Oxidative metabolism study

Oxidative metabolism

The reactive Oxygen Species (ROS) and reactive nitrogen compounds (NOS) produced by stress of cells have many different activities in biological systems. In response, aerobic organisms created defense mechamisms to avoid oxidative stress.
Biosciences Innovations provides a complete line of probes and other assays for reactive OS/NOS species, peroxidation damages and biomarkers of oxidative stress, to study the defense mechanisms and relationships between oxidative damage and stress conditions (UV exposure ot UV, chemicals,…), disease (cancer, infections) or aging processes.
Find here a selection of probes and assay kits for oxidative research from brands such as Fluoprobes, Trevigen, Cayman, Dojindo and AAT.

● standard and superior FluoProbes probes for ROS/NO oxidative species: Hydrogen Peroxide, Hydroxyl radical, Hypochlorous acid, Peroxyl radical, Peroxynitrite anion, Nitrite, Nitrate
● unique probes and assay kits for lipid, nucleic acid and protein peroxidation: DNA damage (abasic sites, 8-oxoguanines, 8-nitroguanosine), and lipid peroxide.
● ELISA kits employs well-established methods to assay for superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase, and glutathione.
+

More products can be found at www.interchim.eu (oxidative-metabolism page).

Go to : ■ Reactive Oxygen Species ■ Peroxidation ■ Nitric/Nitrate species ■ Oxidation protection (Catalase, GSH) ■ Damage to proteins, lipids, nucleic acids ■ Glycolysis ■ Krebs cycle / Oxidative phosphorylation ■ Oxidative metabolism inhibitors

Technical tip - Oxidative metabolism – generalities

Oxidative metabolism is the biochemical processes in cell leading to break down of molecules into energy, or adenosine triphosphate (ATP, and alternatively to undesired nocive compounds and effetcs (oxidative damages). It is hence the first part of catabolism, contrasting to anabolism processes that use the chemical energy to build molecules such as tissues and organs. Aerobic cellular respiration, a process requiring the use of oxygen, is the most efficient form of ATP production. ATP can also be produced anaerobically, without the presence of oxygen.

Oxidative metabolism begins with the breakdown of organic nutrients such as carbohydrates, sugars, proteins, vitamins and fats. ***Glucose***, the most common nutrient resulting from digestion of sugars, is broken down in the **glycolysis** process, or glucose metabolism. It produces two ***pyruvate*** molecules that enter the mitochondria of the cell where the Krebs cycle produce through the respiratory chain a redox potential and ATP that supplies cellular energy to the rest of the cell.

The **Krebs cycle**, referred to as the citric acid cycle as well as the tricarboxylic acid (TCA) cycle, performs the oxidation against the reduction of electrons releasing of energy and carbon dioxide.
This cycle begins with one pyruvate molecule that, after a series of chemical reactions, is input into the **cycle as oxaloacetic acid**. The cycle begins and ends with oxaloacetic acid, which undergoes a series of enzyme-initiated chemical reactions during the cycle to produce energy.
In the **citric acid cycle**, oxidation of the carbon atoms results in the production of carbon dioxide and energy. There are two pyruvate molecules input into the mitochondria from one glucose metabolism reaction, so the TCA cycle involves two cycle turns for completion. Each turn produces one ATP, and so at the completion, two ATP are produced.
Oxidative phosphorylation (or OXPHOS) designs the process converting redox energy into ADP then ATP in mitochondira. Occuring in almost all aerobic organisms, it is a highly efficient way of releasing energy, compared to alternative fermentation processes such as anaerobic glycolysis. It produces numerous byproducts, known as reaction intermediates, that are almost immediately used for anabolism after catabolism is complete. Yet, it yields by electron addition to Oxygen (O2) the ***oxygen reactive species*** superoxide ($\bullet O^{-}_{2}$) and peroxide anions ($O2^{2-}$), that are harmfull. These ROS are normally neutralized by several processes, the ***antioxidants*** including vitamins such as vitamin C and vitamin E, and antioxidant enzymes such as superoxide dismutase, catalase, and peroxidases.
Oxidative metabolism **inhibitors** include Poisons (i.e. Cyanide, Carbon monoxide, Azide) that Inhibit the electron transport chain by binding more strongly than oxygen to the Fe–Cu center in cytochrome c oxidase, preventing the reduction of oxygen., Antibiotic (i.e. Oligomycin) that inhibits ATP synthase by blocking the flow of protons through the Fo subunit, and ionophores (i.e. CCCP: 2,4-Dinitrophenol) that are oonophores that carries protons across the inner mitochondrial membrane, thus disrupt the proton gradient, uncoupling proton pumping from ATP synthesis, pesticides (i.e. Rotenone) that prevents the transfer of electrons from complex I to ubiquinone by blocking the ubiquinone-binding site; or competitive inhibitor (i.e. Malonate and oxaloacetate – for of succinate dehydrogenase (complex II)).

Oxidative metabolism is affected in diseases such as type 1 diabetes. Type 1 diabetes prevents glucose from entering the cell, and if it is left untreated, there will be no glucose available for normal production of energy via glycolysis. The body will then resort to the breakdown of fatty acids to fuel itself. The breakdown of fatty acids results in an acidic byproduct known as ketone bodies. If let untreated, the quantity of ketone bodies acidifies the potenz hydrogen (pH) of the blood and leads to the life-threatening condition ketoacidosis.

Oxidative metabolism is classically divided in following biochemical processes

- Oxidative processin mitochondria (OxPhos)
- Reactive Oxigen species (ROS)
- Nitrate/nitrite oxidative metabolism
- Lipid/peroxime/peroxidation
- Oxidation protection: Catalase/Glutathione transferase/….

Disturbances in the normal redox state of cells can cause **Oxidative stress** with toxic effects through the production of peroxides and free radicals that damage all components of the cell, including proteins, lipids, and DNA. Further, it can cause disruptions in normal mechanisms of cellular signaling, because some reactive oxidative species act as cellular messengers in redox signaling.
Oxidative stress results from dysfunction of normal pathways, or from external stress (UV exposure, hyperactivity of mussels,… - similar damage occurs under ionizing radiations) or appear with ageing. Involved species are $\bullet O^{-}_{2}$ (superoxide anion), H_2O_2(peroxide), •OH (hydroxide radical), RO•, alkoxy and ROO• peroxy radicals, HOCl, hypochlorous acid, ONOO-, peroxynitrite.
It is thought to be involved in the development of cancer, Parkinson's disease, Alzheimer's disease, atherosclerosis, heart failure, myocardial infarction, fragile X syndrome, Sickle Cell Disease, lichen planus, vitiligo, autism, and chronic fatigue syndrome.
However, reactive oxygen species can be beneficial, as they are used by the immune system as a way to attack and kill pathogens. Short-term oxidative stress may also be important in prevention of aging by induction of a process named mitohormesis.

ROS/NOs/Superoxides study - probes & kits

Fluorescent probes dedicated to the study of Reactive Oxygen Species (ROS) and other oxidative compounds are used for the study of cell oxidative metabolism, notably in mitochondria, but also in peroxysomes and even other cell structures. ROS include severa oxygen radicals generated by the metabolism and are realtd to those generated by NO species. These reactive radicals are normally scavenged by proper processes such a SOD and GST, or accumulate in cells and cause damages to structures and metabolism (DNA, Lipids, Proteins notably enzymes). Such probes are thus important in R&D and diagnostic of many disfunctions, aging, stress (UV,…) but also in agroindustry (food/bewerage oxidation) and in environment.
Selected Mitochondria probes and stains are listed in bellow tables, and featured in following sections.

Technical Tip – **Oxidative metabolism study (ROS, NO)**
The production of free radicals primarily results from O2 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. Moreover, oxidative and nitrative damage of mitochondrial proteins adds to OXPHOS dysfunction further exacerbating electron transfer efficiency decrease and free radical production. Finally also, more cytosolic proteins are damaged.
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 SO2 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, ROS are produced by peroxidase, a common enzyme for signal amplification in immunoassays (EIA).

