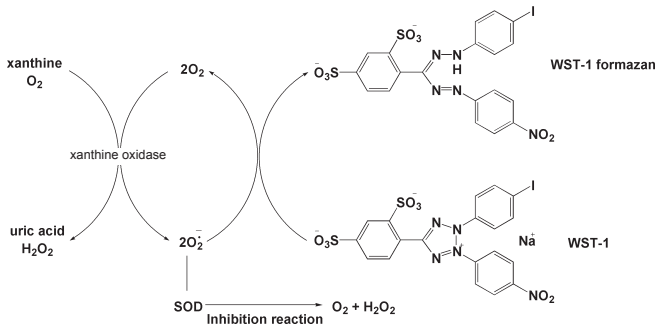


Fig.1 SOD inhibition assay mechanism

1. Preparation of Sample Solutions

► Erythrocytes or Plasma

1. Centrifuge 2-3 ml of anticoagulant-treated blood (such as heparin 10 U/ml with final concentration) at 600 xg 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 xg 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 xg 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 of distilled water, and dilute with 0.25% ethanol 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 78,000 xg for 60 minutes at 4°C, and transfer the supernatant to a new tube.
4. Dilute the supernatant with distilled water to prepare sample solution.

2. Preparations of Solutions (for one 96-well plate)

► WST working solution
Dilute 1 ml of WST Solution with 19 ml of Buffer Solution

► Enzyme working solution
Centrifuge the Enzyme Solution tube for 5 seconds. Mix by pipetting and dilute 15 µl of Enzyme Solution with 2.5 ml of Dilution Buffer.

► Sample solution
Dilute sample solution prepared with Dilution Buffer or Saline.

Contents of the Kit

	100 tests	500 tests
WST Solution	1 ml x 1	5 ml x 1
Enzyme Solution	20 µl x 1	100 µl x 1
Buffer Solution	11 ml x 2	100 ml x 1
Dilution Buffer	10 ml x 1	50 ml x 1

Required Equipment & Materials

Microplate Reader (450 nm filter)
96-well microplate
2-20 µl & 20-200 µl multi-channel pipettes
Incubator(37°C)

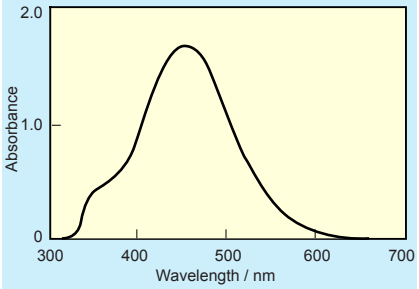


Fig. 2 Absorption spectrum of WST-1 formazan

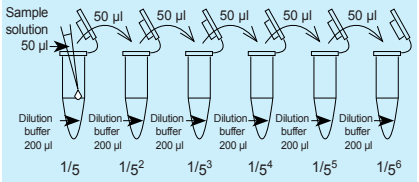


Fig.3 Serial dilution process

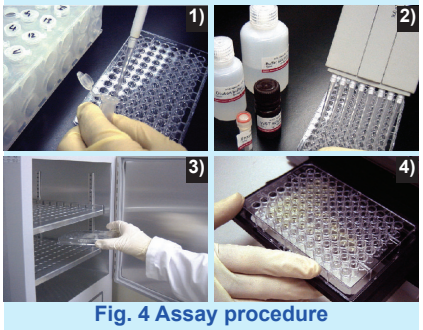


Fig. 4 Assay procedure

e.g.) dilution rate: x1(no dilution), x1/5, x1/5^2, x1/5^3, x1/5^4, x1/5^5, x1/5^6

3. General Protocol (refer to Table 1, Fig. 4 and Fig. 5)

1. Add 20 µl of sample solution to each sample well and blank 2 well, and add 20 µl of ddH_2O(double-distilled water) to each blank 1 and blank 3 well.
2. Add 200 µl of WST Working Solution to each well, and mix by pipetting.
3. Add 20 µl of Dilution Buffer to each blank 2 and blank 3 well.
4. Add 20 µl of Enzyme Working Solution to each sample and blank 1 well.
5. Incubate the plate at 37ºC for 20 minutes.
6. Read the absorbance at 450 nm using a microplate reader.
7. Calculate the SOD activity(inhibition rate %) using the following equation.

SOD activity(inhibition rate %) = $\{[(A_{blank\ 1}-A_{blank\ 3})-(A_{sample}-A_{blank\ 2})]/(A_{blank\ 1}-A_{blank\ 3})\}x100$

	sample	blank 1	blank 2	blank 3
Sample Solution	20 µl	-	20 µl	-
ddH_2O	-	20 µl	-	20 µl
WST Working Solution	200 µl	200 µl	200 µl	200 µl
Dilution Buffer	-	-	20 µl	20 µl
Enzyme Working Solution	20 µl	20 µl	-	-

Table 1 Solution and buffer volumes in each well

4. Inhibition Curve

As Fig. 6 shows, SOD Assay Kit-WST can measure 100% inhibition because WST-1 does not react with the reduced form of xanthine oxidase(XO).

5. Definition of Unit(U)

One unit is defined as a point where a 20 µl of sample solution gives 50% inhibition of a colorimetric reaction between WST-1 and superoxide anion.

* Unit definition differ from the unit definition of Cytochrome c assay.

6. Calculate Unit(U)

1. Figure out a dilution ratio where the inhibition curve gives 50% inhibition.
2. SOD unit in original sample can be calculated by multiplying the dilution rate.

7. Example of Calculating Unit(U): Erythrocytes(x108 dilution sample)

1. Figure out a dilution ratio from the point of IC_{50} in the inhibition curve. Fig. 7 gives the dilution rate at IC_{50} of 1/1.8.
2. According to the definition of unit, 20 µl of this sample is calculated 1.8 U.
3. SOD unit per 1ml of this sample solution can be calculated by the following equation, 1.8 / 0.02 = 90.0 U/ml.
4. Original erythrocytes sample was diluted 108 times at the sample preparation. To calculate the SOD unit in the original, multiply 90.0 U/ml by 108. The SOD unit in the original sample is 9,720 U/ml of blood.

* SOD unit can be calculated as U/gram or U/mg.

8. Distinguish Mn-SOD from Cu/Zn-SOD and EC-SOD

Mn-SOD can be measured by blocking the Cu/Zn-SOD and EC-SOD activity using potassium cyanide(KCN) or Diethyldithiocarbamate(DDC).

9. Interference

Reducing agents, such as ascorbic acids and reduced forms of glutathiones, interfere with the SOD assay. Table 2 shows the concentrations of materials that cause 10% increase in the O.D. value. In case your sample contains the material in the Table 2, dilute the sample to avoid the interference.

10. References

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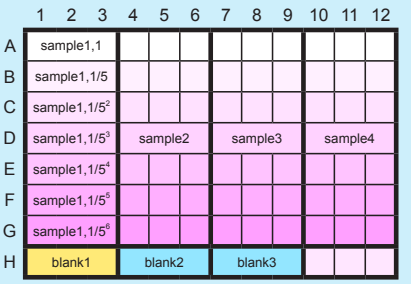

Fig. 5 Sample and blank arrangement on a 96-well microplate

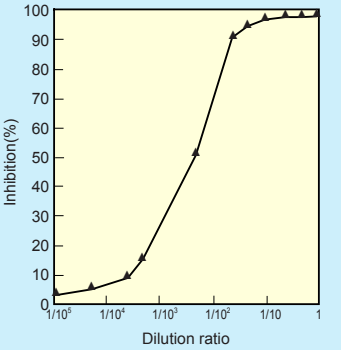

Fig. 6 Inhibition curve of Cu/Zn-SOD

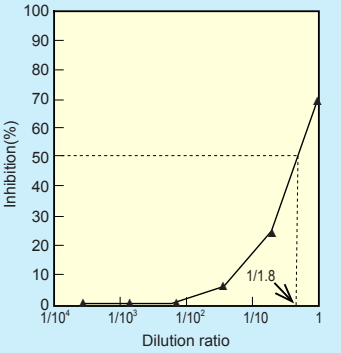

Fig.7 Inhibition curve on erythrocytes sample

Table 2 Minimum Concentrations of Interfering Substances

Detergents	SDS	0.05%
	Tween 20	0.5%
	NP-40	0.5%
	Triton X-100	0.2%
Solvents	Ethanol	25%
	DMSO	5%
Reducing agents	Glutathione, reduced form	1.25 mmol/l
	Ascorbic acid	0.1 mmol/l
Other	EDTA	2 mmol/l
	BSA	1%w/v

Table 3 Measurement Examples

Total SOD	
erythrocyte	9,720 U/ml of blood
serum	355 U/ml of blood
heart(rat)	15,712 U/g (wet)
liver(rat)	142,907 U/g (wet)
HeLa cell	73 U/1x10^7 cells
HL60 cell	226 U/1x10^8 cells

Total Glutathione Detection

Total Glutathione Quantification Kit

Product Code: T419

Glutathione (GSH) is the most abundant thiol (SH) compound in animal tissues, plant tissues, bacteria, and yeast. GSH plays different roles such as protection against reactive oxygen species, and maintenance of protein SH groups. During these reactions, GSH is converted into glutathione disulfide (GSSG: oxidized form of GSH). Since GSSG is 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 for the detection of thiol compounds. In 1985, Dr. Anderson suggested that the glutathione recycling system by DTNB and glutathione reductase created a highly sensitive glutathione detection method. DTNB and glutathione (GSH) react to generate 2-nitro-5-thiobenzoic acid and glutathione disulfide (GSSG). Since 2-nitro-5-thiobenzoic acid is a yellow colored product, GSH concentration in a sample solution can be determined by the absorbance measurement at 412 nm. GSH is generated from GSSG by glutathione reductase, and reacts with DTNB again to produce 2-nitro-5-thiobenzoic acid. Therefore, this recycling reaction improves the sensitivity of total glutathione detection (Fig. 1).

