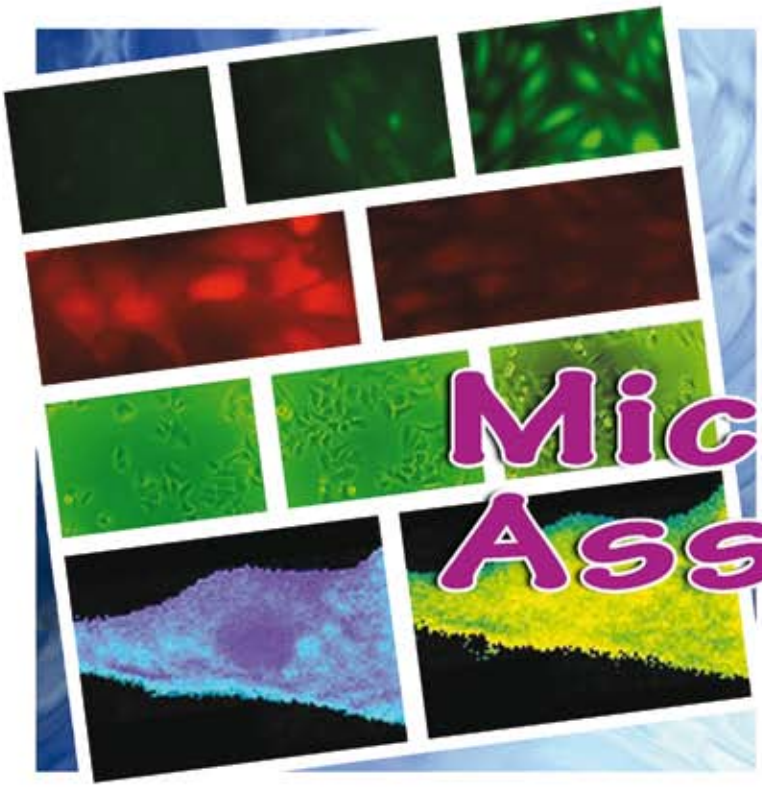
Horizons Nouveaux

Interchim Innovations

11-2008

211 bis avenue J.F. Kennedy - BP 1140 - 03103 Montluçon Cedex - Tel 33 (0)4 70 03 88 55 - Fax 33 (0)4 70 03 82 60 - email interchim@interchim.com - www.interchim.com



Special Microplate Assays

Techniques and solutions for....

- Reporter Gene Assays
- Enzymes Detection
- Calcium Assays
- Viability & Cytotoxicity Assays
- Apoptosis Assays
- DNA, Proteins & Glucides Biochemistry Assays
- Immunological Assays
- Accessory tools

interchim .com

Edito

This HN Microplates Assays presents a selection for reagents and kits to use in microplate readers. All the applications can be performed on the microplate instruments from our partner BERTHOLD TECHNOLOGIES.

Focus on fluorescence and luminescence technologies, FluoProbes® provides useful tools from gene expression studies, cell cytotoxicity to protein and DNA assays.
With the continuous development of fluorescent technologies, FluoProbes® offers the state-of-the-art dyes like Fluo-8 for calcium assays. Also our production capacities enable us in undertaking bulk requirements of our clients and competitive pricing.

Jean Boch, PhD President

Lionel Boch, PDG

How to order

Tel. 33 (0)4 70 03 88 55

Hot Line

BioScience Innovations
33 (0)4 70 03 73 06

Télécopie 33 (0)4 70 03 82 60

Mail

Interchim
211 bis avenue J.F. Kennedy
BP 1140
03103 Montluçon cedex

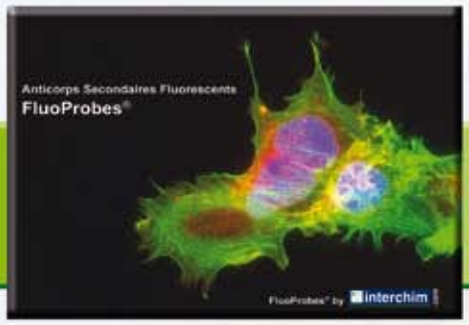
e-mail

Interchim@interchim.com

Order on line

http://www.interchim.com

Secure Web site.
An easy 24h/24 h acces to thousands of Products, Innovations and special offer.
Order On-Line though only few cliks.



Get your personal brochure at interchim.prospect@interchim.com

Interchim and Berthold Technologies collaboration

Interchim, a provider of consummables for life sciences, and Berthold Technologies, a leader in microplate instrumentation technology, have entered into a collaboration agreement to offer complete instrumentation and reagent solutions.

BERTHOLD TECHNOLOGIES provides with the Mithras and TriStar extremely versatile multimode readers for all comprehensive technologies used in today´s laboratory.

Additionally dedicated microplate readers for luminescence, fluorescence and absorbance can be offered for all common microplate formats. Petri dishes and Teraski plates can be measured with respective adapters. Powerful software allows kinetics, scanning, repeated mode, dual ratio measurements etc.

For higher troughput the instruments can be run with the Stacker LB 931. Robot access enables integration into robotic HTS systems.

Mithras LB 940

TriStar LB 941

For instrument informations, please contact :

Berthold France SAS
Parc Technologique des Bruyères
8, route des Bruyères
78770 Thoiry
France

Phone : (+33) 1 34 94 79 00
Fax : (+33) 1 34 94 79 01
E-mail : France@Berthold.com

Summary

Firefly Luciferase

Interchim provides kits and stand-alone reagents to study the expression of Luciferases from different species. Beside the classical *Firefly* and *Renilla* luciferases, we also offer with the new Gaussia luciferases for improved signal intensity :

Comparison of different species luciferases

Species	Luciferase	Size	Quantum Yield	Wavelenght	ATP dependency	Substrate
Photynus pyralis (Firefly)	Fluc	550 aa	>88%	562 nm	YES	D-luciferin
Renilla reniformis (Sea pansy)	RLuc	311 aa	>6%	480 nm	NO	coelenterazine
Gaussia princeps (Copepod)	Gluc	185 aa	1.6 x 10^{16} Qps/mg	480 nm	NO	coelenterazine

Firefly luciferase is widely used as a reporter gene for studying gene regulation and function, and for pharmaceutical screening. It is a very sensitive genetic reporter due to the lack of any endogenous activity in mammalian cells or tissues. The Firefly luciferase is a 62 000 Dalton protein, which is active as a monomer and does not require subsequent processing for its activity. The enzyme catalyzes ATP-dependent D-luciferin oxidation by oxygen into oxyluciferin with emission of light centered on 562 nm (figure 1).

Figure 1 : Bioluminescent reaction catalyzed by Firefly luciferase.

However, the light production resulting from the reaction leads to formation of suicidal adenyloxyluciferin at the enzyme surface. It results in very short half-life of the light emission with a flash-type kinetics. Several substances have been described to prolong light production by regenerating enzyme through removing inhibitory oxyluciferin from the enzyme surface. But the duration (10-15 min) is still too short for batch process screening.

Our luciferase assay kits provide a long lasting signal (steady glow) by preventing the formation of adenyl-oxyluciferin at the enzyme surface.

Technical tip

Microplate Readers

Interchim and Berthold collaboration supports further your works. Many of our fluorescence and luminescence reagents and kits were validated with Berthold Technologies microplate readers.

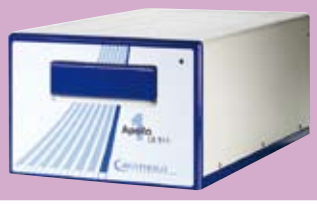
***Mithras LB940 MultiMode Reader.**
Includes a variety of technologies with samples injectors and robot integration module.

. Various formats (from Petri dishes to 1536 well plates)
. Absorbance
. Luminescence Flash & Glow
. Fluorescence
. top and bottom measurement
. Polarisation (FP)
. FRET
. BRET
. AlphaScreen™
. TRF & HTRF® (Time Resolved Fluorescence & Time Resolved FRET)

***Centro XS LB960 Luminometer**
A robust, versatile and sensitive microplate luminometer (lowest crosstalk) exists also in a Clinical version (LB961).

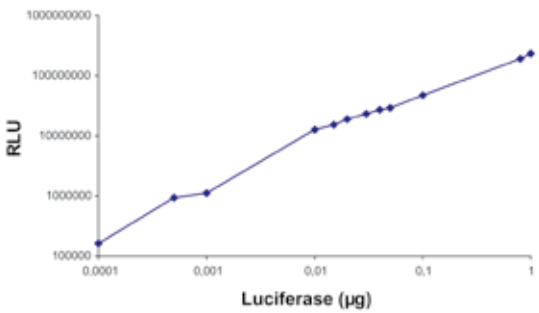
***Twinkle LB970 Fluorometer**
Reading from above and below, from Petri dishes to 864 well microplates. Ideal for sensitive FRET assays

***Apollo LB912 Absorbance Reader**
96 wells microplate in 340-800 nm range, with 8-channels



Firefly Luciferase 1-Step Assay Kit, 2 h reading

- Linear range – Assay **linear over seven orders** of magnitude
- Limit of detection – less than **1 fg of luciferase** per sample
- **No disposal problems or hazards** are associated with the use of this luciferase assay kit
- Reproducibility – CV less than 5%



Sensibility study on the Mithras luminometer from Berthold Technologies : Different quantities of Firefly luciferase on the range of 0.0001-1 µg (0.005-50 µg/ml) have been assayed.

Luciferase 1-Step assay system is a homogeneous high sensitivity firefly luciferase reporter gene assay kit with a half-life of 2 hours for the quantification of firefly luciferase expression in mammalian cells. This kit is specially designed for batch processing systems using microplates such as 96-well plates. In addition, Interchim Luciferase 1-Step assay kit offers higher sensitivity and wider dynamic range for detecting luciferase activity within mammalian cells, consistent reproducibility and cost effectiveness along with the added convenience of a one step assay.

Description	P/N :	Qty
Firefly Luciferase 1-Step Assay Kit	**FP-BX0320**	100 ml (1000 tests in 96-well plate)
	FP-BX0321	100 ml (1000 tests in 96-well plate)

Firefly Luciferase Stable Assay Kit, 3-5 h reading

- **Simple :** single step assay
- **High sensitivity :** higher sensitivity than others steady substrates
- **Suitable for HTS** - batch processing

This kit is a homogeneous high sensitivity firefly luciferase reporter gene assay kit with a half-life of 3-5 hours for the quantification of firefly luciferase expression in mammalian cells. It is specifically designed for batch processing systems using high-density microplates such as 384- and 1536-well plates, in high throughput environments.

Description	P/N :	Qty
Firefly Luciferase Stable Assay Kit, 3-5 h reading	**FP-BU6870**	10 ml
	FP-BU6871	100 ml
	FP-BU6872	1000 ml

Kit contents (10/100/1000 ml) :
1 vial (2.5 /25 /250 mg) of D-Luciferin
1 bottle (10 /100/ 1000 mL) Firefly Assay Buffer
10 ml are sufficient for 100, 400 and 3,300 assays in 96-well, 384-well and 1536-well microplates.

Firefly Luciferase, recombinant, from Photinus pyralis

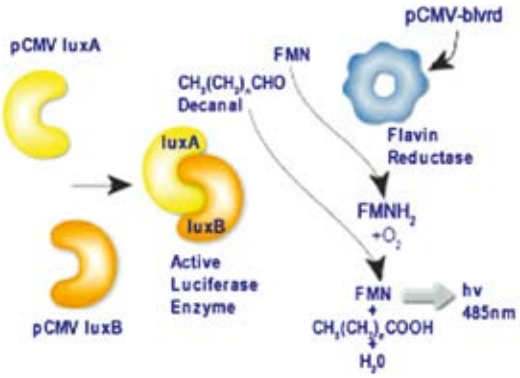
Luciferase can be used to detect trace amounts of ATP (signalling biological contamination). Less than or equal to one femtomole of ATP can be detected using 0.2 µg of luciferase.
Recombinant Firefly luciferase can also be used to prepare standard curve of reporter genes for the study of gene expression.

Description	P/N :	Qty
Firefly Luciferase, recombinant, from *Photinus pyralis*	**FP-D1826B**	1 mg

■ Mammalian Expression Vectors : pCMVLuxA & pCMVLuxB

A single reporter to monitor expression of two cloned genes

The light-emitting reaction of the marine bioluminescent bacterium *Vibrio harveyi* is catalyzed by the bacterial luciferase enzyme which exists as an alpha-beta heterodimer encoded by the luxA and luxB genes with subunit molecular weights of 42K and 37K respectively. The enzyme catalyzes a reaction with FMNH2, oxygen and a long-chain aldehyde as substrates to yield visible light at 490 nm. A new luciferase marker gene detection system has been developed based upon this bacterial luciferase isolated from *Vibrio harveyi*. Sequences encoding the two luciferase subunits, luxA and luxB have been cloned into two separate vectors. These vectors also include a CMV promoter for expression in mammalian cells as well as an ampicillin resistance gene (100 ug/mL ampicillin resistance) for selection and amplification, the SV40 polyadenylation sequence and the SD/SA-RNA splice donor and acceptor sequence for maximum expression. In addition, the LuxA or LuxB gene can be excised using the flanking NotI sites to allow the insertion of other genes to be expressed under the same regulatory elements in mammalian cells. These systems are being developed to monitor regulation of expression for two independent vector constructs, upon the dual expression.



Description	P/N :	Qty
pCMVLuxA Mammalian LuxA Expression Vector	**DO8130**	20 µg
pCMVLuxA Mammalian LuxB Expression Vector	**DO8140**	20 µg

■ Firefly luciferase siRNA constructs : siFLuc

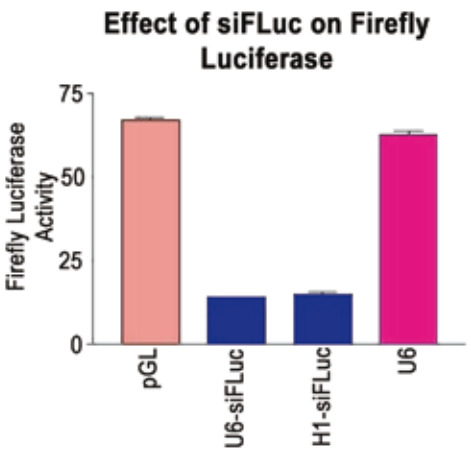
siFLuc are siRNA constructs designed to knock down Firefly luciferase.
In our control experiments, this siRNA can knock down Firefly luciferase activity by ~80%.
The target sequence matches pGL3-control, and is designed to silence Firefly luciferase expressed by a co-transfected pGL3-control vector.

Description	P/N :	Qty
pRNA-U6.1/Neo/siFLuc (positive control in mammalian transfection)	**BG9670**	10 µg

pRNA-U6.1/Neo/siFLuc is a siRNA expression vector designed for mammalian transfection. It uses U6 promoter for siRNA expression. This vector contains siRNA construct for firefly luciferase, and can be used as a positive control. It can also be used as a siRNA vector using BamH I and Hind III for siRNA insertion.

Please contact us for others promoters available :

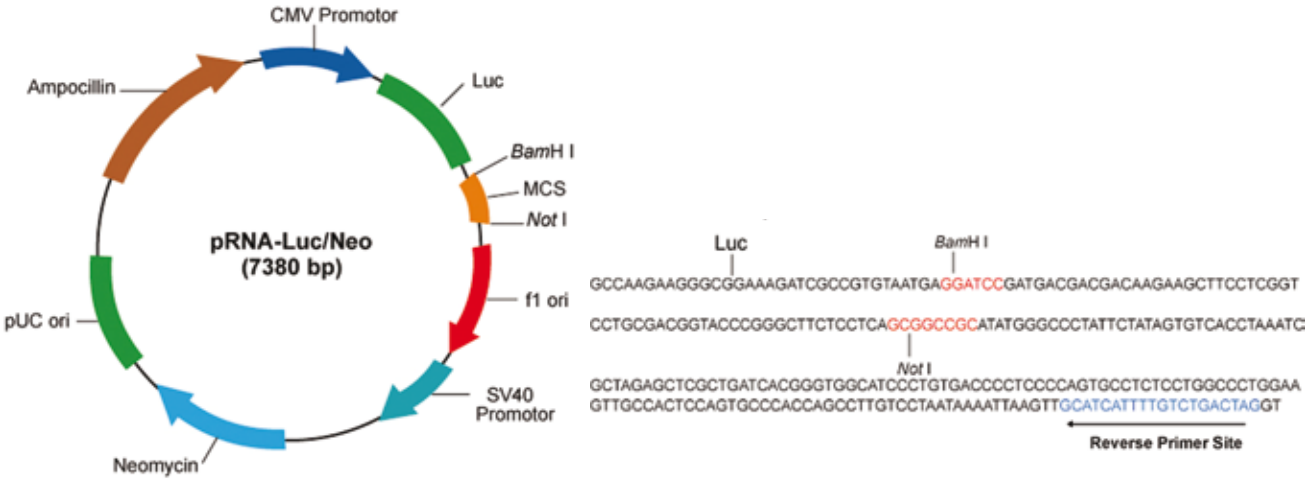
- pGL : HEK293 cells transfected with pGL3-control (0.16 ug) and pRL-TK (0.16 ug)
- U6-siLuc : HEK293 cells transfected with pGL3-control (0.16 ug), pRL-TK (0.16 ug), and 1.6 ug of pRNA-U6.1/Neo/siLuc
- H1-siLuc : HEK293 cells transfected with pGL3-control (0.16 ug), pRL-TK (0.16 ug), and 1.6 ug of pRNA-H1.1/Neo/siLuc



U6: HEK293 cells transfected with pGL3-control (0.16 ug) and pRL-TK (0.16 ug), and 1.6 ug of pRNA-U6.1/Neo empty vector.

pRNA Luc/Neo for monitoring transcriptional activity

The pRNA-Luc/Neo includes a modified coding region for firefly (*Photinus pyralis*) luciferase that has been optimized for monitoring transcriptional activity in transfected eukaryotic cells. The purpose of this reporter vector is to screen for efficient siRNA for the target gene using Luc activity as a reporter gene. The principle is that when a siRNA silence the target gene by degrading mRNA, the Luc will not be expressed either, because the mRNA for both the gene and Luc as a whole molecule is degraded. The assay of this genetic reporter is rapid, sensitive and quantitative.



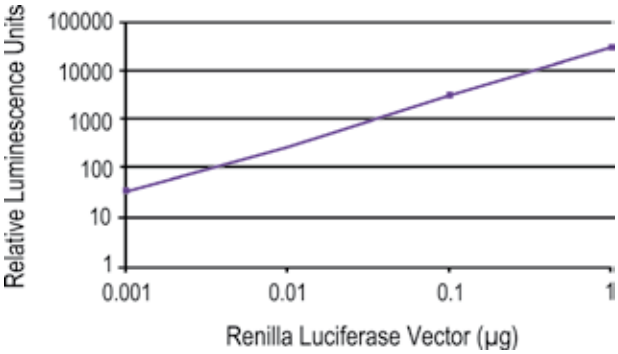
Description	P/N :	Qty
pRNA-Luciferase-Neomycine	**DT3120**	10 µg
Related product		
β-Amyloid (1-40)	**HT8360**	0.5 mg
	HT8361	1 mg

■ Renilla Luciferase Assay Kit

Reporter gene used as normalizing transfection control

- **Sensitivity and Linearity :** Linear correlation between luciferase gene expression and light output for transfection using 1 ng to 1 µg DNA of a Firefly luciferase reporter construct
- **Conveniently packaged** substrate sizes permitting you to run a variable number of assays

Renilla luciferase has been used as a reporter gene for studying gene regulation and function in vitro and in vivo. Recently, Renilla luciferase has been widely used in multiplex transcriptional reporter assays or as a normalizing transfection control for Firefly luciferase assay. Renilla luciferase, a monomeric 36 000 Dalton protein, catalyzes coelenterazine oxidation by oxygen to produce light. The enzyme does not require post-translational modification for its activity, and may function as a genetic reporter immediately following translation. native Coelenterazine is the natural substrate for Renilla luciferase. However, over a dozen of coelenterazine analogs have been synthesized, now commercially available from Interchim. These coelenterazine analogs all function as substrates for Renilla luciferase with different properties in terms of emission wavelength, cell membrane permeability and quantum efficiency.



Coelenterazine also emits light from enzyme-independent oxidation (autoluminescence), enhanced by superoxide anion and peroxynitrite in cells and tissues. Through the use of a specially designed coelenterazine derivative and buffer formulation, the Renilla Luciferase Assay Kit yields reliable, linear results with minimal autoluminescence background and superior sensitivity.

Description	P/N :	Qty
Renilla Luciferase Assay Kit	**FP-BE7930**	150 tests
	FP-BE7931	1000 tests

Kit contents : Coelenterazine, Renilla Luciferase Lysis Buffer, Renilla Luciferase Assay Buffer, Renilla Luciferase Assay Enhancer

Related substrates :
See "Coelenterazines", as stand alone products

■ Renilla Mullerei Luciferase, recombinant

Description	P/N :	Qty
Renilla Mullerei Luciferase, 95% purity (more than 8 x 10^{14} Qps/mg)	**FP-BX6710**	1 mg

■ Renilla Mullerei Luciferase, pUC19 plasmid (pRLuc)

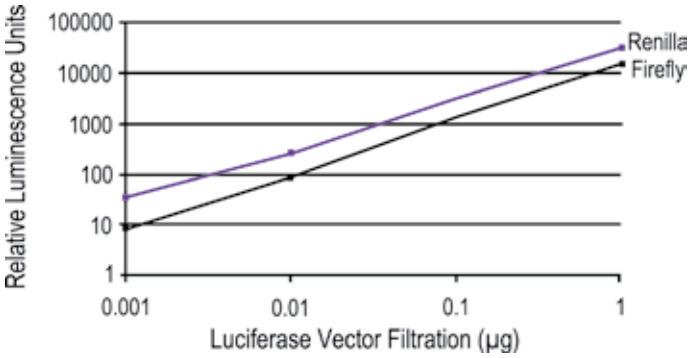
These protein uses coelenterazine and coelenterazine derivatives as substrate.
The Renilla protein expresses well in bacteria.

Description	P/N :	Qty
pUC19 pRLuc	**FP-BS8180**	25 µg

Firefly and Renilla Luciferases Assay Kit

- **Sensitivity and Linearity :** Linear correlation between reporter gene expression and light output for transfection using 1 ng to 1 µg DNA of either Firefly or Renilla luciferase reporter construct.
- **Low Autoluminescence & High Sensitivity :** Reduced autoluminescence background for Renilla luciferase assay and consequently increased sensitivity.
- **Convenient :** One kit for both luciferase assays.

Firefly and Renilla luciferases are widely used as reporter genes for studying gene regulation and function, and for pharmaceutical screening. Renilla Luciferase is often used in conjunction with Firefly Luciferase as a normalizing transfection control or for multiplex transcriptional reporter assays. As with many enzymes, Firefly luciferase and Renilla luciferase follow Michaelis-Menten kinetics and thus maximum light output is not achieved until substrates (above the Km) and co-factor are present in large excess. When assayed under these conditions, light emitted from the reaction is directly proportional to the number of luciferase enzyme molecules. Our Firefly & Renilla luciferase assay kit is designed for detection and quantification of Firefly and Renilla luciferase reporter enzymes from cultured cells in a simple, efficient and linear fashion.



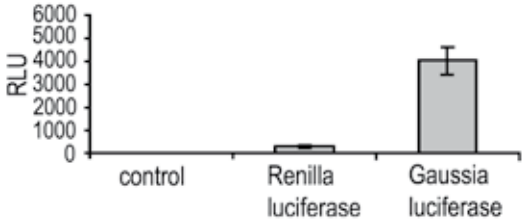
Description	P/N :	Qty
Firefly and Renilla Luciferases Assay Kit	**FP-BE7810**	10 ml
	FP-BE7811	100 ml

Kit contents per 10 ml : 2 x 1 mg D-Luciferin, 100 uL 100X Coelenterazine, 10 mL 5X Passive Lysis Buffer, 10 mL Firefly Luciferase Assay Buffer, 10 mL Renilla Luciferase Assay Buffer, 10 mL Renilla Luciferase Assay Enhancer.

Humanized Gaussia Luciferase

Gaussia luciferase, a novel reporter for gene expression, is the smallest and brightest known luciferase. Recommended for studying weak promoters, hard-to-transfect cells and HTS applications.

- **Greater brightness :** *Gaussia* luciferase expressed in mammalian cells is as much as 750-fold brighter than native *Renilla reniformis* luciferase
- **Avoid cell lysis :** *Gaussia* luciferase with secretion signal is secreted into the media. It is therefore necessary to only assay supernatants for enzyme activity without the need for lysing the cells. Considerable time is saved since time course experiments can be performed sampling the same group of transfected cells without lysing the cell
- **Extremely stable** to elevated temperature : up to 60°C and approx. 20% recovery following a 15 minute incubation at 99°C
- pH resistance : surviving a **pH range of 3-11**
- Resistance to detergents : 1-5% non-ionic detergents (NP-40, Triton X-100, Triton X-114, CHAPSO), cholate, deoxycholate etc
- Ability to recover activity after treatment with 7M guanidine chloride or 8M urea + NP-40



Gaussia luciferase uses coelenterazine and its derivatives to catalyse the oxidative decarboxylation of coelenterazine to produce coelenteramide and light. It has an emission spectral peak at 480 nm.

The specific activity of this luciferase in the presence of high concentrations of coelenterazine (10 µM) is extremely high : 1.24 x 10^{16} Qps/mg (Quanta per second per milligram)

Description	P/N :	Qty
pGluc-basic-1 promoter-less with secretion signal	**BU2550**	25 µg
A promoterless vector with a MCS site upstream of the humanized Gaussia luciferase coding sequence (with secretion signal). This vector is designed for promoter analysis and will express secreted Gaussia luciferase. The transfected cells can be reused for multiple sampling.		
pCMV-Gluc-1 positive control with secretion signal	**BS8160**	25 µg
This positive control vector is very useful in evaluating the efficiency of transgene expression using Gaussia luciferase as a reporter. This vector has both Ampicillin resistance and Neomycin resistance. Therefore it can be easily propagated in *E. coli* and can be used to establish stable cell lines expressing Gaussia luciferase.		
pCMV-KDEL-Basic-1 for intracellular expression	**BU2570**	25 µg
pCMV-KDEL-Gluc-1 for intracellular expression	**BU2560**	25 µg
Related products :		
Anti-Gaussia Luciferase, rabbit titer >1:10000	**CJ3430**	250 µl
UptiFectin-On transfection reagent	**CK5060**	0.5 ml

Gaussia Luciferase Assay kit

The *Gaussia* luciferase assay kit **stabilizes the flash signal** emitted by the *Gaussia* luciferase thus making it possible to use it as a reporter gene for high throughput applications

The humanized *Gaussia* luciferase is secreted into the culture media and only the media needs to be assayed by the addition of native coelenterazine.

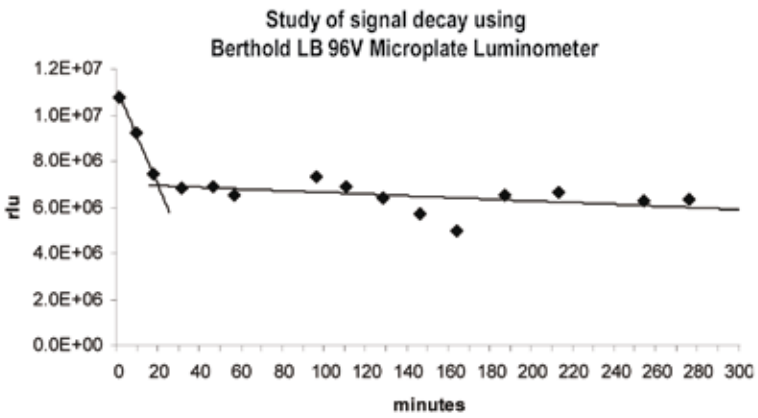
The *Gaussia* Assay Reagent (GAR) is prepared freshly by diluting the coelenterazine stock with the assay buffer. The assay is performed as following :
- Add 50µl of GAR to 20 µl *Gaussia* luciferase sample from microtiter or culture well samples
- Mix well and read in luminometer

Description	P/N :	Qty
Gaussia Luciferase Assay kit	**FP-BY7160**	5 ml (100 tests)
	FP-BY7161	50 ml (1000 tests)
Kit contains : pre-dissolved coelenterazine (100X concentration) and an assay buffer with stabilizers (increase emission up to 45 minutes).		

Coelenterazine – coelenterate luciferin – is the substrate for a number of marine bioluminescent enzymes, including those from marine organisms *Renilla, Gaussia, Pleuromamma* ***(luciferases)*** *Aequorea* ***(aequorin)*** *and Obelia* ***(obelin)***. In some of these reactions it is utilized as a simple substrate being catalytically turned over in the bioluminescent reaction *catalyzed by luciferases)*, while in others, such as aequorin or obelin, it is incorporated as part of the photoprotein.

■ Coelenterazine, native

The native coelenterazine, the luminophore of the native aequorin complex, is the standard substrate used in many applications using luciferase reporter assays. Bioluminescent detection of calcium concentration is highly sensitive in a broad concentration range (0.1µM to >100µM) [1-4]. Monitoring of reporter genes (phot gene and luc gene) using coelenterazine is also a major application. Other uses of coelenterazine include bioluminescence resonance energy transfert (BRET)[5,10] and chemiluminescent detection of superoxide anion and peroxynitrite in cells or tissues [6-9].
Coelenterazine native is recommended when a fast regeneration is important.



This result prompts to prepare FRESH Coelenterazine working solution and then to let it sit for 15-20 minutes at room temperature before use in order to achieve best accurate and high sensitivity.

References
1) Meth. Cell Biol. 40, 305(1994);
2) Meth. Enzymmol. 172, 164, (1989);
3) J. BioChem. 105, 473(1989);
4) J. Chem. Soc. Chem. Commun. 21, 1566(1986)
5) Meth. Enzymol. 57, 271(1978);
6) Tetrahedron Lett. 31, 2963(1973);
7) Nature 256, 236(1975);
8) Anal. BioChem. 219, 169(1994);
9) proc. Natl. Acad. Sci. U SA 96, 151(1999);
10) Molecular Pharmacology, 70:1802-1811 (2006)

Description	P/N :	Qty
Coelenterazine, native	**UP972331**	50 µg
Highest purity	**FP-97233B**	250 µg
	UP972333	1 mg
	UP972334	10 mg

Please contact us for bulk quote at interbiotech@interchim.com
Also available : Coelenterazine native for in vivo applications #FP-BV0730.

■ Coelenterazine 400a

Protein interactions study in BRET with GFP acceptor

Colenterazine 400a, also known as DeepBlue™ C, is a coelenterazine derivative that serves as a substrate for *Renilla luciferase* (Rluc) and generates an emission peak centered around 400 nm. It is the preferred Rluc substrate for BRET studies because it has minimal interference with the emission of the GFP acceptor (GFP vectors are presented in the Bioscience Innovation catalog).

Description	P/N :	Qty
Coelenterazine 400a	**UPBB8391**	50 µg
	FP-BB839B	250 µg
	UPBB8392	1 mg

See other coelenterazines in the Bioscience Innovation catalog.

■ Coelenterazine H (Benzyl-Coelenterazine)

For calcium assay in vitro or protein interactions study in BRET with YFP acceptor

Coelenterazine H works better with Calcium activated photoproteins (Aequorin, obelin) compared to native Coelenterazine; however this is true only *in vitro*. This cell membrane-permeable, very sensitive, specific, intracellular luminophore is useful for measuring changes in Ca^{2+} i.e. in cells that have been transfected with apoaequorin cDNA. In this system, coelenterazine is required for the regeneration of aequorin, a protein that emits light in the presence of calcium, from apoaequorin produced in cells. The luminescence intensity appears to be directly proportional to the Ca^{2+} concentration. Coelentrazine-H exhibits an approximate 16-fold greater luminescence intensity (emission max. : ~ 466 nm ; half-time total of 0.6 - 1.2 sec.) as compared to the native Coelenterazine. Has been used to measure intracellular Ca^{2+} signals in *Dictyostelium discoideum* chemotaxis and in plant wound healing.

The bioluminescence resonance energy transfer (BRET) method, between Renilla luciferase and a variant of GFP, the yellow fluorescent protein (YFP) allows real-time detection of protein-protein interactions in vivo.

Description	P/N :	Qty
Coelenterazine H	**UPR30782**	50 µg
	FP-R3078B	250 µg
	UPR30783	1 mg
	UPR30784	10 mg
Related product :		
BAPTA, AM	**FP-486103**	25 mg
	FP-486104	20 x 1 mg
This Ca^{+2} chelator that delays the peak and increases the duration of light emission from aequorin		

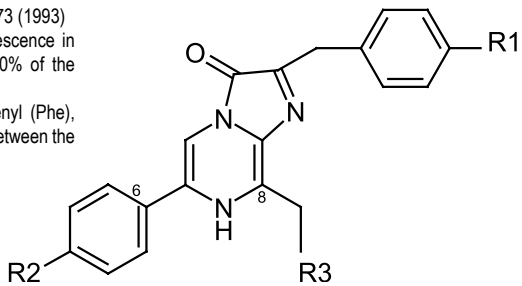
Also available : Coelenterazine H for in vivo #FP-BV0680

■ Other coelenterazines available

Table of Luminescent Properties of Coelenterazine Products with Apoaequorin*
See complete descriptions in the Bioscience Innovation catalog.

