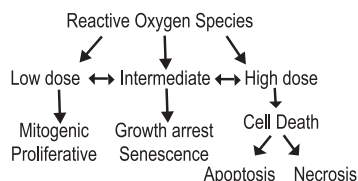
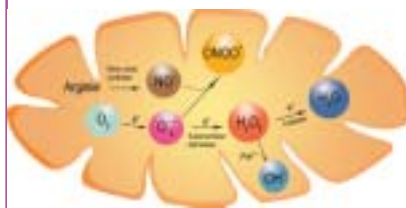


Cell Biology - Study/Probes

Reactive Oxygen Study

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 cause 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, Parkinson's disease, Alzheimers and other vascular-damage-related brain diseases, Cancer, Arteriosclerosis 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, favouring their use for diagnostic analysis. Also, peroxidase is a common enzyme for signal amplification in immunoassays (EIA).

See also fluorescent and chromogenic probes for NO (page E107).
See also immunocapture of OXPHOS complexes for subsequent analysis (WB, MS) (page E135).

Interchim provides several ROS probes for fluorimetry and colorimetry, chromogenic, fluorogenic or luminogenic, as well as for the tissue sections staining and immunoblots.

Selection guide

Fluorescent ROS probes

Cat.#	Probes	Reactive Oxygen Species (ROS)					
		Hydrogen Peroxide H_2O_2	Hydroxy radical $HO\cdot$	Hypochlorous acid $HOCl$	Peroxyl radical $COO\cdot$	Peroxynitrite anion $ONOO\cdot$	Superoxide anion $O_2\cdot$
FP-97895	CM-H2DCFDA	+	+	+	+		
FP-83775	Dihydrorhodamine 123	+		+		+	
FP-46731	H2DCFDA	+			+	+	
FP-46915	Lucigenin*	+					
FP-97233	Coelenterazine					+	+
FP-38544	MCLA*		+				+

See also assays kit for
SOD/GSH (page E172)
 H_2O_2 /Catalase (page E177)

E.100

ROS Probes

abs em. **H₂DCFDA**

2',7'-DichloroDiHydroFluorescein Diacetate

C₂₄H₁₆Cl₂O₇ MW : 487.3

Soluble in DMSO and EtOH

Store at -20°C and protect from light

$\lambda_{exc.}/\lambda_{em.}$ (MetOH) : 258/none ; EC : 11 000

after hydrolysis and oxidation :

$\lambda_{exc.}/\lambda_{em.}$ (hydr.&oxid.) (pH 4) : 495/529 nm ; EC : 38 000 M⁻¹cm⁻¹

$\lambda_{exc.}/\lambda_{em.}$ (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.

Description	Cat.#	Qty
H ₂ DCFDA	FP-467312	100 mg

Other ROS Probes

abs em. **DiHydroEthidium (HydroEthidium)**

C₂₁H₂₁N₃ MW : 315.42

Soluble in DMF, DMSO

Store at -20°C and protect from the light

Useful probe to detect oxidative activities in viable cells. DiHydroEthidium by itself is blue fluorescent

($\lambda_{exc.}/\lambda_{em.}$) : 355/420 nm) in cytoplasm, while the *in situ* oxydized forms ethidium

($\lambda_{exc.}/\lambda_{em.}$ (MeOH) :

518/605 nm) upon DNA fixation. See also DNA probes.

Description	Cat.#	Qty
DiHydroEthidium (HydroEthidium)	FP-52492A	25 mg
	FP-HH9180	1 ml (5 mM in DMSO)

abs em. **DihydroRhodamine123**

$\lambda_{exc.}/\lambda_{em.}$ (oxide) : 505/534 nm

Description of product #83775 page E134.

Description	Cat.#	Qty
DihydroRhodamine123	FP-83775A	10 mg

Coelenterazine (mative)

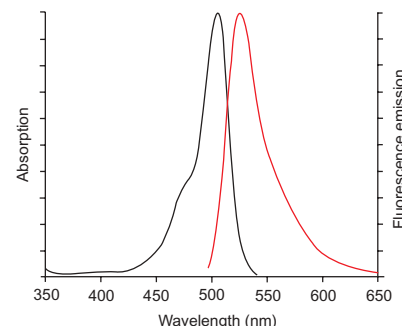
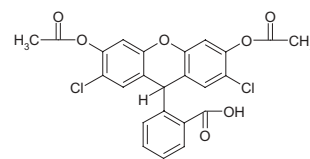
C₂₆H₂₁N₃O₃ MW : 423.47

Store at -20°C and protect from light

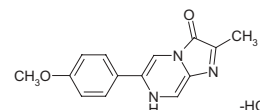
A chemiluminescent probe that emits upon oxydation by superperoxides. It is superior to luminol in this application (pKa).