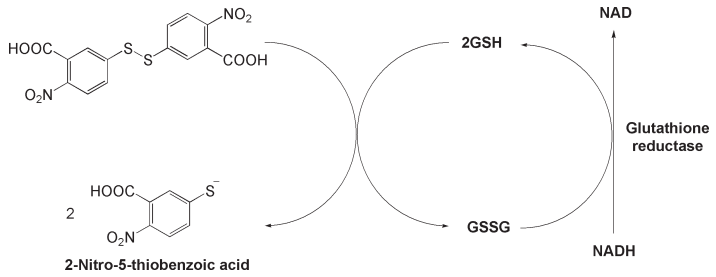

Fig.1 Principal of total glutathione detection

1. Preparation of Sample Solutions

► Cell(adhesive cell: 5 x 10^5 cells, leukocyte cell: 1 x 10^6 cells)
1. Collect the cells by centrifugation at 200 xg for 10 minutes at 4°C, and discard the supernatant.
2. Wash the cells with 300 µl of PBS, and centrifuge at 200 xg for 10 minutes at 4°C. Discard the supernatant.
3. Add 80 µl of 10 mM HCl, and lyse the cells by freezing and thawing(two times).
4. Add 20 µl of 5% SSA, and centrifuge at 8,000 xg for 10 minutes.
5. Transfer the supernatant to a new tube, and use it for the total glutathione assay.

► Tissue(100 mg)
1. Homogenize the tissue in 0.5 to 1 ml of 5% SSA.
2. Centrifuge the homogenized tissue sample at 8,000 x g for 10 minutes.
3. Transfer the supernatant to a new tube, and use it for the total glutathione assay.

► Plasma
1. Centrifuge an anticoagulant treated blood at 1,000 xg for 10 minutes at 4°C.
2. Transfer the top plasma layer to a new tube and add 5% SSA equivalent to 1/2 volume of the plasma.
3. Centrifuge at 8,000 xg for 10 minutes at 4°C.
4. Transfer the supernatant to a new tube, and use it for the total glutathione assay.

► Erythrocyte
1. Centrifuge an anticoagulant treated blood at 1,000 xg for 10 minutes at 4°C.
2. Discard the supernatant and the white buffy layer.
3. Lyse the erythrocytes with 4 vol of 5% SSA.
4. Centrifuge at 8,000 xg for 10 minutes at 4°C.
5. Transfer the supernatant to a new tube, and use it for the total glutathione assay.

*Prior to using the sample solution, please dilute it with water to adjust the concentration of SSA at 0.5 to 1%. High concentration of SSA cause pH changes and interfere with the glutathione assay reaction.

2. Preparations of Solutions

► Substrate working solution

Contents of the Kit

Substrate(DTNB)	2 vials
Enzyme Solution	50 µl x 1
Coenzyme(lyophilized)	2 vials
Standard GSH (lyophilized)	1 vial
Buffer Solution	50 ml x 1

Required Equipment & Materials

Microplate Reader (405 or 415 nm filter)
96-well microplate
20-200 µl multi-channel pipettes
Incubator (37°C)
5-sulfosalicylic acid(SSA)

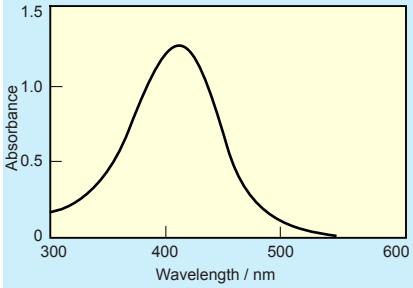

Fig. 2 Absorption spectrum of 5-Mercapto-2-nitrobenzoic acid

Add 1.2 ml of Buffer Solution to one vial of Substrate, and dissolve.
Store the solution at -20°C (stable for 2 months).

► Enzyme working solution
Mix Enzyme Solution using pipette. Take out 20 µl of Enzyme Solution, and mix it with 4 ml of Buffer Solution.
Store the solution at 4°C (stable for 2 months).

► Coenzyme working solution
Add 1.2 ml of ddH_2O to the Coenzyme vial and dissolve. The Coenzyme vial is decompressed. Use a syringe to add ddH_2O, then open the vial.
Store the solution at -20°C(stable for 2 months).

► GSH standard solution
Add 2 ml of 0.5% SSA to Standard GSH vial, and dissolve to prepare 200 µM of GSH standard solution. The Standard GSH vial is decompressed. Use a syringe to add 0.5% SSA, then open the vial.
Store the solution at -20°C(stable for 2 months).
Dilute 100 µl of 200 µM GSH standard solution by serial dilution with 100 ul of 0.5% SSA in plastic tubes as indicated Fig. 4.

3. General Protocol (refer to Fig. 3)

1. Add 20 µl of Enzyme working solution, 20 µl of Coenzyme working solution and 120 µl of Buffer Solution to each well.
2. Incubate the plate at 37°C for 5 minutes.
3. Add 20 µl of GSH standard solution and 20 µl of sample solution to each well.
4. Incubate the plate at 37°C for 10 minutes.
5. Add 20 µl of Substrate working solution, and incubate the plate at room temperature for 10 minutes.
6. Read the absorbance at 405 nm or 415 nm using a microplate reader.
7. Determin concentrations of GSH in the sample solutions using a calibration curve. Since the colorimetric reaction is stable and the O. D. increases linearly over 30 min. A time course of the colorimetric reaction is shown Fig. 5. Typical calibration curves prepared using the pseudo-endpoint method is indicated in Fig. 6.

4. Calculation of total glutathione (GSH and GSSG) concentration

Determine the total glutathione concentration[a] in a sample solution using the following equations.

► pseudo-end point method

$$\textbf{Total glutathione (GSH+GSSG)} = (O.D._{sample} - O.D._{blank})/slope^{b}$$

► kinetic method

$$\textbf{Total glutathione (GSH+GSSG)} = (Slope^{c}_{sample} - Slope^{c}_{blank})/slope^{b}$$

a) Since the values obtained by these equations are the amount of total glutathione in treated sample solutions, further calculations are necessary if the actual concentrations of glutathione in samples need to be determined.
b) slope of the calibration curve
c) slope of the kinetic reaction

5. Interference

Reducing agents such as ascorbic acid, β-mercaptoethanol, dithiothreitol (DTT) and cysteine, or thiol reactive compounds such as maleimide compounds, interfere with the glutathione assay. Therefore, SH compounds, reducing agents and SH reactive materials should be avoided during the sample preparation.

6. Notes

1. Store the kit at 0 to 5°C. It is stable for 6 months at 0 to 5°C.
2. Use the reagents in the kit after the reagents temperature are equilibrated to the room temperature.
3. Triplicate measurements per sample is recommended to obtain accurate data.
4. Since the colorimetric reaction starts immediately after the addition of Substrate working solution to a well, use a multichannel pipette to avoid the reaction time lag of each well.
5. If the concentration range of total glutathione in a sample is unknown, prepare multi-diluted sample solutions.
6. This kit is not for GSSG determination.

7. References

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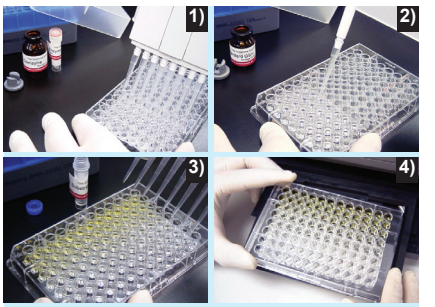


Fig. 3 Assay procedure

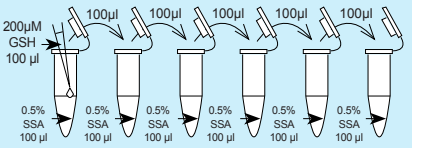


Fig.4 Serial dilution process

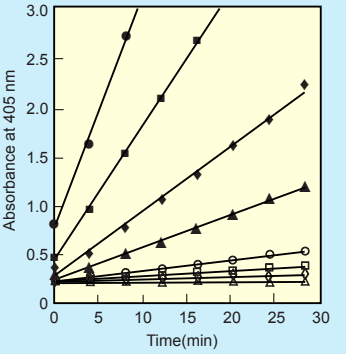


Fig. 5 Time-dependent Abs. increase on eight diferent samples

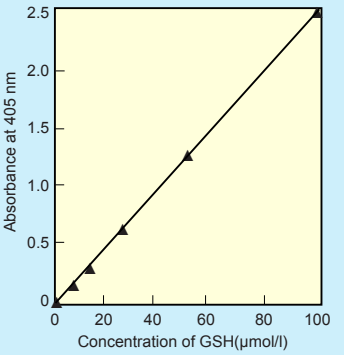


Fig. 6 Calibration curve prepared using pseudo-endpoint method(10 min incubation at room temperature)

Distinguish Measurement of Glutathione

GSSG/GSH Quantification Kit

Product Code: G257

Glutathione (γ-L-glutamyl-L-cysteinylglycine) is a tripeptide present in the body, and it is involved in antioxidation, drug metabolism, and other as enzyme substrate of glutathione peroxidase, glutathione *S*-transferase, and thiol transferase, etc. Glutathione is usually present as reduced form (GSH), but GSH is converted into its oxidized form (GSSG) by stimulation such as oxidative stress. Therefore, the ratio of GSH and GSSG has been noted as index of oxidative stress.
The GSSG/GSH Quantification kit contains Masking Reagent of GSH. The GSH can be deactivated in the sample by adding the Masking Reagent. Therefore, only the GSSG is detected by measuring the absorption (λmax = 412nm) of DTNB (5,5'-dithiobis (2-nitrobenzoic acid) using the enzymatic recycling system. Also, GSH can be determined the quantity by subtracting GSSG from the total amount of glutathione.
The kit can be limited to quantify GSH/GSSG concentration from 0.5 µmol/l to 50 µmol/l and GSSG concentration from 0.5 µmol/l to 25 µmol/l.