Coelenterazine	P/N :	R1 (2) #	R2 (6) #	R3 (8) #	λ max. Emission (nm)	Relative Luminescence capacité §	Relative Intensity §	Half-rise Time(s) §
Coelenterazine Native	**FP-97233A**	OH	OH	Phe	465	1.00	1.00	0.4-0.8
Coelenterazine cp	**FP-R3079A**	OH	OH	CP	442	0.95	20	0.15-0.3
Coelenterazine f	**FP-43876A**	F	OH	Phe	473	0.80	18	0.4-0.8
Coelenterazine fcp	**FP-R4711A**	F	OH	CP	452	0.57	135	0.4-0.8
Coelenterazine h	**FP-R3078A**	H	OH	Phe	464	0.82	10	0.4-0.8
Coelenterazine hcp	**FP-08353A**	H	OH	CP	444	0.67	190	0.15-0.3
Coelenterazine i	**FP-R3080A**	I	OH	Phe	476	0.70	0.03	8
Coelenterazine ip	**FP-R4712A**	I	OH	2P	441	0.54	47	1
Coelenterazine n	**FP-39819A**	Naph	OH	Phe	467	0.26	0.01	5

* All datas from BioChem. J. 261, 913(1989) (other data can be found in O.Shimoraura Cell Calcium 14, 373 (1993)
§ Luminescence capacity is the total light emission of aequorin in saturating Ca2+. Intensity of luminescence in saturating Ca2+ measured at max emission wavelength. Half-rise time is the delay elapsed to get 50% of the maximum emission.
# substituant groups R1, R2 and R3, in positions 2, 6 and 8, are hydrogen (H), hydroxyl (OH), Phenyl (Phe), CycloPentyl (CP), 2-propionyl (2P), Napthyl (Naph), methyl (Met). Coelenterazine e has a -CH2CHbridge between the 6-phenyl-OH and position 2 of the imidazopyrazinone core.



The popular reporter gene, luc gene, encodes the Firefly luciferase. The ATP-dependent oxidation of the substrate D-luciferin by oxygen produces an emission centered around 562 nm. The light output is proportional to luciferase concentration when both D-luciferin and ATP exist in large excess.

Interchim supplies D-luciferin in various forms : free acid, potassium salt and sodium salt and derivatives with acetoxymethyl (AM), methyl ether and DMNPE. The potassium and sodium salt forms are the most popular because they are readily water-soluble. The potassium salt is also the form used in live animal assay. Interchim's D-Luciferins are strictly controlled via several chemical analyses and also via a final enzyme assay to ensure consistency.

Description	P/N :	Qty
D-Luciferin, free acid, >99,0%	**FP-27060A**	25 mg
	FP-27060B	100 mg
	FP-27060C	250 mg
	FP-27060D	1 g
D-Luciferin, K^+ salt, >99,0% Potassium salt is the recommand salt form for in vivo uses.	**FP-M1224A**	25 mg
	FP-M1224B	50 mg
	FP-M1224C	500 mg
	FP-M1224D	1 g
D-Luciferin, Na^{2+} salt, >99,0%	**FP-726045**	10 mg
	FP-72604A	25 mg
	FP-72604B	50 mg
	FP-72604C	1 g
D-Luciferin AM, cell permeant	**FP-M1909A**	5 mg
The cell-permeant D-luciferin AM ester enters easily into live cells, and is well retained once it is cleaved by intracellular esterases to D-luciferin.		
D-Luciferin ethyl ether Cell permeant analog with 30% higher signal intensity	**FP-CF4421**	10 mg
DMNPE-caged D-Luciferin	**FP-21639A**	5 mg
DMNPE-caged D-luciferin is a D-luciferin ester derivative which can cross cell membranes efficiently. Once inside the cells, the ester is continuously hydrolyzed to a supply of D-luciferin. Alternatively a burst of D-luciferin is generated by UV photolysis. References : Luque-Ortega J.R. et al. - In Vivo Monitoring of Intracellular ATP Levels in Leishmania donovani Promastigotes as a Rapid Method To Screen Drugs Targeting Bioenergetic Metabolism, Antimicrobial Agents and Chemotherapy, p. 1121-1125, Vol. 45, No. 4 (2001)		
D-Luciferin 6-methyl ether, Na salt	**FP-M1418A**	10 mg
D-Luciferin methyl ether has been proposed to be a substrate for microsomal dealkylase/cytochrome P450. Demethylation of the substrate generates D-luciferin, and thus can be detected via bioluminescence with extremely high sensitivity. Reference : Denburg J. et al. Substrate-binding properties of firefly luciferase I. Luciferin-binding site. Archs Biochem. Biophys. 134, 381-394 (1969).		
D-Luciferin-6-0-β-D-galactopyranoside β-Galactosidase substrate	**FP-CQ6410**	5 mg
Reference : Yang,Y. et al., Homogeneous enzyme immunoassay modified for application to luminescence-based biosensors, Anal. Biochem 33: 102-107 (2005)		

β-Galactosidase fluorescent substrates sampler Kit

This kit consists of samples of several of our most popular galactosidase substrates and their reference fluorophores allowing multiplexed analysis of lacZ β-Galactosidase activity at a variety of wavelengths. This kit is perfect for those occasions where the preferred wavelength of detection is under development.

Description		P/N :	Qty
β-Galactosidase substrates sampler Kit		**FP-BM8400**	1 kit
Contains :			
Subst. FDG	#52476A, 10 mg		
Fluo.Std. Fluorescein	#193659, 10 mg		
Subst. Res-Gal	#524739, 10 mg		
Fluo.Std. Resorufin	#95432A, 10 mg		
Subst. TFMU-Gal	#M11419, 10 mg		
Fluo.Std. TFMU	#434769, 10 mg		

Each substrate is available separately, and also many other derivatives (inhibitors, activators,...). Please inquire.

Other colorimetric substrates for glycosidases

Description	P/N :	Qty
o-NPG (o-Nitrophenyl-β-D-galactopyranoside, MW : 301.3 ; λ_{abs}(cleaved) : 420 nm)	**UP556683**	5 g
X-GLU (5-Bromo-4-Chloro-3-Indolyl-B-D-Glucopyranoside, MW : 408.6)	**UP193325**	100 mg
X-GAL (5-Bromo-4-Chloro-3-Indolyl-β-D-Galactopyranoside, MW : 408.6)	**UP40534M**	1 g

Phosphatases are a specific class of enzymes that catalyze the removal of phosphate groups from proteins, whereas kinases enzymatically add a phosphate to a protein. Phosphatases are important in signal transduction because they regulate the proteins they are attached to. To reverse the regulatory effect, the phosphate has to be removed. Cell proliferation and differentiation are regulated by phosphatases.

Phosphatases serve also as enzyme markers, allowing to quantify phosphatase activity in different types of cells. Alkaline phosphatase is finally a highly sensitive enzyme for ELISA, immuno-histochemical, Northern, Southern and Western blot applications.

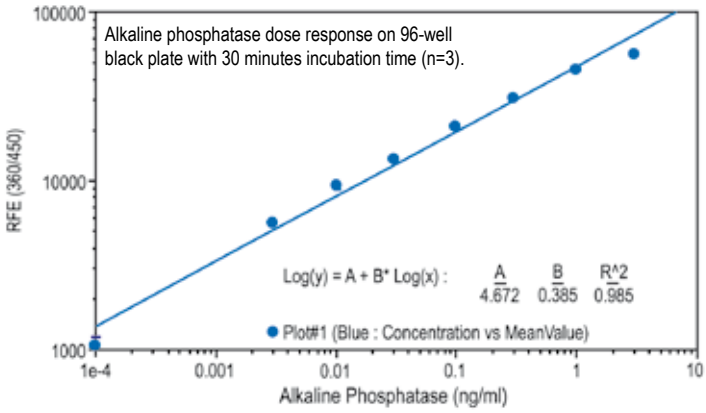
■ Fluorimetric Assay kits

The Alkaline Phosphatase Assay Kits use fluorogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions, in cell extracts, in live cells as well as on solid surfaces (such as PVDF membranes). The kits provide all the essential components with our optimized "mix and read" assay protocol that is compatible with HTS liquid handling instruments.

■ Alkaline Phosphatase Assay Kit, Blue Fluorescence

Substrate : MUP
$\lambda_{ex./em.}$: 360 / 449 nm
Sensitivity : 0.3 pg of alkaline phosphatase

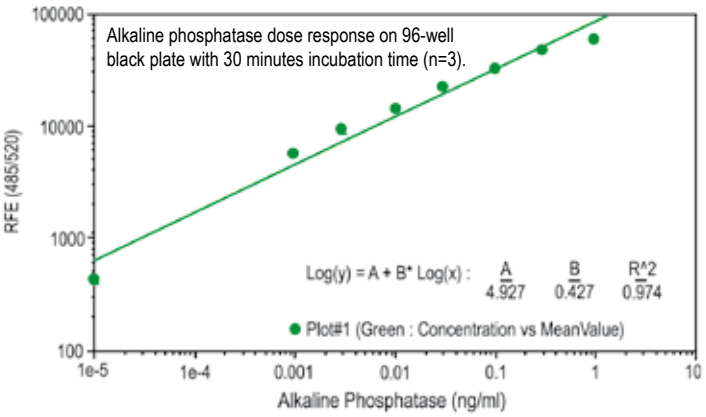
Description	P/N :	Qty
Fluorimetric Alkaline Phosphatase Assay Kit, Blue fluorescence	**JQ6730**	500 tests



■ Alkaline Phosphatase Assay Kit, Green Fluorescence

Substrate : FDP.
$\lambda_{ex./em.}$: 490 / 514 nm
Sensitivity : 0.1 pg of alkaline phosphatase

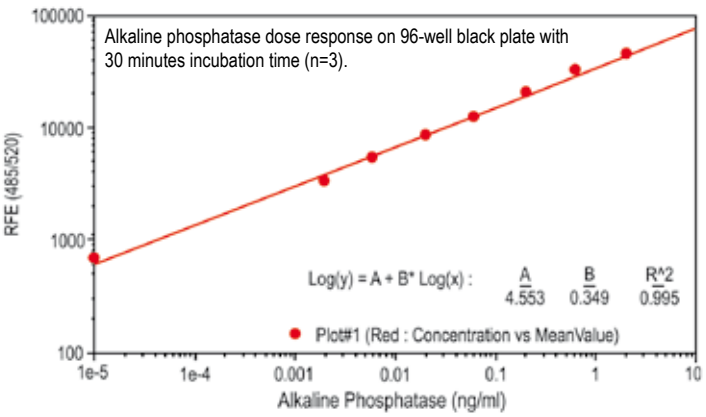
Description	P/N :	Qty
Fluorimetric Alkaline Phosphatase Assay Kit, Green fluorescence	**JQ6740**	500 tests



■ Alkaline Phosphatase Assay Kit, Red Fluorescence

Substrate : Phospholite™ Red
$\lambda_{ex./em.}$: 570 / 590 nm
Sensitivity : 0.2 pg of alkaline phosphatase

Description	P/N :	Qty
Fluorimetric Alkaline Phosphatase Assay Kit, Red fluorescence	**JQ6750**	500 tests

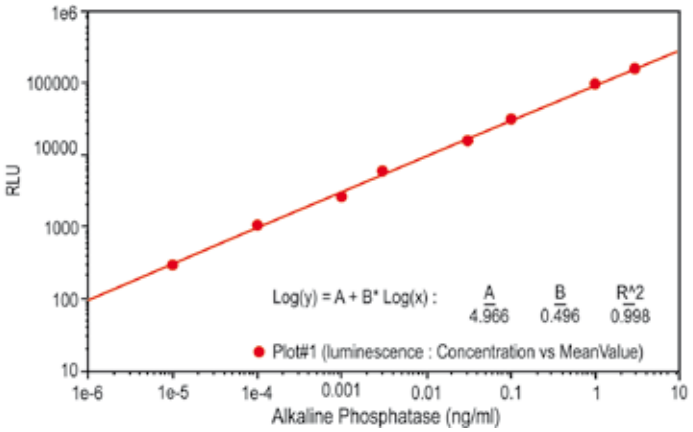


Luminometric AP Assay kit

■ Luminometric Alkaline Phosphatase Assay Kit

Substrate : proprietary luminescent substrate
$\lambda_{em.}$: **560 nm**
Sensitivity : **0.01 pg** of alkaline phosphatase

This Alkaline Phosphatase Assay Kit uses a luminogenic phosphatase substrate, to quantify alkaline phosphatase activity in solutions, in cell extracts, in live cells as well as on solid surfaces (such as PVDF membranes). This proprietary phosphatase substrate generates a luminescent product that produces strong luminescence upon interaction with phosphatase.



Alkaline phosphatase dose response on 96-well black plate with 30 minutes incubation time (n=3).

Description	P/N :	Qty
Luminometric Alkaline Phosphatase Assay Kit	**JQ6760**	200 assays

Colorimetric AP Assay kits

See AP colorimetric assays substrates and kits in Immunoassay reagents section.

Acide phosphatase assays

■ MUP Plus

Although MUP is widely used for detecting phosphatases in solution it is not well suited for living cell or continuous assays since 4-methylumbelliferone, the enzymatic product, which only develops maximum fluorescence at pH value of >10. Thus it is also difficult to use MUP for the detection of phosphatases that have acidic optimal pH range such as acid phosphatases. FluoProbes is pleased to offer MUP Plus that is developed to address this pH limitation associated with MUP substrates. MUP exhibits maximum fluorescence above pH 7.0, thus MUP Plus substrate can be well used for continuous assays. It can also be used for the assays that require acidic pH such as acid phosphatases.

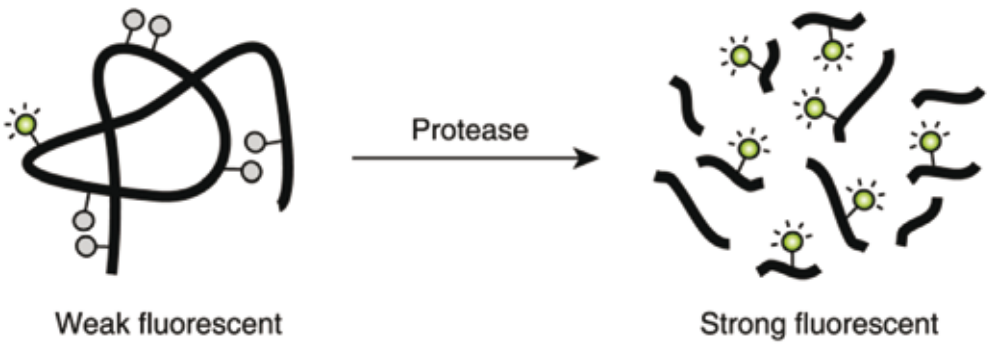
Description	P/N :	Qty
MUP Plus™, sodium salt $\lambda_{ex./em.}$: 360 / 450 nm MW : 300 g.mol^{-1}	**FP-JQ6710**	25 mg

Protease Assays Kits, Green & Red Fluorescence

- Optimized Performance : Optimal conditions for the detection of **generic protease activity**
- **High Speed** : Minimal hands-on time
- **Assured Reliability** : Detailed protocol and references are provided

The Protease Assay Kits are widely used for detection of generic protease activities. The kits use a casein derivative that is heavily labeled with green or red fluorescence, resulting in almost total quenching of the conjugate's fluorescence. Protease-catalyzed hydrolysis relieves this quenching conjugate, yielding brightly fluorescent dye-labeled peptides. The increase in fluorescence intensity is directly proportional to protease activity. The protease assay kits do not require any separation steps and can be used to continuously measure the kinetics of a variety of exopeptidases and endopeptidases.

The kits contains :
• Fluorescent labeled casein with high ratio of dye/protein (pH-insensitive)
• Trypsin (as positive control)
• Assay buffer
• A detailed protocol

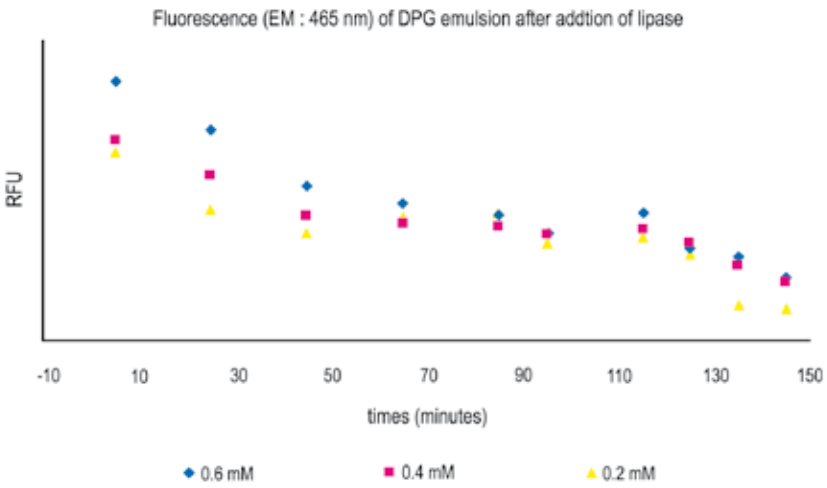


Description	P/N :	Qty
Protease Assay Kit, Green Fluorescence (488/520 nm)	**BK962A**	500 tests*
Protease Assay Kit, Red Fluorescence (546/575 nm)	**BK963A**	500 tests*

Lipase Assay Kit

Fast and easy measurement of lipase activity in vitro, in cell preparations or in vivo

Lipases are a family of enzymes that release fatty acids from triacylglycerols in a site specific manner. Most lipases have optimum activity for the primary ester groups of triglycerides, while some lipases remove fatty acyl groups from either the C-1 or C-3 acyl positions. The substrate is typically not a single molecule, but a nonaqueous phase of aggregated lipid. Lipase activity, ubiquitous among most cells, can be monitored using the new fluorescent substrate 1,2-dioleoyl-3-pyrenyldecanoyl-rac-glycerol (Product # FP-M14031, $\lambda_{exc./em.}$: 342/470 nm) contained in the kit. Upon cleavage, the fluorescent fatty acid pyrenedecanoic acid (Product # FP-37853A, $\lambda_{exc./em.}$: 341/377 nm) is released and activity measurements are easily obtained either in vitro, in cell preparations, or in vivo. The kit contains enough substrate for numerous assays and control experiments, and also contains reference standards and a detailed protocol for use.

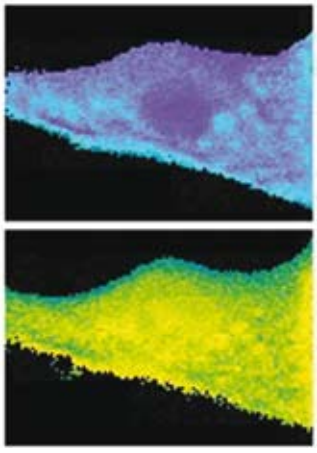


References :
Howard G.T., et al. "Sensitive plate assay for screening and detection of bacterial polyurethanase activity". *Lett. Appl. Microbiol.* 32(3): 211-4 (2001)
Kouker G. and Jaeger K.E., "Specific and sensitive plate assay for bacterial lipases". *Appl Environ Microbiol* 53(1): 211-3 (1987)

Description	P/N :	Qty
Fluorescent Lipase Assay Kit	**BG8440**	1 kit (72 assays in 96 well plate)

Unbound free fatty acids Assay - ADIFAB

The assay can be used in a variety of biochemical and clinical applications including the determination of lipase activity, fatty acid binding to membranes and proteins, and serum unbound free fatty acid (FFAu) levels.
ADIFAB and ADIFAB2 are ideal also for drug screening. They are particularly well-suited for drugs that affect cellular processes as well as those involving purified enzymes.
FFAu probes have been validated for high throughput assay. This includes robotic dispensing of reagents, fluorescence screening and the determination of FFAu levels in 96 and 384 well plates. This system can be used directly to screen for drugs that alter cellular metabolism involving FFA or that alter the behavior of enzymes that either produce or use FFA.
ADIFAB2 is a high affinity version of the original ADIFAB probe. It is formed by acrylodan labeling the Leu72 to Ala mutant of the Intestinal Fatty Acid Binding Protein. ADIFAB2, similarly to ADIFAB, can be used to assay unbound free fatty acid levels but provides greater sensitivity for low concentrations of the FFA levels. For those concentrations below about 400 nM, the increase in the ADIFAB2 emission ratio is about twice that for ADIFAB.
The fluorescence of ADIFAB is measured with the ratio 505/432 upon excitation at 386 nm. ADIFAB2 fluorescence emission spectra occur at longer wavelengths (550/457 nm with excitation at 375 nm). Binding affinities for ADIFAB2 are approximately ten times greater than for ADIFAB. On the other hand, ADIFAB has a wider range of sensitivity than ADIFAB2 and is preferable for higher concentrations of unbound free fatty acids.



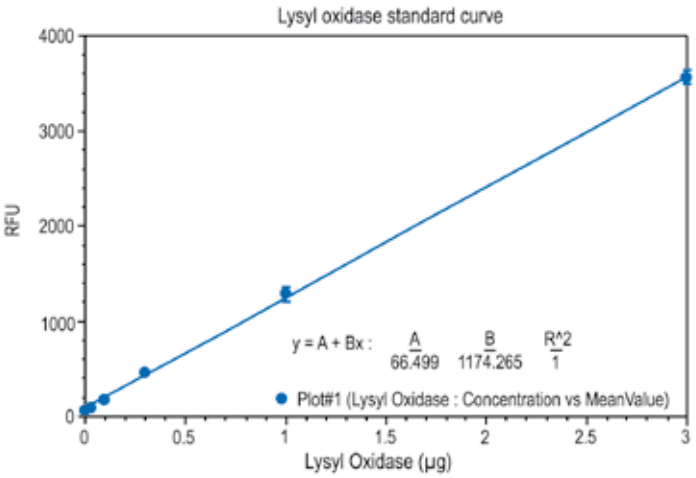
Intracellular FFAu levels measured with ADIFAB micro-injected into fat cells – Copyright FFA biosciences

Description	P/N :	Qty
ADIFAB	**040791**	200 µg
	0470792	1 mg
ADIFAB2	**BB6681**	200 µg
	BB6682	1 mg

■ Lysyl Oxidase Assay Kit, Red Fluorescence

Sensitivity : **40 ng** of lysyl oxidase in solution

Lysyl oxidase is an extracellular enzyme that catalyzes formation of aldehydes from lysine residues in collagen and elastin precursors. These aldehydes are highly reactive, and undergo spontaneous chemical reactions with other lysyl oxidase-derived aldehyde residues, or with unmodified lysine residues. This results in cross-linking collagen and elastin which is essential for stabilization of collagen fibrils and for the integrity and elasticity of mature elastin. Lysyl oxidase has been identified as a possible tumor suppressor. Lysyl oxidase activity in biological samples is traditionally and most reliably assessed by tritium release end-point assays using radiolabeled collagen or elastin substrates involving laborious vacuum distillation of the released tritiated water. This kit offers a sensitive fluorescent assay for lysyl oxidase activity that utilizes 1,5-diaminopentane as substrate, and released hydrogen peroxide is detected using our HRP substrate in HRP-coupled reactions. This method allows the detection of sub ng/mL lysyl oxidase and is much more sensitive than the currently available fluorimetric assay for this enzyme activity. This method eliminates the interference that occurs in some biological samples and can be readily used to detect lysyl oxidase activity in cell culture experiments.



Lysyl oxidase dose response on 96-well black plate with 30 minutes incubation time (n=3). The insert shows the low levels of lysyl oxidase detection.

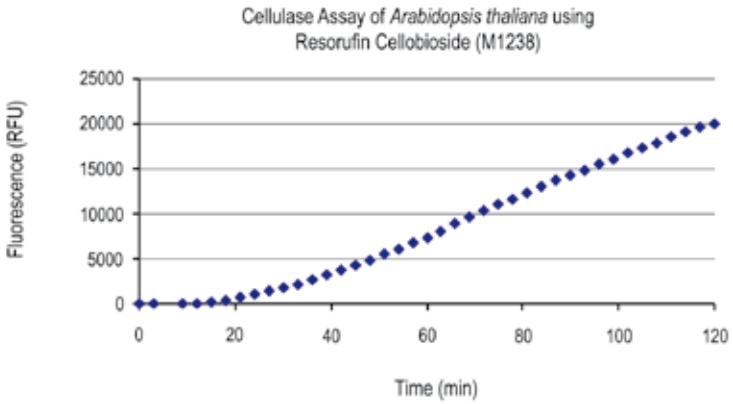
Description	P/N :	Qty
Lysyl Oxidase Assay Kit, red fluorescence	**JQ7270**	500 assays

Cellulase Detection

■ Cellulase Assay Kit, Red Fluorescence

Substrate : Resorufin Cellobioside
Reaction volume : 100 µl
$\lambda_{ex./em.}$: **571/585 nm**

Cellulases are a family of enzymes that include ß-Glucosidases, endoglucanases, and exoglucanases. These enzymes cleave the ß-1,4-D-glycosidic bonds that link the glucose units comprising cellulose. In addition to being produced by plants, cellulase activity is found in many fungi and bacteria, including some plant pathogens. Most animal cells are not known to produce cellulase; cellulolytic activity is often carried out in animals by symbionts. However, recent evidence does suggest cellulase production in some animals, such as insects and arthopods. The study of cellulase activity has many applications in plant molecular biology, agriculture, and manufacturing.



Suspension of flowering buds from two mature *Arabidopsis thaliana* plants in triplicate (50 µL) on a 96-well clear, flat bottom plate read at 3-minute intervals for 120 minutes.

Cellulase is also becoming important in the development of alternative fuel sources, as glucose obtained from cellulose hydrolysis is easily fermented into ethanol. Activity of most cellulases can be monitored using our long wavelength fluorescent substrate, Resorufin Cellobioside, contained in the kit. Upon cleavage, the fluorescent compound, Resorufin, is released and activity measurements are easily obtained in a microtiter plate based assay format.

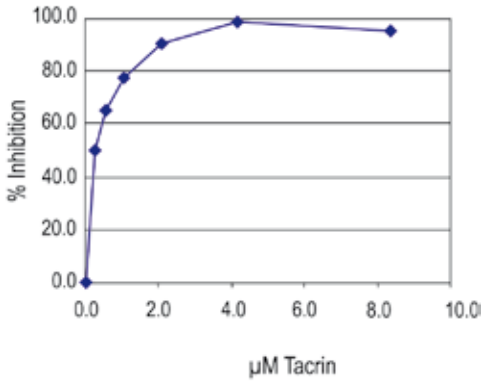
Description	P/N :	Qty
Fluorescent Cellulase Assay Kit	**DO8110**	200 assays
Kit contains : Substrate Reagent, Reference Standard, Reaction Buffer, Stop Buffer, DMSO		

aCella – Acetylcholinesterase Assay

Bioluminescence assay for Monitoring AcetylCholinEsterase Activity

- FAST : Results in **30 seconds - 5 minutes**
- Homogenous : **One-step, no wash assay**
- **Ultra Sensitive** assay to monitor AChE activity
- **Versatile** : Nerve gas, pesticide monitoring ; drug screening applications

Acetylcholinesterase (AChE) is one of the most important enzymes involved in nerve transmission. The enzyme is bound to cellular membranes of excitable tissue (synaptic junction, endoplasmic reticulum, etc). Acute toxicity to humans and animals through inhibition of AChE by both nerve gases and an important class of pesticides has long been a field of intensive scientific investigation. AChE inhibitors have also been used clinically as Alzheimer's treatments (e.g., tacrine (tetrahydroaminoacridine)) and are the subject of increasing interest in various disease processes and treatment strategies. However, both environmental detection of AChE inhibitors and development of modulators of AChE enzymatic activity as drugs have been hampered by the difficulty and complexity of the current assay methods.



Tacrine (a mixed-mode inhibitor of AChE) was serially diluted in DI water. Next 10 µL of the diluted Tacrine (x axis labeling represents µM final concentration of Tacrine) was added to a white opaque 96 well microplate along with 50 µL of component A (AChE enzyme). The samples were incubated for 5 minutes after which 50 µL of component B was added to all the wells. Data was collected using a luminometer. Data shown represents T=2 minutes after the addition of component B.

Assay Principle

Reaction I : AChE + Inhibitor → No Acetate and Choline.

Reaction II : Coupled enzyme reaction + ATP → Reaction does not proceed.

Reaction III : ATP (remaining) + Luciferase/Luciferin → LIGHT

Description	P/N :	Qty
aCella –AchE Assay	**CA6650**	100 tests
	CA6651	500 tests
	CA6652	1000 tests

Kit Contents : Acetylcholinesterase, Detection reagent, acetylcholine and coupled enzyme reaction, Control to measure maximum luminescence

Intracellular Ca^{++} levels have become important indicators for the activation state of ion channels and G-protein coupled receptors as well as for the phases of apoptosis and cell injury. Though the respective kinetics and the absolute amounts of the Calcium levels are different for each of these physiological processes there are common ways for monitoring them. Luminescent labels like Aequorin as well as fluorescent ones are versatile and widely used solutions for microplate assays. Fura 2 and Indo-1 provide ratiometric readout thereby reducing effects caused by leaking or bleached dyes or varying assay conditions.

Product name	MW ($g \cdot mol^{-1}$)	$\lambda_{exc}/\lambda_{em}$ max. [(a)] Free Ca^{2+}(nm)	$\lambda_{exc}/\lambda_{em}$ max. [(a)] High Ca^{2+} (nm)	Kd Ca^{2+} (nM)	Applications
Fluo-3 AM[(b)]	1129	503 / weak	505 / 526	390	. Most classical calcium indicator
Fluo-8 AM	1000	490 / weak	490 / 514	389	. No Wash step needed . 4 times brighter than Fluo-3 . Loading at room temperature
Fluo-8H AM	1100	490 / weak	490 / 514	232	. High Ca^{2+} concentration indicator
Fluo-8L AM	1100	490 / weak	490 / 514	1 860	. Low Ca^{2+} concentration indicator
Rhod-4 AM		530 / weak	530 / 555	525	. Red calcium indicator . 4 times brighter and 10 times larger windows assay than Rhod-2 . Loading at room temperature
Fura-PE3 AM	1258	335 / 495	380 / 495	250	. Leakage resistant form of Fura-2 . Ratio of reads with 2 different $\lambda_{ex.}$. Avoids interference due to dye distribution and photobleaching
Indo-PE3 AM	1266	338 / 480	338 / 410	260	. Leakage resistant form of Indo-1 . Ratio of reads with 2 different $\lambda_{em.}$. Avoids interference due to dye distribution and photobleaching

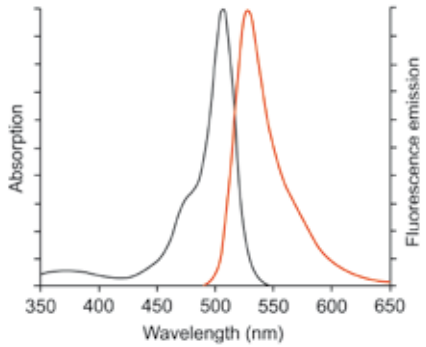
(a) after hydrolysis
(b) **AM ester** are membrane-permeant and thus increases greatly cell loading that can be performed by simple incubation of the cells or tissue preparation in a buffer containing the AM ester. Pluronic® F-127, a mild non-ionic detergent, can facilitate AM esters loading. The AM esters themselves do not bind to Ca^{2+}. However, once they have entered the cells, they are rapidly hydrolyzed by intracellular esterases into the parent Ca^{2+} compounds, thus becoming fully fluorescent upon binding to Ca^{2+}.
Many other ion indicators are available in our Interchim' range of dye. Please contact us for specific application needs.

Fluo-3 AM

Standard Ca^{2+} concentration indicator

- Large dynamic range
- Low compartmentalization
- Appropriate apparent Ca^{2+} binding affinity

Description	P/N :	Qty
Fluo-3 AM	**FP-78932A**	1 mg
$\lambda_{exc.}/\lambda_{em.}$ = 505/523 nm	**FP-R1245A**	1 mg FluoProbes Pure Grade
Kd = 390 nm	**FP-78932B**	10 x 100 µg
	FP-78932C	20 x 50 µg
	FP-M2036A	1 ml (1mM solution in DMSO)
	FP-78932D	50 mg

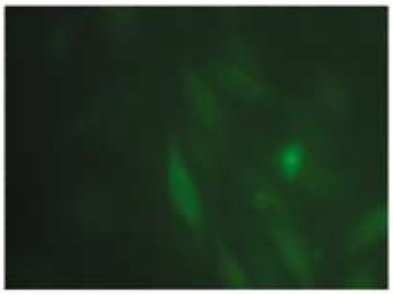


Fluo-3 has an absorption spectrum compatible with excitation at 488 nm by argon-ion laser sources, and a >100 fold fluorescence intensity increase in response to Ca^{2+} binding. Fluo-3 proves to be the generally most applicable Ca^{2+} indicator, even if it is more susceptible to photobleaching than many of the other Ca^{2+} indicators.