Overview of Oxidative metabolism tools

Oxidative Stress Marker Detective	Product lines
ROS	Fluorescent probes Luminescent probes Spin-trapping probes
NOs	Fluorescent probes Luminescent probes Spin-trapping probes
Peroxidation (proteins, lipids, nucleic acids)	Mitophilic Peroxide probes , incl. MitoPeDD
Lipid damage	LipidPeroxide Assay Kit
Protein damage	
DNA Damage	Abasic site of DNA probes and assay kits Nitroguanosine HT 8-oxo-dG BPDE
Glycolysis, Krebs	
Mitochondria	Mitochondria OXPHOS/PDH Assays
SOD	SOD probes and assy kits
Glutathione	SOD probes and assy kits
AGE	ACE assay kits

■ ROS Probes tables

●**Fluorescent Probes** for Reactive Oxygen Species (ROS)

Cat.#	Probes	Hydrogen Peroxide H_2O_2	Hydroxy radical H0-	Hypochlorous acid HOCl	Peroxyl radical COO-	Peroxynitrite anion ONOO-	Superoxide anion O2-
FP-46731	H2DCFDA	+			+	+	
FP-97895	CM-H2DCFDA	+	+	+	+		
FP-83775	Dihydrorhodamine 123	+		+		+	+
FP-46915	Lucigenin*	+					
FP-97233	Coelenterazine					+	+
FP-T8889	Methyl Coelenterazine						
FP-38544	MCLA*		+				+
FP-52492	Dihydroethidium (Hydroethidium)						
1I8940	MitoDePP*	-	-	-	-	-	-

*MitoPeDD is not a ROS probes but detects lipid peroxidation (effect of the ROS probes) very specifically as it does not detect ROS species. See section 'Peroxidation'.

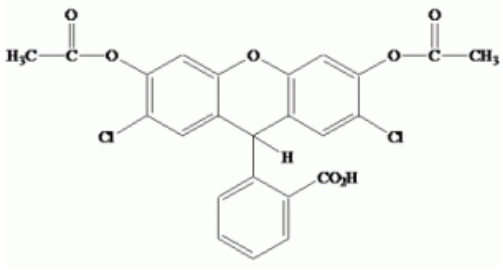
●**Spin-trap Probes** for Reactive Oxygen Species (ROS)

ROS Detector and method type	Product Name	P/N
super oxide, O-, C-, S-, and N-centered free radicals spin-trapping by ESR	**DMPO** CAS:3317-61-1 ; MW:113.2	U24692-D048-10
Superoxide anion (O2-) and Hydroxyl Radicals spin-trapping	**BMPO** CAS:387334-31-8 ; MW:199.25	PIY020-D048-10

Featured ROS probes

H_2DCFDA **FP-467312 100 mg**

FP-46731A, 50 mg **FP-467312, 100 mg**
FP-46731C, 250 mg **FP-46731D, 500 mg**



2',7'-DichloroDiHydroFluorescein Diacetate; CAS: 4091-99-0]; MW : 487.3 (M)
Soluble in DMSO and EtOH; λexc./λem. (MetOH) : 258/none ; EC : 11 000 after hydrolysis and oxidation :
λexc./λem. (hydr.&oxid.) (pH 4) : 495/529 nm ; EC : 38 000 M-1cm-1
λexc./λem. (hydr.&oxid) (pH 8) : 504/529 nm ; EC : 107 000 M-1cm-1
The standard probe to detect reactive oxygen species (COO-, ONOO-) in cells (neutrophils, macrophages). Colorless 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 (FP46629).
Applications : ROS detection, viability and cytotoxicity assays, apoptosis.
Can be used with Propidium iodide to follow oxidant production and nuclear injury.

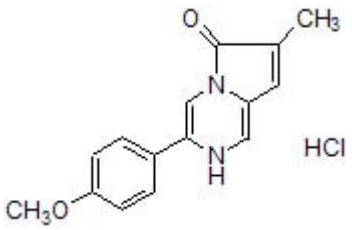
1. Plant Growth Regul (2010) doi: 10.1007/s10725-010-9545-y
2. Physiologia Plantarum (2010) doi: 10.1111/j.1399-3054.2010.01400.x
3. Biochemistry 39, 1040 (2000).
4. Cancer Res. 60, 219 (2000).
5. Free Rad Res 32, 57 (2000).
6. J Immunol Meth 159, 173 (1993).
7. J Immunol Meth159, 131 (1993).
8. Cytometry 13, 615 (1992).

Technical sheet (incl. H_2DCFDA-SE, Carboxy-H_2DCFDA, 6-Carboxy-H_2DCFDA, AM)

CM-H_2DCFDA **FP-97895, inquire**

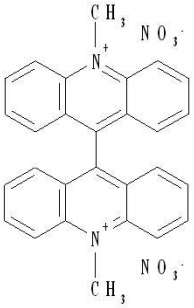
Technical sheet

MCLA **FP-38544A, 5 mg**



2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-one, HCl; CAS [128322-44-1]; MW : 291.74 (M)
Soluble in DMSO, DMF, Water; λexc./λem. (MeOH) : 430/546 nm ; EC : 8 400 M-1cm-1
A chemiluminescent probe that emits at 455 nm upon oxyidation by superperoxides. It is superior to luminol in this application (pH optimum closer to neutral range of cells.
Technical sheet

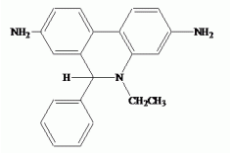
Lucigenin **FP-46915A, 10 mg**



9,9'-bis-N-methylacridinium nitrate, CAS: [22103-92-0]; W : 510.50 (M)
Soluble in water, DMSO; $\lambda_{exc}/\lambda_{em.}$ =455/505 nm;
A chemiluminescent probe that emits at 470 nm (QY : 0.6) upon oxydation with superoxide in basic solution. Widely used, but see MCLA for superior performence in ROS detection in cells. Also a Cl- indicator as it is efficiently quenched by Cl-.
Technical sheet

Dihydroethidium **FP-524923, 5x1mg**

FP-52492A, 25mg **FP-52492B, 100mg**
HH9180 1 ml (5 mM in DMSO)



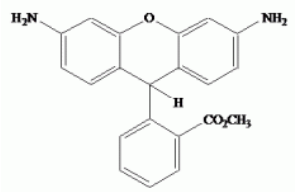
Off-white to light brown solid soluble in DMF or DMSO
CAS: 38483-26-0; MW: 315 (M)
Dihydroethidium (also called hydroethidium) is the chemically reduced form of the commonly used DNA dye ethidium bromide. The probe is useful to detect oxidative activities in viable cells, including respiratory burst in phagocytes. Dihydroethidium itself has blue fluorescence (λ Ex/λ Em: 355/420nm) in cells, while the oxidized form ethidium has red fluorescence (λ Ex/λ Em: 518/605 nm) upon DNA intercalation.
Technical sheet

1. J Applied Technology (2010) DOI 10.1002/jat.1599
2. J Immunol Meth170, 117 (1994).
3. FEMS Microbiol Lett 122, 187 (1994).
4. FEMS Microbiol Lett 101, 173 (1992).
5. J Histochem Cytochem 34, 1109 (1986).

Dihydrorhodamine 123 **FP-83775A, 10mg**

White solid soluble in DMSO
CAS: 109244-58-8; MW: 346 (M)

Dihydrorhodamine 123 is the reduced form of rhodamine 123 (FP-47372A), which is a commonly used fluorescent mitochondrial dye. Dihydrorhodamine 123 itself is non-fluorescent, but it readily enters most of the cells and is oxidized by oxidative species or by cellular redox systems to the fluorescent rhodamine 123 that accumulates in mitochondrial membranes (1). Dihydrorhodamine 123 is useful for detecting reactive oxygen species including superoxide (in the presence of peroxidase or cytochrome c) (2,3) and peroxynitrite (4,5). Also see dihydrorhodamine 123 dihydrochloride (10056), a more stable and water soluble form of dihydrorhodamine 123. Technical sheet



1. Br J of Pharmacol (2010) doi: 10.1111/j.1476-5381.2010.01120.x
2. J Immunol Meth 178, 89 (1995).
3. Biochemistry 34, 3544 (1995).
4. Eur J Biochem. 217, 973 (1993).
5. Arc Biochem Biophys 302, 348 (1993).