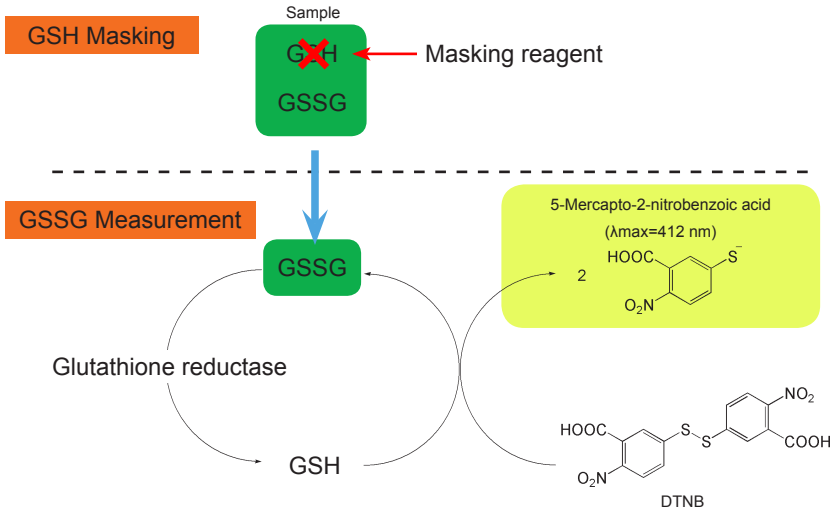


Fig.1 Principal of GSSG/GSH detection

1. General Protocol

► Preparation of Sample Solution
Please refer to Total Glutathione Quantification, page 5.

► Determination of GSSG concentration

1. Add 4 µl of Masking Solution to sample solution and 200 µl of GSSG standard solution diluted with 0.5% SSA respectively, then transfer 40 µl of the solution to each well.
2. Add 120 µl of Buffer Solution to each well and incubate for 1 hour at 37°C.
3. Add 20 µl of Substrate working solution to each well, then add 20 µl of Coenzyme working solution and Enzyme working solution to each well respectively.
4. Incubate for 10 minutes at 37°C and read the absorbance at 405 or 415 nm using a microplate reader.
5. Determine concentrations of GSSG in the sample solution using a GSSG calibration curve (Fig. 2).

► Determination of total glutathione concentration

1. Add sample solution and 40 µl of GSH standard solution diluted with 0.5% SSA to each well.
2. Add 120 µl of Buffer solution to each well and incubate for 1 hour at 37°C.
3. Add 20 µl of Substrate working solution to each well, then add 20 µl of Coenzyme working solution and Enzyme working solution to each well respectively.
4. Incubate for 10 minutes at 37°C and read the absorbance at 405 or 415 nm using a microplate reader.
5. Determine concentrations of total glutathione in the sample solution using a GSH calibration curve(Fig. 3).

► Calculating the concentration of GSH
GSH(conc.) = total Glutathione(conc.) - 2 x GSSG(conc.)

Contents of the Kit

Enzyme Solution	50 µl x 1
Coenzyme	2 vials
Buffer Solution	60 ml x 1
Substrate (DTNB)	4 vials
Standard GSH	1 vial
Standard GSSG	1 vial
Masking Reagent	20 µl x 1

Required Equipment & Materials

Microplate Reader (405 or 415 nm filter)
96-well microplate
20-200 µl multi-channel pipettes
Incubator (37°C)
5-sulfosalicylic acid (SSA)
Ethanol

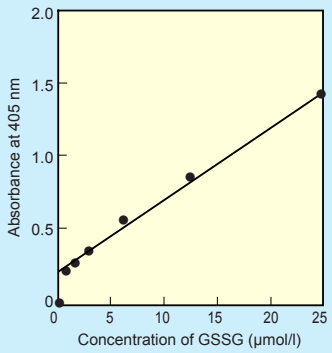


Fig. 2 Determination of the concentration of GSSG

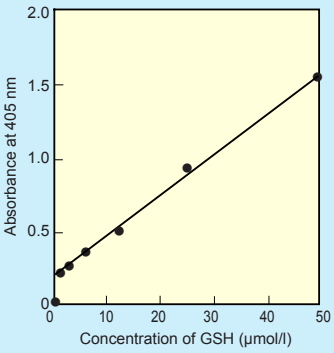


Fig. 3 Determination of the concentration of total glutathione

DNA Damage Detection

DNA Damage Quantification Kit-AP Site Counting-

Product Code: DK02

Oxidative damage to DNA is a result of the interaction of DNA with reactive oxygen species (ROS), in particular, the hydroxy radical which is converted from superoxide and hydrogen peroxide by the Fenton reaction. Hydroxy radicals produce a multiplicity of modifications in DNA. Oxidative attack by hydroxy radical 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. It has been estimated that endogeneous ROS can result in about $2x10^5$ base lesions per cell per day.
Aldehyde Reactive Probe (ARP) reagent (N'-aminooxymethylcarbonylhydrazino-D-biotin, Fig. 1) reacts specifically with an aldehyde group which is the open ring form of the AP sites. This reaction makes it possible to detect DNA modifications that result in the formation of an aldehyde group. After treating DNA containing AP sites with ARP reagent, AP sites are tagged with biotin residues. By using an excess amount of ARP, all AP sites can be converted to biotin-tagged AP sites. Therefore, AP sites can be quantified using avidin-biotin assay followed by a colorimetric detection of peroxidase or alkaline phosphatase conjugated to the avidin. DNA Damage Quantification Kit contains all the necessary solutions, enabling the determination of 1 to 40 AP sites per $1x10^5$ bp.

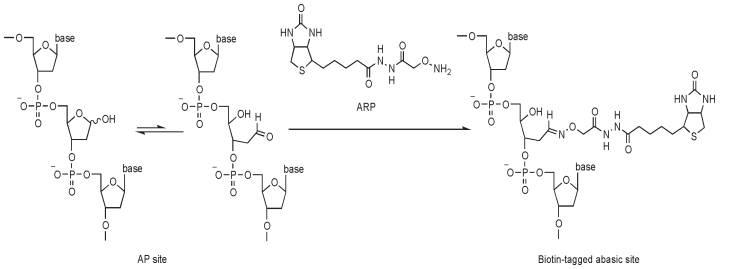


Fig. 1 Reaction of AP site with ARP

1. Purification of genomic DNA

Several different methods and products are available for the isolation of genomic DNA from samples such as membrane binding method, guanidine/detergent lysis method, and polyelectrolyte precipitation method. Among these methods, the guanidine/detergent lysis method is simple, and it gives highly purified genomic DNA for the ARP-based abasic sites detection. During the purification process, avoid heating of the DNA solution. Determine the concentration and purity of the purified genomic DNA using the spectrophotometer* and agarose gel electrophoresis. Dissolve the genomic DNA in TE at the concentration of 100 µg/ml. It is important for an accurate assay that the DNA concentration is adjusted exactly to 100 µg/ml.

* 1 $OD_{260\ nm}$ = 50 µg/ml. The ratio of $OD_{260\ nm}/OD_{280\ nm}$ of highly purified DNA solution is 1.8 or higher. Protein contamination in the sample solution may cause a positive error.

2. General Protocol

► ARP reaction

1. Mix 10 µl of purified genomic DNA solution(100 µg/ml) and 10 µl of 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 of TE to the reaction solution, and transfer the solution to the Filtration Tube.
4. Centrifuge the Filtration Tube at 2,500 xg for 15 minutes, and discard the filtrate solution.
5. Add 400 µl of TE to the Filtration Tube and resuspend the DNA on the filter with a pipette.
6. Centrifuge the Filtration Tube at 2,500 xg for 15 minutes.[a)]
7. Add 200 µl of TE to the Filtration Tube to resuspend the DNA on the filter with a pipette.
8. Transfer the DNA solution to the 1.5 ml tube, and add 200 µl of TE again to the Filtration Tube to transfer the ARP-labeled DNA on the filter completely to the 1.5 ml tube.[b)]
9. Store the ARP-labeled genomic DNA solution at 0 to 5°C.

a) If the DNA solution still remains on the filter after the centrifugation, spin for another 5 minutes.

b) Recovery rate of DNA using the filtration tube is 90%, so the concentration of the ARP-labeled DNA is 2.25 µg/ml. For more accurate determination of the number of

Contents of the Kit

5 samples	
ARP Solution (10mM ARP)	100 µl x 1
ARP-DNA Standard Soln.*	250 µl ea.
(0, 2.5, 5, 10, 20, 40 AP sites/100,000 bp)	
DNA Binding Solution	10 ml x 1
Substrate Solution	10 ml x 1
TE Buffer	15 ml x 1
HRP-Streptavidin	25 µl x 1
Washing Buffer	1 pack
Filtration Tube	5 tubes
96-well Microplate/ U bottom	1 plate

20 samples	
ARP Solution (10mM ARP)	250 µl x 1
ARP-DNA Standard Soln.*	250 µl ea.
(0, 2.5, 5, 10, 20, 40 AP sites/100,000 bp)	
DNA Binding Solution	10 ml x 1
Substrate Solution	10 ml x 1
TE Buffer	40 ml x 1
HRP-Streptavidin	25 µl x 1
Washing Buffer	1 pack
Filtration Tube	20 tubes
96-well Microplate/ U bottom	1 plate

Required Equipment & Materials

Microplate Reader (650 nm filter)
10 µl, 100-200 µl and 1 ml pipettes
50-250 µl multi-channel pipettes
Incubator(37°C)
0.5 ml and 1.5 ml tube
Centrifuge
Paper Towel

abasic sites in the sample DNA, we recommend measuring the DNA concentration.