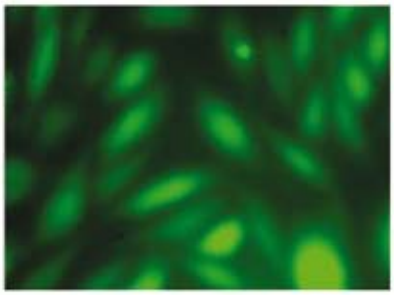
Fluo-8 AM - NoWash

The next generation calcium indicator for automated screening (HTS) applications

Fluo-3



Fluo-4

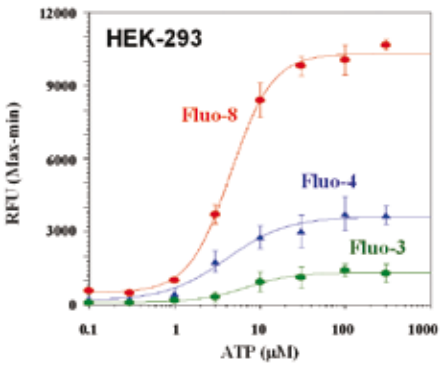


Fluo-8

- **Increased signal intensity**
- **Rapid dye loading** : dye loading at RT (rather than 37°C required for Fluo-4 AM)
- **Convenient and robust** : No wash step needed.
- Performed in 96 or 384-well microtiter-plate

The **Fluo-8 NW** (No Wash) can cross cell membrane. Once inside the cell, the lipophilic blocking groups of Fluo-8 NW are cleaved by esterases, resulting in a negatively charged fluorescent dye that stays inside cells and its fluorescence is greatly enhanced upon binding to calcium. When cells stimulated with agonists, the receptor signals the release of intracellular calcium, which greatly increase the fluorescence of Fluo-8 NW. The characteristics of its long wavelength (490/514 nm), high sensitivity, and >100 times fluorescence enhancement (when it forms a complex with calcium) make Fluo-8 NW an ideal indicator for measurement of cellular calcium.

Description	P/N :	Qty
Fluo-8 No Wash Calcium Assay Kit, Medium removal[1]	**CJ2560**	10 plates
	CJ2561	100 plates
Fluo-8 No Wash Calcium Assay Kit, 1% FBS Medium[2]	**CJ2550**	10 plates
	CJ2551	100 plates
Fluo-8 AM	**CP7501**	5 x 50 µg
$\lambda_{exc.}/\lambda_{em.}$: (Hydr.,Ca^{2+}) : 505/523 nm ; Kd(Ca^{2+}) = 390 nM ; MW : 1000		
Related products :		
Probenecid, Cell culture tested, to suppress efflux of dyes	**FP-288652**	10 x 150 mg
Probenecid, water soluble	**FP-288653**	10 x 150 mg
Ionomycin, Ca^{2+} ionophore	**FP-53989A**	1 mg
Ionomycin, Ca^{2+} ionophore	**FP-53989B**	5 mg



ATP dose responses in HEK-293 cells

Note 1 : It is important to remove the growth medium in order to minimize background fluorescence, and compound interference with serum or culture media.
Note 2 : Alternatively, one can grow the cells in growth medium with 0.5-to 1% FBS to avoid medium removal step.

■ Fluo-8H AM

High cytosolic Ca^{2+} concentration indicator

High Ca^{2+} concentrations, present in some organelles (mitochondria, vacuoles) and in excitable cells (fibroblast i.e.), were hardly detected : standard dyes Fluo-3, Fluo-4 and Rhod-2 have too high affinity for Ca^{2+}.

The measurement of cytosolic free Ca^{2+} ion concentration with low affinity Ca^{2+} indicators has advantages for kinetic studies of cytosolic [Ca^{2+}] transients when compared with more commonly used high affinity Ca^{2+} indicators. Their dynamic range and linearity are better suited to measurement of high-localised transient concentration changes that exist near sites of influx or release, and the additional buffering introduced by the indicator is minimised.

Description	P/N :	Qty
Fluo-8H AM (490/514 nm)	**FP-CP7531**	10 x 50 µg
$\lambda_{exc.}/\lambda_{em.}$: (Hydr.,Ca^{2+}): 490/514 nm ; Kd(Ca^{2+})=232 nM ; MW : 1100	**FP-CP7530**	1 mg
Related products		
Mag-Fura-2 AM ($\lambda_{exc.}/\lambda_{em.}$: 369, 329/510 nm)	**FP-35374C**	20 x 50 µg

■ Fluo-8L AM

Low cytosolic Ca^{2+} concentration indicator

Description	P/N :	Qty
Fluo-8L AM	**FP-CP7551**	10 x 50 µg
$\lambda_{exc.}/\lambda_{em.}$: (Hydr.,Ca^{2+}): 490/514 nm ; Kd(Ca^{2+})=1.86 µM	**FP-CP7550**	1 mg

■ Rhod-4™ AM - No Wash (NW)

The brighest red fluorescent calcium indicator

Rhod-2 is most commonly used among the red fluorescent calcium indicators. However, Rhod-2 AM is only moderately fluorescent in live cells upon esterase hydrolysis, and has very small cellular calcium responses. **Rhod-4™** has been developed to **improve cell loading** and calcium response while maintaining the spectral wavelength of Rhod-2. In CHO and HEK cells Rhod-4™ AM has cellular calcium response that is 10 times more sensitive than Rhod-2 AM.

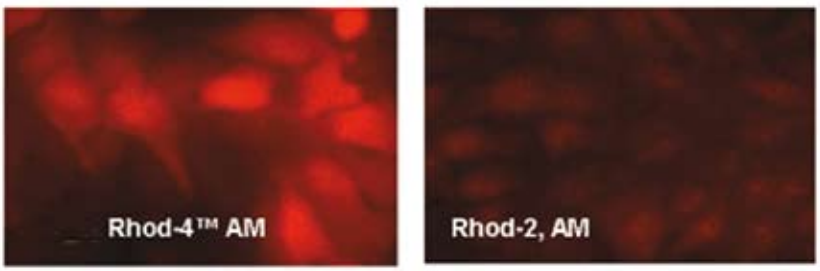


Figure 2. Rhod-4 AM vs Rhod-2 AM in U2OS. U2OS cells were seeded overnight at 40 000 cells per 100 µl per well in a 96 wells black wall/clear bottom costar plate. The growth medium was removed, and the cells were incubated with 100 µl of 5uM Rhod-4 AM or Rhod-2 AM in HHBS at 37°C, 5% CO^2 incubator for 1 hour. The cells were washed with 2 times with 200 µl HHBS, then imaged under fluorescent microscope using Tritc channel.

Rhod-4™ is the brightest red calcium indicator available for HTS screening. Once inside the cell, the lipophilic blocking groups of Rhod-4™ are cleaved by non-specific cell esterase, resulting in a negatively charged fluorescent dye that stays inside cells, and its fluorescence is greatly enhanced upon binding to calcium. When cells stimulated with screening compounds, the receptor signals release of intracellular calcium, which greatly increase the fluorescence of Rhod-4. The characteristics of its long wavelength, high sensitivity, and >250 times fluorescence increases (when it forms complexes with calcium) make Rhod-4™ an ideal indicator for measurement of cellular calcium. This Rhod-4 NW Calcium Assay Kit provides an optimized assay method for monitoring G-protein-coupled receptors (GPCRs) and calcium channels. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation.

The **Rhod-4 NW Calcium Assay Kit** provides a homogeneous fluorescence-based assay for detecting the intracellular calcium mobilization, and a preferred method in drug discovery for screening. Cells expressing a GPCR of interest that signals through calcium are pre-loaded with our proprietary Rhod-4 NW which can cross cell membrane.

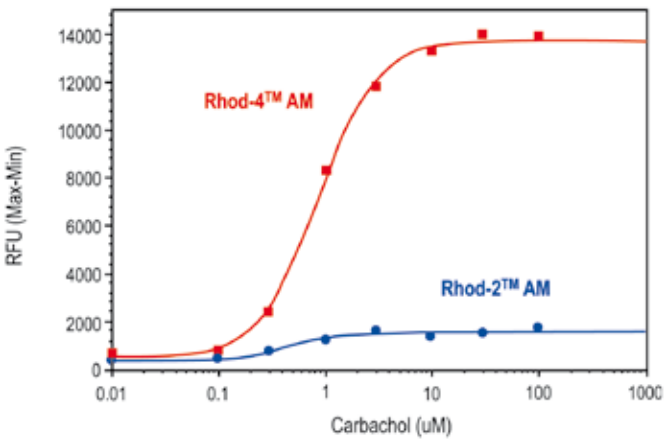


Figure 1. Carbachol Dose Response in HEK-293 cells measured with Rhod-4 NW Calcium Assay kit and Rhod-2 AM. HEK-293 cells were seeded overnight at 40 000 cells per 100 µl per well in a 96-well black wall/clear bottom costar plate. The growth medium was removed, and the cells were incubated with 100 µl of the Rhod-4 NW calcium assay kit, or 5 µM Rhod-2 AM at 37°C, 5% CO_2 incubator for 1 hour. Carbachol (25 µl/well) was added by NOVOstar (BMG LabTech) to achieve the final indicated concentrations. The EC^{59} of rhod-4 NW is about 0.8 uM.

Description	P/N :	Qty
Rhod-4™ NW Calcium Assay Kit, Medium Removal	**CQ6080**	1 plate
	CQ6081	10 plates
	CQ6082	100 plates
Rhod-4™ NW Calcium Assay Kit, 1% FBS Growth Medium	**CQ6090**	1 plate
	CQ6091	10 plates
	CQ6092	100 plates
Rhod-4™ AM $\lambda_{exc.}/\lambda_{em.}$: (Hydr.,Ca^{2+}) : 530/555 nm ; Kd(Ca^{2+}) = 525 nm	**CQ6061**	5 x 50 µg
	CQ6062	10 x 50 µg
	CQ6063	20 x 50 µg
	CQ6064	1 mg

■ Fura-PE3 AM & Fura-2 AM

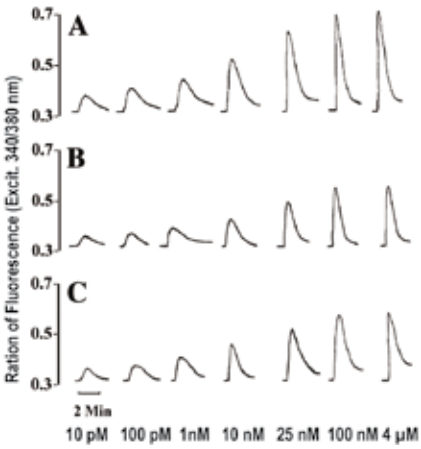
Leakage resistant form of Fura-2, a popular Setrometric Ca^{2+} indicator

Fura-2 AM may be useful in microplate studies, where cell lines with different properties are compared or where screening treatments lead to differences in the number of cells or dye loading. Some of the limitations in the use of Fura-2 appear to be overcome by the use of glass bottom microplates (See page 78).

Reference : Robinson JA et al., Ratiometric and non-ratiometric Ca^{2+} indicators for the assessment of intracellular free Ca^{2+} in a breast cancer cell line using a fluorescence microplate reader, J Biochem Biophys Methods. 2004 Mar 31;58(3):227-37.

Fura-PE3 is an improved version of Fura-2, that reduces cell leakage and thus increases dye loading accuracy.

Description	P/N :	Qty
Fura-2 AM	**FP-42776A**	1 mg
	FP-42776C	20 x 50 µg
	FP-85312A	1 ml (1 mM)
Fura-PE3 AM	**FP-AM603A**	500 µg



Representative experiment of angiotensin II (ANG II)-evoked changes in the ratio of fura 2 fluoresence (340/380 nm) in adherent neonatal rat cardiomyocytes (NRC). Primary cultures of NRC loaded with Fura 2 were stimulated with increasing concentrations of ANG II (10 pM-1 µM) either in the absence (control ; A) or presence of AA-861 (10 µM ; B) or MK-571 (100 nM ; C).

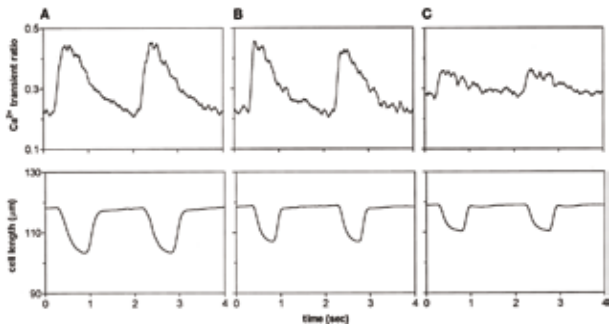
■ Indo-1 AM

Indo-1 AM has a shift in the emission from 485 nm to 405 nm in the presence of calcium. Indo-1 AM can be used with a single argon-ion laser for excitation and to monitor two different emissions.

Description	P/N :	Qty
Indo-1 AM	**FP-427755**	500 mg
	FP-42775A	20 x 50 µg
	FP-98180A	1 ml (1 mM)
Related products :		
4-bromo A-23187, Ca^{2+} ionophore for UV light-excited dyes	**FP-372221**	1 mg
A-23187 (calcimycin or Calcium Ionophore III) to equilibrate intracellular and extracellular $[Ca^{2+}]$	**FP-28362B**	5 mg

Related product

Description	P/N :	Qty
Calcium Calibration kit	**FP-21527A**	1 kit
Pluronic® F-127	**FP-37361A**	1 g
Pluronic® F-127, 20% solution (in DMSO)	**FP-69806A**	1 ml



Representative tracings from indo 1-loaded myocytes show simultaneous Ca^{2+} transients (top panels) and cell length (bottom panels). Myocytes were stimulated at 0.5 Hz after 6 h of no treatment (control) (A), LPS (10 ng/ml) (B), or LPS with ANG II (100 nM) (C).

■ Indo-PE3 AM

Leakage resistant form of Indo-1

Description	P/N :	Qty
Indo-PE3 AM	**FP-AM602A**	500 µg

Cell counting, viability & proliferation

Cell counting is required

- To monitor cells during cell cultures
- For cell preparation or any cell experiment
- To standardize cell samples for analysis.
- Cell proliferation
- Cytotoxicity assays

Several methods have been proposed, each fitting more or less to each specific application : counting dead cells may be acceptable for the preparation of cell extracts or desired when one do not want to operate with hazardous cells or for cytotoxicity study. At the opposite dead cells counting is generally precluded for cell culture and bioassays. It may be useful to quantitate only viable cells, or only fast proliferating cells.

Interchim provides a large choice of cell assays covering standard as well as innovative methods for general to specific cell assays.

Technical tip

MicroPlate readers & Imaging systems

Interchim and Berthold collaboration supports further your works. Many of our fluorescence and luminescence reagents and kits were validated with instruments.

BERTHOLD TECHNOLOGIES

***NightOWL LB983 NC100**

***Mithras LB940 MultiMode Reader**

Selection guide

Probe	Principle	Detection Method	Dead	Viable	Proliferating	Features/Advantages - Drawbacks
Trypan blue	Membrane exclusion	Colorimetric Microscopy	++	++	++	Cheap, but time consuming, not scalable. Do not state on viability.
Hoechst	DNA probe exclusion	Fluorimetric	++	++	+++	Cheap, Scalable, Non toxic. Do not state on viability. More rapid than MTT/XTT ; unfixed or fixed samples.
MTT	Formazan dye, orange precipitate.	Colorimetric	-	++	+++	Popular method. Sensitive, Scalable. Non toxic Increased solubility and performance from MTT to XTT and WST.
XTT	Same as MTT but more soluble.	Colorimetric	-	++	+++	
WST	Formazan dye, soluble & not toxic		-	++	+++	
UptiBlue	ratiometric blue probe for cell redox	Colorimetric Fluorimetric	-	+++	+++	No solubilization step (unlike MTT). Applyalso to adherent cells. Sensitivity similar to MTT/XTT, but easier to use Fluorimetry/Superior sensitivity to MTT / XTT.
Calcein-AM	Calcein accumulation in cytoplasm	Fluorimetric	-	+++	++	No solubilization step (unlike MTT/XTT). Adaptable to a wide variety of techniques, including : microplate assays, in vivo cell tracing. Do not work for bacteria. May alter some cell functions.
GAPDH	Release of GAPDH coupled to ATP assay	Bioluminescence	-	+++	+	Measurement of Cell-Mediated (T Cells, ADCC, NK) or Complement-Mediated Cytolsis.
CFSE	Fluorescein protein labeling	Fluorimetric	++	++	++	Useful when other method do not work properly. Do not state on viability.
AnnexinV	AnnexinV/PhosphoSerine	Fluorimetric	+	+++	+	Useful for Apoptosis study.
LDH	convertion in colored product		-	++	+	Recommended for cytotoxicity assays Serum Interference.
Luciferin Syst.	ATP measure	Luminescence	-	+	+++	Pros : sensitivity / linearity. Cons : signal depends on each cell line, on temperature
-3H Thymidine	DNA incorporation of radioactivity	Radioactivity	-	+	+++	Cons : hazardous (radioelements).
BRDU	DNA incorporation	Immunoassay	-	+	+++	
^{51}Cr release EU^{3+}	Release of radioactivity by cytoplasm	Radioactivity	-	-	+++	Recommended for cytotoxicity assays. Cons : hazardous (radioelements).
Propidium Iodide, AAD	**7-**Membrane permeability	Fluorimetric	+++	-	-	Used in combinaison of green fluorescence dye like. Annexin V-FP488 to discriminate dead cells from alive cells.

Dual staining to detect live and dead cells

■ Live/Dead Mammalian Viability Assay Kit

Two-color fluorescent staining of live (green) and dead cells (red)

- Dual Detection : Detect both live and dead cells simultaneously.
- Simple & Fast : Require only a 30-min dye loading time and then measure without washing.
- Economical : Perform viability and cytotoxicity assays at the same time.
- Versatile : Analyze with flow cytometers, fluorescence microscopes or fluorescence plate readers.

The Viability/Cytotoxicity Assay Kit for Live / Dead Cells provides a two-color fluorescence staining on both live and dead cells using two probes that measure two recognized parameters of cell viability — intracellular esterase activity and plasma membrane integrity [Papadopoulos, 1994]. The kit is suitable for use with fluorescence microscopes, fluorescence multiwell plate scanners and flow cytometers and other fluorescence detection systems. The assay principles are general and applicable to most eukaryotic cell types, including adherent cells [Vaughan, 1995] and certain tissues [Poole, 1993], but not to bacteria or yeast. This fluorescence-based method of assessing cell viability can be used in place of trypan blue exclusion, ^{51}Cr release and similar methods for determining cell viability and cytotoxicity. It is generally faster, less expensive, safer and a more sensitive indicator of cytotoxic events than alternative methods. EthD-III shares the same property with EthD-I used in Live/Dead Viability/Cytotoxicity Assay Kit #486301 and is 40% brighter at intensity compared to EthD-I. Validity of the Live/Dead Viability/Cytotoxicity assay for animal cell applications has been established by several publications.

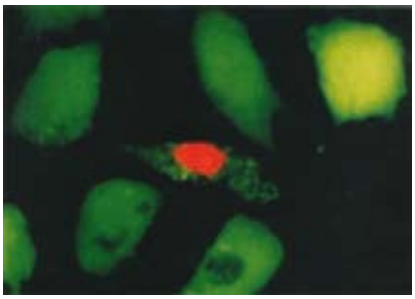
Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually nonfluorescent cell-permeant calcein AM to the intensely fluorescent calcein. The polyanionic dye calcein is well retained within live cells, producing an intense uniform green fluorescence in live cells (ex/em ~495 nm/~515 nm). EthD-III enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells (ex/em ~495 nm/~635 nm). EthD-III is excluded by the intact plasma membrane of live cells. The determination of cell viability depends on these physical and biochemical properties of cells. Cytotoxic events that do not affect these cell properties may not be accurately assessed using this method. Background fluorescence levels are inherently low with this assay technique because the dyes are virtually nonfluorescent before interacting with cells.

If cells are first fixed, and then stained, the Live/Dead Bacterial Viability/Cytotoxicity kit can also be considered. To replace the dye Calcein AM that will only stain the live cells, the DMAO; a DNA-binding dye, will stain both intact and damaged cell membranes.

References :

J Immunol Methods, 177, 101 (1994).
J Cell Sci, **106**, 685 (1993).
J Neurosci, **15**, 5389 (1995).

Description	P/N :	Qty
Live/Dead Mammalian Viability Assay Kit	**FP-BF4710**	1000 tests in microplate reader
Related products :		
DMAO, nuclei stain for live cells, 2 mM soln in DMSO	**FP-CA8150**	1 ml
Ethidium Bromide III, 1 mM solution	**FP-BP9341**	200 µl
MTT ($\lambda_{abs.}$(cleaved) : 650 nm (550-600 nm))	**FP-65939A**	1 g
Live/Dead Yeast Viability Assay Kit based on calcein-AM and PI.	**486301**	1 kit

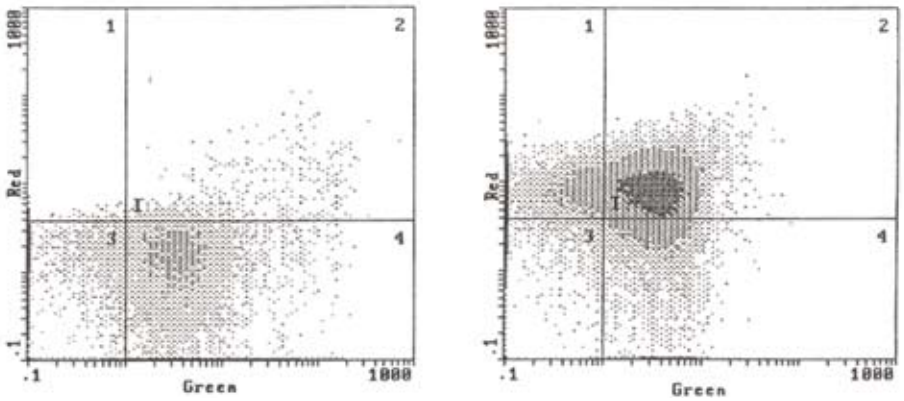


Hela Cells incubated with assay solution.

Live/Dead Bacterial Viability/Cytotoxicity kit

Two color fluorescence staining on both live bacteria (green) and dead bacteria (red)

- Dual Detection : Detect live and dead bacteria cells in a cell population simultaneously.
- Simple & Fast : 15 min dye loading and measure without washing.
- Economic : Perform viability and cytotoxicity assays at the same time.
- Versatile : Analysis compatiable with flow cytometers and fluorescence microscopes using popular settings for fluorescein and propidium iodide.



Viability/Cytotoxicity Assay Kit for Bacteria Live & Dead Cell Staining Kit provides two-color fluorescence staining on both live (green) and dead (red) bacteria using two probes, DMAO and EtD-III. DMAO is a green-fluorescent nucleic acid dye that stains both live and dead bacteria with intact and damaged cell membranes. EtD-III is a red-fluorescent nucleic acid dye that stains only dead bacteria with damaged cell membranes. With an appropriate mixture of DMAO and EtD-III, bacteria with intact cell membranes is stained fluorescent green, whereas bacteria with damaged cell membranes is stained fluorescent red. The kit is suitable for use with fluorescence microscopes and flow cytometers. The assay principles are general and applicable to most bacteria types.

A common criterion for bacterial viability is the ability of a bacterium to reproduce in suitable nutrient media that is referred to as growth assays. This kit yields results that correlate well with growth assays in liquid or solid media. Under certain conditions, however, bacteria having damaged membranes may be able to recover and reproduce — such bacteria may be scored as "dead" in this assay. Conversely, some bacteria with intact membranes may be unable to reproduce in nutrient medium, and yet these may be scored as "alive". Therefore, these situations need to be considered if a vast difference of live and dead bacteria counts is observed between this assay and growth assays.

This kit can also be considered if cells like mammalian cells, are first fixed, and then stained.

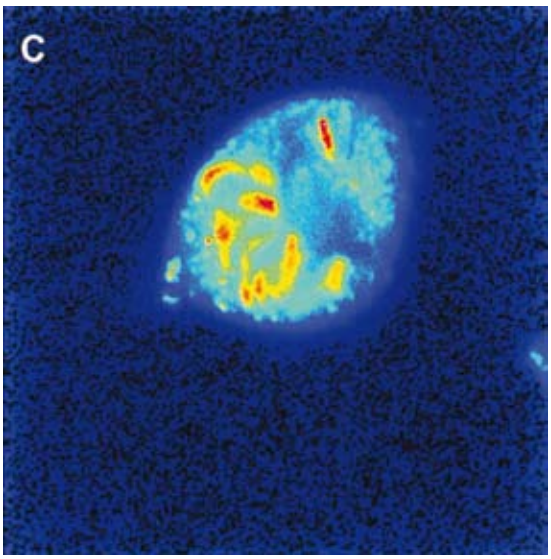
Description	P/N :	Qty
Live/Dead Bacterial Viability/Cytotoxicity	**FP-BU1040**	1000 tests in microplate reader
Related products :		
Live/Dead Yeast Viability Assay Kit based on WST-8 formazan dye. Read at 450 nm (450-490 nm)	**486301**	1 Kit

Dual staining to detect live and dead cells

■ Ethidium monoazide, bromide (EMA)

Selectively and covalently labels membrane-damaged or metabolically compromised cells in the presence of live cells

Ethidium monoazide bromide is a red fluorescent nucleic acid stain with a photoaffinity label. The dye, after photolysis, binds covalently to nucleic acids.[1] After photocrosslinking to DNA, the wavelengths ($\lambda_{exc.}$ / $\lambda_{em.}$ = 504 / 600 nm) are compatible with a simultaneous observation of another green indicator. The dye has been used to "footprint" drug binding sites on DNA[2] to modify plasmid DNA,[3,4] and to determine hemopoietic cell phenotype, function and position in the cell cycle.[5] A particularly useful application of the dye is to selectively and covalently label dead cells in the presence of live cells. Since ethidium monoazide bromide is relatively impermeant to live cells, it selectively labels DNA in dead cells in a mixed population of live and dead cells. Photolysis following the dye application renders the dead cell DNA covalently labeled with the dye. One can then wash and fix the cell preparation and exam it by microscopy fluorescence plate reader or flow cytometry. The major advantage of this method is that researchers can avoid extensive manipulation of live pathogenic organisms.[6] At the difference of propidium iodide, the ethidium monoazide binds covalently, and, when applied to cells before fixation, provides an indication of what fraction of the unfixed population were membrane-damaged or metabolically compromised.



Two photon fluorescent image of live PTK2 cells vitally stained with EMA : A late prometaphase cell illustrating the high selectivity of the stain for the chromosomes.

References :
1)J. Mol. Biol. 92, 319(1975)
2) Euro. J. Biochem. 182, 437(1989)
3)J. Biol. Chem. 257, 13205(1982)
4) J. Biol. Chem. 259, 11090(1984)
5) Cytometry 11, 610(1990)
6)Cytometry, 12, 133(1991)
7) PNAS, 97 , no. 17, p. 9504-9507 (2000)

Description	P/N :	Qty
Ethidium monoazide, bromide (EMA) $\lambda_{ex.}/\lambda_{em.}$(DNA bound) : 504/600 nm ; MW : 420.3	**FP-48256A**	5 mg

■ Propidium monoazide (PMA)

Selectively and covalently labels dead cells in the presence of live cells

PMA™ is a derivative of EMA, but it has significantly higher DNA binding affinity and is cell impermeant. As EMA, after photolysis, the dye is converted to a fluorescent DNA stain covalently bound to DNA.

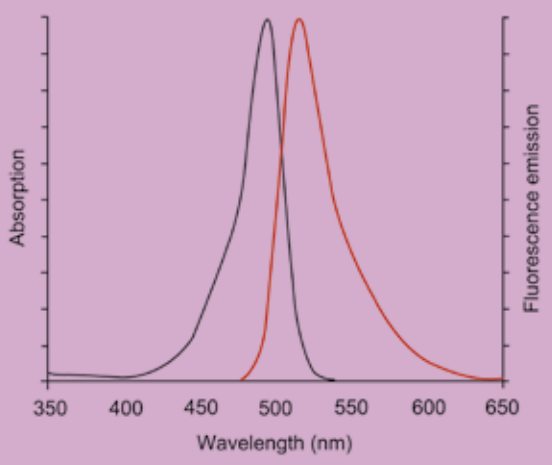
Description	P/N :	Qty
Propidium monoazide (PMA) $\lambda_{ex.}/\lambda_{em.}$(DNA bound) : 510/610 nm ; MW : 512	**FP-BZ9340**	1 mg

References :
Nocker, A. *et al.,* Comparison of propidium monoazide with ethidium monoazide for differentiation of live vs. dead bacteria by selective removal of DNA from dead cells. J. *Microbio Meth. 67(2)*, 310-320 (2006).
Nocker A. *et al.,* Use of Propidium Monoazide for Live/Dead Distinction in Microbial Ecology, *Applied and Environmental Microbiology*, p. 5111-5117, Vol. 73, No. 16 (2007).
Pan Y., Breidt F., Enumeration of *Listeria monocytogenes* by Real-Time PCR with Propidium Monoazide and Ethidium Monoazide in the Presence of Dead Cells, *Appl. Environ. Microbiol.* doi:10.1128/AEM.01198-07 (2007).

Technical tip

Calcein dye is a polyanionic derivate of fluorescein that exhibits fluorescence that is essentially independent of pH between 6.5 and 12. The excitation and the emission wavelengths of calcein are 485 nm and 535 nm, respectively. It is well retained in cells. These features have made it a popular and versatile dye for various applications, including cell volume changes in neurons and other cells, endocytosis, gap junctional communication, membrane integrity and permeability, angiography, liposomes…
It is worthy to notice that calcein is strongly quenched by several ions, including Fe^{3+}, Co^{2+}, Cu^{2+} and Mn^{2+} at physiological pH (not by Ca^{2+} or Mg^{2+} ions). Ions levels should thus be monitored.

AM ester is membrane-permeant and enters readily cell membranes. Intracellular esterases convert it into calcein. The DMSO solution is more convenient (time saving, reduces solubilization variability) especially for more reproductible screening assays.



Fluorescence of calcein at pH9.0

■ Calcein AM Cell Counting & Viability Assay Kit

The Calcein-AM Kit provides a simple, rapid and accurate method to measure cell viability and/or cytotoxicity. The kit utilizes calcein AM for the fluorometric determination of living cell numbers. The amount of a fluorescent dye read at 512 nm, calcein, hydrolyzed by esterases in cells, is directly proportional to the number of viable cells in culture media. The 96-well microplate assay has a detection range of less than 50 cells to more than 25 000 cells per well. It can be used for 384-well plates by adding 5 µl (instead of 10 µl) assay solution to 50 µl PBS solution per well. Since esterases and phenol red in the culture medium interfere with the fluorescence measurement, replacing the cell culture medium with PBS is necessary prior to adding the Calcein-AM assay solution. An incubation of 10 to 30 minutes gives sufficient fluorescence intensity for the cell viability determination.

Features :

- Suitable for proliferating and non-proliferating cells
- Ideal for both suspension and adherent cells
- Non-radioactive microplate
- Rapid (no solubilization step as in an MTT assay)
- Ideal for high-throughput assays
- Better retention and brightness compared to other fluorescent compounds (i.e. fluorescein)

Applications :

- Cell adhesion, chemotaxis, multidrug resistance, cell viability, apoptosis, cytotoxicity,...
- Microplate assays, immunocytochemistry, flow cytometry, and in vivo cell tracing

Description	P/N :	Qty
Cell Counting Kit, calcein-AM based	**876981**	500 tests
	876982	2 x 500 tests
Calcein AM	**FP-895514**	1 mg
$\lambda_{ex}/\lambda_{em}$ (cleaved) : 494/517 nm ; MW : 994.9	**FP-895515**	20 x 50 µg
Calcein AM, 1 mg/ml in anhydrous DMSO	**FP-855422**	1 ml
Calcein AM, 4 mg/ml in anhydrous DMSO	**FP-FI9820**	100 µl
Calcein AM, 5 mM in anhydrous DMSO, Pure Grade	**FP-JQ8140**	200 µl
Related products :		
Annexin V-FluoProbes 488, Flow Cytometry Grade (495/519 mm)	**FP-BH9390**	100 tests
Propidium iodide, 1 mg/ml	**FP-36774A**	10 ml

■ CFDA, SE for microbial and cell enumeration

5-(and 6-)-carboxyfluorescein diacetate, succinimidyl ester (CFDA, SE) efficiently stains gram-negative and gram-positive bacterial genera without causing undesirable effects on cell adhesion or viability.
The high throughput method using microplate spectrofluorometry has a detection limit of mid-10^5 CFDA-stained cells/ml.
CFDA, SE tracking technique has applications in bacterial transport, public health microbiology, allowing the movement of pathogen to be monitored in terrestrial, aquatic, and even food-processing environments. The technique may also be useful for studying infection and colonization by pathogens in vivo using animal models.

