# Superoxide probes: MCLA, Lucigenin

## Product Information

**Generation of chemiluminescence by reaction with superoxide \( \text{O}_2^- \) or singlet oxygen.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Catalog Number</th>
<th>Structure</th>
<th>Molecular Weight</th>
<th>Solubility</th>
<th>Absorption / Emission</th>
<th>EC:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MCLA</strong></td>
<td>FP-38544A, 5 mg</td>
<td>( \text{C}<em>{14}\text{H}</em>{13}\text{ClN}_3\text{O}_2 ); CAS [128322-44-1]</td>
<td>( \text{MW}= 291.73 )</td>
<td>DMSO, DMF and water</td>
<td>( \lambda_{\text{exc}}\lambda_{\text{em}} ) (methanol) = 430 / 549 nm</td>
<td>8 400 ( \text{M}^{-1} \text{cm}^3 )</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Name</th>
<th>Catalog Number</th>
<th>Structure</th>
<th>Molecular Weight</th>
<th>Solubility</th>
<th>Absorption / Emission</th>
<th>Storage:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lucigenin</strong></td>
<td>FP-46915A, 10mg</td>
<td>( \text{C}<em>{28}\text{H}</em>{22}\text{N}_4\text{O}_6 ); CAS: [22103-92-0]</td>
<td>( \text{MW}= 510.51 )</td>
<td>water</td>
<td>( \lambda_{\text{exc}}\lambda_{\text{em}} ) (methanol) = ( . ) / 470 nm ( \text{QY}:0.6 )</td>
<td>( \text{–20°C} ) (M) Protect from light and moisture. Air sensitive, use under a ( \text{N}_2 ) or ( \text{Ar} ) atmosphere.</td>
</tr>
</tbody>
</table>

## Technical information

**Superoxide radical** (\( \text{O}_2^- \)) 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 (Coelenterazine), 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 multidecctions).

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 (LuCI, \textit{Igor 2001}) has frequently been used to assess the formation of superoxide. However, several question have benne raised and limitations have been described: 1/lucigenin may...
FluoProbes®

FT-38544 undergo redox cycling in purified enzyme-substrate mixtures. Lucigenin was reported to enhance superoxide formation[1]. 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 \(10^5 \text{M}^{-1}\text{s}^{-1}\).

Coelenterazine (FP-97233, see related products) is a sensitive probe for the detection of superoxide and peroxynitrite, without interference from H2O2 or azide[2]. Coelenterazine is the preferred probe for luminescent detection of superoxide in experiments where quantitative determination of superoxide production is required[3].

**Directions for use - Lucigenin**

**References - Lucigenin**


**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**


Info@fluoprobes.com
Technical-support@fluoprobes.com
Order-online@fluoprobes.com
Contact your local distributor
FluoProbes®

Related products

- Dihydroethidium, FP-52492A
- Xanthine solution, FX9200
- Superoxide Dismutase Assay Kit, S5310
- Coelenterazine: UP972333
- NBT, UP143456
- 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.