► Determination of the number of AP site in DNA

Day 1

1. Dilute 90 µl of the ARP-labeled genomic DNA with 310 µl of TE.
2. Add 60 µl of ARP-DNA Standard Solution per well. Use three wells per 1 standard solution.
3. Add 60 µl of the diluted ARP-labeled genomic DNA solution per well. Use at least three wells per 1 sample.
4. Add 100 µl of the DNA Binding Solution to each well, then allow the plate to remain at room temperature overnight.

Day 2

1. Prepare stock solutions
   - » Washing Buffer: Dissolve the contents of the Washing Buffer packet in 1 L of deionized or distilled water. Store this Washing Buffer solution at room temperature.
   - » HRP-Streptavidin solution: Dilute HRP-Streptavidin with Washing Buffer to prepare 1/4000 diluted working solution.*
   - » 1/4000 dilution preparation: Centrifuge HRP-Streptavidin tube for 30 seconds. Add 10 µl of HRP-Streptavidin into 40 ml of Washing Buffer solution, and mix well.

   * Since this working solution is not stable, always use freshly prepared solution.
2. Discard the DNA Binding Solution in the wells, and wash the well with 250 µl Washing Buffer 5 times.
3. Add 150 µl of diluted HRP-Streptavidin solution to each well, and incubate the plate at 37°C for 1 hour.
4. Discard the solution in the well, and wash the well with 250 µl Washing Buffer 5 times.[b)]
5. Add 100 µl of Substrate Solution to each well, and incubate at 37°C for 1 hour.
6. Measure the O.D. at 650 nm within 1 hour after the incubation is finished, and prepare a calibration curve using the data obtained with ARP-DNA Standard solutions.
7. Determine the number of abasic sites in the genomic DNA using the calibration curve.

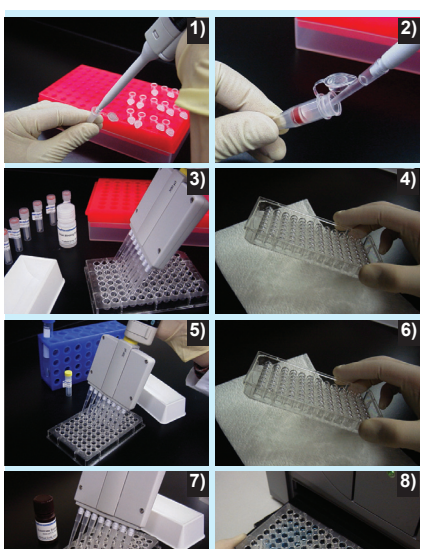


Fig. 2 Assay procedure

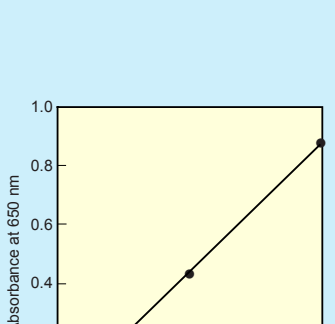


Fig. 3 Typical calibration curve of DNA Damage Quantification Kit

3. Notes

1. Please store the kit at 0-5°C. Do not freeze. Store Washing Buffer solution at room temperature.
2. AP-DNA is not stable. Please treat it with ARP and purify with Filtration Tube after the isolation of genomic DNA from a sample.
3. Purified ARP-DNA solution in TE buffer is stable over one year at 0-5°C storage.
4. After the spinning of Filtration Tube for ARP-labeled DNA purification, add 200 µl TE immediately. If the DNA stays in Filtration Tube for more than 30 minutes after the spinning, the DNA recovery ratio may decline.
5. γ-Ray-sterilized tubes may cause DNA binding on the surface of the tube during the mixing of the DNA solution with DNA Binding Solution. If you prefer to mix ARP-DNA solution with DNA Binding Solution in a tube rather than mixing them in a well, please avoid using g-ray-sterilized tubes.
6. If the 650 nm filter is not available for the measurement of O.D. after the color development, transfer 50 µl of the solution in each well to a well of a new plate (not provided). Then, add 50 µl of 1 M sulfuric acid, and measure the O.D. at 450 nm.
7. Remaining solution in a well may cause error, so please remove the solution thoroughly by tapping the plate on a paper towel in each step.

4. 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.

Advanced Glycation End-products(AGEs) Research

3-Deoxyglucosone Detection Reagents

Product Code: D536

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.
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.

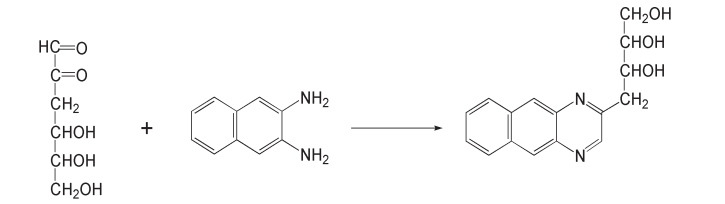


Fig. 1 Principal of 3-DG detection

2. General Protocol

► HPLC Method: Human Serum

1. Add 60% perchloric acid solution to 1 ml human serum and spin at 3,000 xg 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-hexanedione 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.
   * Normal serum 3-DG level: 12.8±5.2 ng/ml
   * Serum 3-DG level of diabetic patient: 31.8±11.3 ng/ml

3. 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.

Contents of the Kit

DAN [2,3-Diaminonaphthalene] 10 mg x 1

3-DG/DAN adduct [2-(2,3,4-Trihydroxybutyl)benzo[g]quinoxaline] 1 mg x 1

3-DG standard

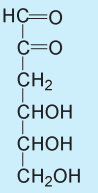
3-Deoxyglucosone

Product Code: D535

3-DG can be utilized for AGE production or as a standard for 3-DG level detection in plasma or serum samples.

1. Specification

► Appearance: white or white pale yellow solid
► Purity: ≥99.0%(HPLC)



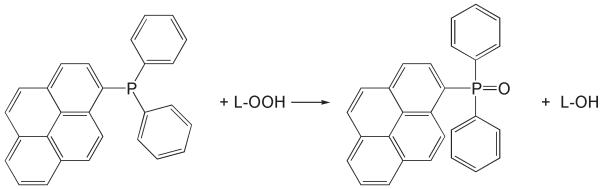
3-Deoxyglucosone
3-Deoxy-D-erythro-hexos-2-ulose
$C_6H_{10}O_5$ = 162.14
CAS No. [4084-27-9]
Unit: 1 mg

Lipid Peroxide Detection

DPPP

Product Code: D350

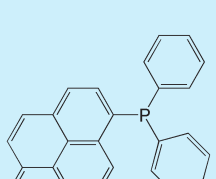
Lipid peroxides are derived from unsaturated lipids, phospholipids, glycolipids and cholesterol esters. In food industry, lipid peroxides have been considered as one of a major cause of food deterioration. Meanwhile, there are many ongoing studies today that investigate the mechanism of lipid oxidation in human diseases, disorders, and aging. Hence, measuring amount of lipid peroxide in biological samples is significant, and an accurate method for detecting low level of lipid peroxides is eagerly anticipated. TBARS(Thiobarbituric acid reactive substances) assay is a well-established method and widely used for measuring lipid peroxidation. However, MDA(Malondialdehyde), one of a end-product generated in the lipid peroxidation, is not reflected in actual level of peroxidation because there are other source of MDA.
DPPP, Diphenyl-1-pyrenylphosphine, was developed by Dr. Meguro, *et al*. as a fluorescent probe for detecting lipid peroxide. It selectively reacts with hydroperoxides to generate DPPP oxide that emits fluorescence at 380 nm(ex: 352 nm), and makes it possible to quantify 0.1 to 7 nmol of hydroperoxide. In addition, the range of 1 to 2 pmol of lipid peroxide can be selectively detected with the combination of HPLC separation and the post-column reaction with DPPP.