Dihydrorhodamine 123, diHCl **FP-AM352A, 10mg**

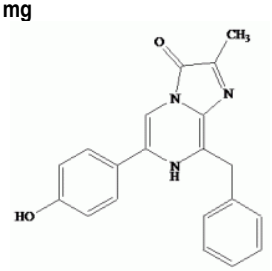
BTM.10056
CAS:-; MW: 419 (M) ; White solid soluble in DMSO

Dihydrorhodamine 123 dihydrochloride is functionally equivalent to dihydrorhodamine 123 (10055) but with increased stability toward air oxidation and light during storage. Technical sheet

Methyl coelenterazine **UPT88890, 50µg** **UPT88890, 1mg**
FluoProbes grade **FP-T8889A, 50µg**

Yellow solid soluble in MeOH or EtOH; CAS: -; MW: 331.37 (M)

Methyl coelenterazine (Coelenterazine, 2-methyl analog) has been reported to be a superior antioxidant for cells against reactive oxygen species (ROS) such as singlet oxygen and superoxide anion (1). The coelenterazine derivative is membrane-permeant, nontoxic and highly reactive toward ROS. As oxidative stress is believed to be a mediator of apoptosis (2), methyl coelenterazine should be another important tool for studies of apoptosis.
Coelenterazine products have been used to detect superoxide and peroxynitrite via chemiluminescence (3,4). Technical sheet



1. Biochem Pharmacol 60, 471 (2000).
2. Immunol Today 15, 7 (1994).
3. Anal Biochem 206, 273 (1992).
4. Circ Res 84, 1203 (1999).

Coelenterazine (native) **FP-97233B, 250 µg** **UP972332, 500µg** **UP972333, 1mg**
972334, 10mg **UP972335, 100mg**

CAS:55779-48-1; MW : 423.47 (M)

A chemiluminescent probe that emits upon oxidation by superperoxides. It is superior to luminol in this application (pKa). Also a Ca2+ indicator as this ion is needed for the reaction. Technical sheet

Coelenterazine-WS **BE8130, 1mg**

Coelenterazine-WS is a β-cyclodextrin complex of coelenterazine and its water solubility at neutral pH is drastically improved over native coelenterazine that has poor water solubility under physiological conditions, and is adsorbed to cell membranes

Other probes for superoxide detections

Superoxide radical ($O_2^{\bullet}$) are secreted by cells where they accumulates and exhibits decreased antioxidant enzyme activity. Hence, it causes directly or indirectly damages as enzymatic deficiencies. Their accumulation is involved in various biological processes including carcinogenesis, vascular disease and senescence.

Tetrazolium salts are chromogenic probes for superoxide detection based on the generation of water-insoluble blue formazan dye upon reaction with superoxide. They are however more widely used HRP based immunoassays (**NBT**, UP143456) and for detecting redox potential of cells for viability, proliferation and cytotoxicity assays (**MTT**, FP-69939A).

Compared with chromogenic probes (NBT, MTT), fluorescent probes offer high photon output/signal, allows multicolor detection, yet reduced photodamages may however occur depending on excitation wavelength and light intensity. Chemiluminescent assays (**Coelenterazin**), based on direct reaction with superoxide or mediated by a bioluminescence process, offer the combined advantages of high sensitivity thanks invariable low background, and excellent cell permeability. Coelenterazine (FP-97233, see related products) is a sensitive probe for the detection of superoxide and peroxynitrite, without interference from H2O2 or azide[1]. Coelenterazine is the preferred probe for luminescent detection of superoxide in experiments where quantitative determination of superoxide production is required[2].
However they have lower photon output, and imaging is more difficult (limited possibility of multidetections).

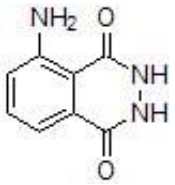
Lucigenin-amplified chemiluminescence (LuCL Igor 2001) has frequently been used to assess the formation of superoxide. However, 1/lucigenin may undergo redox cycling in purified enzyme-substrate mixtures, 2/ Lucigenic was reported to enhances superoxide formation Igor 2001 3/it was revealed that lucigenin stimulated oxidant formation. Lucigenin should therefore be avoided in quantitative applications, or used only alongside careful controls.

Although **luminol** (FP-04247A) is not useful for detecting superoxide in live cells, it is commonly employed to detect peroxidase- or metal ion–mediated oxidative events. Used alone, luminol can detect oxidative events in cells rich in peroxidases, including granulocytes ref and spermatozoa.ref This probe has also been used in conjunction with horseradish peroxidase (HRP) to investigate reoxygenation injury in rat hepatocytes.ref In these experiments, it is thought that the primary species being detected is hydrogen peroxide. In addition, luminol has been employed to detect peroxynitrite generated from the reaction of nitric oxide and superoxide.ref

In contrast, the chemiluminescent probe **MCLA**, chemically very similar to coelenterazine, has no significant effect on hydrogen peroxide release. MCLA reversibly reacts with superoxide, forming an adduct whose irreversible decay generates light (~465 nm). The apparent rate constant of this reaction is ~10^5 M^{-1} s^{-1}. Hence the MCLA chemiluminescence is a sensitive marker for detecting superoxide. It is used notably in research of leucocyte function.

● luminescent probes for oxidation studies:

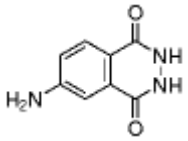
Luminol **FP-04247A, 1g** **FP-04247C, 10g**



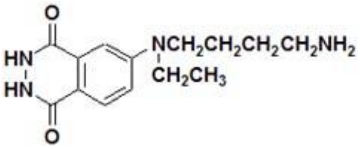
5-Amino-2,3-dihydro-1,4-phthalazinedione ; 3-Amino-phthalhydrazide ; 1,4-phthalazinedione, 5-amino-2,3-dihydro
CAS: [521-31-3] ; MW : 177.16
UV 254 nm) (in 0.1 N NaOH) λmax 1 : 347 nm & λmax 2 : 300 nm; EC(at λmax 1): 7650 L/mol x cm
λabs./λem.(MetOH): 355/413nm
Solubility: poorly in water; 2 % in 1N NaOH; 50 mg in 2-propanol/ammonia/water 7:1:2
Appearance: pale yellow to tan to greenish powder
Commonly employed to detect peroxidase- or metal ion–mediated oxidative events, but not in living cells (see MCLA for superior performance).

