

■ 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 ($\lambda_{ex} \lambda_{em} = 496/520 \text{ nm}$).

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-IETD)₂-R110 contains two IETD tetrapeptides and is completely hydrolyzed by the enzyme in two successive steps. The fluorogenic substrate (Ac-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-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-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-LEHD) ₂ -R110 , Enzyme inhibitor Ac-LEHD-CHO, R110		
Related products :		
Annexin V-FluoProbes 488, FCM grade (495/519 nm)	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-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-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-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 apoptosis.

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 apoptosis. Granzyme B that is inactive in lysosomal granules, is activated in the presence of Target cells during (degranulation), extremely early (before Perforin).

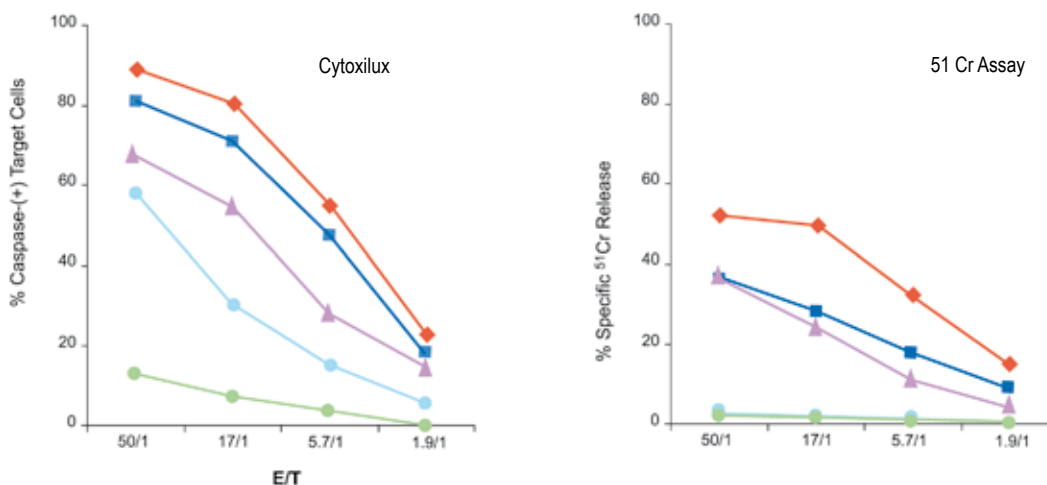
Literature : Nature Med. 8:185-189 (2002); Methods Mol. Biol. 263:125-140 (2004); J. Immunol. 171:27-31 (2003)

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 xenobiotics, physiology and fate of effector cells
- ▶ **More rapid** : co-incubation of 0.3-2 H (vs. 4 H for ^{51}Cr release assay)
- ▶ **More sensitive** than the ^{51}Cr 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 ^{51}Cr method

Comparison 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

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.