DPPP
non-fluorescent

DPPP oxide(1)
fluorescent

Fig. 1 Reaction scheme of DPPP with hydroperoxide



DPPP
Diphenyl-1-pyrenylphosphine
$C_{28}H_{19}P$ = 386.42
CAS No. [110954-36-4]
Unit: 10 mg

1. General Protocol

► HPLC detection of hydroperoxides in plasma sample

1. Prepare 10 mg/ml BHT(Butyl hydroxytoluene)/$CHCl_3$-Methanol(2:1) solution.
2. Dissolve a sample in 100 µl of the solution above.
3. Add 50 µl of DPPP solution(1 mg/10 ml in $CHCl_3$:Methanol=1:1) to the solution prepared in step 2.
4. Incubate the solution under dark condition for 60 minutes at 60°C.
5. Cool down the solution and measure the fluorescence intensity by HPLC.

► Determination of hydroperoxides on cell membrane(*in vivo*)

1. Disolve DPPP in DMSO and prepare 5 mM DPPP/DMSO solution.
2. Add the solution above to cell suspension(1 x 10^7 cells/ml) to the final concentration of 50 µM DPPP.
3. Incubate the cells at 37°C for 10 minutes.
4. Wash the cells twice with Hank's solution.
5. Stimulate the cells by adding H_2O_2 or Methyl linoleate hydroperoxide and measure flueorescence intensity.

2. 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.

3. Specification

► Appearance: slightly yellow powder
► Purity: ≥97.0%(HPLC)

Lipid Peroxide Detection

Spy-LHP

Product Code: S343

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 or 4-hydroxynonenal are derivatives from lipid hydroperoxide prepared by oxidation with reactive oxygen species. Thiobarbituriic acid and 1-Methyl-2-phenylindole are used for the derivertization of 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. Since the oxidized Spy-LHP emits strong fluorescence (quantum yield: ~1) with maximum wavelength at 535 nm when excited at 524 nm, damage to a 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, or alkylperoxy radicals.

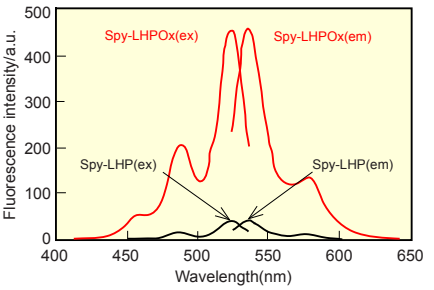


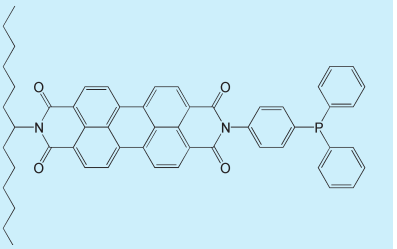
Fig.1 Fluorescence spectra of Spy-LHP and Spy-LHPOx

1. 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.

2. Specification

- Appearance: reddish black crystalline powder
- Purity: ≥90.0%(HPLC)



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

$C_{55}H_{49}N_2O_4P$ = 832.96

Unit: 1 mg

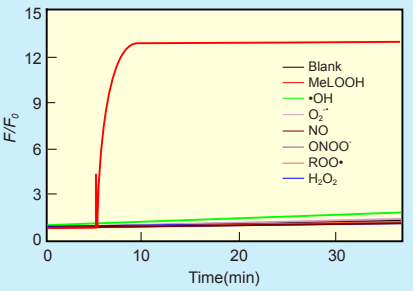


Fig. 2 Selectivity of Spy-LHP (Time course)

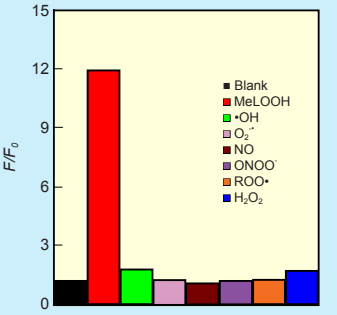


Fig. 3 Selectivity of Spy-LHP (Fluorescence intensity)

8-Nitroguanosine, 8-Nitroguanine Detection

Anti-Nitroguanosine Antibodies

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.

Anti-Nitroguanosine antibodies have been developed jointly by Dr. Akaike at Kumamoto University and Dojindo Laboratories.

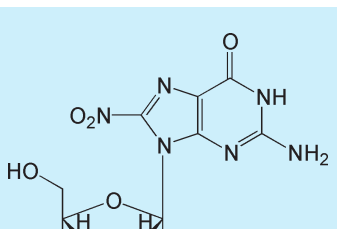


Fig. 1 Structure of 8-Nitroguanosine

8-Nitroguanosine, 8-Nitroguanine Detection

Anti-Nitroguanosine monoclonal antibody (Clone# NO_2G52)

Product Code: AB02

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-hydroxydeoxy-guanosine, 3-nitrotyrosine, xanthine, or 2-nitroimidazole.

The specificity of NO_2G52 was determined by a competitive ELISA using an 8-nitroguanosine-BSA-coated plate. As shown in the figures below, NO_2G52 has very high affinity for 8-nitroguanine and 8-nitroguanosine, and it slightly cross-reacts with 8-bromoguanosine, 8-bromoguanine, and 8-chloroguanine.

strongly react(10 µmol/l)			
8-NO_2-guanosine	8-NO_2-guanine	8-NO_2-cGMP	8-NO_2-Xanthine
slightly react(>1 mmol/l)			
8-Br-guanosine	8-Br-guanine	8-Br-cGMP	8-Cl-guanine
no reaction			
guanosine	guanine	8-OH-guanine	
cytosine	xanthine	adenine	
adenosine	thymine	deoxythymidine	
uracil	uridine	3-NO_2-tyrosine	
2-NO_2-imidazole	8-OH-deoxyguanosine		

Table 1 Reactivity of monoclonal antibody

Unit: 50 µg

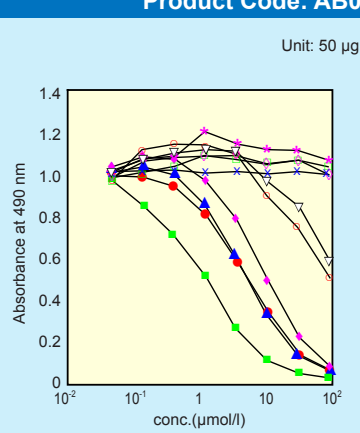


Fig.2 Reactivity of Anti-Nitroguanosine monoclonal antibody$NO_2G52(IC_{50})$

1. Specification

- Species: mouse(BALB/c)
- Clonality: monoclonal
- Isotype: IgG1
- Concentration: 1 mg/ml in PBS, contains 0.1% ProClin as a preservative

8-Nitroguanosine, 8-Nitroguanine Detection

Anti-Nitroguanosine polyclonal antibody

Product Code: AB01

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.

strongly react(10 µmol/l)		
8-NO_2-guanosine	8-NO_2-guanine	
no reaction		
guanosine	guanine	8-OH-guanine
3-NO_2-tyrosine		

Table 2 Reactivity of polyclonal antibody

Unit: 50 µg

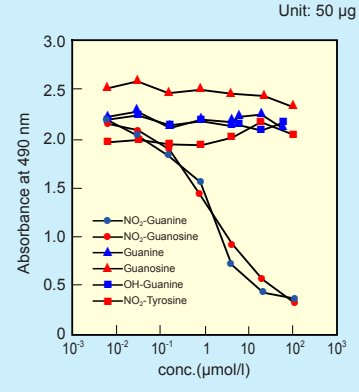


Fig.3 Reactivity of Anti-Nitroguanosine polyclonal antibody

1. Example of Immunostaining(Fig. 4)

► Sample
Influenza virus-infected mouse lung

► Immunostaining
1. Fix the mouse lung with 2% periodate-lysine-paraformaldehyde.
2. Add anti-nitroguanosine antibody(10 µg/ml) to the lung sample.
3. Add alkaline phosphatase-conjugated secondary antibody.
4. Stain the sample with Vector red substrate kit I.

2. Notes

1. Freeze and thaw cycles can cause degradation of the antibody. After opening, store in the refrigerator.
2. If 8-Nitroguanosine staining was observed with polyclonal antibody, it is reccomended to confirm the experimental verification as follows,
   » no staining is observed by the competition with 8-Nitroguanine standard
   » no staining is observed by treating the sample with reducing agent, such as Sodium hydrosulfite
3. Monoclonal antibody can be used on human samples due to the high activity and selectivity.

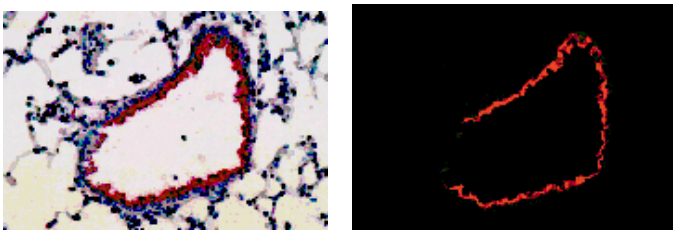


Fig. 4 Tissue staining with Anti-Nitroguanosine antibody

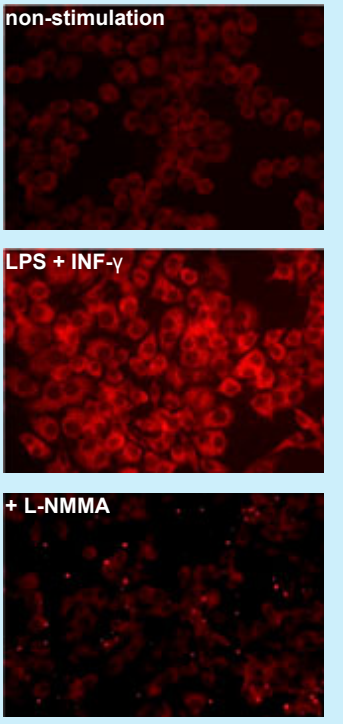


Fig.5 Detection of guanine nitration on RAW264.7 cell

3. Specification

► Species: mouse(BALB/c)
► Clonality: monoclonal
► Isotype: IgG1
► Concentration: 1 mg/ml in PBS, contains 0.1% ProClin as a preservative

4. References

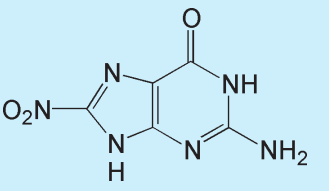
1. T. Akaike, *et al*., 8-nitroguanosine formation in viral pneumonia and its implication for pathogenesis, *PNAS*. 2003;**100**:685-690.
2. J. Yoshitake, *et al*., Nitric oxide as an endogenous mutagen for Sendai virus without antiviral activity. *J Virol*. 2004;**78**:8709-8719.
3. T. Sawa, *et al*., 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 Standard

8-Nitroguanine(lyophilized)

Product Code: N455

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. 8-Nitroguanine/PBS solution 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.