Also available
Luminol, FluoPure grade **FP-57578A, 1g**
Highest purity grade; greatest relative intensity at 425 nm, with optimal pH 9-10.3
Luminol, hemihydrate FP-BG077
3-Amino-phthalhydrazide Na salt; CAS: [206658-90-4] – MW: 217.16
Luminol, Na salt FP-CA9611
3-Amino-phthalhydrazide Na salt; CAS: [20666-12-0] – MW: 199.15
Luminol, HCl FP-BG076
3-Amino-phthalhydrazide HydroChloride; CAS: [74165-64-3] – MW: 213.62
Isoluminol, monohydrate FP-07624A, 1g
4-Aminophthalhydrazide monohydrate; – MW: 195.18
Isoluminol FP-E9095
4-Aminophthalhydrazide; CAS: [3682-14-1]– MW: 117.16 (Xi)
Isoluminol ABEI FP-60404A, 5mg
4-Aminophthalhydrazide monohydrate; CAS: [66612-29-1] – MW: 276.34

isoluminol FP-DT3701



isoluminol ABEI FP-07624A



● chromogenic probes for oxidation studies:

NBT **143457, 1g**
Technical sheet
MTT **FP-65939A, 1g**
Thiazoyl Blue tetrazolium bromide. CAS: 298-93-1; MW: 414,32 (L)
λabs = 550 nm. See description in cell viability probes sections, or in the Technical sheet
XTT **FP-40936A 100 mg**
(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, disodium salt); CAS: 111072-31-2; MW : 675.53
See description in cell viability probes sections, or in the Technical sheet
WST1 (Water Soluble Tetrazolium) **F98880, 1g**
2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium; CAS: 150859-42-8; Technical sheet (with other WSTs)
λexc.(WST): 651.35 >21 600(244nm); EC(formazan): >37 000(438nm)

● other:
CelRox ROS probes Inquire

ROS generators
(descruiption a rechercher: CAS, MW (L)… sur ?560
t-BHP: tert-butylhydroperoxide
Generates ROS radicals
PMS (Phorbol Myristate Acetate)
Generates O2- ˙ radicals
NOC7 (1-Hydroxy-2-oxo-3-(N-methyl-3-aminopropyl)-3-methyl-1-triazine)
Generates NO radicals
SIN-1 (3-(Morpholinyl)sydnonimine, hydrochloride)
Generates ONOO- radicals. See description in 'NO donnors' section.

ROS inducers
Inquire. In progress

Pyocyanin (ROS/SO inducer)
TBHP (ROS inducer)
AMA (superoxide inducer)

■ Peroxidation (of protein, lipids, nucleic acids) study

Peroxidation caused by ROS species that are produced by oxidative stress alter proteins, lipids, and nucleic acids. Below are useful tools to study these effects See also 'Oxidative Damages' section for protecting systems (SOD, GSH,…).

Overview

Oxidative Stress Marker Detective	Products Names	
Lipid peroxide	MitoPeDPP, Liperfluo, Spy-LHP, DPPPP, TMRE	
Lipid peroxide	Liperfluo , DPPP , Spy-LHP	
Lipophilic Peroxides probes	MitoPeDPP	

Featured products

See below: DPPP, MitoPeDPP, Spy-LHP and LiperFluo

Peroxynitrite Donor (Release)

See the section 'NO Donnors (release)' for SIN-1 #077332 (used to estimate the effectiveness of NO and peroxynitrite with other NO donors)

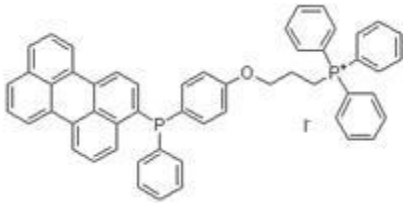
Lipophilic Peroxides probes (for mitochondria)

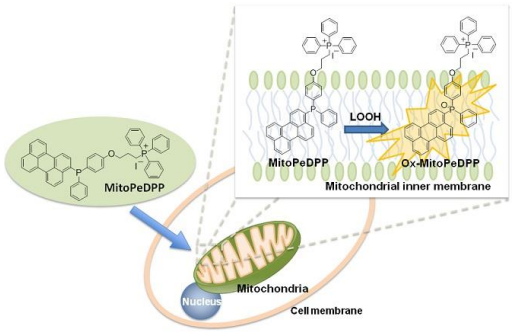
Probes for lipophilic include classic DPPP, and the remakably specific probes MitoPeDD, Spy-LHP and LiperFluo.
Applications: Mitochondria oxidation studies.

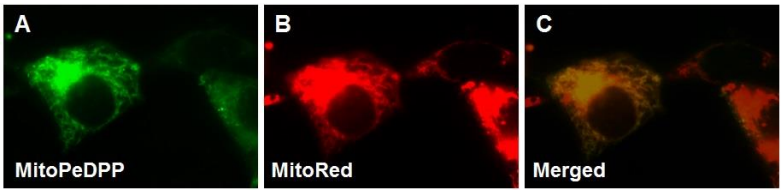
●MitoPeDPP probe for mitochondria peroxidation

MitoDeDPP (Lipophilic Peroxides probe in Mitochondria) **1I8940, 3x5µg**

3-[4-(Perylenylphenylphosphino)phenoxy]propyltriphenylphosphonium iodide
CAS:[] [(L)], MW: 882.74,
abs/em.:452 nm and 470 nm
MitoPeDPP is a cell-membrane-permeable probe, Perylene-based dye. It specifically localizes in mitochondria due to the triphenylphosphonium moiety introduced.



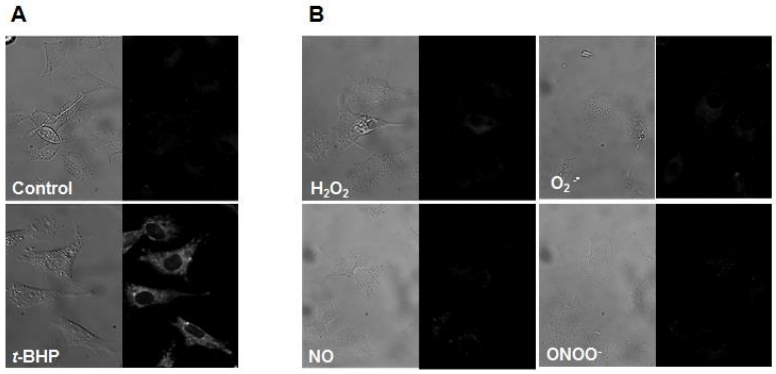




A: MitoPeDPP stained Mitochondria with *t*-BHP treatment
B: MitoRed stained Mitochondria
C: Merged Image (A/B)

MitoPeDPP reacts in homogeneous systems with various peroxides (H_2O_2, *t*-BHP, $ONOO^-$), but it is specifically-oxidized by *t*-BHP in mitochondria (A) but not with ROS and RNS (B).

As the excitation and emission wavelength of MitoPeDPP are 452 nm and 470 nm, respectively, the probe can be applied for lipophilic peroxide imaging in living cells.



A: MitoPeDPP stained cells with t-BHP treatment (t-BHP) and without (control).
B: MitoPeDPP stained cells with ROS or RNS exposure.
ROS generators used in the experiment were PMA (O2- ・), NOC7(NO), and SIN-1(ONOO-)

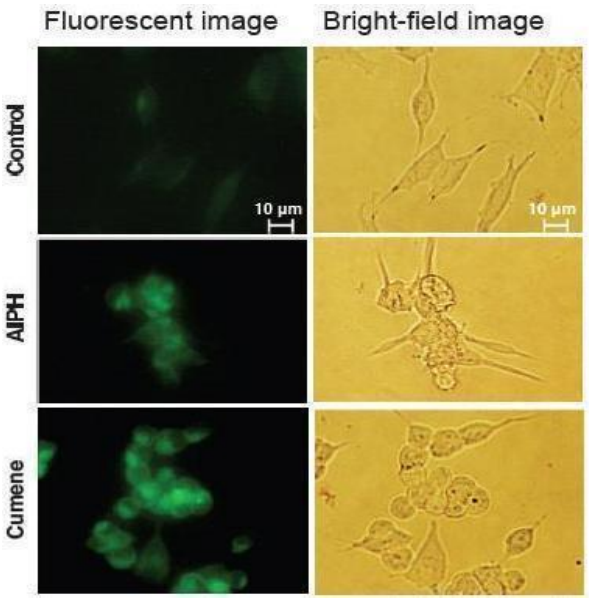
*This probe has been developed by Dr. Shioji et al. at Fukuoka University, Department of Chemistry

