

## MitoSOX™ Red mitochondrial superoxide indicator \*for live-cell imaging\* (M36008)

### Quick Facts

#### Storage upon receipt:

- $\leq -20^{\circ}\text{C}$
- Protect from light
- Desiccate
- Avoid freeze-thaw cycles

**Ex/Em:** 510/580 nm

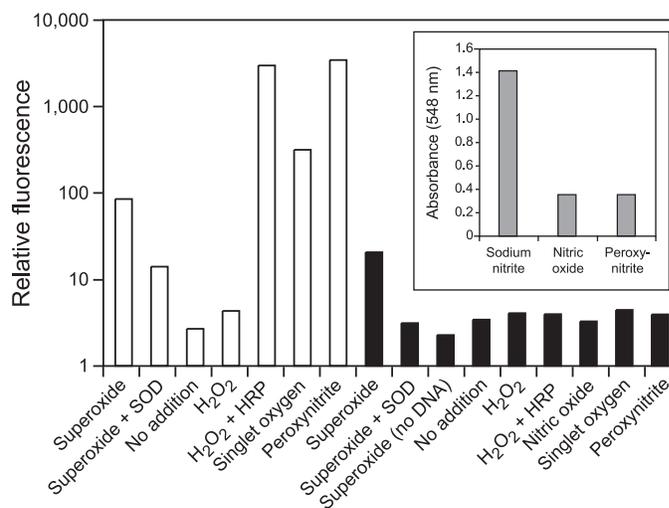
**Note:** To preserve the activity of the MitoSOX™ Red reagent, minimize exposure to air.

### Introduction

Mitochondrial superoxide is generated as a byproduct of oxidative phosphorylation. In an otherwise tightly coupled electron transport chain, approximately 1–3% of mitochondrial oxygen consumed is incompletely reduced; those “leaky” electrons can quickly interact with molecular oxygen to form superoxide anion, the predominant reactive oxygen species (ROS) in mitochondria.<sup>1–4</sup> Increases in cellular superoxide production have been implicated in cardiovascular diseases, including hypertension, atherosclerosis, and diabetes-associated vascular injuries,<sup>5–7</sup> as well as in neurodegenerative diseases such as Parkinson’s, Alzheimer’s, and amyotrophic lateral sclerosis (ALS).<sup>8–12</sup>

The assumption that mitochondria serve as the major intracellular source of ROS has been based largely on experiments with isolated mitochondria rather than direct measurements in living cells. MitoSOX™ Red mitochondrial superoxide indicator is a novel fluorogenic dye for highly selective detection of superoxide (Figure 1) in the mitochondria of live cells. MitoSOX™ Red reagent is live-cell permeant and is rapidly and selectively targeted to the mitochondria. Once in the mitochondria, MitoSOX™ Red reagent is oxidized by superoxide and exhibits red fluorescence. MitoSOX™ Red reagent is readily oxidized by superoxide but not by other ROS- or reactive nitrogen species (RNS)-generating systems, and oxidation of the probe is prevented by superoxide dismutase. The oxidation product becomes highly fluorescent upon binding to nucleic acids.

This reagent may enable researchers to distinguish artifacts of isolated mitochondrial preparations from direct measurements of superoxide generated in the mitochondria of live cells. It may also provide a valuable tool in the discovery of agents that modulate oxidative stress in various pathologies.



**Figure 1.** Selectivity of the MitoSOX™ Red mitochondrial superoxide indicator. Cell-free systems were used to generate a variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS); each oxidant was then added to a separate 10  $\mu\text{M}$  solution of MitoSOX™ Red reagent and incubated at 37°C for 10 minutes. Excess DNA was added (unless otherwise noted) and the samples were incubated for an additional 15 minutes at 37°C before fluorescence was measured. The Griess nitrite determination kit (for nitric oxide, peroxynitrite, and nitrite standards only; gray bars) and dihydrorhodamine 123 (DHR 123; white bars) were employed as positive controls for oxidant generation. Superoxide dismutase (SOD), a superoxide scavenger, was used as a negative control for superoxide. The results show that the MitoSOX™ Red probe (black bars) is readily oxidized by superoxide but not by the other oxidants.