8-Nitroguanine(lyophilized)
$C_5H_4N_8O_3$ = 196.12
Unit: 100 µg

Radical Scavenger, ESR probe for Superoxide and Hydroxyradical

DMPO

Product Code: D048

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 when 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.

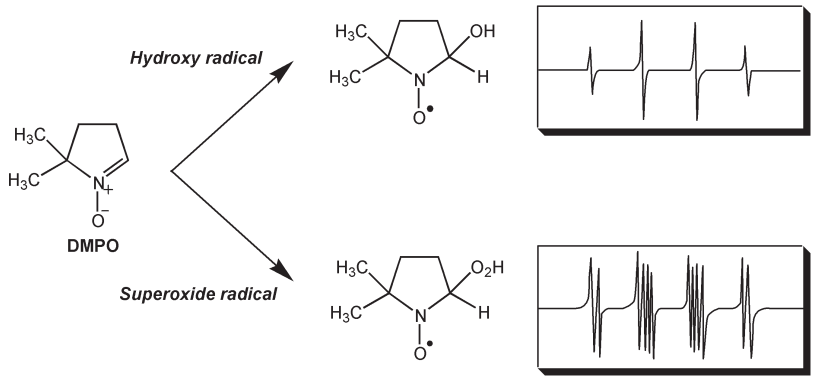


Fig. 1 ESR Spectera of DMPO Adducts

1. General Protocol

► Evaluation of superoxide scavenging activities

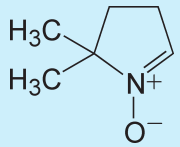
1. Add 15 µl of DMPO and 50 µl of 5 mM hypoxanthine to 35 µl of 0.1 M Phosphate buffer(pH 7.8).
2. Add 50 µl of SOD standard or samples to be tested and voltex for 1-2 seconds.
3. Add 50 µl of 0.4 U/ml xanthine oxidase and voltex immediately.
4. Transfer the solution to ESR sample tube and measure ESR spectra after certain time of period, e.g. 1 minute.
5. Calculate relative intensity(DMPO-O_2^-/Mn^{2+}) from the peak height.

2. 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.

3. Specification

► Appearance: colorless liquid
► Purity: ≥99.0%(GC)
► ESR spectrum: to pass test
► IR spectrum: authentic



DMPO
5,5-Dimethyl-1-pyrroline *N*-oxide
$C_6H_{11}NO$ = 113.16
CAS No. [3317-61-1]
Unit: 1 ml

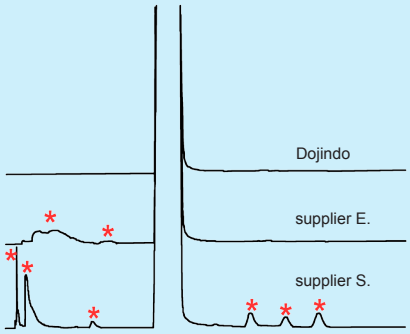


Fig. 2 Purity comparison in HPLC spectra
(*: impurities)

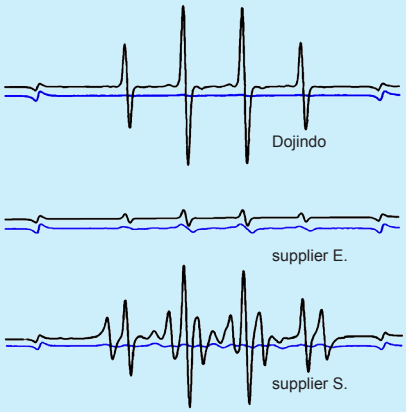


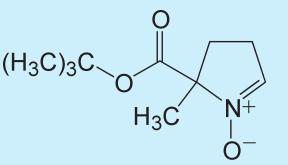
Fig. 3 Purity comparison of ESR spectra
(black: fenton reaction, blue: blank)

Radical Scavenger, ESR probe for Superoxide and Hydroxyradical

BMPO

Product Code: B568

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 ($t_{1/2}$=24 min) than other spin trap reagents. It gives us reproducible and steady results. Because BMPO is highly soluble in water, hydrophilic sample is applicable for analyzing the free radicals.



BMPO
5-*tert*-Butoxycarbonyl-5-methyl-1-pyrroline *N*-oxide
$C_{10}H_{17}NO_3$ = 199.25
Unit: 50 mg

1. General Protocol

► Measuring hydroxy radical from Fenton reaction

1. Add 15 µl of BMPO solution, 75 µl of 1 mM H_2O_2 and 75 µl of 100 µM $FeSO_4$ to 50 µl of ddH_2O.
2. Transfer the solution to ESR sample tube and measure ESR spectra after certain time of period, e.g. 1 minute.
3. 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(pH 7.4)(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.

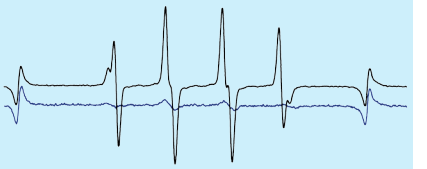


Fig. 1 ESR Spectra of hydroxy radical adduct
(black: fenton reaction, blue: blank)

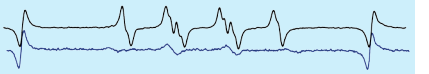


Fig. 2 ESR Spectra of superoxide radical adduct
(black: XO reaction, blue: blank)

2. References

1. H. Zhao, J. Joseph, H. Zhang, H. Karoui and B. Kalyanaraman, *Free Radic Biol Med*. 2001;**31**:599-606.
2. G. M. Rosen, P. Tsai, J. Weaver, S. Porasuphatana, L. J. Roman, A. A. Starkov, G. Fiskum and S. Pou, *J Biol Chem*. 2002;**277**:40275-40280.

3. Specification

► Appearance: white crystal or crystalline powder
► Purity: ≥99.0%(HPLC)

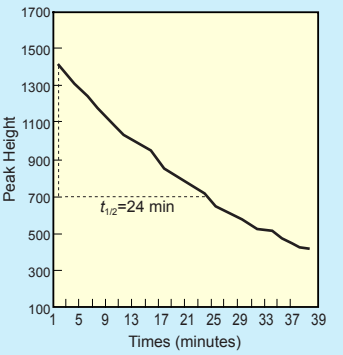


Fig. 3 Half-life of BMPO

NO Scavenger, ESR probe for NO

Carboxy-PTIO

Product Code: C348

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 NG-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.

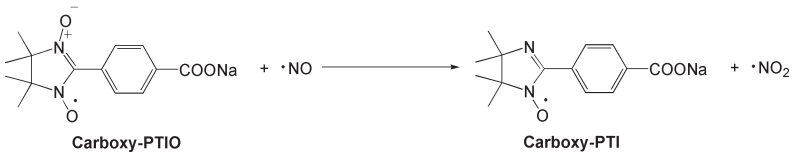


Fig. 1 Reaction of NO trapping

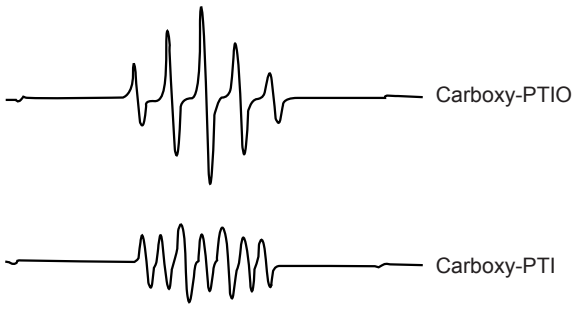
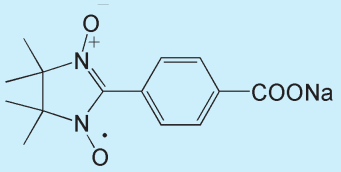


Fig. 2 ESR Spectra of Carboxy-PTIO and Carboxy-PTI



Carboxy-PTIO
2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, sodium salt
$C_{14}H_{16}N_2NaO_4$ = 299.28
CAS No. [148819-93-6]
Unit: 10 mg

2. 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.

3. Specification

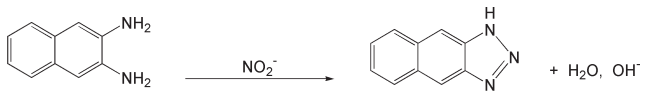
- Appearance: dark blue powder
- Purity: ≥97.0%(TLC)

Fluorescence NO probe

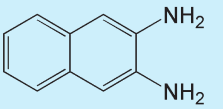
2,3-Diaminonaphthalene (for NO detection)

Product Code: D418

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.