Reference :
Mark F. et al. - Development of a Vital Fluorescent Staining Method for Monitoring Bacterial Transport in Subsurface Environments, Applied and Environmental Microbiology, October 2000, p. 4486-4496, Vol. 66, No. 10

Description	P/N :	Qty
CFDA-SE (CFSE, Green Cell Tracking reagent) $\lambda_{ex./em.}$ (cleaved) : 495/519 nm ; MW : 557	**FP-52493A**	25 mg

■ UptiBlue Cell Viability Assay Kit

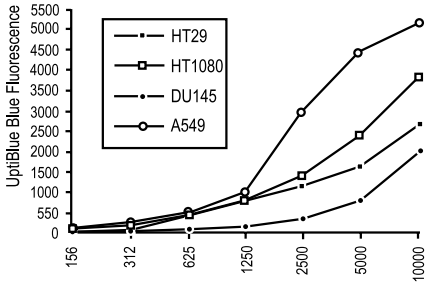
Substrate : Resazurin
$\lambda_{ex./em.}$: 540 / 590 nm
Sensitive : 100 cells

Principle : the UptiBlue dye enters readily into cells, where it elicits a wavelength shift of absorbance and a strong fluorescence related to redox potential in cell, informing on cell energetic state.

UptiBlue shows excellent correlation to formazan and tritiated thymidine techniques, while being much easier and safer to use. It especially replaces advantagely MTT/XTT in many applications, from cell counting to proliferation assay and cytotoxicity testing. Furthermore it allows longer studies.

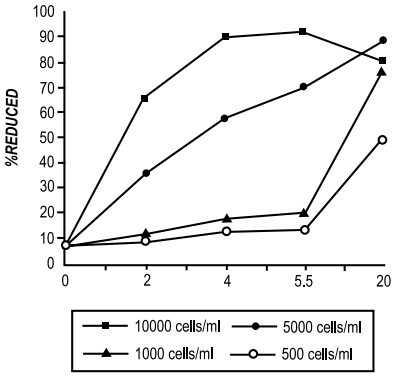
Applications

Cell proliferation assay



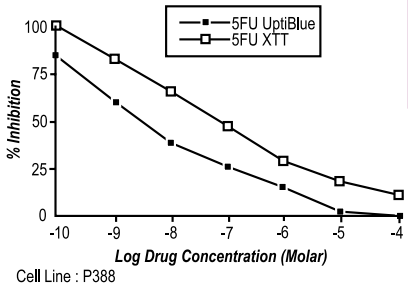
Detection of cell Growth of 4 Cell Lines using UptiBlue

Kinetic / long term assays



Kinetic reduction curve with UptiBlue with plating density from 500 to 10000 cells A549/ml.

Cytotoxicity assay



Cell Line : P388
Cell density 1250 cells/well, 5 days exposure to5-Fluorouracï
4 hours incubation with UptiBlue or XTT (+ menadione).

Determination of Doxorubicin LD_{50} using UptiBlue and XTT

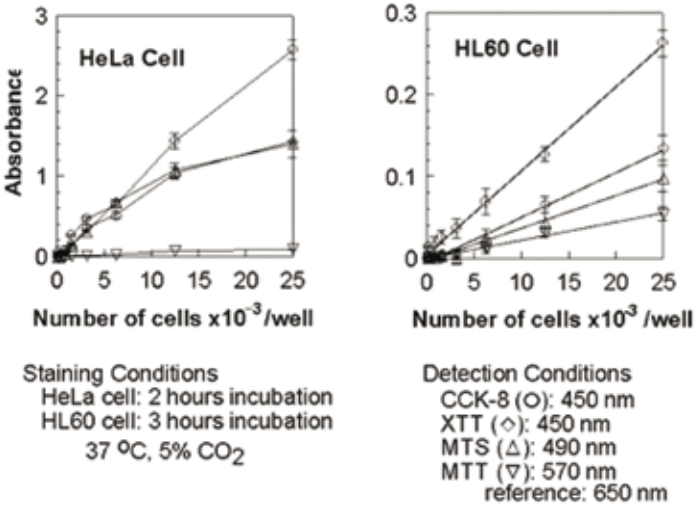
Description	P/N :	Qty
UptiBlue Cell Viability Assay Kit	**UP669412**	25 ml
	UP669413	100 ml

WST-8 Cell Proliferation and Cytotoxicity Assay Kit

- Colorimetric microplate assay
- **Ready-to-use** one-bottle solution
- **Safe** : no radioisotope or organic solvent required
- **No toxicity** to cells
- **Easy and fast** : no harvesting, washing or solubilization step required
- **More sensitive** than MTT, XTT, MTS and WST-1

Reduced toxicity of assay solution :

Cell proliferation assay :



CCK-8 consists of WST-8 and 1-methoxy PMS as an electron mediator. After the plate is incubated for 1-4 hours in the incubator, the absorbance is measured in 96 or 384-well plate. The wavelength range for the measurement of the absorbance is between 450 nm and 490 nm. The amount of the yellow colored formazan dye generated by dehydrogenases in cells is directly proportional to the number of viable cells in a culture medium. The sensitivity using CCK-8 is higher than that using MTT or the other tetrazolium salts that produce water-soluble formazan dyes such as XTT or MTS for HeLa cells and HL60 cells. Furthermore, the cell proliferation assay data using CCK-8 correlates with that using the ^{3}H-thymidine incorporate assay.

Description	P/N :	Qty
CKK-8 Cell Counting Kit	**899650**	1000 tests
	899651	3000 tests
	899654	10000 tests
Also available :		
MTT Cell Proliferation Assay Kit	**45547A**	1000 tests
MTT UltraPure	**FP-65939A**	1 g
XTT Cell Proliferation Assay Kit	**FX873A**	1000 tests
XTT UltraPure	**FP-409036A**	1 g
WST-1 Cell Proliferation Assay Kit	**KS0790**	96 tests
WST-1 as stand alone product	**F98883**	100 mg

Technical tip

Formazan based Cell Viability Assay Kit

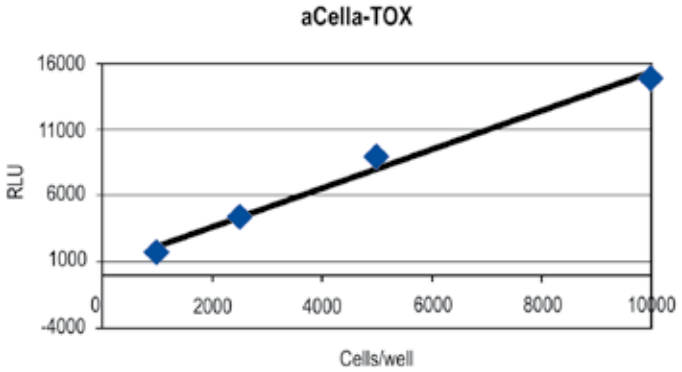
MTT based assay is probably the most popular cell viability assay. It has several drawbacks including toxicity, poor solubility that requires an extraction step and limited sensitivity. Interchim provides those kits as well (see related products) but recommends strongly the WST-8 assay kit, or alternatively the UptiBlue reagent.

■ aCella™ - TOX Bioluminescence Cytotoxicity Assay (GAPDH)

Measurement of Cell-Mediated (T Cells, ADCC, NK) or Complement-Mediated Cytolysis

- **Versatile :** Assay can be run in serum supplemented media.
- **Homogenous** - One-step, no wash assay. Assay can be run in same plate as samples.
- **FAST** - Results in 3-5 minutes.
- **Highly Sensitive** - Can detect fewer than 500 cells/well.
- Works with PRIMARY CELLS for determining cell Cytotoxicity.
- **Non-destructive** assay allows monitoring of additional parameters.

aCella-TOX provides a new and highly sensitive assay using a patented coupled luminescent technology for the detection of cytotoxicity[1]. This assay quantitatively measures the release of Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) from Primary Cells, mammalian cell lines, bacterial cells[1,2,3]. aCella-TOX can work in different media formulations and allows overnight assays while retaining its sensitivity. The sensitivity of aCella-TOX is also greatly enhanced by the coupled luminescent signal-amplification system (3-Phosphoglyceric Phosphokinase/ATP/Luciferase), which yields a strong luminescent signal from even small amounts of released enzyme.



Jurkat cells were plated at various cell concentrations per well. NP-40 cytotoxic agent was added to each well. The aCella-TOX kit was used to detected G3PDH enzyme release. Data points show average RLU in triplicate.

In the aCella-TOX reaction scheme the release of GAPDH is coupled to the activity of the enzyme 3-Phosphoglyceric Phosphokinase (PGK) to produce ATP. ATP is detected via the luciferase, luciferin Bioluminescence methodology. Further, aCella-TOX is a homogeneous cytotoxicity assay ; alternatively in dual mode, aCella-TOX can measure cytotoxicity and cell viability in the same plate. Culture supernatants can also be removed from the original plate and assayed in a different plate, allowing kinetics runs to be set up. The assay is non-destructive, allowing the monitoring of additional parameters such as gene expression.
The method is highly general, since all known cells express copious amounts of GAPDH, and, unlike other enzymes, GAPDH is very readily released from the cytoplasm upon cell lysis. Using specially adapted formulations, the sensitivity of the method can be driven below 1 eukaryotic cell [2].

Applications :
The aCella-TOX method has been tested with many modes of cytolysis, including :

- Cellular cytotoxicity (T cells)
- Complement[2,3], pore-forming agents
- Antibiotic-mediated lysis of bacteria
- Detergent mediated and mechanical lysis

References :

1. Methods and compositions for coupled luminescent assays. United States Patent 6,811,990 Corey and Kinders, issued November 2, 2004.
2. Corey, M. J. and Kinders, R. J. (2005), Drug Discovery Handbook, Ed. Shayne Cox Gad, pp. 689-731
3. Corey, M.J., et al Journal of Immunological Methods 207:43-51, 1997.
4. Corey, M. J., et al., Journal of Biological Chemistry 275: 12917-12925, 2000.
5. Ogbomo H., et al. - Biochemical and Biophysical Research Comunications 339 (2006) pp375-379.
6. Corey, J. and Kinders, J. (2005), Drug Discovery Handbook, Ed. Shayne Cox Gad, pp. 689-731

Description	P/N :	Qty
aCella-Tox bioluminescent Cytotoxicity Assay	**CA4670**	500 tests
Kit Content : 4x Enzyme Assay Reagent, 1x Enzyme Assay Diluent, Glyeraldehyde 3-Phosphate (G3P), 50x Detection Reagent, 5.5x Detection Assay Diluent, Lytic Agent		

■ ATP Assay kit, 0.1 to 100 pmol

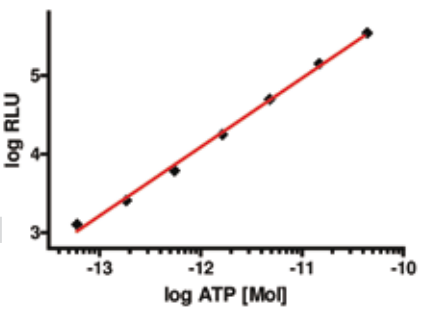
To detect ATP in biological samples or monitore ATP dependent enzyme assays

Substrate : luciferin with stabilizer
$\lambda_{em.}$: **560 nm**
Sensitivity : **0.1 to 100 pmol ATP** - 30 min. signal

The ATP Determination Kit, sensitive assay, offers a convenient bioluminescense assay for quantitative determination of small amounts of ATP. Catalysed by firefly luciferase the substrate D-luciferin is oxidized in an ATP-dependent process generating chemiluminescence at 560 nm (pH 7.8) :

luciferin + ATP+O_2 → (Mg^{2+}, luciferase) → **Oxyluciferin + ATP + pyrophospate + CO_2 + light**

The sensitive assay is optimized for fast determination of low levels of pre-existing ATP or ATP formed in kinetic systems. After a 10 min incubation of the assay reagent, ATP concentrations down to 0.1 pmol can be exactly determined using the linear luminescent signal of the luciferase reaction. Loss of luminescent signal and sensitivity is observed after incubation times of more than 30 minutes. If you are interested in a time-stable assay (i.e. for high throughput screenings) with nearly constant luminescence signals over a period of up to four hours, use our Steady Glow ATP Assay Kit.



Linear luminescence signal for ATP concentrations down to 0.1 pmol using the ATP Determination Kit, sensitive assay.

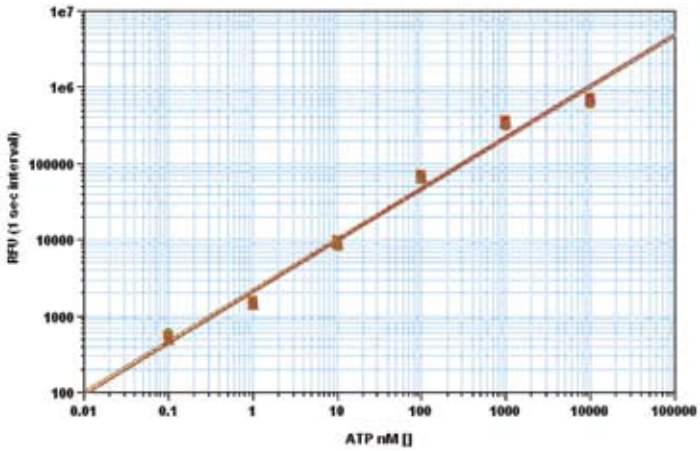
Description	P/N :	Qty
ATP Assay Kit, 0.1 to 100 pmol sensitive	**FP-S2841A**	200-1000 assays (10 ml)
Each kit contains :	**FP-S2841B**	600-3000 assays (30 ml)
Component A : Firefly Luciferase (ready to use glycerol stock solution)	**FP-S2841C**	2000-10000 assays (100 ml)
Component B : D-Luciferin (to dissolve in reaction buffer)		
Component C : Dithiothreitol DTT (to dissolve in reaction buffer)		
Component D : Reaction Buffer (ready to use buffer)		

Related products :

ATP disodium salt	**00064A**	25 g
Reaction Buffer as stand alone product (Component D of ATP Assay Kit)	**CA3920**	30 ml
Reaction Buffer as stand alone product (Component D of ATP Assay Kit)	**CA3921**	100 ml
ARL-67156 Ecto-ATPase inhibitor	**CG2331**	10 mg

■ ATP Assay Kit, Steady Glow

Substrate : luciferin with stabilizer
$\lambda_{em.}$: **560 nm**
Sensitivity : **10 cells/well** - 10 uM to 0.1 nM ATP - 4 h signal



Adenosine triphosphate (ATP) plays a fundamental role in cellular energenics, metabolic regulation and cellular signaling. The ATP Assay Kit provides a fast, simple and homogeneous luminescence assay for the determination of cell proliferation and cytotoxicity in mammalian cells. The assay can be performed in a convenient 96-well and 384-well microtiter-plate format. The high sensitivity of this assay permits the detection of ATP in many biological systems, environmental samples and foods. This ATP Assay Kit has the stable luminescence signal as long as 4 hours. It has stable luminescence with no mixing or separations required, and formulated to have minimal hands-on time.

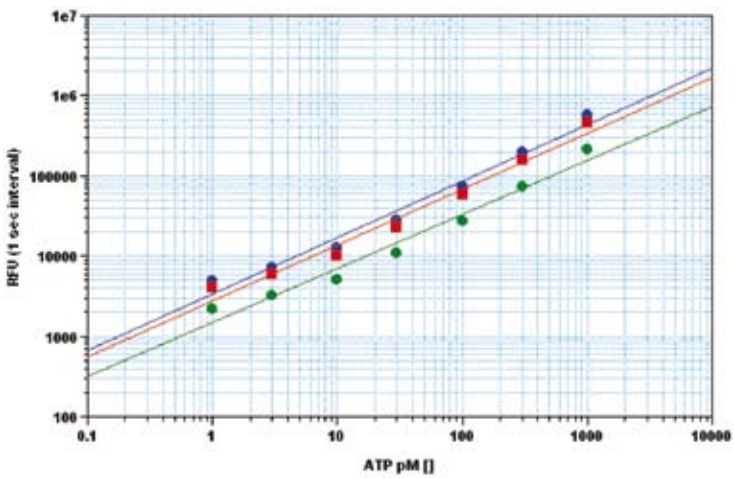
Linear luminescence signal for ATP concentrations from 10 µM to 0.1 nM was detected up to 5 h (Z' factor = 0.7) without signal decayed (above fig shows 20 min, 1, 2, 3, 4, and 5 hr signal). The integrated time was 1 sec.

Description	P/N :	Qty
ATP Assay Kit, Steady Glow	**FN0630**	96 assays

ATP Assay Kit, Bright Glow

Substrate : luciferin with stabilizer
$\lambda_{em.}$: **560 nm**
Sensitivity : **10 cells/well** - 3 pmol ATP 2 h incubation time

Adenosine triphosphate (ATP) plays a fundamental role in cellular energenics, metabolic regulation and cellular signaling. The ATP Assay Kit provides a fast, simple and homogeneous luminescence assay for the determination of cell proliferation and cytotoxicity in mammalian cells. The high sensitivity of this assay permits the detection of ATP in many biological systems, environmental samples and foods. This ATP Assay Kit can detect as low as 10 cells/well. It has stable luminescence with no mixing or separations required, and formulated to have minimal hands-on time.

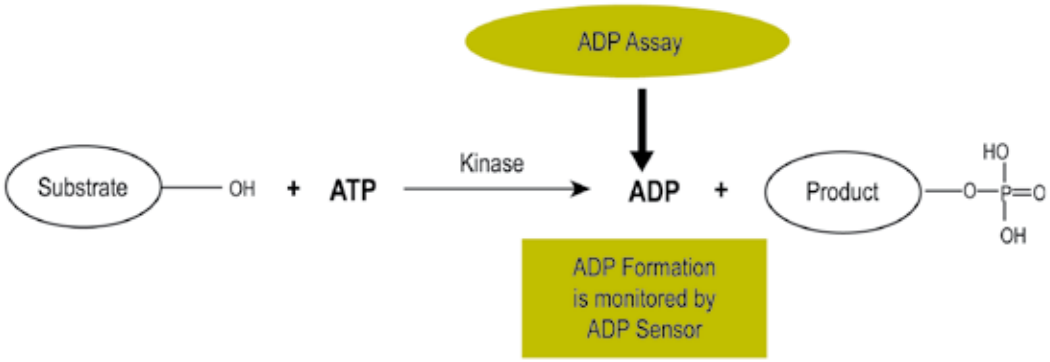


ATP dose response on 96-well white plate using 2 h incubation time (Z' factor = 0.6, Blue 30 min, red 1 h, and green 2 h). The integration time was 1 sec. The half life is more than 1.5 h.

Description	P/N :	Qty
ATP Assay Kit, Bright Glow	**FN0640**	96 assays

Universal Fluorimetric Kinase Assay Kit, Red Fluorescence

Most of commercial protein kinase assay kits are either based on monitoring of phosphopeptide formation or ATP deletion. For the kinase assay kits that are based on detection of phosphopeptides one has to spend time and efforts to identify an optimized peptide substrate while the ATP depletion method suffers various interferences due to the use of luciferase that are inhibited or activated by various biological compounds. The Universal Kinase Assay Kit is based on the monitoring of ADP formation, which is directly proportional to enzyme phoshphotransferase activity and is measured fluorimetricaly. This kit provides a fast, simple, and homogeneous assay for measure kinases activities. The characteristics of its high sensitivity (<0.2 uM ADP), broad ATP tolerance (1-300 uM), non-antibody based, non-radioactive and no-wash method to detect the amount of ADP produced as a result of enzyme activity make it an ideal kit for determining kinase Michaelis-Menten kinetics and for screening and identifying kinase inhibitors.

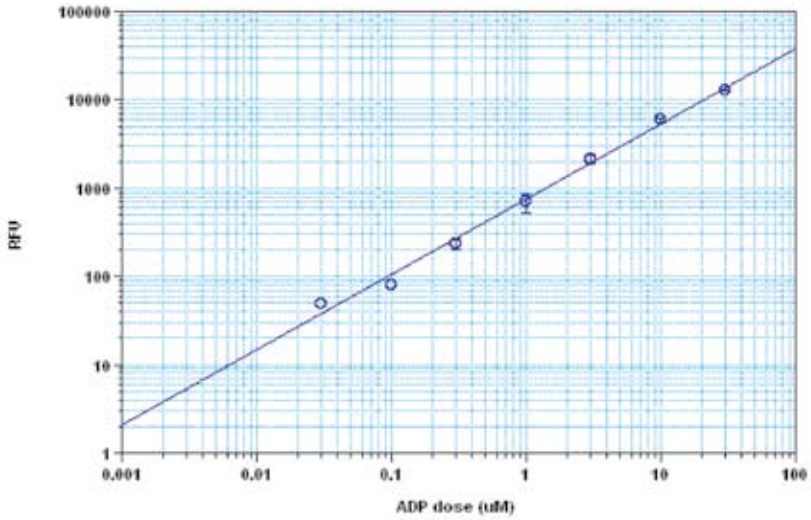


- Universal : Can be used for any kinases that used ATP as phosphate donor.
- Continuous : Easily adapted to automation with no mixing or separation protocols.
- Use of Native substrates : Substrates can be proteins, peptides or sugars.
- Non-Antibody-Based : No antibody is used in the kit.

Description	P/N :	Qty
Universal Fluorimetric Kinase Assay Kit (540/590 nm)	**CL9170**	250 assays
Contains : ADP sensor buffer, ADP Sensor, ADP standard, ADP Assay Buffer		

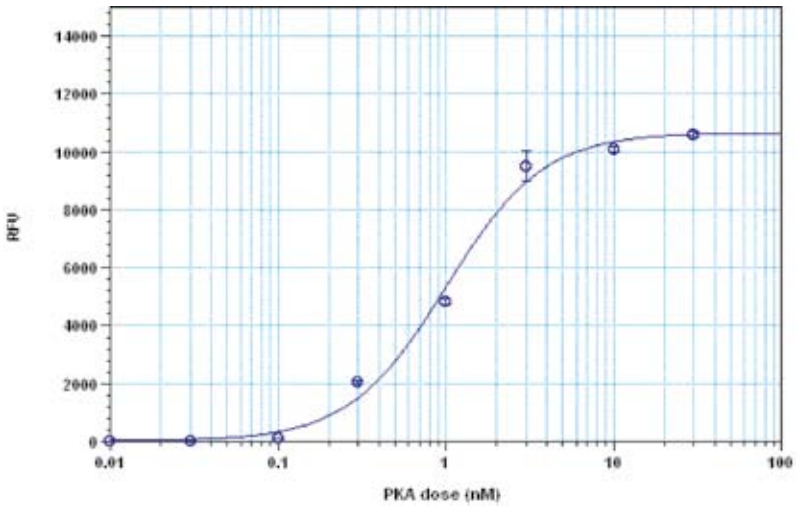
■ ADP Assay Kit, Red Fluorescence

Substrate : red fluorescent substrate
$\lambda_{ex./em.}$: **571 / 585 nm**
Sensitivity : **0.2 µM ADP**
Large Range of ATP Tolerance : 1-300 µM.



ADP dose response on 384-well black plate with 15, 30 minutes and 1 hour incubation time (Z' factor =0.65).

ADP is involved in many biological reactions such as protein kinases. Our ADP assay kit can be used for assaying protein kinase reactions universally by monitoring ADP formation, which is directly proportional to enzyme phosphotransferase activity and is measured fluorimetrically. This kit provides a fast, simple and homogeneous assay for measuring ADP formation or depletion. The assay is continuous, and can be easily adapted to automation. The kit is convenient, requiring minimal hands-on time. Protein kinases are of interest to researchers involved in drug discovery due to their broad relevance to diseases such as cancer and other proliferative diseases, inflammatory diseases, metabolic disorders and neurological diseases. Most of commercial protein kinase assay kits are either based on monitoring of phosphopeptide formation or ATP deletion. Our ADP Assay Kit is based on the monitoring of ADP formation, which is directly proportional to enzyme phoshphotransferase activity and is measured fluorimetricaly. This kit provides a fast, simple, and homogeneous assay for measure kinases activities.



Protein kinase A detection with incubation of the kinase in the presence of ATP and kemptide peptide substrate for 30 minutes.

Description	P/N :	Qty
ADP Assay Kit, Red Fluorescence	**CI4171**	100 assays

■ Phosphate Assay Kit, Blue Fluorescence

Substrate : proprietary substrate
$\lambda_{ex./em}$: **370 / 420 nm**

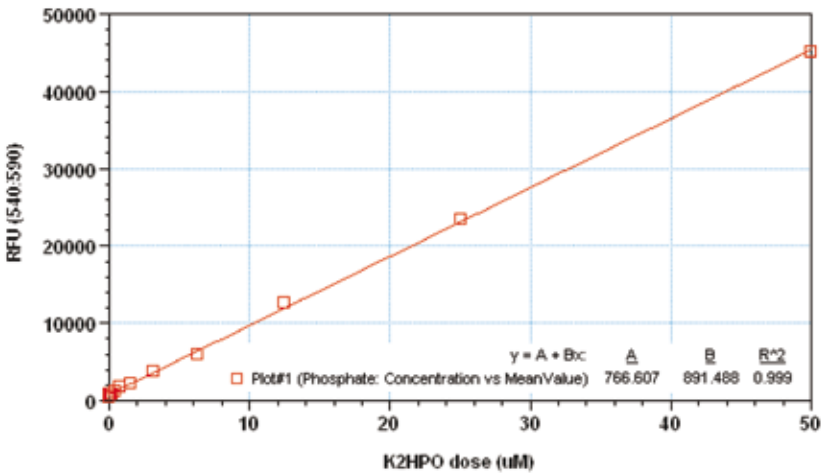
Phosphate is involved in many biological reactions. For example, phosphatases, ATPases and several other enzymes catalyze reactions in which inorganic phosphate (Pi) is released from a substrate. This Phosphate Assay Kit has been developed for measuring the activity of any Pi-generating enzyme. The kit is formulated to give the simplest detection of Pi, neither coupling enzymes nor hazardous radioactive methods are involved. The measurement of Pi is based on our proprietary fluorescent sensor that has its fluorescence intensity proportionally dependent on phosphate concentration. Unlike other phosphate assays, this kit is easy to use. It is a mix and read format, and compatible with all the biological buffers.

Description	P/N :	Qty
Phosphate Assay Kit, Blue Fluorescence	**JQ8120**	1 kit

■ Phosphate Assay Kit, Red Fluorescence

Substrate : proprietary substrate
$\lambda_{ex./em.}$: **540 / 590 nm**
Sensitivity : **0.1 µM phosphate**

Cells utilize a wide variety of phosphate (Pi) and polyphosphate esters as enzyme substrates, second messengers, membrane structural components and vital energy reservoirs. Phosphate is involved in many biological processes. For example, phosphatases, ATPases and several other enzymes catalyze biochemical reactions in which inorganic phosphate is released from a phosphoester substrate. Detection of many phosphoester–metabolizing enzymes is difficult because suitable substrates are not available. It usually has been necessary to determine inorganic phosphate release using tedious colorimetric assays or radioisotope-based methods. This Fluorimetric Phosphate Assay Kit has been developed for measuring the activity of any Pi-generating enzyme using our red fluorescent phosphate sensor. The measurement of Pi is based on the change in the absorbance and fluorescence of our new phosphate sensor. Our kit provides all the essential reagents including phosphate sensor, phosphate standards and assay buffer. It can be used to measure the kinetics of phosphate release from phosphatases (such as GTPases and ATPases) by coupling the two enzymatic reactions. The assay can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation with no separation steps required.



Phosphate dose response on 96-well black plate with 1 hr incubation time

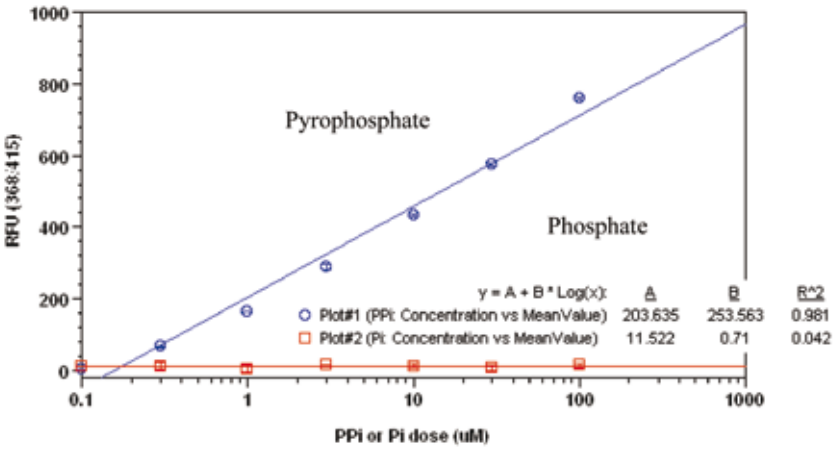
Description	P/N :	Qty
Phosphate Assay Kit, Red Fluorescence	**CI4161**	100 assays
Also available : colorimetric phosphate assays*		
Phosphate Assay, MG method Original molybdate and malachyte green dye method. 600-660 nm reading.	**IS2790**	1kit (600 assays)
Phosphate Assay, MG Plus method Improved end-point stable signal (not prone to precipitation)	**CI4211**	1kit (1000 assays)

*The kit can also be used to estimate the phosphate content of proteins (phosphoserine or phosphothreonine post-translational modifications, after alkaline hydrolysis.

■ Pyrophosphate Assay Kit, Blue Fluorescence

Substrate : proprietary substrate
$\lambda_{ex./em.}$: **370 / 420 nm**
Sensitivity : **0.3 µM** (30 pmoles) pyrophosphate

Pyrophosphate (PPi) are produced by a number of biochemical reactions, such as ATP hydrolysis, DNA and RNA polymerizations, cyclic AMP formation by the enzyme adenylate cyclase and the enzymatic activation of fatty acids to form their coenzyme A esters. The Pyrophosphate Assay Kit provides the most robust spectrophotometric method for measuring pyrophosphate. This kit uses our proprietary fluorogenic pyrophosphate sensor that has its fluorescence intensity proportionally dependent upon the concentration of pyrophosphate. Our assay is much easier and more robust than the enzyme-coupling pyrophosphate methods that require at least two enzymes for their pyrophosphate detections. The kit provides all the essential components for assaying pyrophosphate.



Description	P/N :	Qty
Pyrophosphate Assay Kit, Blue Fluorescence	**JQ8080**	200 assays

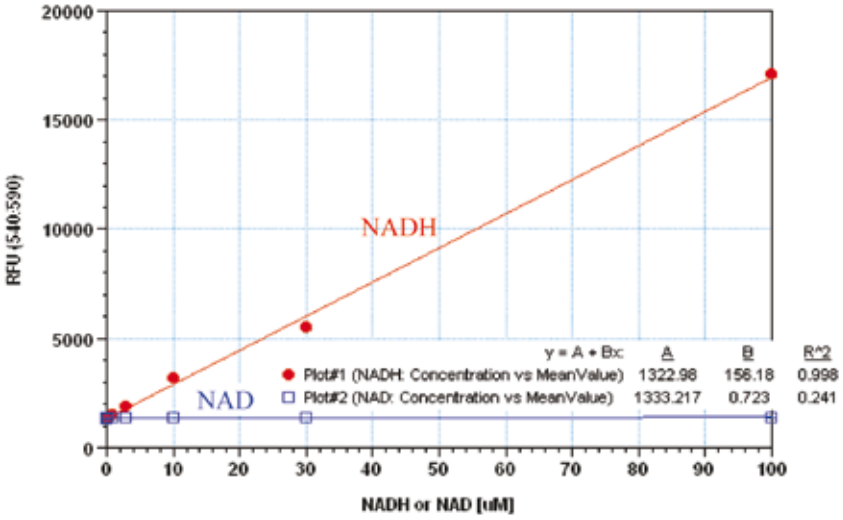
NAD/NADH, NADP/NADPH Assay Kits

Nicotinamide adenine dinucleotide (NAD+) and nicotinamide adenine dinucleotide phosphate (NADP+) are two important cofactors found in cells. NADH is the reduced form of NAD+, and NAD+ is the oxidized form of NADH. It forms NADP with the addition of a phosphate group to the 2' position of the adenyl nucleotide through an ester linkage. NADP is used in anabolic biological reactions, such as fatty acid and nucleic acid synthesis, which require NADPH as a reducing agent. In chloroplasts, NADP is an oxidizing agent important in the preliminary reactions of photosynthesis. The NADPH produced by photosynthesis is then used as reducing power for the biosynthetic reactions in the Calvin cycle of photosynthesis. The traditional NAD/NADH and NADP/NADPH assays are done by monitoring of NADH or NADPH absorption at 340 nm. This method suffers low sensitivity and high interference since the assay is done in the UV range that requires expensive quartz microplate. Theses NAD/NADH & NADP/NADPH Assay Kits provide a convenient method for sensitive detection. In addition, this assay has very low background since it is run in the red visible range that significantly reduces the interference from biological samples. The assay has demonstrated high sensitivity and low interference with 570 nm excitation 590 nm emission.