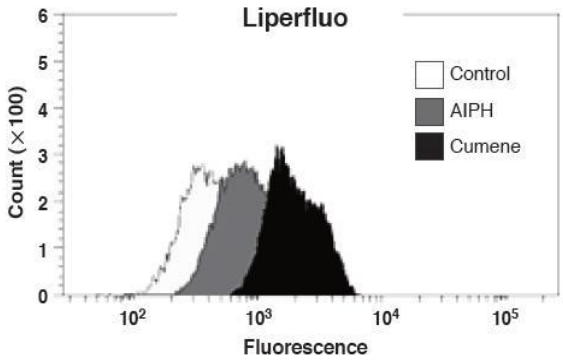
●Other Mitochondria peroxidation probes

LiperFluo (Lipid Peroxides ***selective***) **PIX950, 5x50µg**
N-(4-Diphenylphosphinophenyl)-N'-(3,6,9,12-tetraoxatridecyl)perylene-3,4,9,10-tetracarboxydiimide; MW: 840.85 (L)
λexc./em.(oxidized): 524 nm / 535 nm

Liperfluo, a perylene derivative containing oligooxyethylene, is designed for specific detection of lipid peroxides. It emits intense fluorescence upon oxidation by a lipid peroxide in organic solvents such as ethanol. Among fluorescent probes that detect Reactive Oxigen Species(ROS), Liperfluo is the only compound that can specifically detect lipid peroxides. Since the excitation and emission wavelengths of the oxidized Liperfluo are 524 nm and 535 nm, respectively, both a photo-damage against a sample and an auto-fluorescence from the sample can be minimized. The tetraethyleneglycol group linked to one end of diisoquinoline ring helps its solubility and dispersibility to aqueous buffer. Though Liperfluo oxidized form is almost nonfluorescent in an aqueous media, it emits fluorescence in lipophilic sites such as in cell membranes. Therefore it can easily be applied to lipid peroxide imaging by a fluorescence microscopy and a flow cytometric analysis for living cells.

References K. Yamanaka and N. Noguchi et al., "A novel fluorescent probe with high sensitivity and selective detection of lipid hydroperoxides in cells", RSC Advances, 2012, 2, 7894.
N. Soh, et al., Swallow-tailed perylene derivative: a new tool for fluorescent imaging of lipid hydroperoxides. Org Biomol Chem. 2007;5:3762-3768.





Data was kindly provided from Dr. N. Noguchi, Doshisha University, System Life Science Laboratory.

Live cell imaging of lipid peroxide

Procedure
1. Innocurate SH-SY5Y cells(6.0 x 105 cells/well) to a 6-well plate.
2. Incubate the plate at 37 ºC for overnight.
3. Add Liperfluo, DMSO solution(final conc. 20 µM) and incubate at 37 ºC for 15 min.
4. Add either Cumene Hydroperoxide(final conc. 100 µM) or AIPH*(final conc. 6 mM).
5. Incubate at 37 ºC for 2 hours.
6. Observe fluorescent by microscope**.

* AIPH: 2,2'-azobis-[2-(2-imidazolin-2-yl)propane]dihydrochloride
** Olympus IX-71 epifluorescent microscope, mirror unit: U-MNIBA3, exposure time: 10 sec, ISO: 800

Data was kindly provided from Dr. N. Noguchi, Doshisha University, System Life Science Laboratory.
K. Yamanaka and N. Noguchi et al., "A novel fluorescent probe with high sensitivity and selective detection of lipid hydroperoxides in cells", RSC Advances, 2012, 2, 7894.

Flow cytometric analysis of lipid hydroperoxides in live cell

Procedure
1. Innocurate SH-SY5Y cells(6.0 x 105 cells/well) to a 6-well plate.
2. Incubate the plate at 37 ºC for overnight.
3. Add Liperfluo, DMSO solution(final conc. 20 µM) and incubate at 37 ºC for 15 min.
4. Add either Cumene Hydroperoxide(final conc. 100 µM) or AIPH*(final conc. 6 mM).
5. Incubate at 37 ºC for 2 hours.
6. Wash cells with PBS.
7. Collect cells with PBA and analyse by flow cytometer**.

* AIPH: 2,2'-azobis-[2-(2-imidazolin-2-yl)propane]dihydrochloride** BD FACSAriaTM I

K. Yamanaka and N. Noguchi et al., "A novel fluorescent probe with high sensitivity and selective detection of lipid hydroperoxides in cells", RSC Advances, 2012, 2, 7894.

Properties of Dye

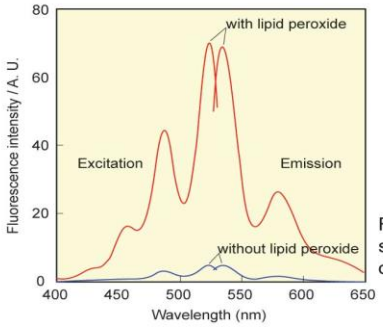


Fig 1. Excitation and emission spectra of Liperfluo with or without lipid peroxide in ethanol.

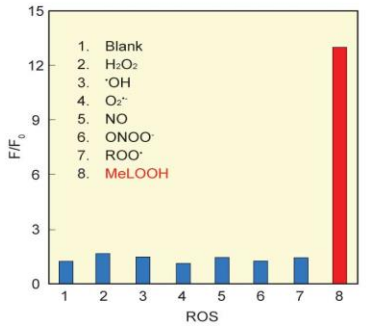


Fig 2. Reaction selectivity of Liperfluo against the various reactive oxygen species.

DPPP (standard Lipophilic Peroxides probe) **69157A, 10µg**
Diphenyl-1-pyrenylphosphine; CAS: 110954-36-4; MW: 386.42 (Z)
λexc./em.(oxidized): 352 nm / 380 nm

DPPP is a non-fluorescent triphenylphosphine compound. It reacts with hydroperoxide to generate DPPP Oxide that emits fluorescence at 352 nm excitation and 380 nm emission wavelengths. Post-column HPLC method is used to determine phospholipid peroxide in sample solutions.
Technical sheet

Spy-LHP, Lipid Peroxides Detection Reagent **CEW910, 1mg**
2-(4-Diphenylphosphanyl-phenyl)-9-(1-hexyl-heptyl)-anthra[2,1,9-def,6,5,10-d'e'f']diisoquinoline-1,3,8,10-tetraone
MW: 832.96 (Z)
λexc./em.(oxidized): 535 nm / 524 nm
Spy-LHP is a low-fluorescent compound, becoming a high fluorescent when oxidized with lipid hydroperoxide. Unlike the similar product, DPPP, which the UV excitation significantly damages live cells, the oxidized Spy-LHP emits strong fluorescence (quantum yield: ~1) with maximum wavelength at 535 nm when excited at 524 nm, so damage to live cells is very small. Spy-LHP has two alkyl chains to improve the affinity to the lipid bilayer. Spy-LHP is highly selective to lipid hydroperoxide and does not react with hydrogen peroxide, hydroxy radicals, superoxide anion, nitric oxides, peroxynitrite, and alkylperoxy radicals.
References
1. N. Soh, et al., Novel fluorescent probe for detecting hydroperoxides with strong emission in the visible range. Bioorg Med Chem Lett. 2006;16:2943-2946.
2. N. Soh, et al., Swallow-tailed perylene derivative: a new tool for fluorescent imaging of lipid hydroperoxides. Org Biomol Chem. 2007;5:3762-3768.

Related products:
See also 'Mitochondria probes / staining' section[BA030a].
See also Lipophilic probes in the CellBiology Assay catalog.

Lipid peroxidation assay kits

Inquire. In progress
#
LIPID PEROXIDASE ASSAY, LIPERFLUO Selective Measurement PIX950-L248-10, 5 x 50 ug

See the lipid catalog.

■ NOs (Nitrite, Nitrate) study

Nitrite (NO_2^-) and Nitrate (NO_3^{2-}) are the main Nitric Oxide species involved in oxidative metabolism of cells.