2,3-Diaminonaphthalene *Naphthalenetriazole*



2,3-Diaminonaphthalene(for NO detection)
2,3-Diaminonaphthalene
$C_{10}H_{10}N_2$ = 158.20
CAS No. [771-97-1]
Unit: 10 mg

1. General Protocol

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.
3. Incubate the mixture at room temperature for 10-15 minutes.
4. Add 5 µl 2.8 M NaOH solution to the reaction solution.[b)]
5. 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.
6. Prepare a calibration curve using this data where the X-axis is $NaNO_2$ 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.

2. References

1. W. R. Tracey, *et al.*, Comparison of spectrophotometric and biological assays for nitric oxide (NO) and endothelium-derived relaxing factor (EDRF): nonspecificity of the diazotization reaction for NO and failure to detect EDRF. *J Pharmacol Exp Ther.* 1990;**252**:922-928.
2. J. S. Pollock, *et al.*, Purification and characterization of particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells. *PNAS*. 1991;**88**:10480-10484.
3. S. Archer, Measurement of nitric oxide in biological models. *FASEB J.* 1993;**7**:349-360.
4. J. H. Wiersma, 2,3-Diaminonaphthalene as a Spectrophotometric and Fluorometric Reagent for the Determination of Nitrite Ion. *Anal Lett.* 1970;**3**:123-132.
5. C. R. Sawicki, Fluorimetric Determination of Nitrate. *Anal Lett.* 1971;**4**:761-775.
6. P. Damiani, *et al.*, Fluorometric determination of nitrite. *Talanta*. 1986;**8**:649-652.
7. T. P. Misko, *et al.*, A fluorometric assay for the measurement of nitrite in biological samples. *Anal Biochem.* 1993;**214**:11-16.
8. G. L. Wheeler, et al., Rapid determination of trace amounts of selenium (IV), nitrite, and nitrate by high-pressure liquid chromatography using 2,3-diaminonaphthalene. *Microchem J.* 1974;**19**:390-405.

3. Specification

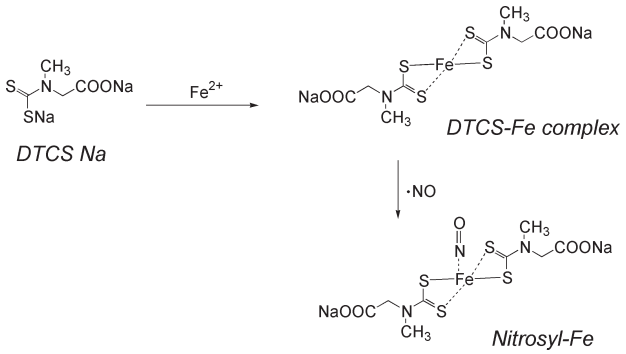
- ► Appearance: white or pale yellowish-brown powder
- ► Melting Point: 185°C to 200°C

ESR probe for NO

DTCS Na

Product Code: D465

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 spin-trapping reagent for biochemical research. 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.



1. General Protocol

► 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 water[a] to prepare 50 mM DTCS solution.
3. Mix 1 ml DTCS Na solution with 8.8 ml buffer solution[a] (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 by dissolved oxygen in the solution. However, the brown solution can still be used for NO trapping.

► Preparation of NO-Fe(II)-DTCS Complex

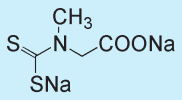
1. Under argon gas flow, add 200 µl of $FeSO_4$ solution to 9.4 ml buffer solution (pH 7 or higher). Then introduce NO into the solution by bubbling NO gas through a glass capillary for 15 minutes.
2. Add 400 ml of DTCS Na solution to the $FeSO_4$ solution, and continue to introduce NO by bubbling for 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.

2. 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.

3. Specification

► Appearance: white or pale yellow powder



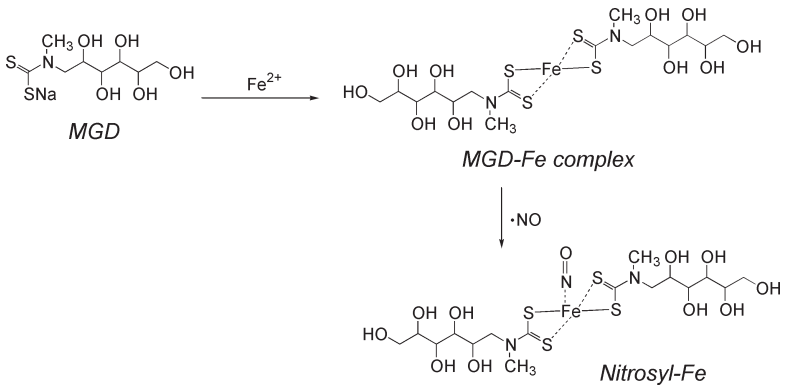
DTCS Na
N-(Dithiocarboxy)sarcosine, disodium salt, dihydrate
$C_4H_5NNa_2O_2S_2 \cdot 2H_2O$ = 245.23
CAS No. [13442-87-0
Unit: 100 mg, 500 mg

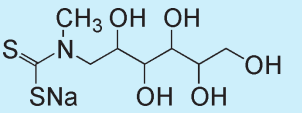
ESR probe for NO

MGD

Product Code: M323

MGD is a highly water-soluble dithiocarbamate-type chelator that generates many transitional metal complexes 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.





MGD
N-(Dithiocarbamoyl)-*N*-methyl-D-gluca-mine, sodium salt
$C_8H_{16}NNa_2O_5S_2$ =293.34
CAS No. [94161-07-6(free acid)]
Unit: 500 mg

1. General Protocol

► 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. 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.

3. Specification

► Appearance: white crystalline powder
► Purity: ≥98.0%(HPLC)

NO Donor

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. It is also confirmed that the by-products do not possess any significant bioactivities even though the NO release mechanism of NOR has not been completely determined. 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.

NO Donors			
NOR 1	NOR 3	NOR 4	NOR 5
1.8 min	30 min	60 min	20 hrs

Fig.1 Half-life of NOR donors(pH 7.4)

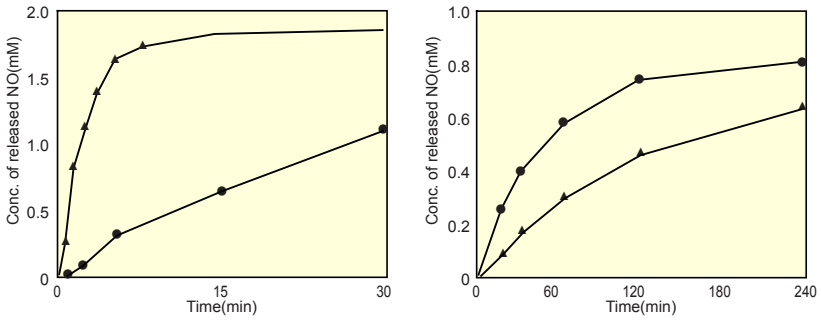


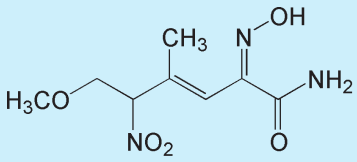
Fig.2 Time course of releasing NO from NOC compounds

1. References

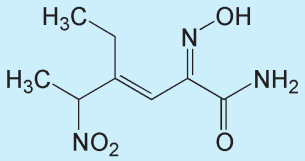
1. S. Shibata, *et al.*, Characteristics of the Vasorelaxing Action of (3E)-4-Ethyl-2-hydroxyimino-5-nitro-3-hexamide FK409, a New Vasodilator Isolated from Microbial Sources, in Isolated Rabbit Arteries. *J Cardiovasc Pharmacol.* 1991;**17**:508-518.
2. Y. Kita, *et al.*, Antianginal Effects of FK409, 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.

2. Specification

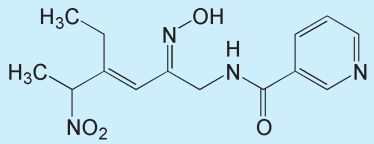
- ► Appearance: White or slightly yellow powder(NOR 1, NOR 4, NOR 5), White crystalline powder(NOR 3)
- ► Purity: ≥98.0%(HPLC)



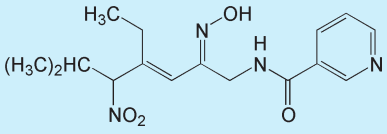
NOR 1 (Product code: N388)
(±)-(E)-4-Methyl-2-[(E)-hydroxyimino]-5- nitro-6-methoxy-3-hexenamide
$C_8H_{13}N_3O_5$ =231.21
CAS No. [163032-70-0]
Unit: 10 mg



NOR 3 (Product code: N390)
(±)-(E)-4-Ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide
$C_8H_{13}N_3O_4$ =215.21
CAS No. [163180-49-2]
Unit: 10 mg



NOR 4 (Product code: N391)
(±)-*N*-[(E)-4-Ethyl-2-[(Z)-hydroxyimino]-5-nitro-3-hexene-1-yl]-3-pyridinecarboxamide
$C_{14}H_{18}N_4O_4$ =306.32
CAS No. [162626-99-5]
Unit: 10 mg



NOR 5 (Product code: N448)
(±)-*N*-[(E)-4-ethyl-3-[(Z)-hydroxyimino]-6-methyl-5-nitro-3-heptenyl]-3-pyridine-carboxamide
$C_{16}H_{22}N_4O_4$ = 334.37
Unit: 10 mg

NO Donor

NOC Compounds

NOCs are stable NO-amine complexes that spontaneously release NO, without co-factors, and under physiological conditions. The rate of NO release depends on the chemical structure of 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.