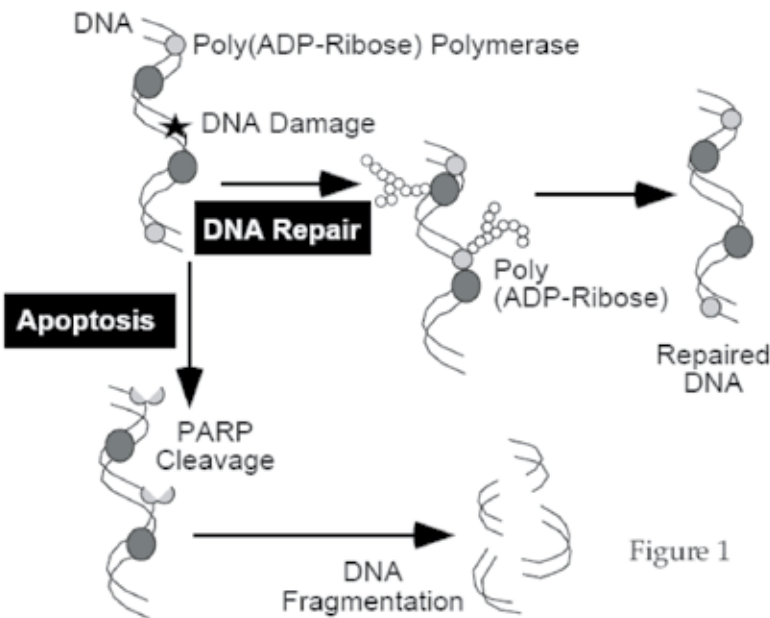


Figure 1

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

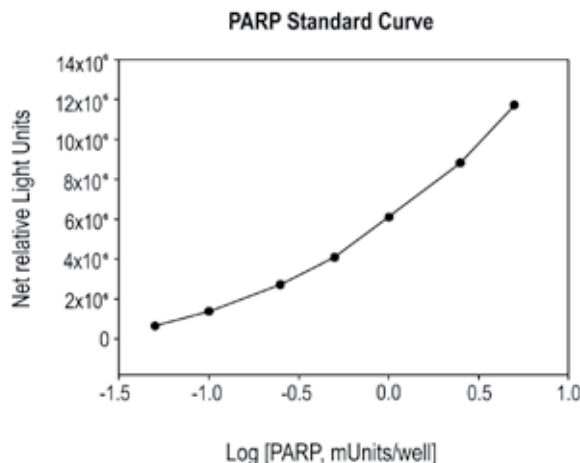
Description	P/N :	Qty
Universal Chemiluminescent PARP Assay Kit w/ Histone Reagent	HP9090	96 tests
Universal Chemiluminescent PARP Assay Kit w/ Histone Coated	HP9130	96 tests
Related product :		
FITC-NAD	FX8241	250 µl

■ 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.



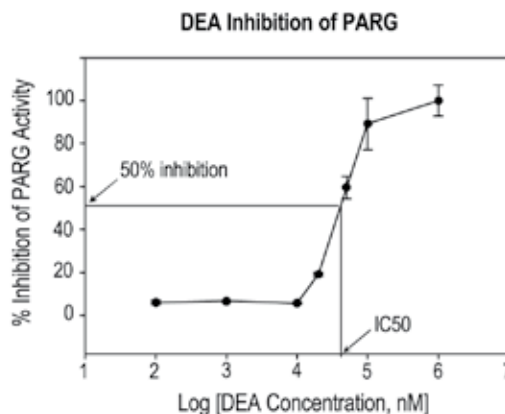
Graphical representation of an example Chemiluminescent readout of a PARP standard curve.

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 :

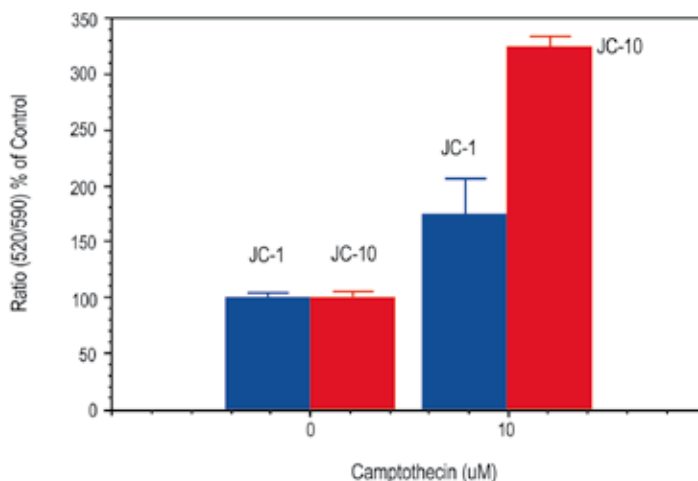
Debbasch C, et al., Mitochondrial activity and glutathione injury in apoptosis induced by unpreserved and preserved beta-blockers on Chang conjunctival cells. *Invest Ophthalmol Vis Sci.* 2001 Oct;42(11):2525-33.

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 μ M 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 Camptothecin induced mitochondria membrane potential change in Jurkat cells. Jurkat cells were treated with 10 μ M 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

Mitochondrial events

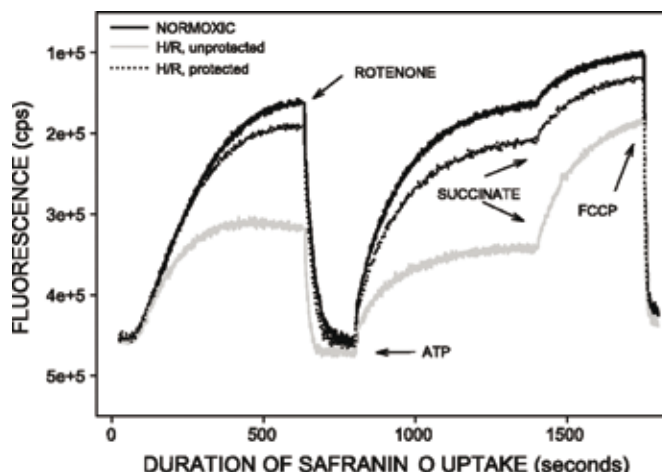


Safranin O

Dynamic and quantitative approach of changes of mitochondrial membrane potential ($\Delta\psi(m)$) $\Delta\psi m$:

- Suitable for **dynamic studies** of energization
- Allows **direct assessment** of both substrate-dependent, electron transport-mediated $\Delta\psi m$ and ATP hydrolysis-supported $\Delta\psi m$.
- **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, safranin O was rapidly and completely released when mitochondria were de-energized with FCCP. The decreased fluorescence of safranin O resulting from quenching of safranin O after uptake by energized mitochondria means that low fluorescence corresponds to high $\Delta\psi m$ (Feldkamp, 2004).



Description	P/N :	Qty
Safranin 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
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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 nonfluorescent 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
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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

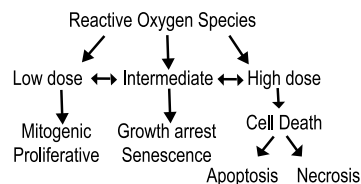
Oxidative metabolism study (ROS, NO)

The production of free radicals primarily results from O_2 caught 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, Artherosclerosis 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, favourising 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

P/N :	Probes	Reactive Oxygen Species (ROS)					
		Hydrogen Peroxide	Hydroxy radical	Hypochlorous acid	Peroxyl radical	Peroxynitrite anion	Superoxide anion
		H2O2	HO•	HOCl	COO•	ONOO•	O2•-
FP-83775	Dihydrorhodamine 123	+		+		+	
FP-46731	H2DCFDA	+			+	+	+
FP-46915	Lucigenin*	+					
FP-97233	Coelenterazine					+	+
FP-38544	MCLA*		+				+
	Dihydrocalcein AM	+	(O_2)		+	+	
	Dihydroethidium					-	+
24200A	tMPV						
CA7170	HPF		+			+	
CA7270	APF	-	+	+	-	+	-
U3238A	ADHP	+					

*ROS-generating Enzyme Detection kits

MyeloPeroxidase detection kit

Catalase detection kit

Cis-Parinaric acid

Malachite Green IT

SuperOxideDismutase (SOD)

Hemoprotein of PMNs cells / Cl⁻ oxidation to HOCl
antioxidant enzyme / decomposes H_2O_2
fatty acid to monitor lipid peroxidation
produce a Hydroxyl radical burst upon irradiation
converts $O_2^{\bullet -}$ into H_2O_2 and O_2

*Other oxidative species : aldehydes

SSAO detection kit

MonoAmineOxidase (MAO A&B)

deamination / formaldehyde, methylglyoxal
oxidate a variety of neurotransmitters / aldehydes



■ H₂DCFDA, Carboxy-H₂DCFDA, H₂DCFDA-SE

H₂DCFDA 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-H₂DCFDA is an indicator for ROS. On penetration into the cells, carboxy-H₂DCFDA is deacetylated by intracellular esterases, resulting in an enhanced cellular retention of the probe. The fluorescence of the oxidized form of carboxy-H₂DCFDA is measured with a fluorescence excitation of 485 nm and emission at 535 nm.

H₂DCFDA, SE is an amine reactive form of the H₂DCFDA 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 NH₂-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 ₂ DCFDA)	FP-467312	100 mg
Carboxy-2',7'-Dichlorodihydrofluorescein diacetate (CH ₂ DCFD)	FP-46634A	25 mg
2',7'-Dichlorodihydrofluorescein diacetate, succinimidyl ester (H ₂ DCFDA-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 peroxyxynitrite. 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 cyclomaltononaoase (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	FP-38544A	5 mg
2-Methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-one, hydrochloride		

■ 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 peroxynitrite ($ONOO^-$)

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 ($E_m = 465$ 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

Reactive Oxygen Species (ROS)