■ NADH Assay Kit, Red Fluorescence

Sensitivity : **10 nanomoles** of NADH in solution

The enzymes in the system specifically recognize NADH in an enzyme cycling reaction which significantly increases detection sensitivity.



NADPH dose response on 96-well black plate was measured with 1 hour incubation time (n=3) while there is no response from NADP (the insert shows the lower detection limit).

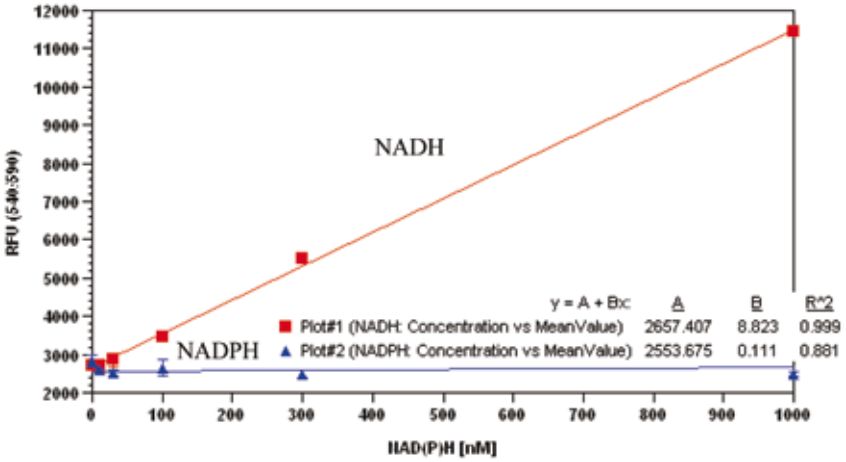
Description	P/N :	Qty
Fluorimetric NADPH Assay Kit	**JQ7320**	400 assays

■ NAD/NADH Assay Kit, Red fluorescence

Sensitivity : **100 nM** (10 pmol/well) of NADH in solution

$\lambda_{exc./em.}$: **570/590 nm**

The enzymes in the system specifically recognize NAD/NADH in an enzyme cycling reaction. There is no need to purify NAD/NADH from sample mix. The enzyme cycling reaction significantly increases detection sensitivity.



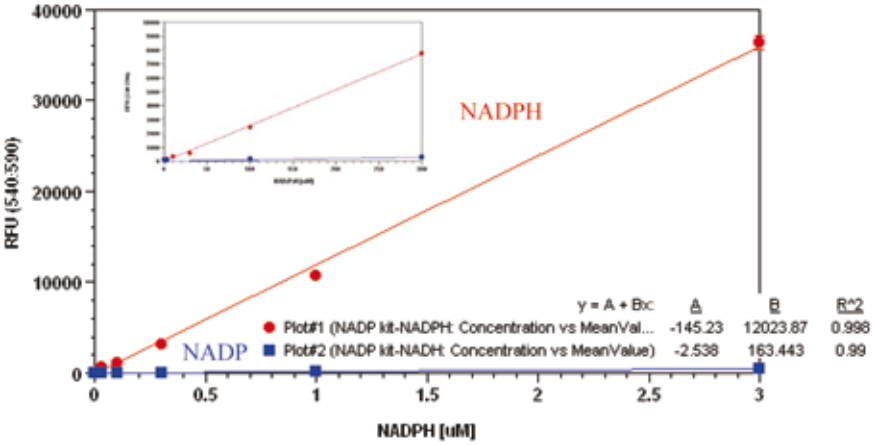
NADH dose response on 96-well black plate was measured with 1 hour incubation time (n=3) while there is no response from NADPH.

Description	P/N :	Qty
NAD/NADH Assay Kit, Red fluorescence	**JQ7280**	400 assays

NADPH Assay Kit, Red Fluorescence

Sensitivity : **30 nM** (0,3 nmol/well) of NADPH in solution

The enzymes in the system specifically recognize NADPH in an enzyme cycling reaction. The enzyme cycling reaction significantly increases detection sensitivity.



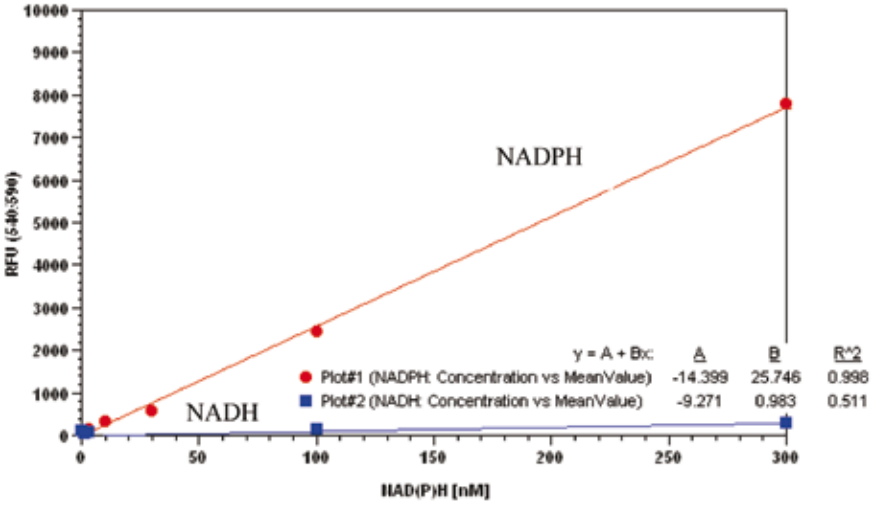
NADPH dose response on 96-well black plate was measured with 1 hour incubation time (n=3) while there is no response from NADP (the insert shows the lower detection limit).

Description	P/N :	Qty
Fluorimetric NADPH Assay Kit, Red fluorescence	**JQ7330**	400 assays

NADP/NADPH Assay Kit, Red fluorescence

Sensitivity : **10 nM** (1 pmol/well) of NADPH in solution

This NADP/NADPH Assay Kit provides a convenient method for sensitive detection of NADP, NADPH and their ratio. The enzymes in the system specifically recognize NADP/NADPH in an enzyme cycling reaction. There is no need to purify NADP/NADPH from sample mix. The enzyme cycling reaction significantly increases detection sensitivity.



NADPH dose response on 96-well black plate was measured with NADP/NADPH Assay Kit with 30 min incubation time (n=3) while there is no response from NADH.

Description	P/N :	Qty
NADP/NADPH Assay Kit, Red fluorescence	**JQ7300**	400 assays

Caspases Fluorometric HTS Assay Kits

- **HTS-compatible** : Single-step homogenous assay specifically designed for HTS-based detection.
- **Fast** : Fast enzyme kinetics.
- **Sensitive** : The enzymatic reaction forms intensely green fluorescent rhodamine 110 (R110) product. The long wavelengths of R110 excitation and emission minimize cellular autofluorescence ($\lambda_{ex}\backslash\lambda_{em}$ = 496/520 nm).

Caspases play important roles in apoptosis and cell signaling. Caspases Fluorometric HTS Assay Kits are specifically designed for HTS-based assays. The kits provide a homogenous assay system for fast and highly sensitive detection of specific caspase activity by fluorescence in enzymatic reaction or mammalian cells.

The assay kits include a caspase inhibitor and can be used as a negative control. Also, R110 dye is provided in the kit for generating a standard curve, which can be used for quantifying caspase activity.

The fluorogenic substrate R110-labeled contains two specific tetrapeptides and is completely hydrolyzed by the enzyme in two successive steps [3, 4]. Cleavage of the first peptide results in the monopeptide intermediate, which has absorption and emission wavelengths similar to those of R110, but has only about 10% of the fluorescence of the latter. Hydrolysis of the second peptide releases the dye R110, leading to a substantial fluorescence increase.

The fluorogenic substrate $(Ac\text{-}IETD)_2$-R110 contains two IETD tetrapeptides and is completely hydrolyzed by the enzyme in two successive steps.
The fluorogenic substrate $(Ac\text{-}LEHD)_2$-R110 contains two LEHD tetrapeptides and is completely hydrolyzed by the enzyme in two successive steps.

Reference :

1. *Cell Death Diff.* 6, 99(1999); 2) *J. Biol. Chem.*. 274, 11549(1999); 3) *J. Biol. Chem.* 275, 288(2000); 4) *Biochemistry*, 38, 13906(1999)

Description	P/N :	Qty
Caspase-3 Fluorometric HTS Assay Kit, DEVD-R110	**FP-BR4930**	1 ml
	FP-BR4931	10 ml
	FP-BR4932	100 ml
Kit Components : Cell lysis/assay buffer , Enzyme substrate $(Ac\text{-}DEVD)_2$-R110 , Enzyme inhibitor Ac-DEVD-CHO, R110		
Caspase-8 Fluorometric HTS Assay Kit, IETD-R110	**FP-BX1510**	1 ml
	FP-BX1511	10 ml
	FP-BX1512	100 ml
Kit Components : Cell lysis/assay buffer, Enzyme substrate $(Ac\text{-}IETD)_2$-R110, Enzyme inhibitor Ac-IETD-CHO, R110		
Caspase-9 Fluorometric HTS Assay Kit, LEHD-R110	**FP-BX1530**	1 ml
	FP-BX1531	10 ml
	FP-BX1532	100 ml
Kit Components : Cell lysis/assay buffer , Enzyme substrate $(Ac\text{-}LEHD)_2$-R110 , Enzyme inhibitor Ac-LEHD-CHO, R110		
Related products :		
Annexin V-FluoProbes 488, FCM grade (495/519 mm)	**FP-BH9390**	100 tests
Staurosporine (apoptosis inducer)	**74146D**	100 µg
	74146E	1 mg

See BioScience Innovations catalog, Membrane apoptosis events.

Caspases Fluorimetric and Colorimetric Assay Kits

Continuous measurement of the caspase activity

- Fast enzyme kinetics
- Sensitive : Rhodamine 110 (496/520 nm) minimizes cellular autofluorescence
- Versatile : Compatible with both fluorometric and colorimetric detection systems.

The principle is the same as for the Caspases Fluorometric HTS Assay Kits.

Although fluorometric detection of the end products is preferred because of the superior sensitivity, detection by absorbance is also possible. In fact, the extinction coefficient of R110 is 10 times higher than that of p-nitroaniline (pNA), a dye commonly used in chromogenic substrates, making R110-based substrates significantly more sensitive than pNA-based substrates, even by colorimetric detection.

The assay kit includes a caspase inhibitor and can be used as a negative control. Also, R110 is provided in the kit for generating a standard curve, which can be used for quantifying caspase activity.

Description	P/N :	Qty
Caspase-3 Fluorometric and Colorimetric Assay Kit, z-DEVD-R110	**FP-85785C**	25 tests
	FP-85785B	100 tests
Kit Components : Cell lysis buffer, Assay buffer, Enzyme substrate $(Ac\text{-}DEVD)_2$-R110, Enzyme inhibitor Ac-DEVD-CHO, R110		
Caspase-8 Fluorometric and Colorimetric Assay Kit, IETD-R110	**FP-BR4940**	25 tests
	FP-BR4941	100 tests
Kit Components : Cell lysis buffer, Assay buffer, Enzyme substrate $(Ac\text{-}IETD)_2$-R110, Enzyme inhibitor Ac-IETD-CHO, R110		
Caspase-9 Fluorometric and Colorimetric Assay Kit, LEHD-R110	**FP-BX1520**	25 tests
	FP-BX1521	100 tests
Kit Components : Cell lysis buffer, Assay buffer, Enzyme substrate $(Ac\text{-}LEHD)_2$-R110, Enzyme inhibitor Ac-LEHD-CHO, R110		
Related products :		
Staurosporine, protein kinase inhibitor	**74146D**	100 µg
Annexin V-FluoProbes 488	**FP-BH4140**	500 µl

See BioScience Innovations catalog, Membrane apoptosis events

■ Caspase-6 and GranzymeB based apoptosis/toxicity Assays

Cytotoxicity is measured as functions of fundamental biochemical pathways leading to cell death :
-in the **CyToxiLux®** kit, cleavage of a cell permeable fluorogenic very specific substrate of caspase-6, an established initial activation step in apotosis.
Literature : Nature Med. 8:185-189 (2002)

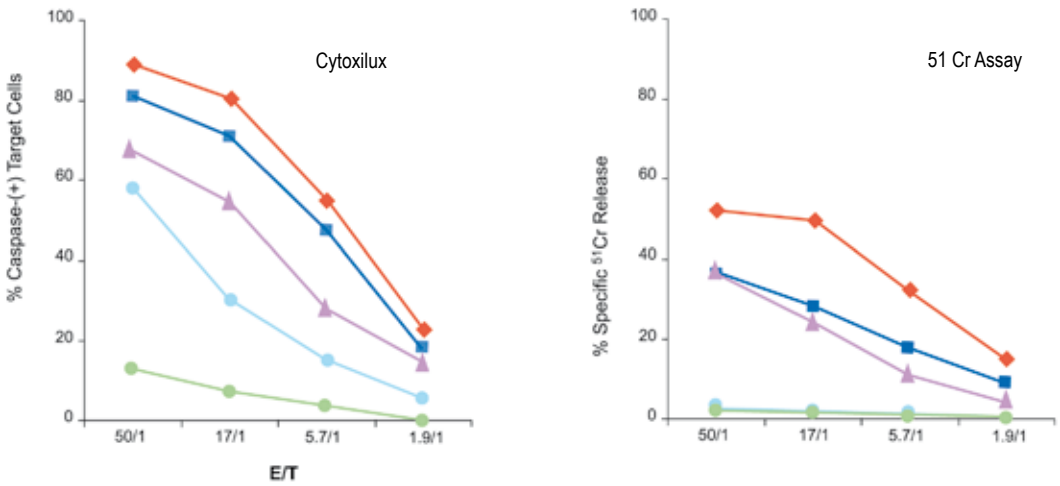
-in the **GranToxiLux™** kit, cleavage of a cell permeable substrate for Granzyme B, involve in a early event of cell-mediated apotosis. Granzyme B that is inactive in lysosomal granules, is activated in the presence of Target cells during (degranulation), extremely early (before Perforin).
Literature : Nature Med. 8:185-189 (2002); Methods Mol. Biol. 263:125-140 (2004): J. Immunol. 171:27-31 (2003)

These assays provide an extremely early quantitative assessment of caspase-6 and cell-mediated cellular cytotoxicity. Especially Grantoxilux replaces advantageously the classic ^{51}Cr release assay is an end stage of cell mediated cytotoxicity, i.e. after cell lysis. Furthermore that can be used by FCM and microscopy yielding single cell measurements in complex populations.

Benefits :

- **More versatile in applications** : suits CTL/NK and other factor mediated cytotoxicity, cytotoxicity induced by intracellular agents or xenobioteics, physiology and fate of effector cells
- **More rapid** : co-incubation of 0.3-2 H (vs. 4 H for 51Cr release assay)
- **More sensitive** than the 51Cr method : can detect relatively weak CTL responses against subdominant epitopes whereas the latter cannot.
- **Large study period** : hour to days allow long term studies, that is useful for non- or slow proliferating cells
- compatible with multiparametric FCM & Microscopy analysis at the cell level, even in mixed populations
- **No seric interferences** : avoid this limitation of LDH and Formazan methods
- **No pre-labeling of cells** : avoid this limitation of 51Cr method

Comparaison between Cytoxilux® and ^{51}Cr Release Assays with a Panel of MHC Class I-Restricted Viral Epitopes



TFL- Labeled EL-4 cells were pulsed with LCMV peptides NP396-404, GP33-42, GP276-286, NP 205-212 or polyoma virus peptide MT 246-254. Following coculture with splenocytes from day 8-post LCMV-infected C57BL/6 mice, Cytoxilux cell permeable fluorogenic caspase substrate was added, cells were washed and subsequently analysed by flow cytometry.

Description	P/N :	Qty
Grantoxilux cytotoxicity assay (Fluo.) Measure the GranzymeB (path of cell-mediated apoptosis)	**BP8891**	50 tests
Cytoxilux cytotoxicity assay (Fluo.) Measure the caspase-6 (classic path of apoptosis)	**BP8881**	50 tests

Each kit contains sufficient reagents for 50 assays i n FCM. It may be applied also for microscopy with some modifications (Caspase-6 or GranzymeB Substrate solution, Target cell marker for use with single laser instruments (Ar ion(488 nm), Target cell marker for use with dual laser instruments (Ar ion(488 nm) and Red (633 nm)), Resuspension medium , Wash Buffer bottle, Assay/Culture medium.

Universal Chemiluminescent PARP Assay Kit

- **Chemiluminescent**, non-radioactive format
- **Higher throughput** 96 test size
- Sensitivity **down to 0.0025 units of PARP** per well
- Available either with a histone coated plate or histone reagent

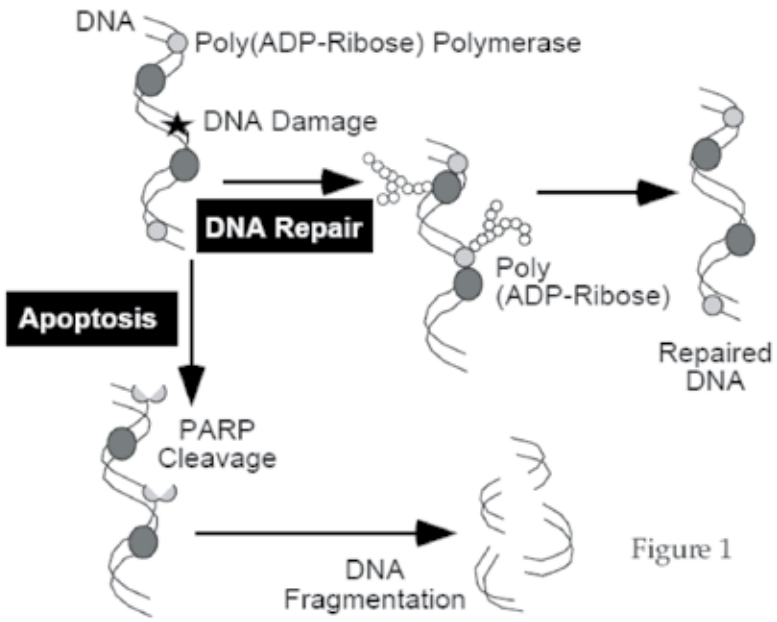


Figure 1

Poly ADP-ribosylation of nuclear proteins is a post-translational event that occurs in response to DNA damage. Poly (ADP-ribose) polymerase (PARP) catalyzes the NAD-dependent addition of poly (ADP-ribose) to adjacent nuclear proteins. PARP plays an important role in DNA repair but can also lead to apoptosis by depleting the cellular NAD pool. Experimental models have shown that PARP inhibition prevents tissue damage in animal models of myocardial and neuronal ischemia, diabetes, septic shock, and vascular stroke. Recent data implicate a synergistic function of Ku80 and PARP-1 in minimizing chromosome aberrations and cancer development. Universal 96-well PARP Assay Kit measures the incorporation of biotinylated Poly (ADP-ribose) onto histone proteins in a 96-well plate format. This assay is ideal for the screening of PARP inhibitors and for measuring the activity of PARP in cell and tissue extracts.

Applications :

- Identify inhibitors and activators of PARP activity
- Measure caspase inactivation of PARP
- Quantitate levels of DNA damage in cells caused by a variety of genotoxic agents
- Measure activity of PARP in cell and tissue extracts

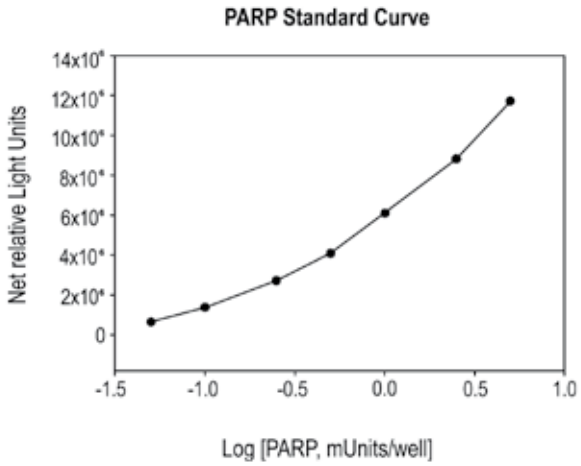
Description	P/N :	Qty
Universal Chemiluminescent PARP Assay Kit w/ Histone Reagent	**HP9090**	96 tests
Universal Chemiluminescent PARP Assay Kit w/ Histone Coated	**HP9130**	96 tests
Related product :		
FITC-NAD	**FX8241**	250 µl

HT Chemiluminescent PARP/Apoptosis Assay

ELISA assay kit for monitoring PARP activity before and during apoptosis

Sensitivity **down to 0.1 mUnits of PARP** - less than 500 cells/well

During apoptosis, PARP-1 which catalyzes the NAD dependent addition of poly (ADP-ribose) (PAR) onto various cytoplasmic and nuclear proteins, is cleaved from about 116 kDa to 85 kDa. HT PARP/Apoptosis Assay is ideal for measuring the activity of PARP in cell extracts before and during apoptosis. The HT PARP/Apoptosis Assay is an ELISA which semi-quantitatively detects PAR deposited onto immobilized histone proteins in a 96-well format. An anti-PAR monoclonal antibody, goat anti-mouse IgG-HRP conjugate, and HRP substrate are used to generate a chemiluminescent signal. Thus, absorbance correlates with PARP activity. Etoposide is a topoisomerase II inhibitor that stabilizes this enzyme after it cleaves DNA. It is included as a control apoptosis inducer.



Graphical representation of an example Chemiluminescent readout of a PARP standard curve.

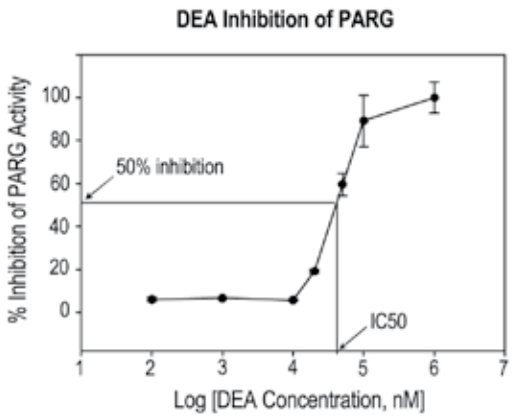
Description	P/N :	Qty
HT Chemiluminescent PARP/Apoptosis Assay	**CP1990**	96 tests

Kit content : PARP Buffer, activated DNA, PeroxyGlow™ A & B, Histone coated strip wells, PARP HSA, NAD, Antibody diluent, anti-PAR monoclonal antibody, HRP conjugate diluent, Etoposide.

HT Chemiluminescent PARG Assay Kit

- **Chemiluminescent** format
- **Higher throughput** 96 test size
- Sensitivity **down to 50 pg** of PARG per well

Poly(ADP-ribose) glycohydrolase (PARG) degrades poly(ADP-ribose) (PAR) polymers synthesized by poly(ADP-ribose) polymerase (PARP1). When activated by DNA strand breaks, PARP1 uses NAD as a substrate to form ADP-ribose polymers on itself and on specific acceptor proteins such as histones, DNA polymerases, DNA ligases, p53, and Fos. These polymers are in turn rapidly degraded by PARG, a ubiquitously expressed exo- and endoglycohydrolase. Excessive activation of PARP1 leads to NAD depletion and cell death during ischemia and other conditions that generate extensive DNA damage. PARG may maintain the active state of PARP1 by continuously removing inhibitory ADP-ribose residues from PARP1. The regulation of PARG activity may therefore, influence PARP-mediated cell death. First, PARG inhibition could slow the turnover of PAR and thereby limit NAD depletion. Second, PARG inhibition could prevent the removal of PAR from PARP1. Because PARP1 is inhibited by extensive poly-(ADP-ribosyl)ation, PARG inhibitors could thereby indirectly inhibit PARP1 activity. Prior work has shown that the PARG inhibitor gallotannin can markedly reduce death of astrocytes after oxidative stress.



HT Chemiluminescent PARG Assay Kit measures the loss of biotinylated PAR from histones attached to strip wells in a 96 well format and is ideal for the screening of PARG inhibitors and for measuring the activity of PARG in cell extracts.

Applications :

- Identify inhibitors and activators of PARG activity
- Measure activity of PARG in cell and tissue extracts

Description	P/N :	Qty
HT Chemiluminescent PARG Assay Kit	**JZ4060**	96 tests

■ FlowTACS™ Apoptosis Detection Kits

Identify and quantitate apoptotic cells in culture

DNA fragmentation is a committed step in apoptosis, and the labeling of 3' ends provides an easy measure of cells undergoing apoptosis. Cells may also be analyzed for DNA content using the included propidium iodide/RNase A solution. The FlowTACS™ Kit also provides TACS-Nuclease™ to generate positive controls for calibration. The FlowTACS™ Kit uses fixed cells, allowing you to safely work with cells that are infected with biohazardous agents. Also, samples may be stored conveniently during time-course experiments.

This complete kit provides all the reagents required for labeling including two permeabilization reagents, labeling and stop buffers, labeling and detection reagents, and TACS-Nuclease for generating positive controls with your own samples.

Features :

- **Fast.** Requires less than 3 hours to complete.
- Exclusive, **non-toxic** TACS Safe TdT™ buffer - sodium cacodylate free.
- Unique buffer system produces more **consistent labeling**.
- **Works on fixed cells.**
- Includes exclusive Cytonin™ permeabilization reagent.
- Includes TACS-Nuclease solution for preparing sample-dependent positive controls.

Description	P/N :	Qty
FlowTACS™ Apoptosis Detection Kits	**512510**	60 samples
Kit content : Permeabilization reagents, Optimized cation, TACS-Nuclease, Labeling buffer, Detection reagents, Propidium iodide/RNase solution, Nucleotide mix, Fluorescent label, Labeling enzyme, Counterstain		

■ Hoechst 33342

Cell-permeant bis-benzimide that binds to DNA with fluorescence enhancement ($\lambda_{exc.}/\lambda_{em.}$: 350/461 nm).

Reference :

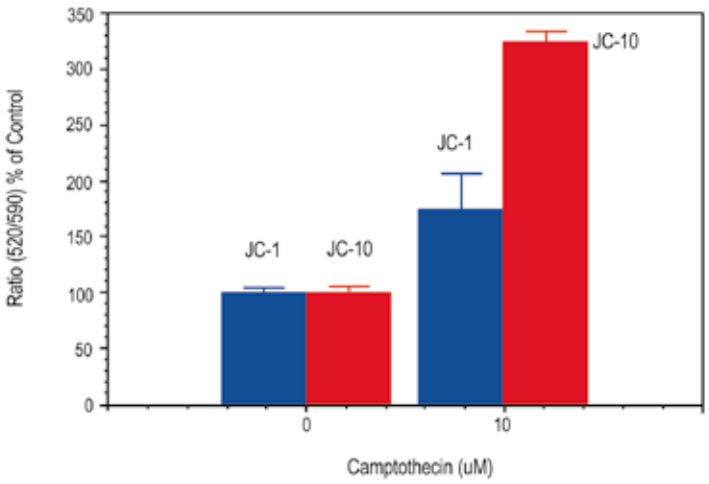
Debbasch C, et al., Mitochondrial activity and glutathione injury in apoptosis induced by unpreserved and preserved beta-blockers on Chang conjunctival cells. *Invest Ophthalmol Vis Sci.* 2001 Oct;42(11):2525-33.

Description	P/N :	Qty
Hoechst 33342, 10 mg/ml in water	**FP-59046A**	10 ml

JC-10 Mitochondria Membrane Potential Assay Kit

- Increased Signal Intensity : Larger assay window
- Increased solubility : Much better water solubility than JC-1
- Convenient and Robust : Formulated to have minimal hands-on time
- Versatile applications : Compatible with many cell lines and targets

Although JC-1 is widely used in many labs, its poor water solubility makes it hard to use for some applications. Even at 1 µM concentration, JC-1 tends to precipitate in aqueous buffer. JC-10 has been developed to be a superior alternative to JC-1 where high dye concentration is desired. Compared to JC-1, our JC-10 has much better water solubility. JC-10 is capable of entering selectively into mitochondria, and changes reversibly its color from green to orange as membrane potentials increase. This property is due to the reversible formation of JC-10 aggregates upon membrane polarization that causes shifts in emitted light from 520 nm (i.e., emission of JC-10 monomeric form) to 570 nm (i.e., emission of J-aggregate). When excited at 490 nm, the color of JC-10 changes reversibly from green to greenish orange as the mitochondrial membrane becomes more polarized. This JC-10 Mitochondrial Membrane Potential Assay Kit enable you to monitor mitochondrial membrane potential changes using a simple microplate reader while all the other commercial JC-1 assay kits require the use of a flow cytometer. Our kit provides the most robust method to monitor mitochondrial membrane potential changes, and can be readily used for screening a large compound library.



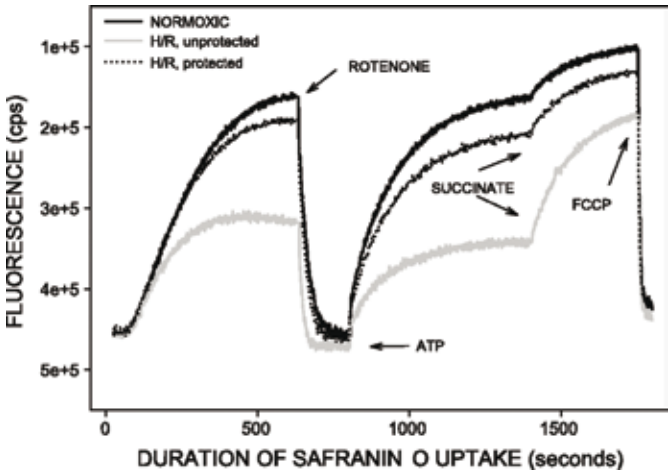
JC-10 and JC-1 Comparison on Effect of Campotothecin induced mitochondria membrane potential change in JurKat cells. Jurkat cells were treated with 10 µM camptothecin for 4 hours. JC-1 and JC-10 dye loading solution was then added to the wells for 30 minutes. The fluorescent intensity for both J-aggregates and monomeric forms of JC-1 and JC-10 was measured at Ex 485 nm/Em 520 and 595 nm.

Description	P/N :	Qty
JC-10 Mitochondria Membrane Potential Assay Kit	**DT2420**	5 plates (96- or 384-well)
JC-10	**CL0440**	5 mg
Related products :		
CCCP	**091640**	500 mg
JC-1, as stand alone product	**FP-52314A**	5 mg

Safranine O

Dynamic and quantitative approach of changes of mitochondrial membrane potential (DeltaPsi(m)) ΔΨm :

- Suitable for **dynamic studies** of energization
- Allows **direct assessment** of both substrate-dependent, electron transport-mediated ΔΨm and ATP hydrolysis-supported ΔΨm.
- **Avoid limitation of JC-1** uptake when the plasma membrane potential decreases



Fluorescence is followed at 485 nm excitation and 586 nm emission. Unlike the behavior of JC-1, safranine O was rapidly and completely released when mitochondrial were de-energized with FCCP. The decreased fluorescence of safranine O resulting from quenching of safranine O after uptake by energized mitochondria means that low fluorescence corresponds to high ΔΨm (Feldkamp, 2004).

Description	P/N :	Qty
Safranine O	**N12820**	25 g
	N12821	100 g

Reference : Feldkamp T. *et al.* – Assessment of mitochondrial membrane potential in proximal tubules after hypoxia/reoxygenation, AM. J. Physiol. Renal Physiol., 288: F1092 - F1102 (2005)

Related products :

Ouabain	**050538**	1 g

Glutathione Detection Kit, Monochlorobimane

GSH depletion follow up during early stage of mitochondrion-associated apoptosis

- **Simple** : Compatible with a standard or fluorescence plate reader.
- **Fast** : Around 1 hour assay time.

Diminished cellular glutathione (GSH) level occurs at the early stage of mitochondrion-associated apoptosis pathway due to GSH efflux. GSH depletion further leads to cytochrome c release and caspase 3 induction (1,2) MCB Glutathione Detection Kit utilizes a thiol-reactive dye monochlorobimane (MCB), which is essentially monfluorescent until it reacts with a thiol to form a blue fluorescent product ($\lambda_{abs}/\lambda_{em}$ = 380/461 nm). By incubating cellular lysate with MCB, the intensity of the fluorescent signal generated from the assay reflects the amount of GSH present in the cells.

Reference :
1) FASEB J. 12(6), 479(1998); 2) Biochem. Soc. Trans. 28, 56(2000).