1. General Protocol

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.

		NO Donors			
		NOC 5	NOC 7	NOC 12	NOC 18
pH	7.0	12 min	2.2 min	40 min	13 hrs
	7.2	20 min	3.8 min	1.2 hrs	18 hrs
	7.4	25 min	5 min	100 min	21 hrs
	7.6	42 min	8.2 min	3 hrs	34 hrs
	7.8	66 min	12.4 min	4.6 hrs	45 hrs

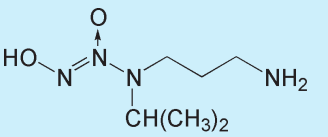
Fig.1 Half-life of NOC donors

2. References

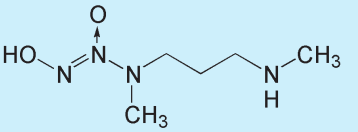
1. K. Hayashi, *et al.*, Action of Nitric Oxide as a Antioxidant Against Oxidation of Soybean Phosphatidylcholine Liposomal Membrane. *FEBS Lett.* 1995;**370**:37-40. (Noc 12)
2. S. Shibuta, *et al.*, Intracerebroventricular Administration of a Nitric Oxide-releasing Compound, NOC-18, Produces Thermal Hyperalgesia in Rats. *Neurosci Lett.* 1995;**187**:103-106. (NOC 18)
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)

3. Specification

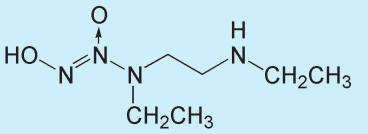
- ► Appearance: White powder
- ► Purity: ≥90.0%(HPLC)



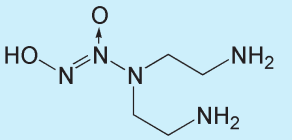
NOC 5 (Product code: N380)
1-Hydroxy-2-oxo-3-(3-aminopropyl)-3-isopropyl-1-triazene
$C_6H_{16}N_4O_2$ = 176.22
CAS No. [146724-82-5]
Unit: 10 mg, 50 mg



NOC 7 (Product code: N377)
1-Hydroxy-2-oxo-3-(*N*-methyl-3-amino-propyl)-3-methyl-1-triazene
$C_5H_{14}N_4O_2$ = 162.19
CAS No. [146724-84-7]
Unit: 10 mg, 50 mg



NOC 12 (Product code: N378)
1-Hydroxy-2-oxo-3-(*N*-ethyl-2-aminoethyl)-3-ethyl-1-triazene
$C_6H_{16}N_4O_2$ = 176.22
CAS No. [146724-89-2]
Unit: 10 mg, 50 mg



NOC 18 (Product code: N379)
1-Hydroxy-2-oxo-3,3-bis(2-aminoethyl)-1-tria-zene
$C_4H_{13}N_5O_2$ = 163.18
CAS No. [146724-94-9]
Unit: 10 mg, 50 mg

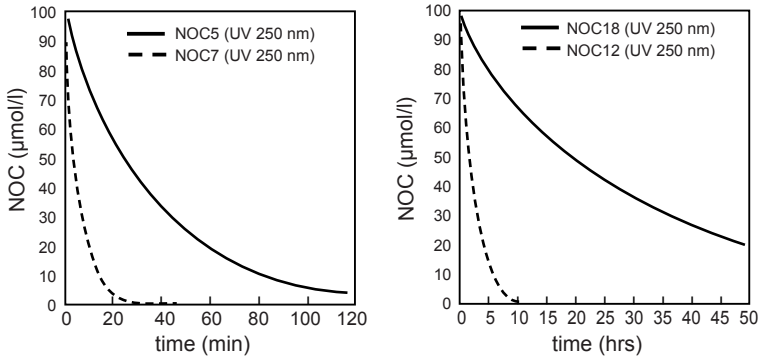


Fig. 1 Time course of NOC degradation
(100 µM NOCs in 100 mM PBS, pH7.0, 37°C)

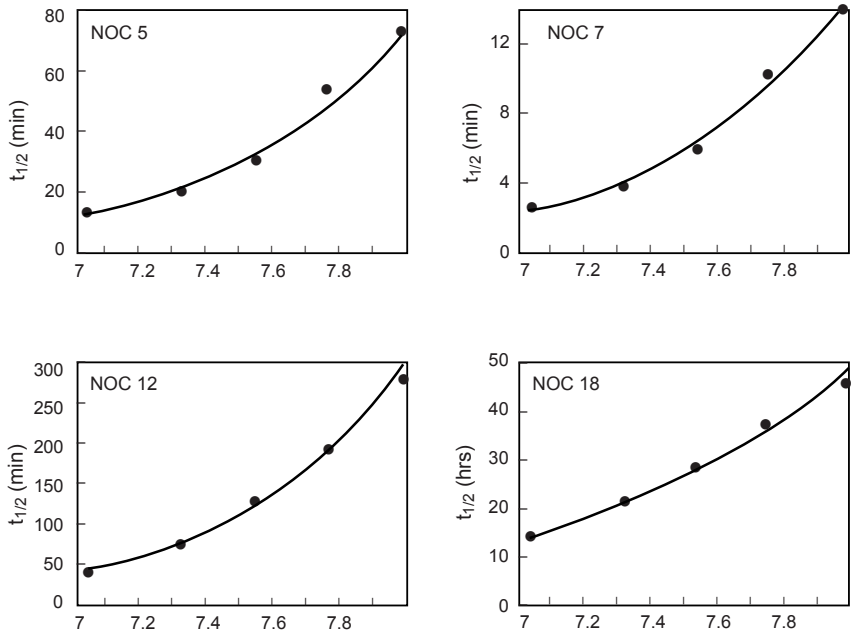


Fig. 2 Half life of NOC compounds in 100mM PBS(37°C)
under the various pH conditions

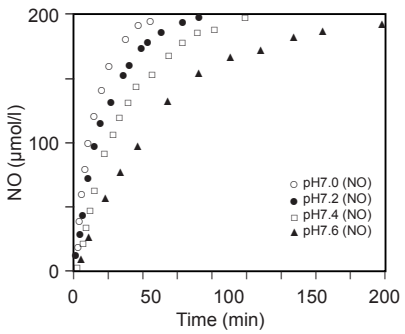


Fig. 3 pH dependency of releasing NO from NOC 5
(0.1 mM NOC 5, in 100 mM PBS at 37°C)

NO Donor

S-Nitrosoglutathione

Product Code: N415

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 NO; however, this does not seem to be the current majority view. Though 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

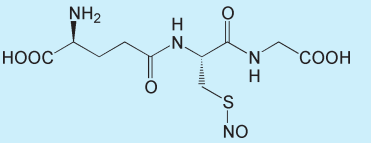
This reaction is accelerated by light and heat. If GSNO is incubated at 37°C without light, NO will not be released spontaneously. Metal ions, such as Cu(II), Cu(I), and Hg(II), also accelerate the reaction. Thus, masking reagents such as EDTA prevent the releasing reaction. Another important characteristic 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 the platelet aggregation.



S-Nitrosoglutathione
N-(*N*-L-γ-Glutamyl-*S*-nitroso-L-cysteinyl)glycine
$C_{10}H_{16}N_4O_7S$ = 336.32
CAS No. [57564-91-7]
Unit: 25 mg, 100 mg

1. 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.
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5. D. Barrachina, *et al.*, Nitric Oxide Donors Preferencially Inhibit Neuronally Mediated Rat Gastric Acid Secretion. *Eur J Pharmacol.* 1994;**262**:181-183.
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2. Specification

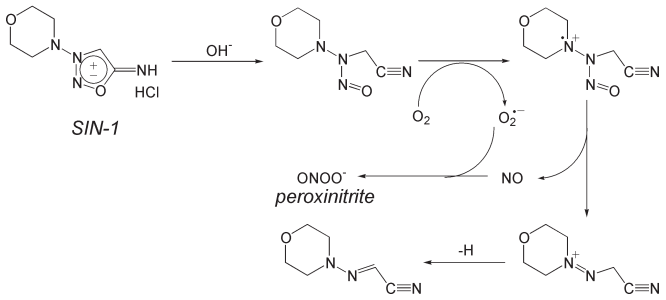
- ► Appearance: Pink powder
- ► Purity: ≥90.0%(HPLC)

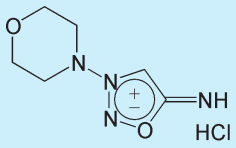
Peroxinitrite Donor

SIN-1

Product Code: S264

SIN-1, a metabolite of the vasodilator molsidomine, is utilized to individually 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: 3.7×10^{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.





SIN-1
3-(4-Morpholinyl)sydnonimine, hydrochloride
$C_6H_{11}ClN_4O_2$ = 206.63
CAS No. [16142-27-1]
Unit: 25 mg

1. References

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2. Specification

- Appearance: White needles or slightly yellowish-white crystalline powder
- Purity: pass test(TLC)
- Melting Point: 180°C to 190°C