Also a Ca²⁺ indicator as this ion is needed for the reaction (see page E50).

Description	Cat.#	Qty
Coelenterazine	FP-97233B	250 µg



Absorption and fluorescence emission spectra of FP-46629 (final product of H₂DCFDA) in pH 9.0 buffer.



See more coelenterazines page E50.

Cell Biology - Study/Probes

Reactive Oxygen Study

abs em. MCLA

2-methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-one, hydrochloride
 $C_{14}H_{14}ClN_3O_2$ MW : 291.74
 Soluble in DMSO

Store at -20°C and protect from light

$\lambda_{exc.}/\lambda_{em.}$ (MeOH) : 430/546 nm ; EC : 8 400 M⁻¹cm⁻¹

A chemiluminescent probe that emits at 455 nm upon oxydation by superperoxides. It is superior to luminol in this application (pH optimum closer to neutral range of cells).

Description	Cat.#	Qty
MCLA	FP-38544A	5 mg

abs em. Lucigenin

9,9'-bis-*N*-methylacridinium nitrate

$C_{28}H_{22}N_4O_6$ MW : 510.50

Soluble in water

Store at -20°C and protect from light

A chemiluminescent probe that emits at 470 nm (QY : 0.6) upon oxydation in basic solution. Widely used, but see MCLA for superior performances in ROS detection. Also a Cl⁻ indicator as it is efficiently quenched by Cl⁻.

Description	Cat.#	Qty
Lucigenin	FP-46915A	10 mg

Other Oxido-Reduction probes

MTT

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

$C_{18}H_{16}BrN_5S$; MW : 414.32

MTT is a tetrazolium salt that is commonly used to detect reductive metabolism in cells for viability, proliferation and cytotoxicity assays. On reduction, tetrazolium salts turn into deeply colored products that can be easily quantified by colorimetric measurement. MTT forms a purple formazan product that needs to be solubilized prior to quantification. It is particularly useful for HTS of antiviral and anticancer compounds and for the studies of cytokine effect on cell proliferation.

See also MTT Cell Proliferation Assay Kit (#455470, page E152).

Description	Cat.#	Qty
MTT	FP-65939A	1g

XTT

(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide, disodium salt)

$C_{22}H_{12}N_7NaO_{13}S_2$; MW : 675.53

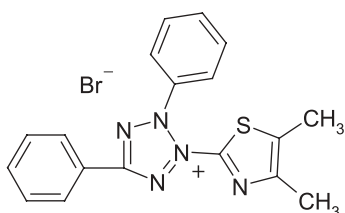
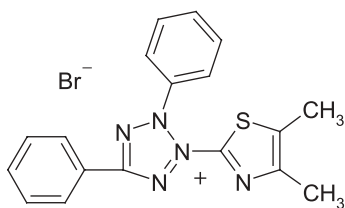
Soluble in water ; Irritant

$\lambda_{exc.}/\lambda_{em.}$ (pH 9) =

A tetrazolium derivative used widely in cell viability and proliferation testings. The material is readily reduced in viable cells to a highly water-soluble orange colored product ($\lambda = 475$ nm). Applications of the reagent include studies of : antifungal susceptibility, drug sensitivity of cells, parasitic nematode viability and tumor cell cytotoxicity.

See also XTT Cell Proliferation Assay Kit (#FX8710, page E152).