Description	P/N :	Qty
Glutathione Detection Kit, Monochlorobimane	**FP-BU1410**	100 tests
Kit content : Cell lysis buffer, Monochlorobimane (MCB), GST positive control		

Related product :

Monochlorobimane, as stand alone product	**FP-38980A**	25 mg

Live Cell Glutathione Transferase Activity Kit

$\lambda_{abs}/\lambda_{em}$: 380/461 nm
Sample size : 200 cells

This new kit provides reagents and methods to simply and quickly measure glutathione transferase activity in live cells, tissues or cell lysate samples. It allows intracellular glutathione S-transferase detection by simply adding a fluorogenic reagent mCB to the cell culture medium or lysate to form GSH-mCB complexes. Unlike other bimanes such as monobromobimane, monochlorobimane appears to form an adduct exclusively with GSH. This procedure has been used to measure GSH content of cultured neural cells and in tissue homogenates and, indeed, several laboratories have used this approach to measure the GSH content of the cytosolic fraction of liver or in intact tissues. It has been found that monochlorobimane readily enters cells to form a fluorescent GSH mono-chlorobimane adduct that can be measured fluorometrically and that this reaction is catalyzed by glutathione S-transferase.

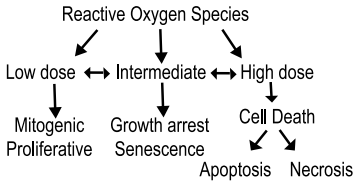
Description	P/N :	Qty
Live Cell Glutathione Transferase Activity Kit	**BQ2350**	100 assays
Kit contains : Monochlorobimane, Cell Lysis Buffer, L-Glutathione, Glutathione S-transferase		

Technical tip

Oxidative metabolism study (ROS, NO)

The production of free radicals primarily results from O_2 catched by cells and reduced in mitochondria. 98% is fully utilized by cytochrome c oxidase to form water, but this enzyme can release partly reduced species. Other respiratory chain enzymes, and in particular complexes I and III, also produce partly reduced oxygen species including superoxide. These reactive oxygen species can react with nitric oxide to produce reactive nitrogen species including peroxynitrite. A significant proportion of the reactive oxygen and nitrogen species diffuse with controlled rate into the cytosol, where they react with various molecules, lipids, proteins, sugars and nucleotides. But a major portion remains in the mitochondrion where they causes oxidative damage. When the electron transfer efficiency decreases, more radicals are produced, and so more cytosolic proteins are damaged. Moreover, oxidative and nitrative damage of mitochondrial proteins adds to OXPHOS dysfunction further exacerbating free radical production. A protective mechanism against ROS is SOD metabolism.

Enhanced oxidative stress occurs in number degenerative diseases. In human, ROS are considered to be one of the main causes of aging-related diseases, Parkinsons disease, Alzheimers and other vascular-damage-related brain diseases, Cancer, Artherioschlerosis and diabetes.
In plants, the SOD activity is increased by the use of herbicides such as paraquat, by the SO_2 concentration in the atmosphere, by drought, or by exposure to high concentration of zinc and magnesium.
ROS probes have high selectivity and sensitivity in enzymatic oxidation reactions, favorising their use for diagnostic analysis. Also, peroxidase is a common enzyme for signal amplification in immunoassays (EIA).



Interchim provides several ROS probes for fluorimetry and colorimetry, chromogenic, fluorogenic or luminogenic.

Selection guide

Fluorescent ROS probes

		Reactive Oxygen Species (ROS)					
P/N :	Probes	Hydrogen Peroxide	Hydroxy radical	Hypochlorous acid	Peroxyl radical	Peroxynitrite anion	Superoxide anion
		H202	H0-	HOCl	COO-	ONOO-	O2-
FP-83775	Dihydrorhodamine 123	+		+		+	
FP-46731	H2DCFDA	+			+	+	+
FP-46915	Lucigenin*	+					
FP-97233	Coelenterazine					+	+
FP-38544	MCLA*		+				+
Dihydrocalcein	AM	+	($^1O_2$)		+	+	
Dihydroethidium						-	+
24200A	tMPV						
CA7170	HPF		+			+	
CA7270	APF	-	+	+	-	+	-
U3238A	ADHP	+					

***ROS-generating Enzyme Detection kits**

MyeloPeroxidase detection kit	Hemoprotein of PMNs cells / Cl- oxidation to HOCl
Catalase detection kit	antioxidant enzyme / decomposes H_2O_2
Cis-Parinaric acid	fatty acid to monitor lipid peroxidation
Malachite Green IT	produce a Hydroxyl radical burst upon irradiation
SuperOxideDismutase (SOD)	converts $O2^{-\bullet}$ into H_2O_2 and O_2

***Other oxidative species : aldehydes**

SSAO detection kit	deamination / formaldehyde, methylglyoxal
MonoAmineOxidase (MAO A&B)	oxidate a variety of neurotransmitters / aldehydes

H_2DCFDA, Carboxy-H_2DCFDA, H_2DCFDA-SE

H_2DCFDA is widely used to detect reactive oxygen species (hydrogen peroxide, $ONOO^-$) in cells (neutrophils, macrophages). Colorless ($\lambda_{exc.}/\lambda_{em.}$: 258/none ; EC : 11 000) and nonfluorescent until the acetate groups are hydrolyzed by intracellular esterases and oxydation occurs within the cell, giving the highly green fluorescent 2',7'-dichlorofluorescein (DCF #FP46629; $\lambda_{abs.}/\lambda_{em.}$: 495/529 nm). Applications include ROS detection, viability and cytotoxicity assays, apoptosis. It can be used with Propidium iodide to follow oxidant production and nuclear injury.
Carboxy-H_2DCFDA is an indicator for ROS. On penetration into the cells, carboxy-H_2DCFDA is deacetylated by intracellular esterases, resulting in an enhanced cellular retention of the probe. The fluorescence of the oxidized form of carboxy-H_2DCFDA is measured with a fluorescence excitation of 485 nm and emission at 535 nm.
H_2DCFDA, SE is an amine reactive form of the H_2DCFDA to prepare oxidation-sensitive conjugates, like dextran. This allows detection of oxidative burst in phagocytic cells.

Reference :
Jiyoung K. and Raghubir S., Calcium-Mediated Activation of c-Jun NH_2-Terminal Kinase (JNK) and Apoptosis in Response to Cadmium in Murine, *Macrophage, Toxicological Sciences* 2004 81(2):518-527

Description	P/N :	Qty
2',7'-Dichlorodihydrofluorescein diacetate **(H_2DCFDA)**	**FP-467312**	100 mg
Carboxy-2',7'-Dichlorodihydrofluorescein diacetate **(CH_2DCFD)**	**FP-46634A**	25 mg
2',7'-Dichlorodihydrofluorescein diacetate, succinimidyl ester **(H_2DCFDA-SE)**	**FP-59031A**	5 mg
$\lambda_{exc.}/\lambda_{em.}$ (hydr.&oxid.) (pH 4) : 495/529 nm ; EC : 38 000 $M^{-1}cm^{-1}$		
$\lambda_{exc.}/\lambda_{em.}$ (hydr.&oxid.) (pH 8) : 504/529 nm ; EC : 107 000 $M^{-1}cm^{-1}$		

Coelenterazine (native)

Coelenterazine is a sensitive chemiluminescent marker for detecting both superoxide and peroxynitrite. It has no significant effect on xanthine oxidase–dependent oxygen consumption, endothelial cell hydrogen peroxide release, or endothelium-dependent relaxation. Coelenterazine emits chemiluminescence (Em = 466 nm) on oxidation by superoxide.

Reference :
Margaret T. et al.- Chemiluminescent Detection of Oxidants in Vascular Tissue, Lucigenin But Not Coelenterazine Enhances Superoxide Formation, *Circulation Research.* 84:1203-1211 (1999)

Description	P/N :	Qty
Coelenterazine (native)	**UP972333**	1 mg

MCLA

Superoxide or singlet oxygen chemiluminescent probe

MCLA like coelenterazine is a superior alternative to lucigenin for superoxide detection. Lucigenin can reportedly sensitize superoxide production, leading to false-positive results. An additional advantage of MCLA is that its pH optimum for luminescence generation is closer to the physiological near-neutral range than are the pH optima of luminol and lucigenin. MCLA generates chemiluminescence (Em = 455 nm) upon reaction with superoxide.

Reference :
. Teranishi K, *et al.* Enhanced chemiluminescence of 6-(4-methoxyphenyl)imidazo[1,2-a]pyrazin-3(7H)-one by attachment of a cyclomaltooligosaccharide (cyclodextrin). Attachment of cyclomaltononaose (delta-cyclodextrin)." *Carbohydr Res* 338, 987-93 (2003)
. Kondo M. *et al.*, The ability of neonatal and maternal erythrocytes to produce reactive oxygen species in response to oxidative stress.", *Early Hum Dev* 66, 81-8 (2002)
. Sakurai T. *et al.*, Superoxide production in the islet of Langerhans detected by the MCLA chemiluminescence method." *Methods Mol Biol* 196, 203-9 (2002)

Description	P/N :	Qty
MCLA 2-Methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-one, hydrochloride	**FP-38544A**	5 mg

■ Dihydroethidium (hydroethidine)

Measurement of O_2^-

The superoxide oxidizes dihydroethidium to a specific fluorescent product (oxyethidium) that differs from ethidium by the presence of an additional oxygen atom in its molecular structure. Exposure of dihydroethidium to hydrogen peroxide or peroxynitrite caused no formation of oxyethidium from dihydroethidium. Fluorescence detection at 590 nm (emission) and 530 nm (excitation) is used to monitor oxyethidium production.

Reference :

Debbasch C, et al., Mitochondrial activity and glutathione injury in apoptosis induced by unpreserved and preserved beta-blockers on Chang conjunctival cells. *Invest Ophthalmol Vis Sci.* 2001 Oct;42(11):2525-33.

Description	P/N :	Qty
Dihydroethidium, special air-free packaging	**FP-52492A**	25 mg
	FP-52492B	10 x 1 mg Dihydroethidium, 5 mM in DMSO
	FP-R5919A	1 ml Dihydroethidium
	FP-524929	20 x 50 µg

■ Dihydrorhodamine-1,2,3 (DHR 123)

Measurement of peroxonitrite ($ONOO^-$)

The level of $ONOO^-$ can be measured by monitoring the oxidation of dihydrorhodamine-1,2,3 (DHR 123), using microplate reader at excitation and emission wavelengths of 485 nm and 530 nm, respectively. It is nonfluorescent until oxidized to the mitochondrial probe rhodamine 123.

Reference :

Ji Young Lee, *et al.*, Induction of endothelial apoptosis by 4-hydroxyhexenal, *Eur. J. Biochem.* 271, 1339-1347 (2004)

Description	P/N :	Qty
DiHydroRhodamine 123, 5 mM stabilized solution in DMSO	**FP-R6805A**	1 ml
Dihydrorhodamine 123	**FP-83775A**	10 mg
Dihydrorhodamine 123, air-free packaging	**FP-IT397A**	10 x 1 mg
Dihydrorhodamine 123, 5 mM in DMSO	**FP-R6805A**	1 ml

■ Dihydrocalcein A

This cell permeant probe is oxidized to the calcein with better cell retention than H_2DCFDA.

Reference :

Keller A, *et al.* Analysis of dichlorodihydrofluorescein and dihydrocalcein as probes for the detection of intracellular reactive oxygen species. *Free Radic Res.* 38: 1257–1267 (2004)

Description	P/N :	Qty
Dihydrocalcein AM	**FP-BA1970**	1 mg
	FP-T7996A	20 x 50 µg

■ trans-1-(2'-Methoxyvinyl)pyrene (tMPV)

Sensitive singlet oxygen chemiluminescence probe

trans-1-(2'-Methoxyvinyl)pyrene is the most sensitive singlet oxygen probe that could be used to detect picomole quantities of singlet oxygen in chemical and biological systems. Furthermore, this highly selective probe does not react with other activated oxygen species such as hydroxyl radical, superoxide or hydrogen peroxide. It generates chemiluminescence (Em = 465 nm in 0.1 M SDS) upon reaction with $^1O_2$.

Reference :

Nat Biotechnol 24, 95-9 (2006).
Plant and Cell Physiology 46(6):947-954 (2005)
Methods Enzymol 133, 569-584 (1986)

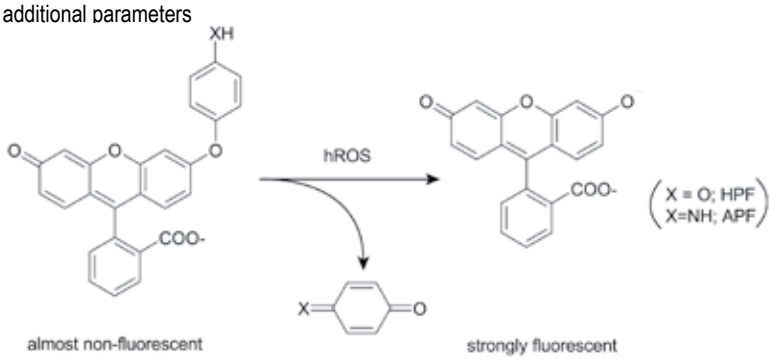
Description	P/N :	Qty
trans-1-(2'-Methoxyvinyl)pyrene (353/401 nm)	**FP-24200A**	1 mg

■ Hydroxyphenyl fluorescein (HPF) Assay Kit

Fluorescent Hydroxyl (•OH) / Peroxynitrite ($ONOO^-$) assay

- Quenched Cell permeable dye
- Can be used with cell lysates, tissue homogenates
- One step, no wash, homogenous assay
- Can monitor multiple time points to follow real time kinetics
- Non-destructive cell based assay allows monitoring of additional parameters

A novel probe, Hydroxyphenyl fluorescein (HPF) is a highly selective probe for the detection of highly Reactive Oxygen Species (hROS). It is a cell permeable highly sensitive fluorescent probe for hydroxyl radical (OH•), and peroxynitrite ($ONOO^-$) detection. It has little reactivity towards other hROS such as : hypochlorite (^-OCl), singlet oxygen ($0_2{}^1$), superoxide ($0_2{}^-$•), hydrogen peroxide (H_20_2), nitric oxide (NO•), and alkyl peroxide (RO2•).



Reference :

Ken-ichi Setsukinai, *et al.*: *Development of Novel Fluorescence Probes That Can Reliably Detect Reactive Oxygen Species and Distinguish Specific Species.* J. of Bilogical Chemistry, Vol. 278, No. 5, Issue of January 31, pp. 3170–3175, 2003

Description	P/N :	Qty
Hydroxyl / Peroxynitrite Detection Kit (HPF)	**CA7170**	150 tests
Related product :		
FeTPPS, specific peroxynitrite scavenger	**888810**	250 mg

■ APF (for hROS detection)

Selective Indicator for Highly Reactive Oxygen Species

- Can monitor multiple time points to follow **real time kinetics**
- Quenched cell permeable dye
- **One-step, no wash** assay
- Adaptable for **High Throughput** format
- **Non-destructive** cell based assay allows monitoring of additional parameters
- **Applications** - Fluorescence Plate Reader / Fluorescent Microscope / Flow Cytometry

A new novel probe, Aminophenyl fluorescein (APF) developed by Tetsuo Nagano et. al. [(1)], is a general selective indicator for the detection of highly reactive oxygen species (hROS). The probe has little reactivity towards other ROS such as: singlet oxygen ($O_2{}^1$), superoxide ($O_2{}^-$•), hydrogen peroxide (H_2O_2), nitric oxide (NO•), and alkyl peroxide (RO_2•) (see table below)[1]. APF is a cell permeable indicator that can be used to detect Hydroxyl Radical (^-OH), Peroxynitrite: ($ONOO^-$) and hypochlorite (^-OCl) production in cells.

ROS (RFU)	APF (RFU) Ex : 499 Em : 515	DCFH-DA (RFU) Ex : 500 Em : 520
Hydroxyl Radical : •OH	1200	7400
Peroxynitrite : $ONOO^-$	560	6600
Hypochlorite : ^-OCl	3600	86
Oxygen Radical :$1O_2$	9	26
Superoxide : $O_2{}^-$•	6	67
Hydrogen Peroxide : H_20_2	<1	190
Nitric Oxide : NO	<1	150
Alkylperoxyl Radical : ROO.	2	710
Autoxidation	<1	2000

Reference :

1- Setsukinai K., *et al.*, Development of Novel Fluorescence Probes That Can Reliably Detect Reactive Oxygen Species and Distinguish Specific Species. The journal of Biological Chemistry Vol. 278, No. 5, Issue of January 31, pp. 3170–3175, 2003

Description	P/N :	Qty
Aminophenyl Fluorescein (APF)	**CA7270**	150 tests

■ Hypochlorite Detection Kit

Fluorescent OCl (Hypochlorite) assay

- Quenched Cell permeable dye
- Can be used with Cell Lysates, Tissue Homogenates
- One Step, No wash Homogenous assay
- Can monitor multiple time points to follow real time kinetics
- Non-destructive cell based assay allows monitoring of additional parameters

The two novel probes, Aminophenyl fluorescein (APF) and Hydroxyphenyl fluorescein (HPF) are selective for the detection of highly reactive oxygen species (hROS). They offer greater selectivity and stability than does H_2DCFDA. Both probes have little reactivity towards other ROS such as: singlet oxygen (0_2^1), superoxide (O_2^{-}•), hydrogen peroxide (H_20_2), nitric oxide (NO•), and alkyl peroxide (RO_2•). HPF/APF are cell permeable and can be used in combination to detect hypochlorite ($^{-}$OCl) production in cells (see fig 1). Hypochlorite can be detected by loading two samples, one with APF and the other with HPF. Hypochlorite production is visualized by increase in fluorescence of APF loaded cells and no increase in fluorescence in HPF loaded cells.

Reactivity Profile of APF/HPF:

ROS (RFU)	HPF (RFU) $\lambda_{ex./em.}$: 499 / 515 nm	APF (RFU) $\lambda_{ex./em.}$: 499 / 515 nm	DCFH-DA (RFU) $\lambda_{ex./em.}$: 500 / 520 nm
Hydroxyl Radical: •OH	730	1200	7400
Peroxynitrite: $ONOO^-$	120	560	6600
Hypochlorite: $^{-}$Ocl	6	3600	86
Oxygen Radical: $^1O_2$	5	9	26
Superoxide: O_2^{-}•	8	6	67
Hydrogen Peroxide : H_20_2	2	1	190
Nitric Oxide: NO	6	1	150
Alkylperoxyl Radical: ROO	17	2	710
Autoxidation	1	1	2000

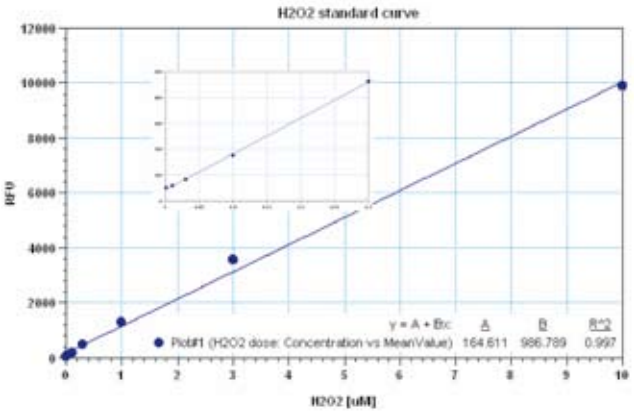
Description	P/N :	Qty
Hypochlorite Detection Kit (HPF, APF)	**CA7250**	150 tests

■ ADHP Hydrogen peroxide Assay Kit

"Read and mix" sensitive H_20_2 Detection Kit

Sensitive : **10 picomoles** of H_2O_2 in solution.

Hydrogen peroxide (H_2O_2) is a reactive oxygen metabolic by-product that serves as a key regulator for a number of oxidative stress-related states. It is involved in a number of biological events that have been linked to asthma, atherosclerosis, diabetic vasculopathy, osteoporosis, a number of neurodegenerative diseases and Down's syndrome. Perhaps the most intriguing aspect of H_20_2 biology is the recent report that antibodies have the capacity to convert molecular oxygen into hydrogen peroxide to contribute to the normal recognition and destruction processes of the immune system. Measurement of this reactive species will help to determine how oxidative stress modulates varied intracellular pathways. Hydrogen Peroxide Assay Kit uses our Red peroxidase substrate to quantify hydrogen peroxide in solutions, in cell extracts and in live cells. It can also be used to detect a variety of oxidase activities through enzyme-coupled reactions. The kit is an optimized "mix and read" assay that is compatible with HTS liquid handling instruments.



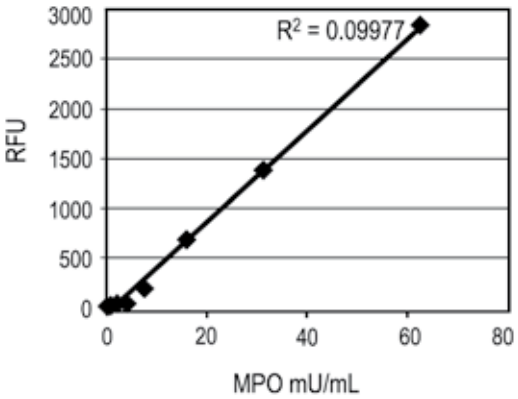
H2O2 dose response on 384-well black plate with 30 minutes incubation time (n=3). The insert shows the low levels of H2O2 detection.

Description	P/N :	Qty
ADHP Hydrogen peroxide Assay Kit	**CL2580**	500 assays
Also available :		
ADHP Hydrogen peroxide/Peroxidase Assay Kit dual mode, can detect H_2O_2 or peroxidase activity.	**U3238A**	500 tests
10-acetyl-3,7-dihydroxyphenoxazine (ADPH) $\lambda_{abs}/\lambda_{em.}$: 563/587 nm	**FP-39423B**	25 mg

Myeloperoxidase Detection Kit

- Readout : **Fluorescence or absorbance**
- Can monitor multiple time points to follow **kinetics**
- **One-step, no wash assay**
- Adaptable for High Throughput format
- **Sensitive**

Myeloperoxidase (MPO) is a highly cationic glycosolated hemoprotein that has a molecular weight of 144kD. The hemoprotein consists of two dimers linked via a disulfide bridge. Each dimmer is composed of a heavy (53kD) and light (15kD) subunit. Each heavy chain contains an independently acting protoporphyrin group containing a central iron. MPO is present in the azurophilic granules of polymorphonuclear leukocytes (PMNs) and is unique to neutrophils and monocytes. However, monocytes contain only one third of the MPO found in PMN's. MPO utilizes H_2O_2 produced by the neutrophils to oxidize a varity of aromatic compounds to give substrate radicals for bactericidal activity. This enzyme is unique however in that it can oxidize chloride ions to produce a strong nonradical oxidant,HOCl. HOCl is the most powerfull bactericidal produced by neutrophils. Excessive production of these radicals can cause oxidative stress leading to oxidative tissue injury.



MPO standard curve was serially diluted in 1X Reaction Buffer. Reaction cocktail (RC) was prepared as described (without EPO inhibitor). Next 50 µL of MPO standard and 50 µL of RC was added to individual well of a 96 well black plates. The plate was incubated at room and temperature in the dark. Data collected Ex. : 530 nm Em. : 590 nm.

H_2O_2 + Detection reagent (non-fluorescent) + MPO fluorescent analog. → **Ex. / Em. : 530-571 / 590-600 nm**

Description	P/N :	Qty
Myeloperoxidase Detection Kit	**CF2980**	500 tests

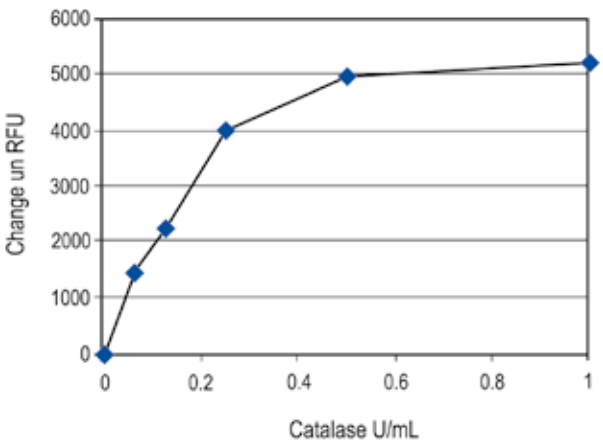
References :
J Neural Transm Suppl **23:**55–72. (1987)
Proc. Nat. Acad. Sci. U.S.A., **8**5, 4934–4938 (1988)
Biochem. Pharmaco., **1**7, 1285–1297. I l (1968)
J. Biochem., **7**9, 1297–1299 (1976)
Biochem. Pharmaco., **2**7, 1995–2000 (1978)

Catalase Detection Kit

Catalase is an antioxidant enzyme that catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. Catalase is ubiquitously expressed in mammalian and non-mammalian aerobic cells containing the cytochrome system. The enzyme has been isolated from various sources, including bacteria and plant cells [1-3]. Catalase activity varies greatly from tissue to tissue. Highest activity is seen in liver and kidney, while lowest activity is seen in connective tissue [3]. In eukarotic cells, catalase in concentrated in organelles called peroxisomes [4].

The production of hydrogen peroxide in eukaryotic cells is an end product result of various oxidases and superoxide dismutase reactions. Accumulation of H_2O_2 can result in cellular damage through oxidation of proteins, DNA and lipids thus resulting in cell death and mutagenisis [8-11]. H_2O_2 role in oxidative stress related diseases have been widely studied [8,12].

The Catalase detection kit is sensitive assay that utilizes a non - fluorescent substrate, 10-Acetyl-3, 7-dihydroxyphenoxazine (ADHP, 530/590 nm), to detect H_2O_2 substrate left over from the catalase reaction [5-6].



Catalase activity detected using the Catalase kit.
The reaction contained 20 µM H_2O_2 (final) per well and the indicated amounts of catalase in 1X reaction Buffer. The reaction was incubated for 30 minutes at room temperature. Next 100 µL of Reaction cocktail was added to each well and the reaction incubated for another 10 minutes in the dark at room temperature.

References :
1. Physiol. Rev., 50, 319-375 (1970).
2. Analytical Biochemistry Vol. 245, Issue 1 ,1 February 1997, Pages 55-60.
3. Physiol. Rev., 50, 319-375 (1970).
4. Progress in Biophys. Mol. Biol., 72, 19-66 (1999).
5. Anal Biochem 253, 162 (1997).
6. J. Immunol Methods 202, 133 (1997).
7. Archives of Biochemistry and Biophysics, 431:138-144 (2004).
8. J. Biol. Chem., Sep 1999; 274: 26217 - 26224
9. FEBS Lett., 442, 65-69 (1999).
10. FEBS Lett., 414, 552-556 (1997).
11. FEBS Lett., 473, 177-182 (2000).
12. Cancer Res., 61, 2766-2733 (2001).

Description	P/N :	Qty
Catalase Detection Kit	**CA7280**	500 tests

Cis-Parinaric Acid (CPA)

Measurement of lipid peroxidation

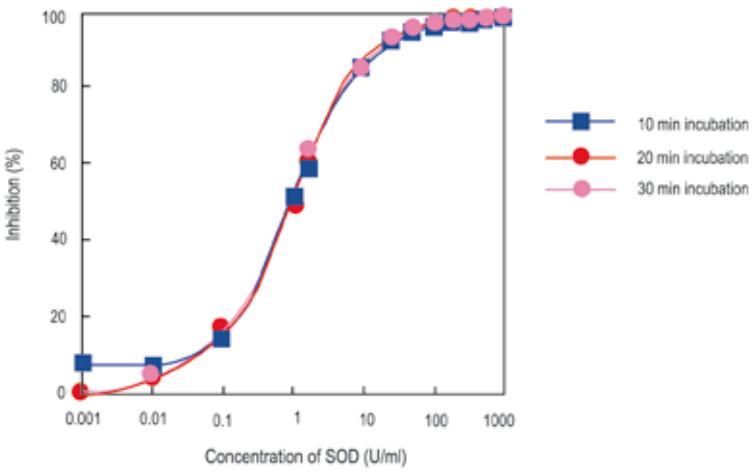
Cis-parinaric acid (CPA) is a fluorescent poly-unsaturated fatty acid used as a probe to directly monitor lipid peroxidation. Fluorescence measurement is using 318 nm excitation and 420 nm emission filters.

Description	P/N :	Qty
Cis-Parinaric Acid	**FP-46900A**	10 mg

SOD Assay Kit with colorimetric substrate WST-1

- Measures **100% inhibition by SOD**
- **pH independent** IC_{50} determination
- **Convenient** 96-well microplate colorimetric assay
- **Low-background** noise measurement

Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion ($O_2^{-}\bullet$) 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 assaying by utilizing highly water-soluble tetrazolium salt, WST-1 that produces a water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with $O_2^{-}\bullet$ are linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD, as shown in the Figure above. Therefore, the IC_{50} (50% inhibition activity of SOD or SOD-like materials) can be determined by measuring the decrease in the color development at 440 nm.

Description	P/N :	Qty
SOD Assay Kit	**S07411**	100 tests
	S07410	500 tests
Related products :		
WST-1 as stand alone product	**F98883**	100 mg
NADPH	**Q91330**	40-wells

Malachite green isothiocyanate

Localized production of hydroxyl radicals by amine-reactive probe

This non-fluorescent photosensitizer probe ($\lambda_{exc./em.}$ 628 nm/none) can be conjugated to specific antibodies. Enzymes and other proteins within ~10 Å of the binding site of the malachite green–labeled antibody can then be selectively destroyed by production of hydroxyl radicals upon irradiation with long-wavelength light.

References :

Stresser DM. et al., Drug Metab Dispos 30, 845-52 (2002)
Tolosa L. et al., Lifetime-based sensing of glucose using energy transfer with a long lifetime donor.". Anal Biochem 250, 102-108 (1997)
Beermann A. et al., "Chromophore-assisted laser inactivation of cellular proteins." Methods Cell Biol 44, 715-732 (1994)

Description	P/N :	Qty
Malachite green isothiocyanate	**FP-98782A**	10 mg

Monoamine Oxidase A&B Detection Kit

Monoamine oxidase (MAO) is a flavin-containing enzyme that catalyses the oxidation of a variety of amine-containing neurotransmitters such as serotonin, norepinephrine, epinephrine and dopamine to yield the corresponding aldehydes. MAO exists in two isoforms, namely MAO-A and MAO-B, which are the products of two distinct genes.
MAO-A and B exhibit different specificities to substrates and inhibitor selectivities. Extensive studies have been preformed to characterize their properties. MAO-A acts preferentially on serotonin and norepinephrine, and is inhibited by clorgyline. MAO-B acts preferentially on 2-phenylethylamine and benzylamine and is inhibited by deprenyl and pargyline.
Localized in the outer mitochondrial membrane, these enzymes are found throughout the body. Often only one form of the enzyme is present in a specific organ and/or within a specific cell type. In addition to their role in regulating neurotransmitters, these enzymes are also involved in processing biogenic amines including tyramine.

Substrate + O2 + H2O + MAO-A/B → **aldehyde + NH^3 + N_2O_2**

H_2O_2 + Detection reagent (non-fluorescent) +Peroxidase → **Resorufin (fluorescent)**

Ex. / Em. : 530-570 / 590-600 nm

Description	P/N :	Qty
Monoamine Oxidase A&B Detection Kit	**CA7290**	500 tests

SSAO Detection Kit

Fluorescent Semicarbazide-Sensitive Amine Oxidase Detection Kit

Semicarbazide-sensitive amine oxidase (SSAO) is a common name for a widely distributed enzyme in nature. In man this enzyme is present in the vascular system and circulates in plasma.
SSAO's functional role has been suggested to be involved in: apoptosis, atherogenesis, cell adhesion, leucocyte trafficking, glucose transport and local production of hydrogen peroxide. Reports of elevated levels of SSAO have been reported in congestive heart failure, diabetes mellitus, alzheimer's disease and various other inflammatory diseases.

Furthermore by-products of SSAO deamination, such as formaldehyde and methylglyoxal, have been proposed to be involved in pathogenesis of cancer, aging and atherosclerosis.

Benzylamine + O_2 + H_2O + SSAO → **Benzaldehyde + NH^3 + N_2O_2**

H_2O_2 + Detection reagent (non-fluorescent) +Peroxidase → **Resorufin (fluorescent)**

Description	P/N :	Qty
SSAO Detection Kit	**CA7310**	500 tests

DAF-2 diacetate

- Cell permeable
- **No wash** homogenous assay
- **Real time detection** of NOS activity
- NO and NO_2^- detection limit : **~5 nM**

NO scavenging can be measured by monitoring 4,5-diaminofluorescein (DAF-2). DAF-2, as a specific NO indicator, selectively traps NO between two amino groups in its molecule, and yields triazolofluorescein, which emits green fluorescence when excited at 490–515 nm. The diacetate form is cell-permeable derivative of DAF-2. DAF-2 diacetate can be used to detect NOS activity in cell culture or tissue sections. This reagent is not species specific and can also be used to detect NOS activity in plant cells (but not in barley aleurone cells[1]).

