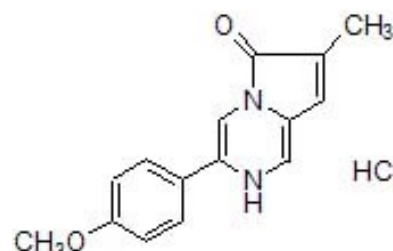


Superoxide probes: MCLA, Lucigenin

Product Information

Generation of chemiluminescence by reaction with super oxide $O_2^{\cdot -}$ or singlet oxygen.

Name :	MCLA 2-Methyl-6-(4-methoxyphenyl)-3,7-dihydroimidazo[1,2-a]pyrazin-3-one, hydrochloride Cypridina Luciferin methoxy-analogue
Catalog Number :	FP-38544A, 5 mg
Structure :	$C_{14}H_{13}ClN_3O_2$; CAS [128322-44-1]
Molecular Weight :	MW= 291.73
Solubility:	DMSO, DMF and water
Absorption / Emission :	$\lambda_{exc} \backslash \lambda_{em}$ (methanol) = 430 / 549 nm Chemiluminescence emission at 455 nm upon reaction with superoxide
EC:	8 400 $M^{-1} cm^{-1}$



Name :	Lucigenin 9,9'-bis-N-Methylacridinium Nitrate
Catalog Number :	FP-46915A, 10mg
Structure :	$C_{28}H_{22}N_4O_6$; CAS: [22103-92-0]
Molecular Weight :	MW= 510.51
Solubility:	water
Absorption / Emission :	$\lambda_{exc} \backslash \lambda_{em}$. (methanol) = . / 470 nm QY:0.6 Chemiluminescence emission at 470 nm upon reaction with superoxide Also used as a Cl- indicator

Storage: $-20^{\circ}C$ (M) Protect from light and moisture. Air sensitive, use under a N_2 or Ar atmosphere.

Technical information

Superoxide radical ($O_2^{\cdot -}$) 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 are involved in various biological processes including carcinogenesis, vascular disease and senescence.

Besides chromogenic probes (NBT, MTT), fluorescent probes (MCLA) offer high photon output/signal, allows multicolor detection and has 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. However they have lower photon output, and imaging is more difficult (limited possibility of multidetections).

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, see related products) and for detecting redox potential of cells for viability, proliferation and cytotoxicity assays (MTT, FP-69939A, see related products).

Lucigenin-amplified chemiluminescence (LuCL ^{Igor2001}) has frequently been used to assess the formation of superoxide. However, several question have benne raised and limitations have been described: 1/lucigenin may

FT-38544

undergo redox cycling in purified enzyme-substrate mixtures, 2/ Lucigenin was reported to enhance 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. And MCLA or coelenterazine is recommended as generally more appropriate probe for the measurement of superoxide production.

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 $\sim 10^5 \text{ M}^{-1} \text{ s}^{-1}$. The MCLA chemiluminescence is a sensitive marker for detecting superoxide. It is used notably in research of leucocyte function.

Coelenterazine (FP-97233, see related products) is a sensitive probe for the detection of superoxide and peroxynitrite, without interference from H₂O₂ or azide¹. Coelenterazine is the preferred probe for luminescent detection of superoxide in experiments where quantitative determination of superoxide production is required².

Directions for use - Lucigenin

References - Lucigenin

Igor B. Afanas'ev, Circulation Research. 2001;89:e46. Lucigenin Chemiluminescence Assay for Superoxide Detection. [Abstract](#)

Directions for use - MCLA

Guidelines for use - MCLA

- Prepare reaction buffer: 5 μM MCLA + buffer

Buffer examples:

. for Cytochrome C: 50 mM Tris-Cl, pH 7.8, 0.1 mM EDTA, 7 mM sodium succinate

. for mitochondria proteins: 125 mM KCl, 10 mM HEPES, 5 mM MgCl₂, 2 mM K₂HPO₄

- Conduct reaction in 100 μl of reaction buffer
- Light emission is detected and quantified using a microplate luminometer with opaque (white) 96-well plates. The photomultiplier can be set to default with an integration time of 1000 ms. The MCLA signal is quantified as an integral of 20 s to 60 s of continuous measurement.
- A positive control can be prepared with the xanthine/xanthine oxidase system: 1 mM xanthine + 0.1 unit/ml xanthine oxidase causes a ~100-fold increase in chemiluminescent signal as compared with xanthine or xanthine oxidase alone, which did not differ significantly from reaction buffer alone. Addition of SOD (CuZn-SOD from erythrocytes) decreases the MCLA signal by over 98%

References - MCLA

- **Daekyung K.** *et al*, Presence of the distinct systems responsible for superoxide anion and hydrogen peroxide generation in red tide phytoplankton *Chattonella marina* and *Chattonella ovata*, *J. Plankton Res.*, **29**: 241 - 247 (2007) [Abstract](#)
- **Goto T.** *et al.*, *Clin. Chim. Acta* **179**, 177 (1989)
- **Koga S.** *et al.*, *Arch. Biochem. Biophys.*, **289**, 223 (1991)
- **Tarpey M.**, *et al.* - Chemiluminescent Detection of Oxidants in Vascular Tissue, Lucigenin But Not Coelenterazine Enhances Superoxide Formation, *Circulation Research.*; **84**:1203-1211 (1999)
- **Weir C. and Robaire B.**, Spermatozoa Have Decreased Antioxidant Enzymatic Capacity and Increased Reactive Oxygen Species Production During Aging in the Brown Norway Rat, *J Androl*; **28**: 229 - 240 (2007) [Abstract](#)

Related products

- Dihydroethidium, [FP-52492A](#)
- Xanthine solution, [EX9200](#)
- Superoxide Dismutase Assay Kit, [S5310](#)
- Coelenterazine: [UP972333](#)
- NBT, [UPI43456](#)
- MTT, [FP-69939A](#)

Ordering information

Catalog size quantities and prices may be found at <http://www.fluoprobes.com>
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

Disclaimer : Materials from FluoProbes® are sold **for research use only**, and are not intended for food, drug, household, or cosmetic use. FluoProbes® is not liable for any damage resulting from handling or contact with this product.