### Materials

#### Contents

MitoSOX™ Red mitochondrial superoxide indicator (MW = 759) is supplied in 10 vials, each containing 50  $\mu\text{g}$ .

#### Storage and Handling

Upon receipt, the MitoSOX™ Red reagent should be stored upright, desiccated, and protected from light at  $\leq -20^{\circ}\text{C}$ . Avoid freeze-thaw cycles. MitoSOX™ Red reagent is packaged with an oxygen scavenging pouch that will extend the shelf life of the product. After removing vials for use, seal the remaining vials into the pouch to preserve the activity of the reagent. Vials should be allowed to warm to room temperature before opening. When stored properly, components should be stable for at least 6 months. Note: MitoSOX™ Red reagent is a derivative of ethidium bromide and should be treated with the appropriate caution.

### **Spectral Characteristics**

MitoSOX™ Red mitochondrial superoxide indicator has excitation/emission maxima of approximately 510/580 nm.

### **Materials Recommended but Not Provided**

Hank's balanced salt solution with calcium and magnesium (HBSS/Ca/Mg, available from Gibco (14025-092)).

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## **Experimental Protocol**

### **Reagent Preparation**

**Prepare 5 mM MitoSOX™ reagent stock solution.** Dissolve the contents (50 µg) of one vial of MitoSOX™ mitochondrial superoxide indicator (Component A) in 13 µL of dimethylsulfoxide (DMSO) to make a 5 mM MitoSOX™ reagent stock solution.

### **Labeling Live Eukaryotic Cells**

This protocol was developed using live bovine pulmonary epithelial (BPAE) cells, MRC5 human lung fibroblasts, and mouse 3T3 fibroblasts adhering to coverslips, but can be adapted for use with other cell types. Recommendations for experimental

protocols should be used as a starting point, and optimal labeling conditions should be determined empirically.

### **1.1 Prepare 5 µM MitoSOX™ reagent working solution.**

Dilute the 5 mM MitoSOX™ reagent stock solution (prepared above) in HBSS/Ca/Mg or suitable buffer to make a 5 µM MitoSOX™ reagent working solution.

Note: The concentration of the MitoSOX™ reagent working solution should not exceed 5 µM. Concentrations exceeding 5 µM can produce cytotoxic effects, including altered mitochondrial morphology and redistribution of fluorescence to nuclei and the cytosol.

**1.2 Load cells.** Apply 1.0–2.0 mL of 5 µM MitoSOX™ reagent working solution (prepared in step 1.2) to cover cells adhering to coverslip(s). Incubate cells for 10 minutes at 37°C, protected from light.

**1.3 Wash cells.** Wash cells gently three times with warm buffer.

**1.4 Prepare cells for viewing.** Stain cells with counterstains as desired and mount in warm buffer for imaging.

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## **References**

1. J Cell Mol Med 6, 175 (2002); 2. J Biol Chem 279, 4127 (2004); 3. J Neurochem 80, 780 (2002); 4. Bioorganic and Medicinal Chemistry 10, 3013 (2002); 5. Am J Physiol Heart Circ Physiol 284, H605 (2003); 6. Bioorganic and Medicinal Chemistry 10, 3013 (2002); 7. J Neurochem 89, 1283 (2004); 8. Trends Neurosci 24, S15 (2001); 9. J Cell Mol Med 6, 175 (2002); 10. J Neurosci 18, 923 (1998); 11. Neurochem Res 28, 1563 (2003); 12. IUBMB Life 55, 329 (2003).

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<b>Cat #</b>	<b>Product Name</b>	<b>Unit Size</b>
M36008	MitoSOX™ Red mitochondrial superoxide indicator *for live-cell imaging* .....	10 x 50 µg

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