Description	Cat.#	Qty
XTT	FP-40936A	100 mg



Resazurin

$C_{12}H_6NNaO_4$ MW : 251.17
 $\lambda_{exc.}/\lambda_{em.}$ (pH 9) = 604 nm/none
 Soluble in DMSO or H₂O

Resazurin is the N-oxide of the fluorescent dye resorufin. It is useful to detect reductive activities in cells and has been widely used for cell proliferation and mitochondrial metabolic activity assays. Resazurin itself is nonfluorescent until it is reduced to the highly red fluorescent resorufin. Usually, NADPH or NADH are the reductants that converts resazurin into resorufin in the presence of diaphorase as enzyme. Thus, resazurin can be used to detect NADH, NADPH, or diaphorase level. Furthermore, the resazurin/diaphorase/NADPH system can be used to detect any biochemical or enzyme activity involved in a biochemical reaction generating NADH or NADPH.

Trace amount of resorufin contaminant often found in commercial resazurin make the material unsuitable for bioassay applications. We supply a high grade resazurin that has a minimal background fluorescence.

See also UptiBlue Cell Proliferation Assay Kit (#UP669412, page E150).

See also HRP substrates (TMB, DAB) page A356

Resazurin is also used as a indicator of pH3.8-6.5.

Description	Cat.#	Qty
Resazurin, Sodium Salt	FP-M1901A	10 mg

EBSELEN

2-phenyl-1,2-benzisoselenazol-3(2H)-one

$C_{13}H_9NOSe$; MW : 274.18

Soluble at >5 mg/ml in ethanol, DMSO, or dimethyl formamide.

Ebselen is an anti-inflammatory, antioxidant compound, shown to be a neuroprotective agent and an inhibitor of free radical induced apoptosis.

Ebselen is a non-selective inhibitor of prostaglandin H synthase (PGHS) 1 and 2, and was shown to inhibit cyclooxygenase and lipoxygenases at micromolar concentrations.

See also Phycobiliproteins, especially R-PE, that were used as antioxidant probes.

Description	Cat.#	Qty
EBSELEN	049411	5 mg
	049412	10 mg
	049413	50 mg

See also other biochemicals for prostaglandins studies
 page E87.

Biochemicals for CycloOxygenases study

Description	Cat.#	Qty
N-(4-acetamidophenyl)-Indomethacin amide	Q87350	1 mg
5-Aminosalicylic Acid	00245D	10 g
9,12-Octadecadiynoic Acid	Q88400	1 mg
Aspirin	608503	5 g
COX-1 (murine) Blocking Peptide	Q89150	1 ea
COX-1 (ovine)	666040	5 Kunit
COX-1 (ovine) Blocking Peptide	Q89140	200 µg
COX-2 (human recombinant)	Q91470	1 Kunit
COX-2 (human) Blocking Peptide	Q89130	200 µg
COX-2 (murine) Blocking Peptide	Q89120	200 µg
COX-2 (ovine)	680790	1 Kunit
Diclofenac (sodium salt)	336676	5 g
DuP-697	Q87460	5 mg
Eicosatetraynoic Acid	549591	5 mg
Eicosatriynoic Acid	Q88290	1 mg
8,11-Eicosadiynoic Acid	Q88210	1 mg
(±)-Flurbiprofen	Q87330	1 g
(±)-Ibuprofen	Q87360	500 mg
Indomethacin	27155A	1 g
Indomethacin heptyl ester	S03560	1 mg
Indomethacin N-octyl amide	S03570	1 mg
Ketorolac (tromethamine salt)	Q87500	500 mg
Meclofenamate (sodium salt)	Q87420	1 g
6-methoxy Naphthalene Acetic Acid	Q87450	5 mg
(S)-Naproxen	Q87370	1 g
N-(2-phenylethyl)-Indomethacin amide	F98380	1 mg
N-(3-pyridyl)-Indomethacin amide	Q87340	1 mg
Niflumic Acid	412320	5 g
Nimesulide	C83560	1 g
Nordihydroguaiaretic Acid	058627	500 mg
NS-398	967750	1 mg
9,12-Octadecadiynoic Acid	Q88400	1 mg
Phenylbutazone	21357A	1 g
PPA	Q87600	500 µg
PPHP	Q87590	500 µg
Resveratrol	E69321	10 mg
SC-560	Q87400	1 mg
SC-58125	S03600	1 mg
TMB (dihydrochloride)	S03580	100 mg
TMPD (dihydrochloride)	S03590	5 g
Tolfenamic Acid	336821	5 g
Valeroyl Salicylate	986860	50 mg