Detection and Bioimaging of Nitric Oxide (NO) Using Multicolor DAX – J2™ Reagents

DAF-2 reagents are frequently used to detect nitric oxide (NO). However, DAF-2 diacetate is spontaneously hydrolyzed in cell culture media. The hydrolyzed DAF-2 is not cell-permeable, thus causing high assay background.
New DAX-J2™ probes are developed as excellent replacements for DAF-2 for the detection and bioimaging of NO. They have longer wavelengths and better stability. Three distinct DAX-J2™ allow multicolor imaging reagents for NO detection.

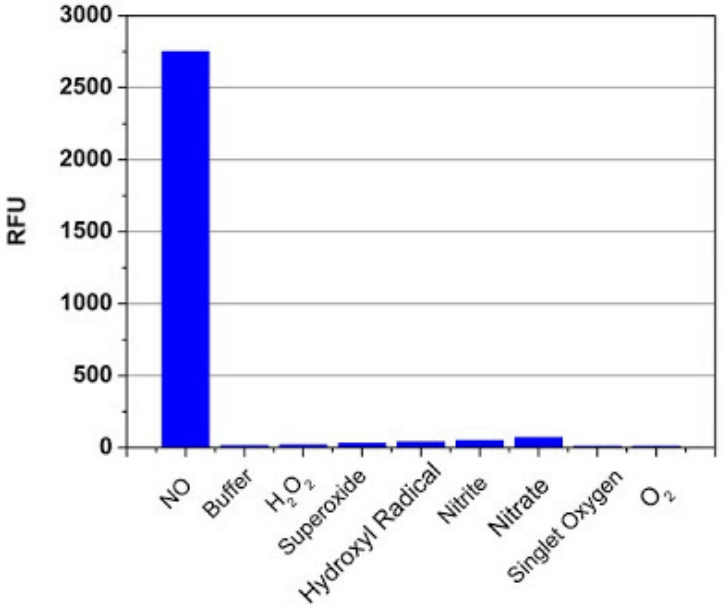
DAX-J2™ Red is a new nitric oxide (NO) sensor that can measure free NO and nitric oxide synthase (NOS) activity in living cells under physiological conditions: unlike DAF-2, it is non-fluorescent cell and permeable reagent, . Once inside the cell, the blocking groups on the DAX-J2™ reagent are released to generate a highly red fluorescent product upon NO oxidation. The DAX-J2™Red fluorescent product can be detected using the filter set of Texas Red® that is equipped with most of flow cytometers and fluorescence microscopes.

DAX-J2™ Orange , similarly to DAX2 Red, can be readily loaded into live cells, and generates a bright orange fluorescent product that has spectra properties similar to those of Cy3® and TRITC. So its fluorescence signal can be conveniently monitored using the filter set of Cy3® and TRITC.

DAX-J2™ IR is a new fluorogenic NO sensor that is highly water-soluble and has near infrared fluorescence. This DAX-J2™ IR reagent enables NO detection *in vivo* using IVIS® Imaging System(Caliper) or Kodak Image Station.

DAX-J2™ IR 16302
Ex (nm): 780nm , Em (nm): 800nm MW: 1016.05 Solvent: DMSO. (M)
DAX-J2™ Red 16301
Ex (nm): 588nm , Em (nm): 610nm MW: 608.77 Solvent: DMSO. (M)
DAX-J2™ Orange 16300
Ex (nm): 545nm , Em (nm): 576nm MW: 476.57 Solvent: DMSO. (M)
Technical sheet

Fig: Fluorescence response of DAX-J2™ Orange (5 µM) to different reactive oxygen species (1 mM) in PBS buffer (pH 7.2). The fluorescene intensities were measured with Ex/Em = 540/570 nm.



NO Oxidative Stress research

NO Detective and method type	Product Name	P/N
By ESR (& Spin Trapping)	**Carboxy-PTIO** CAS:18390-00-6; MW:233.3	199500-
	PTIO CAS:18390-00-6; MW: 299.28	C73371-14982
"	**DTCS Na**	D465-10
"	**MGD**	M323-12
"	**DMPO** CAS:3317-61-1 ; MW:113.2	U24692-D048-10
By Griess Reaction	**2,3-Diaminonaphthalene (for NO detection)**	D418-10
"		

See featured products description in 'Spin-trapping reagents for Oxidative research' (PTIO, DMPO)
See also the section 'Spin Labelling' in chapter 'Biochemistry'.
#

NO Release in Oxidative Stress research

NO Release	Product Name	Product Code
	NOC 7	N377-10
	NOC 12	N378-10
	NOC 18	N379-10
	NOC 5	N380-10
	NOR 1	N388-10
	NOR 3	N390-10
	NOR 4	N391-10

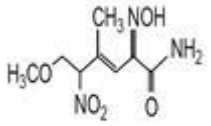
Product Code
N377-12
N378-12
N379-12
N380-12
N388-10
N390-10
N391-10

S-Nitrosoglutathione	Product Name	Product Code	
	S-Nitrosoglutathione	N415-10	N415-12
	"	N415-12	
	NOR 5	N448-10	N448-10
	SIN-1	S264-10	S264-10
Colorimetric Inhibition Activity measurment	Product Name	Product Code	
	ACE Kit - WST	A502-10	A502-10

NO Donnors (Release)

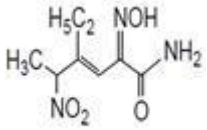
● **NORs** are ideal NO donors with completely different chemical structures from the other NO donors. Although NORs do not have any ONO2 or ONO moiety, they spontaneously release NO at a steady rate. Even though the NO release mechanism of NOR has not been completely determined, it is confirmed that the byproducts do not possess any significant bioactivities. NOR 3, isolated from Streptomyces genseosporeus, is reported to have strong vasodilatory effects on rat and rabbit aortas and dog coronary arteries. Its activity (ED50=1 nM) is 300 times that of isosorbide dinitrate (ISDN). NOR 3 also increases the plasma cyclic GMP levels, whereas ISDN does not. NOR is a potent inhibitor of platelet aggregation and thrombus formation. NOR 3 (IC50=0-7 mM) effectively inhibits 100% of ADP-initiated human platelet Aggregation, whereas ISDN inhibits only 32% of the total aggregation, even at 100 mM concentrations. NOR 3 has also been reported to have antianginal and cardioprotective effects in the ischemia/reperfusion system. In the rat methacholin-induced coronary vasospasm model, NOR 3 suppressed the elevation of the ST segment dose-dependently and significantly at 1 mg per kg. On the other hand, ISDN suppressed it significantly at 3.2 mg per kg. The difference in the NO release rate of NOR reagents was reflected even on the in vivo hypotensive effects. NOR may also be used orally in a 0.5% methylcellulose suspension. NOR is relatively stable in DMSO solution. NOR 1, which has the shortest half-life, is a promising reagent for making NO standard solutions for calibration. For the preparation of the standard solution, a precisely diluted NOR 1/DMSO solution is added to the buffer solutions.

NOR 1
(+)-(E)-4-Methyl-2-[(E)-hydroxyimino]-5-nitro-6-methoxy-3-hexenamide
CAS: 163032-70-0; MW: 231.21, (M)
White or slightly yellow powder



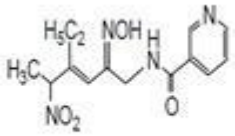
NOR 1

NOR 3
(+)-(E)-4-Ethyl-2-[(E)-hydroxyimino]-5-nitro-3-hexenamide
CAS: 163180-49-2; MW: 215.21, (M)
White crystalline powder



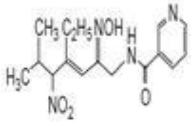
NOR 3

NOR 4
(+)-N-{(E)-4-Ethyl-2-[(Z)-hydroxyimino]-5-nitro-3-hexene-1-yl}-3-pyridinecarboxamide
CAS: 162626-99-5; MW: 306.32, (M)
White or slightly yellow powder



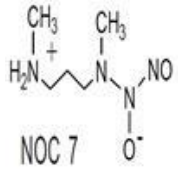
NOR 4

NOC 5
(+)-N-{(E)-4-Ethyl-3-[(Z)-hydroxyimino]-6-methyl-5-nitro-3-heptenyl}-3-pyridinecarboxamide
MW: 334.37, (M)
White or slightly yellow powder