References :
1) Journal of Experimental Botany 2006 57(3):463-470

Description	P/N :	Qty
DAF-2 diacetate	**S03720**	100 µg
NOS Detection kit	**CA7150**	125 µg (2.22 mg/ml)
	CA7151	250 µg
Related products :		
SNAP, photoactivable NO donor	**FP-71646A**	25 mg
Spermine NONOate, pH controlled NO donor	**FP-M16259**	10 mg
Carboxy-PTIO potassium salt, specific NO scavenger	**199500**	5 mg
L-NMMA, NO synthase inhibitor	**FP-85524A**	50 mg

DAF-FM

DAF-FM is important reagent for quantitating low concentrations of nitric oxide in solution. This compound is essentially nonfluorescent until it reacts with NO to form a fluorescent benzotrizole (495/515 nm). The diacetate form is membrane permeant and is deacetylated by intracellular esterases to DAF-FM.

- Fluorescence **independent of pH** above pH 5.5
- Significantly **more photostable** than that of DAF-2
- NO and NO_2^- detection limit : **~3 nM** versus

Description	P/N :	Qty
DAF-FM	**FP-R1227A**	1 mg
DAF-FM diacetate	**FP-R1228A**	1 mg

2,3-Diaminonaphthalene

2,3-Diaminonaphthalene reacts with nitrosonium, which is formed from NO, to form the fluorescent dye 1 H-naphthotriazole (365/415 nm).[1,2] Using this method, 10 nM to 10 µM of nitrite (NO^{2-}) can be detected and the detection is compatible with 96-well format.[3]

References :
Luminescence 14, 283 (1999)
Methods Enzymol. 268, 105(1996)
Anal. Biochem.214, 11(1993)

Description	P/N :	Qty
2,3-Diaminonaphthalene	**FP-04832F**	100 mg

NBD Methylhydrazine for nitrite assay

NBD methylhydrazine (N-methyl-4-hydrazino-7-nitrobenzofurazan) reacts with NO_2^- in the presence of mineral acids leads to formation of fluorescent products (468/537 nm). NBD methylhydrazine has been used to quantitate nitrite in waters.

Reference :
Anal Chem 71, 3003-3007 (1999)

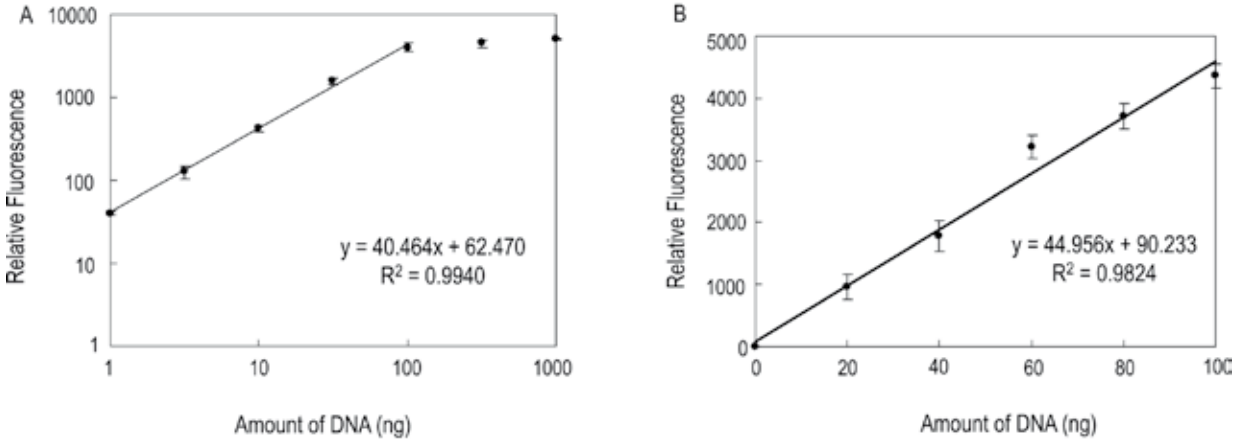
Description	P/N :	Qty
NBD Methylhydrazine	**FP-R1315A**	50 mg

EvaGreen - DNA quantification in solution

- Linear response on the range of **1–100 ng**
- Sample size : 50 µl
- Standard filter set : $\lambda_{exc.}$ / $\lambda_{em.}$: **490/520 nm**
- **Requires small amounts of DNA and dye**

The intensity of fluorescence (relative fluorescence units) is proportional to the total amount of DNA per tube rather than the concentration of DNA in the tube.

Wang W. et al. has used the EvaGreen for DNA quantification on 25 µl sample with linear response from 1 ng to 100 ng (Analytical Biochemistry, Volume 356, Issue 2, Pages 303-305 (2006)) : Figure A and B. A strong linear relationship is observed when the amount of DNA is less than 100 ng (Fig. A), and this is very reproducible (Fig. B).

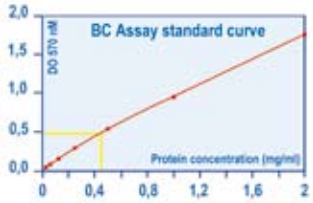
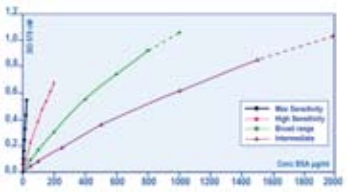
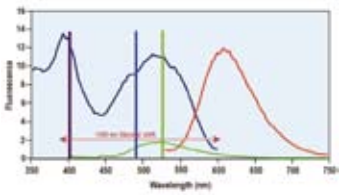


Relationships between fluorescence intensity and the amount of DNA per tube : Triplicate samples of _DNA in the range of 1–1000 ng (A) or 0–100 ng, (B) were added to PCR tubes containing 1.25 µl of EvaGreen (20X concentrate), and water was added to make a final volume of 25 µl.

Description	P/N :	Qty
EvaGreen 20X in PBS	**BI1790**	5 x 1 ml (1000 assays)
EvaGreen 20000X in DMSO	**CA6770**	1 ml (200 000 assays)
Related products :		
DNA from lambda phage, as standard for DNA quantification	**UP947860**	100 µg
Hoechst 33258, for high concentration DNA quantification	**FP-61248A**	100 mg
DNA dosage by UV with only 5 µl sample, using IMAplates	**see description page 72**	

Other application of the EvaGreen in the BioScience Innovation catalog.

Interchim proposes 3 methods to assay proteins in microplates :

	Colorimetric/BCA method : BC Assay & MicroBC Assay	**Colorimetric/Bradford method :** CooAssays ()	**Fluorescent/Epicocconone :** LavaPep method
			
Sensitivity :	0.5 µg/ml (MicroBC Assay) 20 µg/ml (BCA assay)	1-25 µg/ml (max sens.protocol) 50-1500 µg/ml (Broad range protocol)	50-160 pg/ml peptide, proteins
Linear range :	1-250 µg/ml (MicroBC Assay) 20-2000 µg/ml (BCA assay)	1-25 µg/ml (max sens.protocol) 50-1500 µg/ml (Broad range protocol)	100 ng/ml-160 µg/ml
Reading	562 nm	570-610 nm	405-500/560-610 nm
Comments : *advantages*	The most reliable method Compatible with most **detergents**, bases, DNA, lipids **Broader linearity** **Lowest variations** between proteins	**Quick** (1-10 min) Compatible with **reducers**	**Ultimate sensitivity and linearity** Peptide dosage Biodegradable Robust, amenable to N-term sequencing, MS and functionnal assays
Comments : *limitations*	Need 37°C incubation or longer time	Limited compatibility (reducers, acids, some detergents)	Limited compatibility

Description	P/N :	Qty
BC Assay protein determination kit Bicinchoninic acid based method-590nm. Contains : 1L reagent sufficient for 500/5000 tests (tube/µplate), and 10 x 1 ml BSA standard 2 mg/ml	**UP4080A** **UP4080B**	1 kit-1 L 1 kit-250 ml
MicroBC Assay protein determination kit Version of BC Assay with sensitivity 0.5 µg/ml. Contains : 1L reagent sufficient for 500/3400 tests, and 10 x 1 ml BSA standard 2 mg/ml	**UP75860A** **UP75860B**	1 kit-1 L 1 kit-50 ml
Coo Assay protein determination kit Modified Bradford (Coomassie based method). Contains : 1L reagent sufficient for 500/4000 tests (tube/µplate), and 10 x 1 ml BSA standard 2 mg/ml	**UPF86400** **UPF86401**	1 kit-1 L 1 kit-250 ml
LavaPep peptide & proteins assay Epicocconone based method. Contains : Dye concentrate 10X, Buffer concentrate 10X	**CH4191**	1 Kit (up to 2000 tests)

Order together the following reagent to render these protein assays compatible with any interfering substance !

Protein Preparation kit
Réf. : R5594A 500 ml

Biochemistry tests for biologicals samples

Interchim provides a whole range of biochemistry assays for clinical chemistry (Blood, Urine, CerobroSpinal fluids,...) as well as for other samples (food, soil, water...) analysed in agro-alimentary industry or environement study. Here is a short selection of assay kits.

Description	P/N :	Qty
Glucose assay Hexokinase/G6PDH based method. Reading at 500 nm. Linear to 400 mg/dL. For serum, plasma or urine. 5 min procedure.	**BD1850**	1 Kit (120 ml)
Creatinine assay Enzymatic method. Reading at 546 nm. Linear to 30 mg/dL. For serum, plasma and urine samples.	**BP9991**	1 Kit (30 ml)
β-Hydroxybutyrate assay Enzymtic method. Reading at 505 nm. Linear to 4.5 nmol/L. For serum or plasma.	**AL2230**	1 Kit (60 ml)
Glucose assay Hexokinase/G6PDH based method. Reading at 340 nm. Linear 1-80µg/ml. For food stuff and bewerage.	**U67120**	1 Kit (40 tests)
Glycerol assay Enzymatic method. Reading at 330/334/365 nm. One component. For food stuff and bewerage.	**R51065A**	1 Kit (4 x 10 tests)
Cholesterol assay Enzymatic CHOD/PAP method. Reading at 546 nm. For food stuff and bewerage.	**R5756A**	1 Kit (4 x 10 tests)

Contact us for other analytes in biological samples (Calcium, Bilirubin, HDL, Urea, ... / Maltose, Fructose, Starch, Malate, Lactate,...).

ImmunoAssays have been popularized using ELISA technique (Enzyme Linked Immunosorbent Assay), especially using peroxidase (HRP) or alkaline phosphatase (AP) with colorimetric substrates. Interchim offering include not only highly sensitive **colorimetric substrates**, but also **luminescent substrates,** that have spread thanks their incomparable sensitivity, and **fluorescent immunoreagents** that can increase the dynamic range, sensitivity and notably allow for multiplexed analysis.
We finally provide **biotin/streptavidin reagents**, that are recommended to get an amplification of the signal. Additionnally, biotin label is generaly more flexible and quicker to calibrate using few labeled streptavidins for many different assays

ELISA - Luminescent detection (ECL)

■ UptiLight ELISA HRP Chemiluminescent Substrate

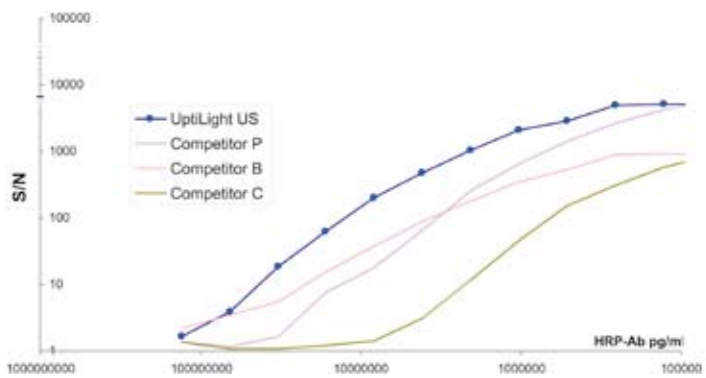
Increase 10 times the net signal of your usual luminol reagent, for ultra-sensitive HRP based immunoassays.

- **High signal** with **minimal background**
- Sensitivity in the **pico and femto range**
- Signal **stable up to 1 hour**
- Great for HTS applications
- Reagent stable >18 months at 4°C
- Easy to use : mix, incubate 5 min, read

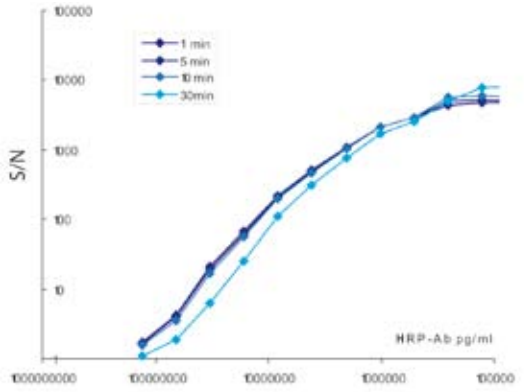
Interchim provides a new chemiluminescent substrate for ELISA techniques, that improves significantly the sensitivity of detection, as well the stability and reproducibility -some crucial points in screening experiments. Not only signal is at equal or higher to your usual reagent, but the background is lower. As a result, UptiLight ELISA substrate achieves unsurpassed Signal to Noise (S/N) ratios.

Technical tip

The luminol was introduced as a convenient and effective chemiluminescent substrate, overcoming the performances (and first, the sensitivity) of classical insoluble chromogenic substrates (incl. OPD, TMB, DAB). The principle consists of the generation of light by the by-products of the chemical reaction from peroxidase upon the substrate. The emission of glow is then recorded by luminometer at 425 nm (in tubes, or in wells of ELISA microplates). The use of luminescent substrates is most recommended for quantitative assays requiring extended dynamic range of detection or qualitative assays requiring the best achievable detection limit (highest sensitivity).



Sensitivity comparison with UptiLight and competitors
Signal to noise in ELISA with UptiLight US ELISA HRP (#996201) compared with competitors PEF, BEF, AEA, and PBP. ELISA was performed with coated Mouse IgG detected by anti Mouse IgG(H+L) - HRP (#UP446330), then the ECL Luminescent substrates, prepared according their respective supplier. Luminescence was recorded with Mithras (Berthold Technolgies) with 0,1 sec integration time, after a 5 min preincubation period. Datas are plotted as Signal to Noise ratios (S/N) for each tested HRP Ab concentration. Reduced background and higher sensitivity was found with UptiLight reagent.



Photostability of light emission with UptiLight US Luminescence was recorded with 0,1 sec integration time after a 1, 5, 10 or 30 min preincubation period.

Description	P/N :	Qty
UptiLight ELISA HRP UltraSensitivity Chemiluminescent Substrate.	**996201**	60 ml
	996202	120 ml
	996203	300 ml
Also available :		
UptiLight ELISA HRP HighSensitivity Chemiluminescent Substrate. A cost effective reagent for routine analysis, when only pico mole detection is required.	**36349A**	200 ml

See also chemiluminescent kits for Assay #JQ6760 page 18.

■ Luminometric Alkaline Phosphatase Assay Kit

Alkaline Phosphatase Assay Kit uses a proprietary luminogenic phosphatase substrate, to quantify alkaline phosphatase activity immobilized on surfaces, i.e. ELISA, as well as in solutions (i.e. cell extracts, live cells; see page 17). This proprietary phosphatase substrate generates a luminescent product (Em (nm) 560 nm) that produces strong luminescence upon interaction with phosphatase. The kit provides all the essential components with our optimized 'mix and read' assay protocol that is compatible with HTS liquid handling instruments. It has extremely high sensitivity, and can be used for the assays that require demanding sensitivity.

Description	P/N :	Qty
Chemiluminescent AP assay kit	**JQ6760**	100 tests

See description in enzyme detection/Alkaline Phosphatase section, page 18.

Related products :

Selected HRP labeled secondary antibodies (anti Igs)

Host [a]	Specificity	Biotin labeled	Qty	HRP labeled	Qty
	***Anti Human antibodies**				
Gt IgG	Anti-Human IgG , min X Bov, Hrs, Ms sr prot.	**UP892650**	0.5 mg	**UP783493**	1 mg
Gt IgG	Anti-Human IgG, Fc fragment specific, min X Bov, Hrs, Ms sr prot.	**722640**	1 mg	**802020**	1 ml
Ms Mab	Anti-Human Kappa Light Chain	**UPB91830**	0.5 mg	**UPB91850**	0.5 mg
Rt Mab	Anti-Human Lambda Light Chain	**UPB91870**	0.5 mg	**UPB91890**	0.5 mg
Rt Mab	Anti-Human IgG Gamma Chain	**UPB92190**	0.5 mg	**UPB92210**	0.5 mg
Rt Mab	Anti-Human IgM Mu Chain	**UPB91910**	0.5 mg	**UPB91930**	0.5 mg
Rt Mab	Anti-Human IgD Delta Chain	**UPB91990**	0.5 mg	**UPB92010**	0.5 mg
Rt Mab	Anti-Human IgE Epsilon Chain	**UPB92030**	0.5 mg	**UPB92050**	0.5 mg
Rt Mab	Anti-Human IgA Alpha1&2 Chain	**UPB92150**	0.5 mg	**UPB92170**	0.5 mg
	***Anti Mouse antibodies**				
Dk IgG	Anti-Mouse IgG(H+L), min X Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu & Rb, Shp sr prot.	**946220**	0.5 ml	**UP973171**	0.5 mg
Gt IgG	Anti-Mouse IgG(H+L), min X Hu, Bov, Hrs, Rb, Sw sr prot.	**668610**	1.5 ml	**UP215731**	1 mg
Rb IgG	Anti-Mouse IgG(H+L), min X Hu sr prot.	**794090**	1 ml	**794560**	1 ml
	Anti Rabbit antibodies				
Dk IgG	Anti-Rabbit IgG(H+L), minX Bov, Ck, Gt, GP, Sy Hms, Hrs, Hu & Ms, Rt, Shp sr prot.	**UP944471**	0.5 mg	**338370**	0.5 ml
Gt IgG	Anti-Rabbit IgG(H+L), min X Hu sr prot.	**812230**	1.5 ml	**UP687714**	1 mg
Gt IgG	Anti-Rabbit IgG(H+L), min X Ms, Rt sr prot.	**UP668621**	1 mg	**UP669631**	1 mg
	***Anti Rat antibodies**				
Gt IgG	Anti-Rat IgG(H+L)	**740470**	2 ml	**UP399892**	1 mg

Secondary antibodies are available with different preadsorbtions, formats (Fab'2), raised in 6 hosts, and against 22 target Ig species. Please inquire.

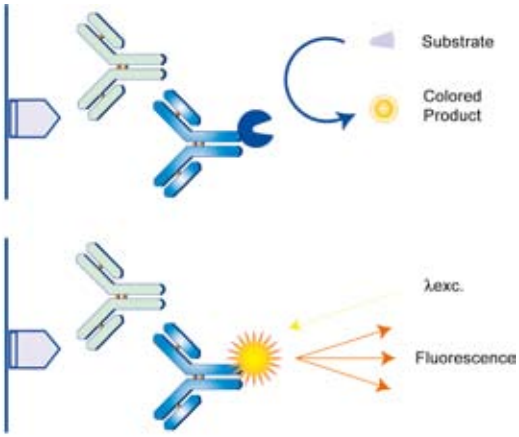
HRP (strep)avidin reagents

Description	P/N :	Qty
HRP labeled Streptavidin	**UP395888**	1 mg
HRP labeled Avidin	**35445A**	2 mg
HRP labeled Neutralized Avidin	**UP36570A**	1 mg

Other Colorimetric & Fluorimetric Enzymatic Substrates for Microplates assays

Peroxidase (HRP) and Alkaline phosphatase (AP) enzymatique labels are widely used in various biological assays including ELISAs, as well as in immunohistochemical techniques and Western blot analyses.
Following are effective ready to use substrates for colorimetric and fluorometric detections in EIA.

Following is a selection of conventional and unique formulations of standard enzymatic substrates to fulfill the requirements of your microplates assays. Below are research catalog quantities, please as for HTS and bulk needs.



Uptima TMB chromogenic substrate for HRP ELISA

Solutions are optimized chromogenic substrates for peroxidase, designed for ELISA techniques, manual or automatic systems. They contain 3,3',5,5'-tetramethylbenzidine (TMB), hydrogene peroxide (H_2O_2), and proprietary catalyzing and stabilizing agents.

- Highest sensitivity
- Reproducible lots
- Ready-to-use

Description	P/N :	Qty
TMB, "Standard" solution	**UP664780**	200 ml
The original formulation, ideal for classic applications	**UP664781**	500 ml
- Highest sensitivity	**UP664782**	1 L
- Stability > 24 months at +4°C		
TMB, "Check" solution	**UPS08170**	100 ml
Recommended for routine assays, to better control failure in reagent distribution.	**UPS08171**	500 ml
Includes a red dye that do not interfere with reaction nor final reading. High sensitivity ; Stability > 1 year at +4°C		
TMB, "Aqueous" solution	**UPS08180**	100 ml
Recommended in particular for diagnostic kits	**UPS08181**	200 ml
Non-hazardous, non-volatile, non-organic, non-toxic	**UPS08182**	500 ml
(does not contain DMF, DMSO)	**UPS08183**	1 L
100% water based formulation to maximize the safety (no regulation concerns). Highest sensitivity ; Stability > 1 year at +4°C.		

ADHP HRP ELISA Assay Kit

ADHP provides higher sensitivity in ELISA that chromogenic substrates. The kit provides the substrate and the stabilized Peroxide buffer in ready-to use convenient format for HTS applications. The fluorescent signal is read at 590 nm ($\lambda_{exc}\backslash\lambda_{em}$ = 530-571 / 590-600 nm) and achieves down femto molar detection sensitivity. It provides the reagents to perform 500 ELISA assays in a 96-well format. The protocol can readily be modified to run assays in a 384-well format.

Description	P/N :	Qty
ADHP HRP ELISA assay kit – ADHP based	**HS6241**	500 tests
Contains ADHP substrate and Peroxide Assay buffer (50 ml)		

Fluorimetric AP ELISA Assay kits

See assay kits #JQ6730 (Blue-MUP), #JQ6740 Green - Red)in section "Apoptosis" page 17.

■ Substrates for horseradish peroxidase

HRP substrates	P/N : [a]	Qty	Format [c]	Type [b]	Comment
OPD tablets of 15 mg	**270861**	50 tabs	Tabs	Chromo.	Each tab contains 15 mg of OPD for quick and easy preparation of substrate solution
OPD, Ultrapure powder CAS: [95-54-5] ; MW : 108.1	**02673F** **02673G**	25 g 50 g	Pow.	Chromo.	(o-Phenylene DiAmine)
ABTS, Liquid substrate	**UP732550**	100 ml	Soln	Chromo.	Ready-to-use solution
ABTS tablets	**42387C**	50 tablets	Tabs	Chromo.	Each tab contains 10 mg of ABTS for quick and easy preparation of substrate solution.
ABTS, Ultra pure grade CAS : [30931-67-0] ; MW : 548.7	**UP423876** **UP423877**	1 g 10 g	Pow.	Chromo.	
TMB solution for ELISA	**UP66478**		Soln	Chromo.	Highest sensitivity. See description page 64.
TMB, Ultrapure powder CAS : [64285-73-0] ; MW : 331.3	**UP15426D** **UP15426E**	1 g 5 g	Pow.	Chromo.	(tetramethylBenzidine)
DAB solution (50X)	**UP732320**	500 ml	Soln	Chromo.	Ready-to-use solution less toxic for manipulation Supplied as a 50x concentrate with a 10x dilution buffer.
DAB tablets of 5 mg	**UP732310**	50 tabs	Tabs	Chromo.	Each tab contains 5 mg of DAB for quick and easy preparation of substrate solution
DAB, Ultrapure powder CAS : [868272-85-9] ; MW : 360.1	**UP01012G** **UP01012H**	5 g 10 g	Pow.	Chromo.	(3.3'-DiAminoBenzidine)
ADHP HRP Assay Kit.	**HS6241**	500 tests	Kit	Fluo.	Page 64.
ADHP, Pure Grade powder CAS : [119171-73-2] ; MW : 257.2	**FP-39423A** **FP-39423B**	5 mg 25 mg	Pow.	Fluo.	Highly and stable fluorigenic substrate $\lambda_{abs}/\lambda_{em.}$: 563/587 nm (10-Acetyl-3,7-Dihydroxyphenoxazine)
Resorufin, Pure Grade CAS:[635-78-9]; MW : 213.04	**FP-95432B**	100 mg	Pow.	Fluo	
Luminol, Pure Grade powder CAS : [521-31-3] ; MW : 177.2	**FP-57578A**		Pow.	Lum.	$\lambda_{abs}/\lambda_{em.}$: 355/413 nm ; EC : 7650 l/mol x cm
Luminol, Na salt CAS : [20666-12-0] ; MW : 199.1	**FP-CA9611**	2.5 g	Pow.	Lum.	See also UptiLight page 62.

[a] all products are available as bulk formats. Please inquire. [b]Chromo. : Chromogenic ; Fluo. : Fluorigenic ; Lum. : Lumigenic
[c]Pow; : powder ; Soln : Solution mono-component or bi-component (2 Soln) See also substrates for reporter assays (B-galactosidase,...) page 16.

■ Substrates for Alkaline Phosphate

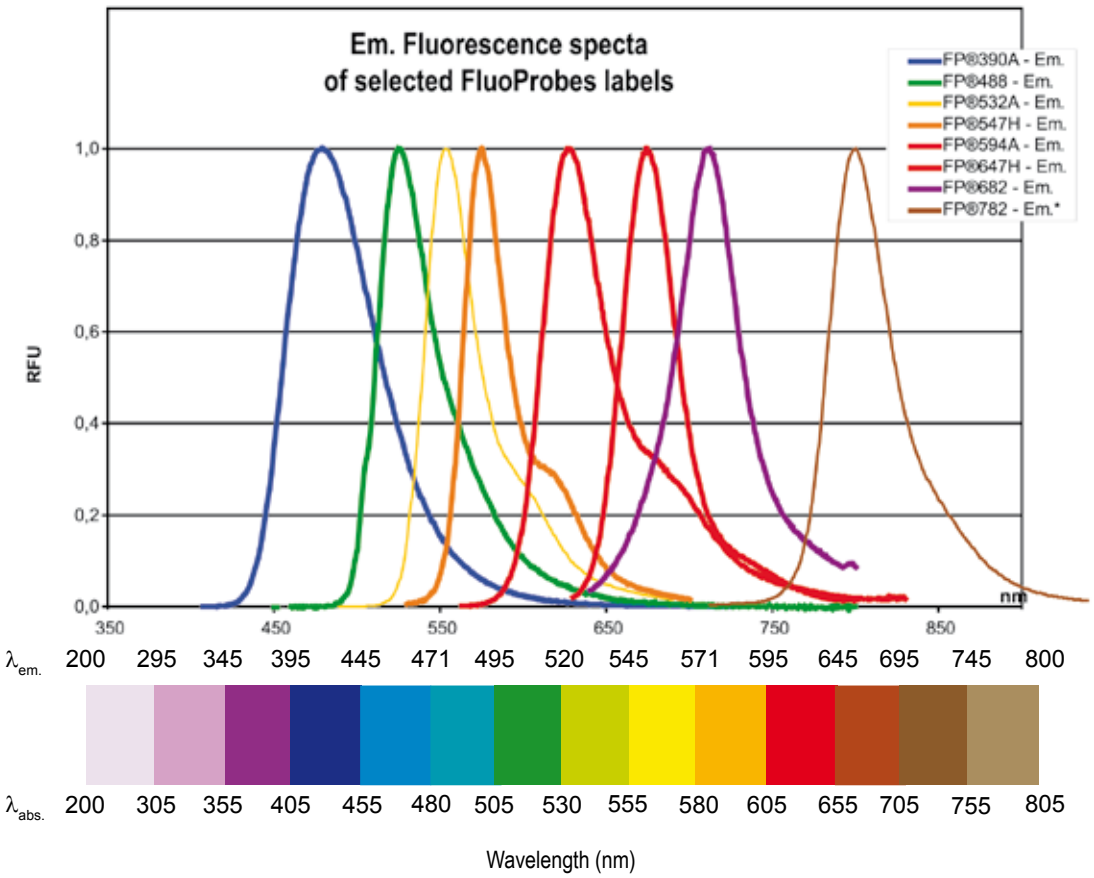
HRP substrates	P/N : [a]	Qty	Format [c]	Type [b]	Comment
pNPP ELISA solution	**BP7080**	500 tests	Kit		Contains Assay buffer, wash buffer, stop solution and AP II Ab.
pNPP tabs of 30 mg	**UP732500** **UP732501**	100 tabs 1000 tabs	Tabs	Chromo	Each tablet contains 30 mg of pNPP for quick and easy preparation of substrate solution.
pNPP tabs of 5 mg	**UP89562G** **UP89562F**	100 tabs 1000 tabs	Tabs	Chromo	Each tablet contains 5 mg of pNPP for quick and easy preparation of substrate solution.
pNPP, Ultrapure powder CAS : [4264-83-9] ; MW : 301.3	**UP89562C** **UP89562D**	25 g 100 g	Pow.	Chromo.	(p-NitroPhenylPhosphate).
MUP Na salt, Ultra Pure grade CAS : [22919-26-2] ; MW : 277.1	**FP-30045A** **FP-30045B** **FP-30045C** **FP-30045D**	100 mg 500 mg 5 g 10 g	Pow.	Fluo.	(MethylUmbelliferyl Phosphate). Used for detecting phosphatases in solution. Maximum fluorescence at pH value of >10.
MUP Plus, Na salt	**FP-JQ6710**	25 mg	Pow.	Fluo.	Maximum fluorescence above pH 7.0, for continuous assays. Also used for the assays that require acidic pH such as acid phosphatases. See description page 18.
MUP Free acid CAS : [3368-04-5] ; MW : 256.1	**FP-24119A**	100 mg	Pow	Fluo	See also MUP based AP assay kit #JQ6730 page 17.
FDP CAS : [217305-49-2] ; MW : 560.4	**FP-72573A**	5 mg	Pow.	Fluo.	(Fluorescein DiPhosphate). See also FDP based AP assay #JQ6740 page 17.
FDP ELISA kit	**HT0790**	1000 tests	Kit	Fluo.	Contains Assay buffer, wash buffer, stop solution and AP II Ab.

Streptavidin conjugates

Streptavidin conjugates are second step reagents for staining with biotinylated antibodies. Interchim covers a wide range of wavelengths. Here is a selection of great FluoProbes dyes, the FluoProbes 782, 682, 647H and R-PE being the most useful in microplates assays :

Description	$\lambda_{exc.}$ (nm)	$\lambda_{em.}$ (nm)	P/N :	Qty
FluoProbes® 782	783	800	**FP-IS1810**	1 mg
FluoProbes® 682	690	709	**FP-BE8050**	1 mg
FluoProbes® 647H	653	675	**FP-CA5640**	1 mg
FluoProbes® 594A	601	627	**FP-CA5620**	1 mg
FluoProbes® 565A	563	592	**FP-CA5610**	1 mg
R-Phycoerythrin	565	576	**FP-77776A**	1 mg
FluoProbes® 547H	557	572	**FP-CA5570**	1 mg
FluoProbes® 488	493	518	**FP-BA2221**	1 mg

Related products
Other labeled (strept)avidin conjugates are available from Interchim



For other wavelengths, please contact us or see in Interchim BioScience Innovations catalog.

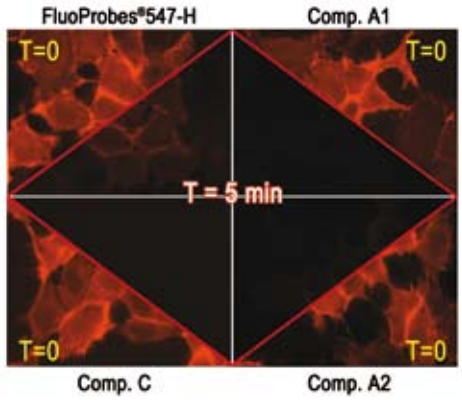
See Fluoprobes dyes descriptions page 67.

References :
Rowell E. et al., Opposing Roles for the Cyclin-Dependent Kinase Inhibitor $p27^{kip1}$ in the Control of $CD4^+$ T Cell Proliferation and Effector Function, The Journal of Immunology, 2005, 174: 3359-3368.

Fluorescent secondary reagents for ELISA (II Abs, Avidin)

■ Secondary Antibodies conjugates

We are offering a range of high quality secondary antibodies conjugated with our fluorophores called FluoProbes. Showing superior fluorescence properties, these fluorophores are an excellent alternative to the conventional dyes such Cyanines and other dyes. Following are selected fluorophores compatible using common exitation sources and standard filters set such as FITC/Cy2, TRITC/Cy3, TR/Cy5, and InfraRed, .