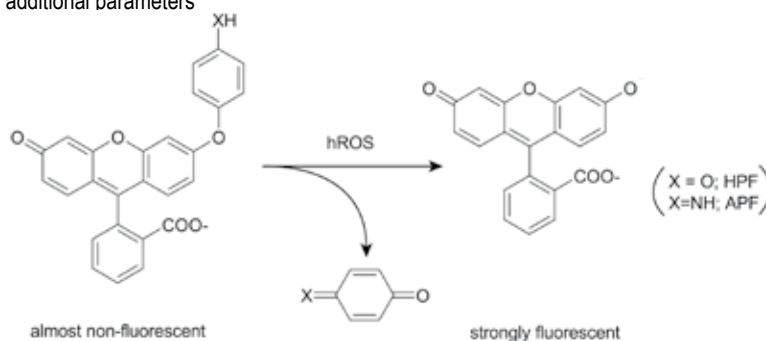
Int

■ Hydroxyphenyl fluorescein (HPF) Assay Kit

Fluorescent Hydroxyl ($\cdot\text{OH}$) / 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 ($\text{OH}\cdot$), and peroxynitrite (ONOO^-) detection. It has little reactivity towards other hROS such as : hypochlorite (OCl^-), singlet oxygen (O_2^1), superoxide ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), nitric oxide ($\text{NO}\cdot$), and alkyl peroxide ($\text{RO}_2\cdot$).



Reference :

Ken-ichi Setsukinai, *et al.*: *Development of Novel Fluorescence Probes That Can Reliably Detect Reactive Oxygen Species and Distinguish Specific Species*. J. of Biological 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 ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), nitric oxide ($\text{NO}\cdot$), and alkyl peroxide ($\text{RO}_2\cdot$) (see table below)¹. APF is a cell permeable indicator that can be used to detect Hydroxyl Radical ($\cdot\text{OH}$), 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 : $\cdot\text{OH}$	1200	7400
Peroxynitrite : ONOO^-	560	6600
Hypochlorite : OCl^-	3600	86
Oxygen Radical : $^1\text{O}_2$	9	26
Superoxide : $\text{O}_2^{\cdot-}$	6	67
Hydrogen Peroxide : H_2O_2	<1	190
Nitric Oxide : NO	<1	150
Alkylperoxyl Radical : $\text{ROO}\cdot$	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 OCI (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 (O_2^1), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), nitric oxide (NO^{\bullet}), and alkyl peroxide (RO_2^{\bullet}). HPF/APF are cell permeable and can be used in combination to detect hypochlorite ($^{\bullet}OCl$) 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: $^{\bullet}OH$	730	1200	7400
Peroxynitrite: $ONOO^{\bullet}$	120	560	6600
Hypochlorite: $^{\bullet}OCl$	6	3600	86
Oxygen Radical: 1O_2	5	9	26
Superoxide: $O_2^{\bullet-}$	8	6	67
Hydrogen Peroxide : H_2O_2	2	1	190
Nitric Oxide: NO	6	1	150
Alkylperoxyl Radical: ROO^{\bullet}	17	2	710
Autoxidation	1	1	2000

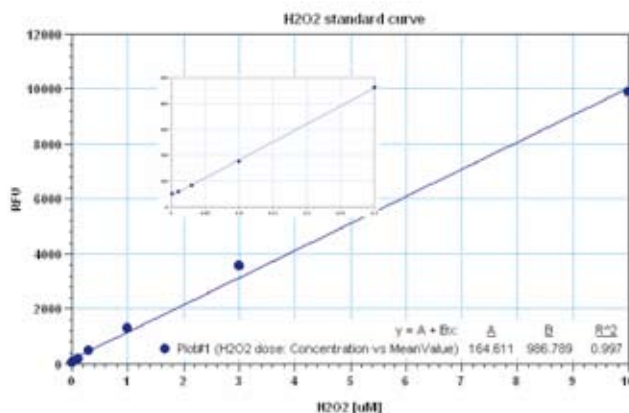
Description	P/N :	Qty
Hypochlorite Detection Kit (HPF, APF)	CA7250	150 tests

■ ADHP Hydrogen peroxide Assay Kit

"Read and mix" sensitive H_2O_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_2O_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

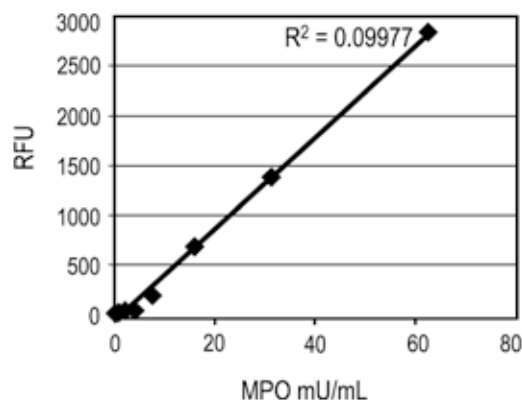
Reactive Oxygen Species (ROS)