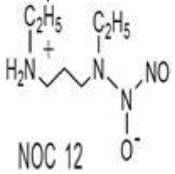
NOR 5

NOC 7
1-Hydroxy-2-oxo-3-(N-methyl-3-aminopropyl)-3-methyl-1-triazene
CAS: 146724-84-7; MW: 162.19, (J)
white powder



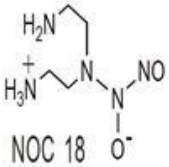
NOC 7

NOC 12
1-Hydroxy-2-oxo-3-(N-ethyl-2-aminoethyl)-3-ethyl-1-triazene
CAS: 146724-89-2; MW: 176.22, (J)
White powder



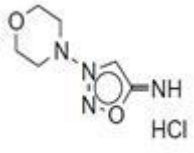
NOC 12

Nor 18
1-Hydroxy-2-oxo-3,3-bis(2-aminoethyl)-1-triazene
CAS: 146724-94-9; MW: 163.18, (J)
White powder



NOC 18

SIN-1 **077332, 10mg**
3-(4-Morpholinyl)sydnonimine, hydrochloride
CAS: 16142-27-1 ; MW: 206.63, C6H11ClN4O2
White or slightly yellowish needles or crystalline powder



SIN-1

● **SIN-1**, a metabolite of the vasodilator molsidomine, is utilized to separately estimate the effectiveness of NO and peroxynitrite with other NO donors. SIN-1 spontaneously decomposes in the presence of molecular oxygen to generate NO and superoxide. Both products bind very rapidly to form peroxynitrite (rate constant k: 3.7×10^{-7} $M^{-1}s^{-1}$). Therefore, SIN-1 is a useful compound that generates peroxynitrite in an efficient manner. Peroxynitrite is a very strong oxidant that generates hydroxyl and nitrosyldioxyl radicals under physiological conditions. Peroxynitrite also decomposes to generate nitrate ion quickly in acidic conditions and slowly in basic conditions. Those species have a different bioactivity from NO.

Biochemicals for DNA damge studies

8-Nitroguanine # 89295 CAS#168701-80-2 A mutagenic nitrative guanine derivative

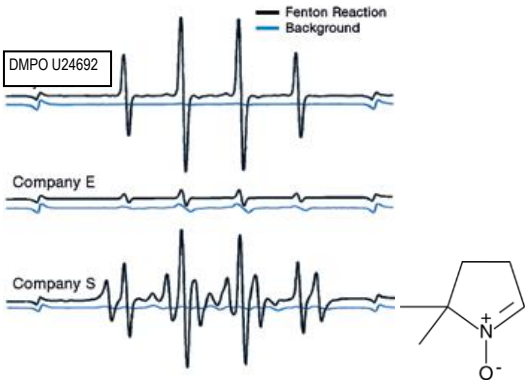
■ Spin-trapping reagents for Oxidative research: DMPO, BMPO,…

Free radicals are highly reactive, short-lived species. Spin traps react with radicals, forming stable adducts that can be further studied.

DMPO and BMPO spin trapping agents (for ESR)

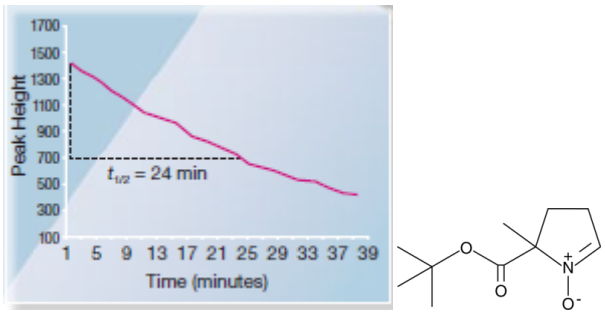
● **DMPO** is the most frequently used spin-trapping reagent for the study of free radicals. It reacts with O-, N-, S-, and C-centered radicals. It is suitable for trapping oxygen radicals and for producing adducts that can be characterized when used in association with electron spin resonance (ESR) patterns and immuno-spin trapping. DMPO is water-soluble, rapidly penetrates lipid bilayers, has low toxicity, and can be used in vitro and in vivo.
Our DMPO is of highest quality, well controlled, and doesn't require any prepurification process unlike the other suppliers' DMPO.
Applications: Spin-trapping studies, EPR (ESR) detection of super oxide, O-, C-, S-, and N-centered free radicals.

Hydroxyl radicals trapping



DMPO **U24692-D048-10, 1ml**
5,5-Dimethyl-1-Pyrroline-N-Oxide; CAS:3317-61-1 ; MW:113.2; λmax:235 nm
Spin Trap Reagent

● **BMPO** is a spin-trapping reagent that can be used for the detection and characterization of thiyl radicals, hydroxyl radicals, and superoxide anions radicals (O2-) in vitro or in vivo. The BMPO-superoxide adduct shows a much longer half-life (t½= 24 min) than other spin-trapping reagents, hence does not rapidly decompose to the hydroxyl adduct in cells. Also, the ESR spectrum of the BMPO-glutathionyl adduct does not fully overlap with the spectrum of its hydroxyl adduct. Purified by crystallization and stored as a solid, BMPO has a longer shelf life than liquid spin traps, hence rovides reproducible and steady results.
Applications: Spin-trapping studies, EPR (ESR) detection of Superoxide anion and Hydroxyl Radicals. Ideal for O2- trapping.

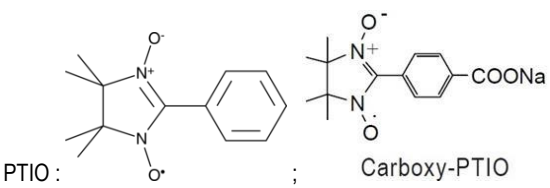


BMPO **PIY020-, 50mg**
BocMPO; CAS:387334-31-8 ; MW:199.25 (M); λmax:239 nm

Ask for the technical notice about DMPO and BMPO applications for spin-trapping of free radicals

PTIO (for NO studies)

PTIO is A spin trap for nitric oxide that forms nitric dioxide and corresponding imino nitroxides. It is useful for examining nitric oxide synthase inhibitory activity.
Carboxy-PTIO is a stable, water-soluble spin-trapl that reacts with NO to form NO2·that can be monitored by electron spin resonance (ESR)
Unlike NO scavengers such as hemoglobin that trap, beside NO, NOS inhibitors such as arginine derivatives and that quench all other NO-related metabolites at the same time, the Carboxy-PTIO does not dramatically affect other NO-related product systems because it transforms NO to NO2, which is a metabolite of NO



PTIO **C73371-14982**
α-Phenyltetramethylnitronyl nitroxide ; CAS:18390-00-6; MW:233.3 (M); λmax:237, 264, 348, 360, 597 nm
Carboxy-PTIO **199500, 10mg** **199501, 50mg**
2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide, sodium salt; CAS: 148819-93-6; MW: 299.28 (M)

Also available:

1110009660 CYPMPO
CAS:934182-09-9; MW:247.2 (M)
A novel spin trap for hydroxyl and superoxide radical detection. Superoxide adducts half-live of 15-51 minutes

110006435 DEPMPO
5-(Diethoxyphosphoryl)-5-methyl-1-pyrroline-N-oxide; CAS:157230-67-6; MW:235.2 (N)
A phosphorylated derivative of DMPO spin trap, reported to produce spin adducts with increased stability particularly for the adduct of superoxide

113251 DEPMPO-biotin (936224-52-1)
CAS: 936224-52-1; MW:591.7 (N)
A biotinylated form of DEPMPO which retains the outstanding persistency of its adducts. Useful for S-nitrosothiols (SNO) monitoring biodistribution in cells, tissues, and organs.