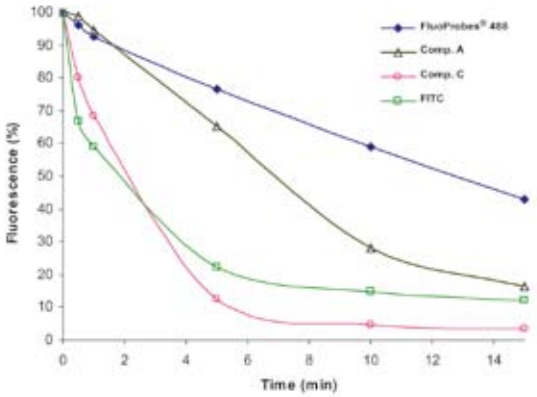
Benefits of FluoProbes-antibody conjugates

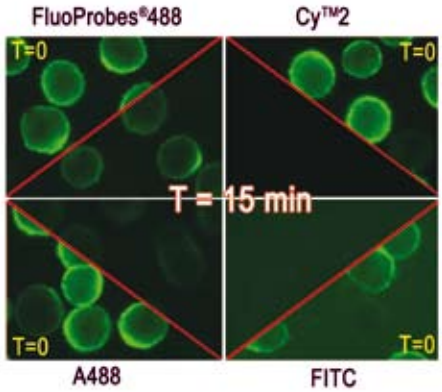
- **High brightness** : FluoProbes® dyes show an enhanced fluorescence compared to other similar conjugates (very high Signal/Noise ratio)
- **High photostability** : Our FluoProbes dies are more photostable than most other conjugates allowing longer reading/scaning.
- **Color choice** : FluoProbes® dyes range in color from green to infra-red.
- **Ready to use solution** : no dissolution step and risk of contamination.
- **High species-specificity** : The secondary antibodies were selected to have highest specificities. It is then possible to have very species-specific secondary antibodies conjugated with the best fluorochromes.

Description	$\lambda_{exc.}$ (nm)	$\lambda_{em.}$ (nm)	P/N :	Qty
Goat anti-Mouse IgG (H+L)				
- FluoProbes® 782	783	800	**FP-BW7970**	1 mg
- FluoProbes® 682	690	709	**FP-BE7250**	1 mg
- FluoProbes® 647H Min x Rat, Hu, Bov, Hrs, Rb sr Prot	653	675	**FP-SC4000**	1 mg
- FluoProbes® 547H Min x Rat, Hu, Bov, Hrs, Rb sr Prot	557	572	**FP-SB4000**	1 mg
- FluoProbes® 488 Min x Rat, Hu, Bov, Hrs, Rb sr Prot	493	518	**FP-SA4000**	1 mg
Goat anti-Rabbit IgG (H+L)				
- FluoProbes® 782	783	800	**FP-BW7980**	1 mg
- FluoProbes® 682	690	709	**FP-BF1690**	1 mg
- FluoProbes® 647H Min X Bov,Ck,Gt,GP,Hms,Hrs,Hu,Ms,Rat,Shp Sr Prot	653	675	**FP-SC5000**	1 mg
- FluoProbes® 547H Min X Bov,Ck,Gt,GP,Hms,Hrs,Hu,Ms,Rat,Shp Sr Prot	557	572	**FP-SB5000**	1 mg
- FluoProbes® 488 Min X Bov,Ck,Gt,GP,Hms,Hrs,Hu,Ms,Rat,Shp Sr Prot	493	518	**FP-SA5000**	1 mg

See all our secondary antibodies items in our catalog Biosciences Innovations.

Related products :
PrimAb™ : a large collection of primary antibodies. Search our web PrimAbs engine tool at http://www.interchim.com/interchim/PrimAb/search.cfm.
Research areas include : CellSignaling (ionic, cytokines, hormones), Apotosis/cell cycle, DNA replications/transcription/Repair, post-translational modifications, Cell adhesion, Enzymes, Membrane study, Lipids, Metabolism, Angio/Histogenesis, Infectious agents, biomarkers for Cancer/Hypoxia, Cardiology, Neurosciences, Drug and resistance, Allergens.





Standard Buffers for Immunoassays

Description	P/N :	Qty
PBS powder pack (makes 10 L)	**UP68723A**	1 pack
TBS powder pack (makes 20 L)	**UP74004A**	1 pack
PBS (Tris Buffered Saline), 20X solution	**N13761**	1 L
PBS tablets (1 Tab.makes 100 ml of 1X solution)	**307150**	100 Tabs
PBS with Tween®, pH 7.5	**N13810**	500 ml
TBS (Tris Buffered Saline), 20X solution	**N14580**	4 L
TBS tablets (1 Tab.=100 ml of 1X solution)	**GS3660**	100 Tabs

Ready to use Buffers for Immunoassays

These powered blends save your time and are high proteomics grade for optimal results in immunodetections.

Description	P/N :	Qty
TBS with Non-Fat Powdered Milk 3%	**GS4160**	5 pk (42 g/1 L)
TBS with BSA 1%	**GS4170**	5 pk (22 g/1 L)
TBS with Tween® 0.05%	**GS4200**	5 pk (12.5 g/1 L)
PBS with Non-Fat Powdered Milk 3%	**GS4180**	5 pk (39.8 g/1 L)
PBS with BSA 1%	**GS4190**	5 pk (19.8 g/1 L)
PBS with Tween® 0.05%	**GS4250**	5 pk (10.4 g/1 L)
Antibody Diluent (Ready to Use)	**HH6690**	125 ml
Ab diluted in this solution can be stored for up to 18 months at +4°C.		

SeaBlock saturating agent (non mammalian serum)

- Non-mammalian nature prevents interactions with immunoreagents (i.e. mammalian antibodies)
- Lower background
- Excellent to saturate high binding surfaces, and Glutaraldehyde activated Amine polystyrene (when BSA, casein and other agents are good but not excellent or even poor blockers).

Description	P/N :	Qty
SeaBlock (standard, excels as a blocker in ELISA)	**UP40301A**	500 ml
SeaBlock, serum free in PBS	**UPAP1370**	500 ml
SeaBlock, serum free in TBS	**UPAP1380**	500 ml

Other saturating agents and Buffers components

Description	P/N :	Qty
BSA powder	**UPQ84170**	100 g
Our standard grade and economic BSA, ubiquitous for most biotechnologies, including immuno-saturations.	**UPQ84171** **UPQ84170**	500 g 1 kg
BSA 30% solution	**UP900100**	50 ml
Using this solution, forget the hassle of weighting and dissolving BSA powder (no agregates !). Save time and money !	**UP900101**	500 ml
Polymerised BSA, 30% solution	**BJ1440**	50 ml
Polymerised BSA improve several detection systems		
BioBlock membrane blocking agent (in PBS)	**N13660**	1 L
An economic standard blocker based on casein, optimized for positively charged nylon or PVDF membranes in nucleic acid or protein blotting applications.	**N13650**	1 L (in TBS)
Non-fat Milk	**768701**	500 g
A popular bloquer		
Gelatin	**N13360**	100 g
CAS [9000-70-8]; Bloom number : 240-270 ; pH(28°C) : 4.5-5.5 ; Water (KF) : <12%; Viscosity : 35-45mpa	**N13361**	500 g
Prionex®, 10% sterile solution	**901770**	100 ml
BSA alternative, strongly reduces unspecific binding of protein to plastic microplate walls		
Tween® 20, pure	**15874A**	1 L
Tween® 20, 20% solution, oxidant free	**UP158740**	5 x 10 ml
Highly pure and packaged in sealed ampuls under argon to increase the accuracy of immunoassays.		

Note : a full range of other grades of BSA and other albumins are also available.

For direct conjugation of primary antibodies or your biomolecules of interest, Interchim provides a large range of biotinylation and fluorescent agents as well complete kits with desalting tools.

For general use, we recommend to use NHS-, Maleimide, or Hydrazide-activated reagents.

Also, complete biotinylation kits are proposed for non experienced investigators, as well as for convenient labeling (spin format) while avoiding to buy separate reagents.

■ Microspin biotinylation labeling kits-NH2 and -SH

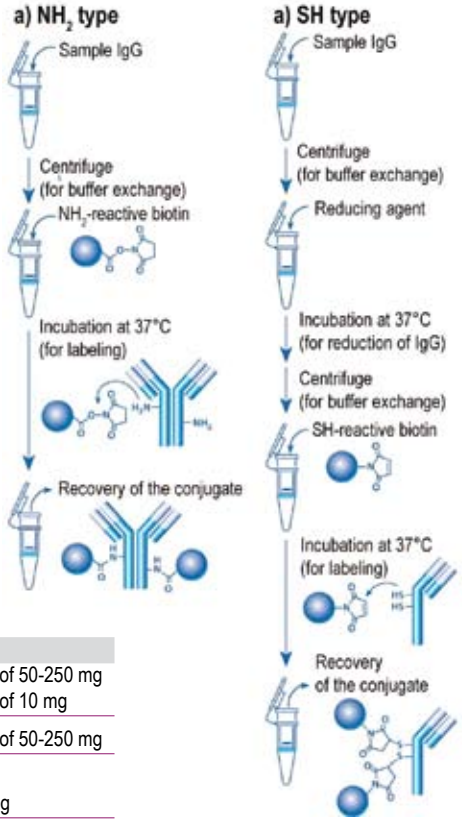
Efficient and convenient biotinylation reagent

- Quick : only 1 hour (/NH_2) or 3 hours (/SH) to get conjugates
- Easy : all processes in a single filtration tube
- Reliable : high recovery of conjugates, even for 500 µg of IgG !

Biotin Labeling Kits are primarily used for the preparation of biotin-labeled IgG for immunoassays. We offer kits with a very convenient format : spin filters where reaction and washes take place, that are available with 2 coupling strategies, and for 2 sample sizes (50-200 µg IgG, or ca 1 mg).

The kit BG767 biotinylates on amines, the most standard strategy. It uses a succinimidyl ester activated biotin, and contains all necessary reagents for labeling 3 samples of IgG antibody (10 µg to 200 µg). It can also be used to biotinylate any protein greater than MW 50 000 Da. The labeling process is simple. Just add the NH_2-reactive biotin to IgG solution on a filter membrane, and incubate at 37 °C for 10 min. On the average, 5 to 8 biotin molecules conjugate to each IgG molecule. Exceeding biotin molecules can be removed using a Filtration tubes included in this kit.

The kit BT3591 biotinylates on sulfhydryls to obtain oriented and defined biotinylation. It uses a maleimide-activated biotin. Features are similar to kit BG7670, except 1/there is an additional step to create a free sulhydryl in those protein (IgG) that do not have one, without loss of affinity ; 2/maleimide incubation occurs at 37 °C for 30 min.

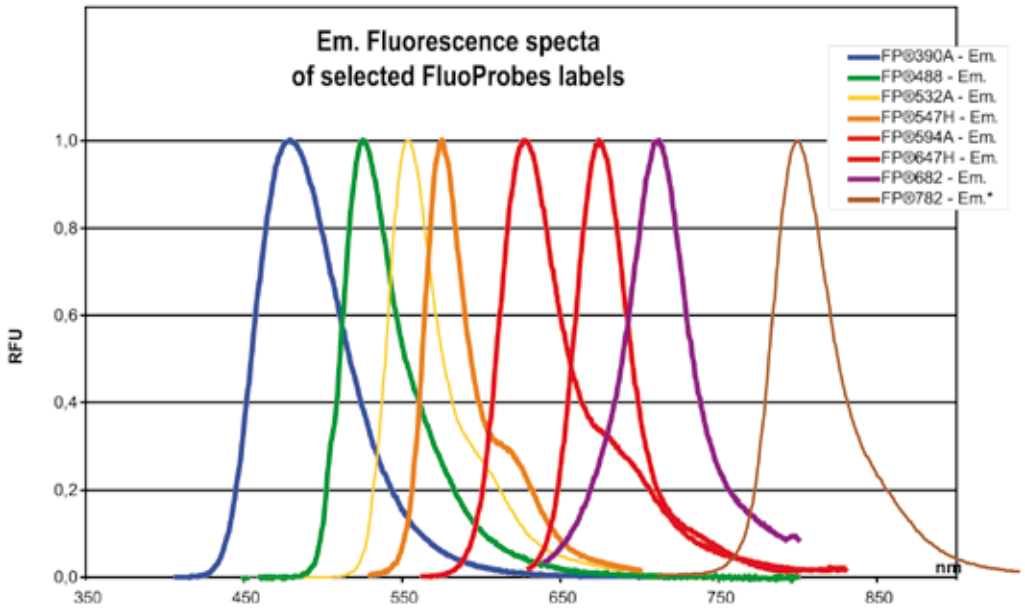


Description	P/N :	Qty
Biotinylation kit-NH_2 reactive	**BG7671**	3 lab. of 50-250 mg
	BG7671	1 lab. of 10 mg
Biotinylation kit-SH reactive	**BT3591**	3 lab. of 50-250 mg
Also available : biotinylation agents as stand alone		
sulfoNHS-lc-Biotin	**UP54398A**	100 mg
NHS-PEO_4-Biotin	**UPR20279A**	50 mg
PEO spacer confers hydrophilicity allowing to reach higher couplig ratio and to yield more bioactive and stable conjugates. It is also available with extended spacer length (PEO12).		
NHS-SS-Biotin cleavable spacer	**UPS073A**	100 mg

■ FluoProbes® Protein Labeling Kits

Easy antibody direct conjugation with the brilliant and photostable FluoProbes dyes !

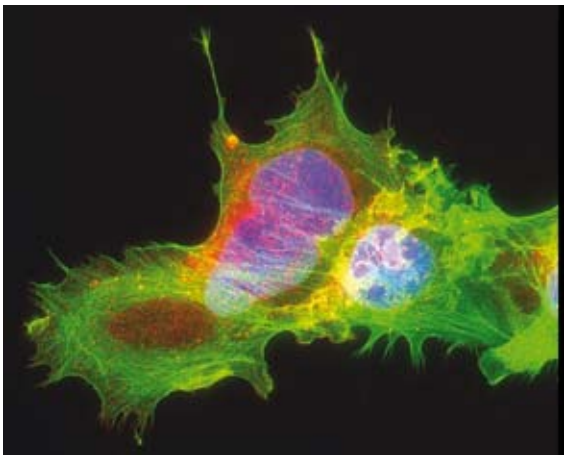
FluoProbes® labeling kits are designed for the easy-to-use and efficient labeling of protein with molecular weights greater than 25 kD, including especially antibodies. They use a succinimidyl ester of fluorescent labels that form a covalent stable linkage. Up 100µg to 1.5 mg of protein (IgG) can be labeled in a 1h30 procedure. They are available with many of our FluoProbes® labels. Following is a list of selected and popular ones (others on inquire).

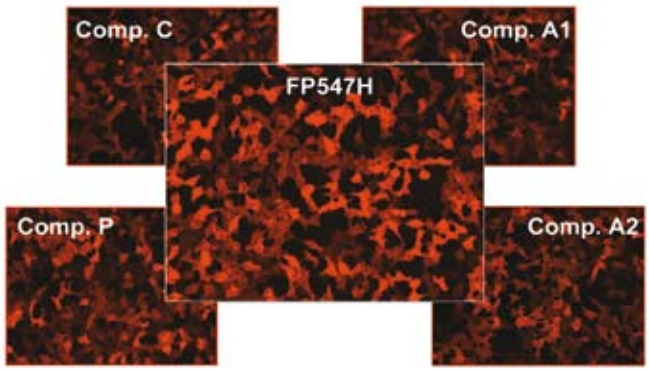


Description	$\lambda_{exc.}$ (nm)	$\lambda_{em.}$ (nm)	P/N :	Qty
FluoProbes® 782	783	800	**FP-CA6070**	1 kit (5 labelings)
FluoProbes® 682	690	709	**FP-BE8280**	1 kit (5 labelings)
FluoProbes® 647H	653	675	**FP-BZ9610**	1 kit (5 labelings)
FluoProbes® 547H	557	572	**FP-BZ9600**	1 kit (5 labelings)
FluoProbes® 488	493	518	**FP-BE3750**	1 kit (5 labelings)

For other wavelengths, please contact us or see in Interchim BioScience Innovations catalog.

See Fluoprobes dyes descriptions page 67.





Microplate innovation enables leading edge screening assays

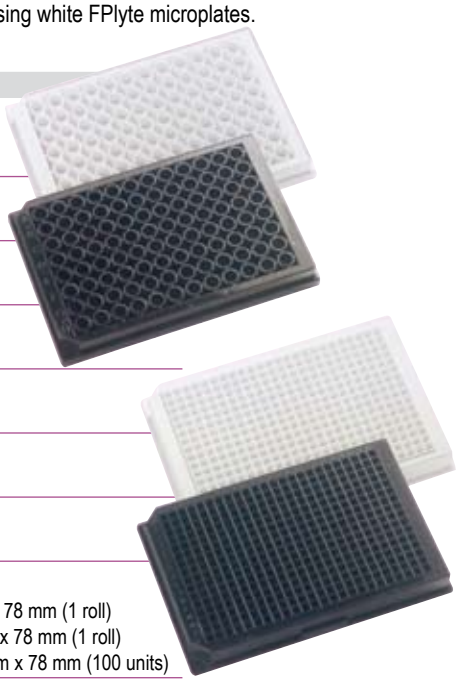
- Long wavelength UV microplates
- Limited well-to-well light cross talk
- Improved cell binding efficiency

The FPlyte microplates are microplates fully compatible with commercially available plate readers, robotic sample processors and automated liquid handling systems.
They are available in 2 formats, 96- and 384-wells and 3 colors :

The black plate provides the all-absorbing background needed to minimise background interference for sensitive fluorescence measurements. The opaque white plate maximises reflectivity enabling even weakly emitting luminescence assays to be routinely undertaken.
In addition to optimised luminescence and fluorescence measurements the unique design offers improved cell binding efficiency and allows the convenience of direct measurements on bottom reading spectrophotometers and inverted microscopes.

FPlyte microplates are ideal for quantitative assays at excitation wavelengths in the long-wavelength UV area between 325 nm – 425 nm. They offer excellent photometric performance down to 325 nm (80%T at 325 nm, 100%T at 335 nm).
Wavelengths below 350 nm are particularly useful for a variety of fluorescence assays such as HNK-1 ($\lambda_{exc./em.}$: 325/380 nm), Thiguanine ($\lambda_{exc./em.}$: 330/410 nm) using black FPlyte microplates, as well as many absorbance assays including Vitamin A (325 nm), retinol and retinyl acetate (325 nm), caspase (325 nm), acid phosphatase (330 nm) and hydroxyproline (335 nm) using white FPlyte microplates.

Description	Colour	P/N :	Qty
96-well FPlyte Microplate, standard	Black	**FP-BA7991**	50 u
	Black	**FP-BA7990**	100 u
	White	**FP-BA7950**	100 u
96-well FPlyte black well, clear bottom	Black	**FP-KT225A**	50 u
	Black	**FP-KT225B**	100 u
96-well FPlyte Microplate, Tissue Culture Treated, with lids	Black	**FP-BA8010**	100 u
	White	**FP-BA7970**	100 u
Hi Bind, 96-well FPlyte Microplate	Black	**FP-BA8000**	100 u
	White	**FP-BA7960**	100 u
Twister™ High Throughput Screening Pack, with lids, 96-well	Black	**FP-BA8020**	80 u
	White	**FP-BA8030**	80 u
384-well FPlyte Microplate, standard	Black	**FP-BA8170**	100 u
	White	**FP-BA8130**	100 u
384-well FPlyte Microplate, Tissue Culture Treated, with lids	Black	**FP-BA8180**	100 u
	White	**FP-BA8160**	100 u
Related products :			
Seal film for fluorescent assays		**FP-CD5130**	25 m x 78 mm (1 roll)
		FP-CD5110	500 m x 78 mm (1 roll)
		FP-CD5150	125 mm x 78 mm (100 units)



Please contact us for other wavelengths of fluorescent reference standards.

Technical tip

Fluorescein detection limit of an instrument
Begin with a hard weigh-out of at least 4-5 mg and solubilize in 100 mM sodium borate (pH9.5). Borate is the NIST buffer used, but it can be replaced by 50 mM phosphate (pH9). The detection limits may vary slightly. To check the absorbance spectrum and back-calculate to confirm the concentration by a known extinction coefficient as calibrated against the NIST standard. Calculate the concentration of fluorescein stock solution by C (M)=(Amax / extinction coefficient) x dilution fold, the light path is 1 cm, A max at 492+-5 nm, extinction coefficient is 78,000 M^{-1} cm^{-1}.
Make a dilution series into the same buffer, starting in the low nM range and dilute down. For most standard curves, triplicate measurements at each concentration are sufficient. But closer to the detection limit, it is recommand to take 8 replicates. This is important for the blank sample, as well. With detection limits using Z-factor analysis, a result > or = -1 is considered to be a detectable signal.
The equation for determining Z-factors is 1-((3*Sample+3*Std Dev Blank)/(Ave. read Sample - Ave. read Blank)).

Description	P/N :	Qty
Fluorescein, standard solution, 100 nM (494/519 nm)	**FP-DO6630**	50 ml
Fluorescein, standard (494/519 nm)	**FP-19365A**	1 g

New applications are under development. Contact us for your special needs.

IMAplate Technology
(Intelligent MultiFunctional Analysis)

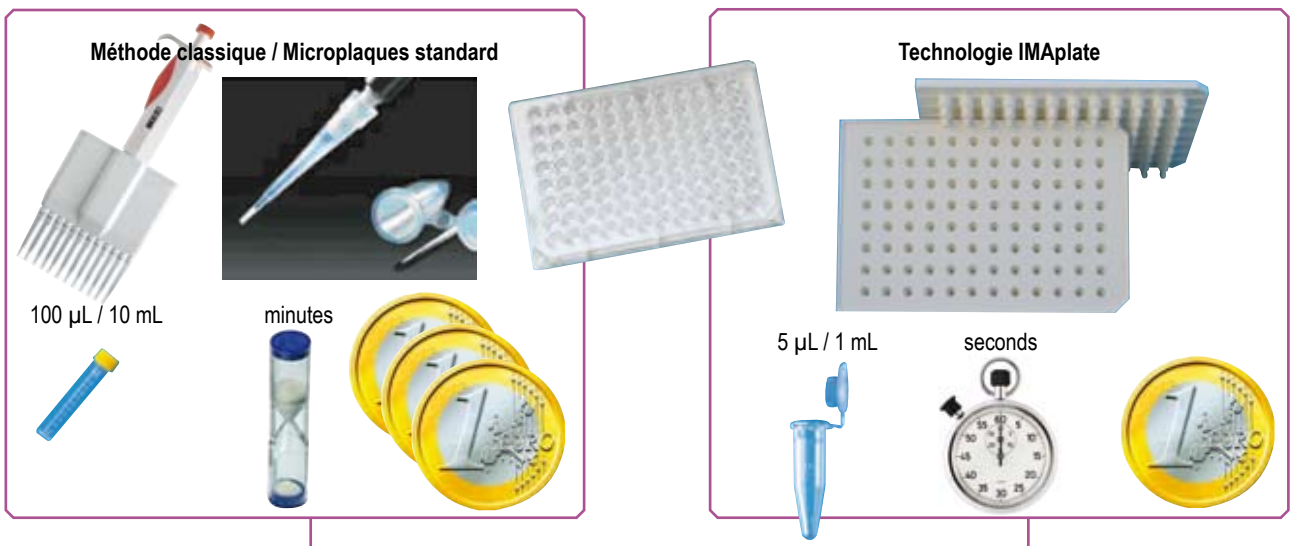
IMAplate is a polystyrene plate of 96 "microcuves". It is used to simplify and accelerate the handling of reagents, AND to make measures of absorbencies in UV, visible or InfraRed, of fluorescence or luminescence, this with any standard reader of microplate, while yielding signals at least equal or superior on microvolumes (5 µL) !

■ Applications

- 96-channel pipetting for **liquid transfer** :
ex. pipette and empty 96 x 5 µL in only 10 seconds !

- 96 bottom-free micro-cuvette array for UV, VIS or IR **spectroscopy** :
ex. assay DNA/RNA and proteins on just 5 µL with your usual microplate reader !

- 96 microwell plate for **parallel reactions, immuno-assays and cell assays** :
ex. do 96 reactions chemical or enzymatic on a unique support, miniaturize ELISAs with only 5 µl of reagents, higher signals and saving time !



Added value of IMAplates :

- Miniaturization : saving limited samples and costly reagents >3-20x / lowered price per test
- Transfer of liquid integrated to assays
- High analysis throughput, even manually
- Robust - highly reproducible
- Reduce reactions times (solid-phase assays)
- Increase sensitivity

Analysis with a microplate reader

Powered by **Interchim** in collaboration with **Berthold** technologies Gmbh

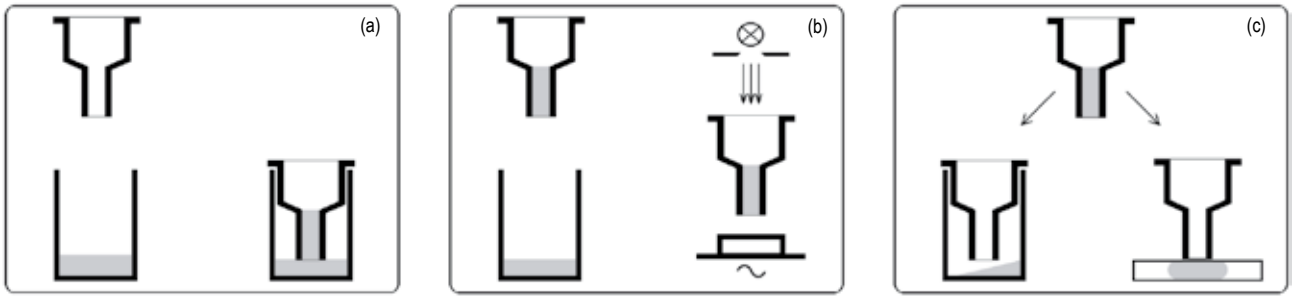
Description	P/N :	Qty
IMAplate™ Start Kit	**DR9621**	1 Kit*
IMAplate™ (96 µcuves) - white§	**DR9611**	5 ea
IMAplate™ (96 µcuves) - black§	**DT5431**	5 ea
IMAplate™ (96 µcuves) - yellow§	**DT5441**	5 ea

* contains 5 IMAplates and Reader adapter

§ White plate are recommended for luminescence measurments, Black for fluorescence measurements, and yellow plates for UV-vis spectrometry and sample handling.

How IMAPlate work?

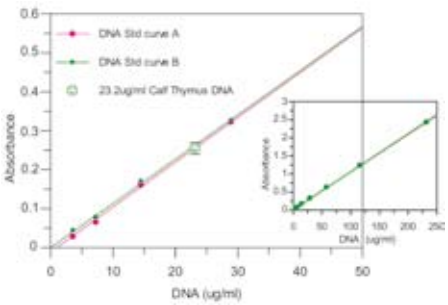
- Loading, un-loading and washes are simplified, accelerated and reliable :
  samples and reagents and buffers are loaded simultaneously by capillary force (a)(precise volume), assayed, then drawn away by an absorbent paper (c) or by centrifugation. ex. 1 plate/ samples can be washed in just 10 seconds, without machine !
- Microcuves of 5 µL save up 20 fold (rare) samples and any (costly) detection reagents (ex in ELISA).
- Reading (b) : the optical path is perfectly defined, and longer to those of standard microplates !
  Hence detection sensitivity is superior.
- The microcuves have no bottom ! Thus no parasite optical absorption take place, and you can work in UV, IR..., with superior sensitivity.
  You even can recover the samples (c).
- The microcuves have a geometry more favorable for immunoenzymatic reactions (surface/volume 3.8x superior), compared with wells of standard microplates : hence kinetic is speeded at each step (ex incubations 2 fold shorter in ELISA).

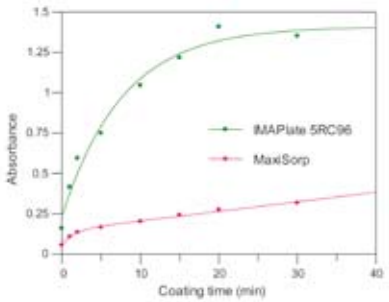


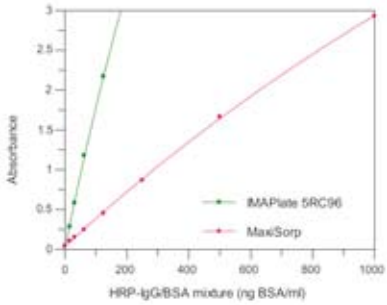
IMAplate technology combines advantageously notably in ELISA, and for multiplexed analysis.

IMAplate offer a solution at the same time more flexible, quicker and cost-effective, when :
- Samples are in limited quantity or precious,
- Reagents are costly (case of commercial kits),
- Several analysis are performed on each sample (multiplex),
- To speed steps and handling with reliability.

Examples of applications particularly appropriate :
- Serological analysis of many analytes in small animals serums
- Multiplex screening (pharma, cosmeto, vaccines)







■ Integrate your different methods/analysis

on a unique support, flexible & efficient, without instrument investment !

SPECTROMETRY
ex :
DNA/RNA dosage (260 nm)

Proteins dosage (UV280 nm)

compound InfraRed analysis

LIQUID HANDLING
ex :
Easy µcuve loading
(10 sec/capillary force, automatable)

Easy µcuve un-loading (10 sec/absorbant, centrifugation)
(automatable)

MicroCuvette : 5 µl volume

IMAplate technology

Reduce times of incubation, transfer, and washes,...

Reduce the volumes & cost of rare samples & costly reagents by 3-20X

Increase the sensitivity of detection

Colorimetric assays - ABS
ex :
Proteins Dosages : BC Assay, Coo Assay

Biochemistry (clinical, IAA) : Tests Glucose...

Cell Biology : MTT, SOD, Thromboxanes...

Fluorimetric Cell Assay - FL
ex :
Assay of Proteins : LavaPep
of DNA/RNA : Evagreen

Calcium Flux : Fluo-3, Fluo-8...

Cell viability : UptiBlue...

Toxicology : LDH, Grantoxilux...

(Chemi)Luminescent Assays - ECL
ex :
ELISA avec HRP / UptiLight ECL

Calcium Flux : Coelenterazine/aequaporin...

Cell viability : ATP/lum...

Reporter assay : Luciferase, Coelenterazine...

Homogenous-Phase assays – CELL Assays

Commercial Kits
Reduce cost/test by 2-5 fold

"home made" Assays
Reduce by 3-20 the cost of reagents

Solid-Phase assays - ELISA

ELISA Commercial Kits
Reduce cost/test by 2-5 fold AND TIME BY 20-50%

"home made" ELISA : TMB, UptiLight ECL...
Reduce by 3-20 the cost of buffers, saturants, antibodies, enz. substrates

Other systems of muliplexed analysis (Luminex, SpotELISA, Micro-Arrays)

■ Antifade Kit for Microplate

When exposed to excitation light, fluorescence intensity of dyes decreases due to their photooxidation or other photoreactions. There are very few fluorescent dyes that completely resist photobleaching. Frequently, when a section has been scanned repeatedly under strong excitation light, dyes could lose significant fluorescence signal before visual evaluation or photography can be accomplished. For examples, the photobleaching of fluoresceins (such as FITC-labeled antibodies) has become a major problem in fluorescence microscopy. In severe cases (such as phycoprotein-labeled bioconjugates), a fluorescence image of high resolution can not even be taken due to the extremely high photobleaching rate. The Antifade Kit is to reduce the dye photobleaching rate, giving researchers longer observation time. The kit contains all the essential components that can be readily applied to imaging experiments. They are all premixed and ready-to-use solutions. This kit is designed for microplate format.

Description	P/N :	Qty
Antifade Kit for Microplate	**FP-CL0530**	1 plate

■ Legals - Trademarks

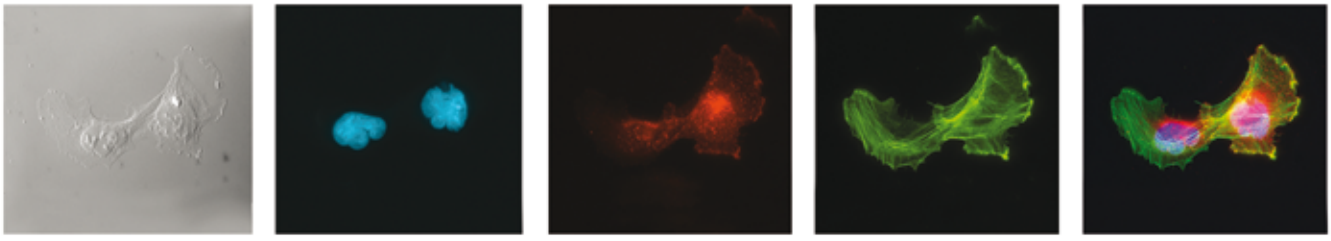
Acella from CellTechnologies
Cytonin, PeroxyGlow, and TACS from Trevigen
CyToxiLux and GranToxiLux from OncoImmun
DeepBlue C from BioSignal Packard
FluoProbes, PrimAb and UptiBlue from Interchim
MUP plus, Phospholite, and Rhod-4 from ABD
PMA from Biotium
Twister from Caliper

NEW

FluoProbes®

New Fluorescent Antibodies

- ✔ Over more efficient new dyes
- ✔ Antibodies with high affinity and specificity
- ✔ Available within 24 hours*



*based on stock availabilty

interchim
211 bis Avenue J.F. Kennedy - BP1140
03103 Montluçon Cedex - France
Tel 33(0)4 70038855 - Fax 33(0)4 70038260
e-mail interbiotech@interchim.com