Biochemicals for Lipoxxygenase study

Description	Cat.#	Qty
Baicalin	Q87440	50 mg
Caffeic Acid	E97050	1 g
Eicosatetraynoic Acid	549590	5 mg
3,4-Dihydroxyphenyl ethanol	196750	5 mg
4,5-dehydro Docosaheaxaenoic Acid	S03800	25 µg
Eicosatriynoic Acid	Q88290	1 mg
(±)5-HETE lactone	Q86330	25 µg
15(S)-HETE	489781	25 µg
15(S)-HETrE	771550	25 µg
5(S)-HpETE	413361	25 µg
12(S)-HpETE	405791	25 µg
5,6-dehydro Arachidonic Acid	277704	25 µg
12-Lipoxygenase (porcine)	964860	1 Kunit
15-Lipoxygenase (soybean P1)	Q86730	15 Munit
15-Lipoxygenase (soybean P1) - Purified	861740	.5 Munit
5-Lipoxygenase (human recombinant)	Q86720	500 units
5-Lipoxygenase (potato)	263831	1 Kunit
5-Lipoxygenase Blocking Peptide	Q89180	200 µg

Description	Cat.#	Qty
5-Lipoxygenase Polyclonal Antiserum	Q88600	1 ea
9,12-Octadecadienoic Acid	Q88400	1 mg
Nordihydroguaiaretic Acid	058627	500 mg
Piriprost (potassium salt)	Q85860	1 mg
REV 5901	Q87430	5 mg

Biochemicals for Oxylipins study

Description	Cat.#	Qty
Cholesteryl Linoleate Hydroperoxides	Q86660	100 µg
Cucurbitic Acid	Q88020	1 mg
5(S),15(S)-DiHETE	406981	25 µg
5(S),6(R)-DiHETE	532282	25 µg
5(S),6(S)-DiHETE	485481	25 µg
8(S),15(S)-DiHETE	495080	25 µg
(±)11,12-DiHETrE	Q86700	25 µg
(±)14,15-DiHETrE	Q86710	25 µg
(±)5,6-DiHETrE	Q86670	25 µg
(±)8,9-DiHETrE	Q86690	25 µg
(±)5,6-DiHETrE lactone	Q86680	25 µg
(±)9,10-DiHOME	AM0730	25 µg
4(5)-EpDPE methyl ester	Q88340	25 µg
(±)11(12)-EpETrE	861391	25 µg
(±)14(15)-EpETrE	861401	25 µg
(±)5(6)-EpETrE	861371	25 µg
(±)8(9)-EpETrE	861381	25 µg
(±)11(12)-EpETrE methyl ester	608350	25 µg
(±)14(15)-EpETrE methyl ester	549600	25 µg
(±)5(6)-EpETrE methyl ester	200730	25 µg
(±)8(9)-EpETrE methyl ester	698190	25 µg
(±)12(13)-EpOME	780990	25 µg
(±)9(10)-EpOME	780980	25 µg
(±)10-HDoHE	Q86220	25 µg
(±)11-HDoHE	Q86230	25 µg
(±)13-HDoHE	Q86240	25 µg
(±)14-HDoHE	Q86250	25 µg
(±)16-HDoHE	Q86260	25 µg
(±)17-HDoHE	Q86270	25 µg
(±)20-HDoHE	Q86280	25 µg
(±)4-HDoHE	Q86190	25 µg
(±)7-HDoHE	Q86200	25 µg
(±)8-HDoHE	Q86210	25 µg
(±)11-HEDE	Q86440	25 µg
(±)15-HEDE	Q86470	25 µg
11(R)-HEDE	Q86450	25 µg
11(S)-HEDE	Q86460	25 µg
15(S)-HEDE	Q86490	25 µg
(±)11-HEPE	Q86150	25 µg
(±)12-HEPE	Q86170	25 µg
(±)15-HEPE	Q86180	25 µg
(±)18-HEPE	S00500	25 µg
(±)5-HEPE	Q86120	25 µg
(±)8-HEPE	Q86130	25 µg
(±)9-HEPE	Q86140	25 µg
11(R)-HEPE	Q86160	25 µg
11(S)-HEPE	648110	25 µg
12(R)-HEPE	876630	25 µg
12(S)-HEPE	681240	25 µg
15(S)-HEPE	680180	25 µg
5(S)-HEPE	643680	25 µg
8(S)-HEPE	186500	25 µg
9(S)-HEPE	674820	25 µg
12(S)-hydroxy-16-Heptadecynoic Acid	Q86100	1 mg
(±)11-HETE	861341	25 µg
(±)12-HETE	608570	25 µg
(±)15-HETE	Q86390	25 µg
(±)5-HETE	489721	25 µg
5(S)-HETE-d8	876570	25 µg
(±)8-HETE	861322	25 µg