110006170 DMPO Nitrone Adduct Polyclonal Antiserum
-

115412 N-tert-butyl-α-Phenylnitrone
CAS: 3376-24-7; MW:177.24
A spin trap

114877 TEMPONE (4-Oxo-2,2,6,6-tetramethylpiperidinooxy)
CAS:2896-70-0; MW:170.23
A 4-oxo derivative of the spin trap TEMPO

See also section "Spin Labeling' in chapter Biochemistry (**proxyl-MTS** #RV9770, **MTSSL** #16463 and **OTMPC-NHS** #16148).

■ SOD Assays (SuperOxide Dismutase)

#

SOD Assay Kit (WST-based)

Superior to NBT assay thanks to highly water-soluble formazan: accurate IC50 mesaurement

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 to use. However, there are several disadvantages to the NBT method such as poor water solubility of the formazan dye and the interaction with the reduced form of xanthine oxidase.
SOD Assay Kit-WST allows very convenient SOD assay by utilizing WST-1 that produces a water-soluble formazan dye upon reduction with superoxide anion. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity, and is inhibited by SOD. Therefore, IC50 (50% inhibition activity of SOD or SOD-like materials) can be determined by a colorimetric method using this kit.

SOD Assay Kit - WST based **S07410-S311-08, 100tests** **S07410-S311-10, 500tests**
TechSheet

Other kits on inquire:

#

SOD Assay Kit - Formazan based (440-460nm) **S53102-706002, 96wells – 480wells**
TechSheet/S

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SOD Assay Kit - NBT based **S53100-7500-100-K**
TechSheet

SOD Assay Kit - WST-1 based **DU1650-7502-100-K**
TechSheet

SOD Assay Kit - WST-1 based, for HTS **JZ4130-7501-500-K**
TechSheet

#

SOD Assay Kit - AMPLITE Blue (550-560nm) **JO4990-**
TechSheet

#

QINI870-KA3782 QIN871-ORB219866 Q53105-CSOD100-2 CH027638 KSD002

See also primary antibodies against SOD (Dismutase)

■ Catalase assays

List of Catalase assays and related products (Inquire for detailled information):
Inquire. In progress

■ Sulfenylation

See more information about **Sulfenylation**/[BE178b] (oxidative damage due to Reactive oxygen species (ROS) react with proteins):

Sulfenylated Protein Cell-Based Detection Kit1B0580
to detect irreversible cysteine oxidation, a hallmark of stress-induced cellular damage

■ Glutathione Assay Kits

List of Glutathione assays and related products (Inquire for detailled information):

#
Total Glutathione Quantification Kit .T419-10 , 100tests
GSSG/GSH Quantification Kit .G257-10 , 200tests
#
Glutathion Reductase Kit
HT Glutathione Assay Kit
HT Glutathione Peroxidase Kit
HT Glutathione Reductase Assay Kit

■ Inhibitors for Oxidative metabolism

Inquire. In progress

■ Misceanous for Oxidative metabolism studies

Inquire. In progress

Applications of oxidative metabolism

The oxidative metabolism of cell is involved in many physiological processes, from cell level and its sub-structures (DNA damage, Krebs cycle in mitochrondria) to organs/tissues and organism level (ageing, Solar irradiation injury/protection, skin burning,..).

■ DNA damage&Repair

DNA Damage Assays

◦DNA Damage Quantification - AP sites Counting

- Determine the number of abasic sites in genomic DNA samples
- Colorimetric microplate assay

Detection range: 1-40 abasic sites per 1x10^5 base pairs DNA

Oxidative damage to DNA is a result of its interaction with reactive oxygen species (ROS), in particular, the hydroxy radical. Hydroxy radicals, which are produced from superoxide anion and hydrogen peroxide by the Fenton reaction, produce multiple modifications in DNA. Oxidative attacks by hydroxy radicals on the deoxyribose moiety will lead to the release of free bases from DNA, generating strand breaks with various sugar modifications and simple abasic sites (AP sites). In fact, AP sites are one of the major types of damage generated by ROS. Aldehyde Reactive Probe (ARP; N'-aminooxymethylcarbonylhydrazin-D-biotin) reacts specifically with an aldehyde group present on the open ring form of the AP sites. This reaction makes it possible to detect DNA modifications that result in the formation of an aldehyde group. After treatment with excess ARP reagent, all of the AP sites on DNA are tagged with a biotin residue. These biotin-tagged AP sites can be quantified using the avidin-biotin assay, followed by colorimetric detection with either peroxidase or alkaline phosphatase conjugated to the avidin.

Assay Procedure

Step 1	Step 2	Step 3	Step 4
Add ARP solution to a sample DNA solution. Incubate at 37°C for 1 hour.	Transfer the ARP reaction mixture to a Filtration tube and spin the tube to purify ARP-labeled DNA.	Discard the solutions and wash wells with Washing buffer. Tap the plate on a paper towel several times to remove as much of buffer as possible.	Add the ARP-DNA standard solution or ARP-labeled sample DNA solution to each well. Add DNA binding solution, and leave the plate at room temperature
Step 5	Step 6	Step 7	Step 8
Add HRP-streptavidin solution to each well and incubate at 37°C for 1 hour.	Discard the solutions and wash well with Washing buffer. Tap the plate on a paper towel to remove as much of buffer as possible.	Add Substrate solution to each well and incubate at 37°C for 1 hour.	Read the O.D. at 650 nm.

DNA Damage Quantification Kit, AP site Counting Q95062-1Kit
Contains ARP solution ARP-DNA standard, filtration tube, washing buffern 96-Well microplate, DA binding solution, TE buffer and HRP-streptavidin
ARP probes (for DNA Damage study) R0756A-10mg , 25mg

Other DNA Damage assays

Inquire:

DNA/RNA Oxidative Damage (Clone 7E6.9) ELISA Kit #01130

Glyco-SPOT DNA Repair Assay Kit # 501280
A multiplexed oligonucleotide cleavage assay for screening the activity of base excision repair enzymes

HT 8-oxo-dG ELISA Kit I & II

Inquire. In progress

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BPDE Competitive ELISA kit

Inquire. In progress

Biochemicals for DNA dammage studies

Aldehyde Reactive Probe (trifluoroacetate salt) #10009350
Oxidative Stress LC-MS Mixture #18701: A mixture of lipids and nucleic acids produced during oxidative stress
8-Hydroxyguanosine # 89300 CAS#3868-31-3 Product of oxidative damage to RNA
8-Hydroxy-2'-deoxyguanosine # 89320 CAS#88847-89-6 8-OH-dG Product of oxidative damage to DNA
Hydroxymethyl Uracil Item #89360 CAS#4433-40-3 Product of oxidative damage to DNA
8-Hydroxyguanine (hydrochloride) # 89290 CAS#1246818-54-1 NSC 22720 8-Oxoguanine Product of oxidative damage to DNA or RNA
+

■ Glycolysis

Inquire. In progress

■ Krebs cycle / Oxidative phosphorylation

Mitochondria OXPHOS/PDH assays

Inquire. In progress

■ Ageing studies (AGE, NAD, NR,…)

Inquire. In progress

•**AGE** (Advanced Glycation End products) are biomarkers of ageing process found on different biomoecuels uch as BSA and DNA.
> List of AGE products and related ones (antibodies,…)

•**NAD+**, beside its important role as a redox carrier and energy path intermediate (NR>NAD+>ATP), has emerged as a key regulator of metabolism (notably cardiometabolism), stress resistance (neuro and muscular degeneration, cellular repair and resiliency) and longevity (i.e. its level declines with age). To theses purposes, it is a cofactor to key enzymes such asPARP and Sirtuins.
>List of NAD+ product and related ones (derivatives, assay kits)
+

•**SIRT** proteins are deacetylase enzymes (6 in humain), a distinct clas of trichostatins.
>List of SIRT proteins and antibodies +

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3-Deoxyglucosone .D535-08, 1mg
3-Deoxyglucosone Detection Reagents .D536-10, 1mg