■ 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 glycosylated hemoprotein that has a molecular weight of 144kD. The hemoprotein consists of two dimers linked via a disulfide bridge. Each dimer 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 variety 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 powerful 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 μ L of MPO standard and 50 μ L of RC was added to individual well of a 96 well black plates. The plate was incubated at room 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 :

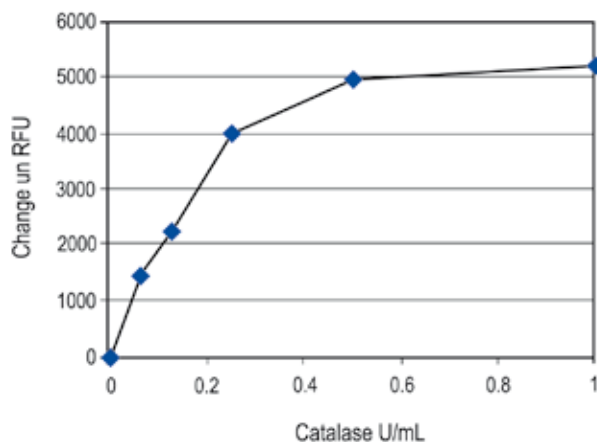
J Neural Transm Suppl 23:55-72. (1987)
 Proc. Nat. Acad. Sci. U.S.A., 85, 4934-4938 (1988)
 Biochem. Pharmacol., 17, 1285-1297. II (1968)
 J. Biochem., 79, 1297-1299 (1976)
 Biochem. Pharmacol., 27, 1995-2000 (1978)

■ 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 eukaryotic cells, catalase is 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 mutagenesis⁽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 μ M 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 μ L 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

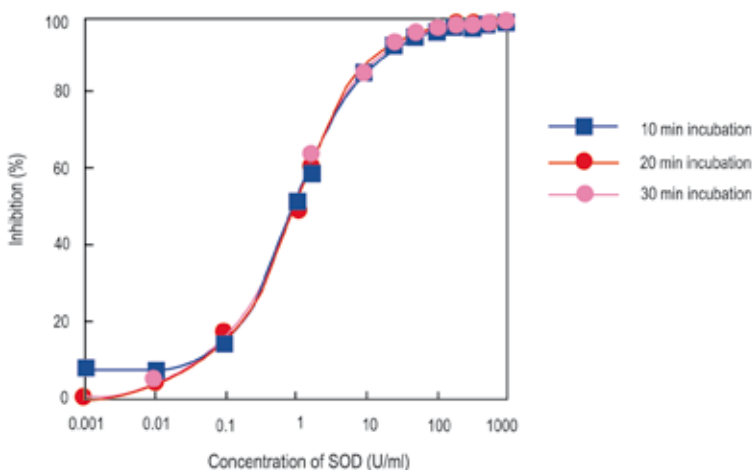
Cis-parinaric acid (CPA) is a fluorescent poly-unsaturated fatty acid used as a probe to directly monitor lipid peroxidation. Fluorescence measurement is using 318 nm excitation and 420 nm emission filters.

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** IC₅₀ determination
- ▶ **Convenient** 96-well microplate colorimetric assay
- ▶ **Low-background** noise measurement

Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion (O₂^{•-}) 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 O₂^{•-} are linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD, as shown in the Figure above. Therefore, the IC₅₀ (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

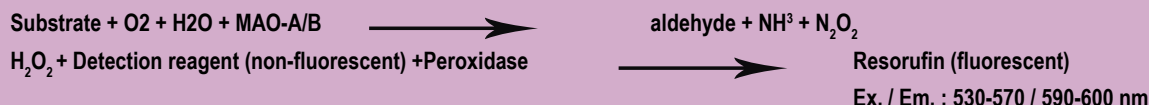


■ Monoamine Oxidase A&B Detection Kit

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 performed 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.



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

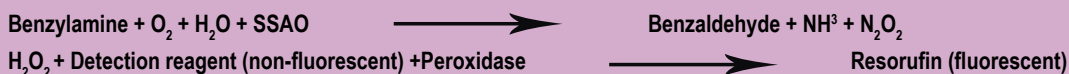
■ SSAO Detection Kit

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.



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 NO₂⁻ 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 triazolo fluorescein, 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 cells¹).

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-NAME, 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 benzotriazole (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 NO₂⁻ 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 (NO₂⁻) 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 NO₂⁻ 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