Cell Biology - Study/Probes

Reactive Oxygen Study

Description	Cat.#	Qty
(±)9-HETE	861330	25 µg
11(R)-HETE	306610	25 µg
11(S)-HETE	127780	25 µg
12(R)-HETE	382010	25 µg
12(S)-HETE	489771	25 µg
15(R)-HETE	307790	25 µg
15(S)-HETE	489781	25 µg
20-HETE	Q88140	25 µg
5(R)-HETE	Q86340	25 µg
5(S)-HETE	489731	25 µg
8(R)-HETE	677350	25 µg
8(S)-HETE	127350	25 µg
9(R)-HETE	Q86360	25 µg
9(S)-HETE	493140	25 µg
(±)5-HETE lactone	Q86330	25 µg
5(S)-HETE lactone	861312	25 µg
(±)5-HETE methyl ester	340271	25 µg
15(S)-HETE-biotin	Q86400	25 µg
12(S)-HETE-d8	876580	25 µg
15(S)-HETE-d8	876590	25 µg
15(S)-HETrE	771550	25 µg
5(S)-HETrE	Q86420	25 µg
8(S)-HETrE	Q86430	25 µg
12(S)-HHTrE	429011	25 µg
9(R)-HODE	876620	25 µg
9(S)-HODE	692360	100 µg
(±)9-HODE cholesteryl ester	Q86510	25 µg
9(R)-HODE cholesteryl ester	Q86520	25 µg
9(S)-HODE cholesteryl ester	Q86530	25 µg
9(S)-HODE-d4	Q89090	25 µg
(±)13-HODE	861361	100 µg
13(R)-HODE	876640	25 µg
13(S)-HODE	692480	100 µg
13(S)-HODE-biotin	Q86580	100 µg
(±)13-HODE cholesteryl ester	Q86550	25 µg
13(R)-HODE cholesteryl ester	Q86560	25 µg
13(S)-HODE cholesteryl ester	Q86570	25 µg
13(S)-HODE-d4	Q89100	25 µg
9(S)-HOTrE	S00560	100 µg
13(S)-HOTrE	Q86610	100 µg
13(S)-HOTrE(.gamma.)	Q86600	25 µg
15(S)-HpEDE	Q86650	25 µg
5(S)-HpEPE	685830	25 µg
12(S)-HpEPE	686400	25 µg
5(S)-HpETE	413361	25 µg
12(S)-HpETE	405791	25 µg
15(S)-HpEPE	686720	25 µg
15(S)-HpETE	409611	25 µg
9(S)-HpODE	519590	100 µg
13(S)-HpODE	698300	100 µg
9(S)-HpOTrE	Q86630	100 µg
13(S)-HpOTrE	831440	100 µg
13(S)-HpOTrE(.gamma.)	Q86640	100 µg
4-hydroxy Hexenal	Q86110	1 mg
Hydroxy Linoleins	Q88070	500 µg
4-hydroxy Nonenal	990970	1 mg
Linolein Hydroperoxides	Q88080	500 µg
5(S),6(R)-Lipoxin A4	104360	25 µg
5-OxoETE	Q86350	25 µg
5-OxoETE-d7	Q89080	25 µg
12-OxoETE	Q86380	25 µg
15-OxoETE	Q86410	25 µg
15-OxoEDE	Q86500	25 µg
9-OxoODE	Q86540	25 µg
13-OxoODE	Q86590	25 µg
PPOH	S03640	1 mg
Tetranor 12(R)-HETE	Q86370	25 µg
6-trans Leukotriene B4	578140	25 µg
6-trans-12-epi Leukotriene B4	861421	25 µg
Traumatic Acid	156054	500 mg
n-Triacontanol	Q88030	25 mg