

MitoSOX™ Green and MitoSOX™ Red Mitochondrial Superoxide Indicators

Mitochondrial, live-cell staining with MitoSOX™ Green and MitoSOX™ Red Mitochondrial Superoxide Indicators

Catalog Numbers M36005, M36006, M36007, M36008, and M36009

Pub. No. MAN0028459 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Invitrogen™ MitoSOX™ Green and MitoSOX™ Red Mitochondrial Superoxide Indicators are novel fluorogenic dyes for the highly selective detection of superoxide in the mitochondria of live cells (see Figure 1 and Figure 3). MitoSOX™ Green (MSG) reagent and MitoSOX™ Red (MSR) reagent are live-cell permeant which rapidly and selectively target the mitochondria. Once in the mitochondria, the MSG reagent or MSR reagent are oxidized by superoxide and exhibit bright green or red fluorescence, respectively. The production of superoxide by mitochondria can be visualized in fluorescence microscopy (see Figure 3). The MSG reagent or MSR reagent are readily oxidized by superoxide but not by other reactive oxygen species (ROS)- or reactive nitrogen species (RNS)-generating systems, and oxidation of the probe is prevented by superoxide dismutase as well as superoxide scavengers.

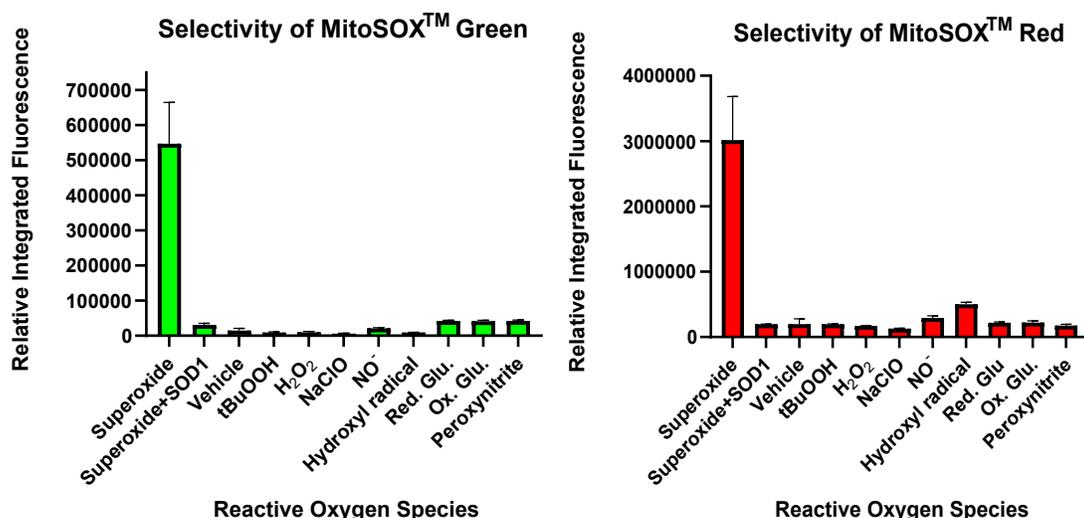


Figure 1 Selectivity of MitoSOX™ Green and MitoSOX™ Red Mitochondrial Superoxide Indicators.

Selectivity of the MSG reagent and MSR reagent in a cell-free environment. Cell-free systems were used to generate ROS. Each ROS was added to separate solutions of 10 μM MSG and MSR and incubated for 30 mins at ambient temperature. Excess DNA was added to the solutions with MSR. Superoxide dismutase was used as a negative control for superoxide. Superoxide was generated using potassium superoxide for MSG, and the xanthine/xanthine oxidase system was used to generate superoxide for MSR. The results show that both MSG and MSR are selectively oxidized by superoxide compared to ROS.

Spectral properties

- MSG reagent absorbs and emits optimally at 488 nm and 510 nm, respectively (see Figure 2). Fluorescence is visualized with a standard FITC or GFP filter.
- MSR reagent absorbs and emits optimally at 396 nm and 610 nm, respectively (see Figure 2). Fluorescence is visualized with an appropriate filter set. The spectral peak at 396 is utilized for accurate detection of superoxide.

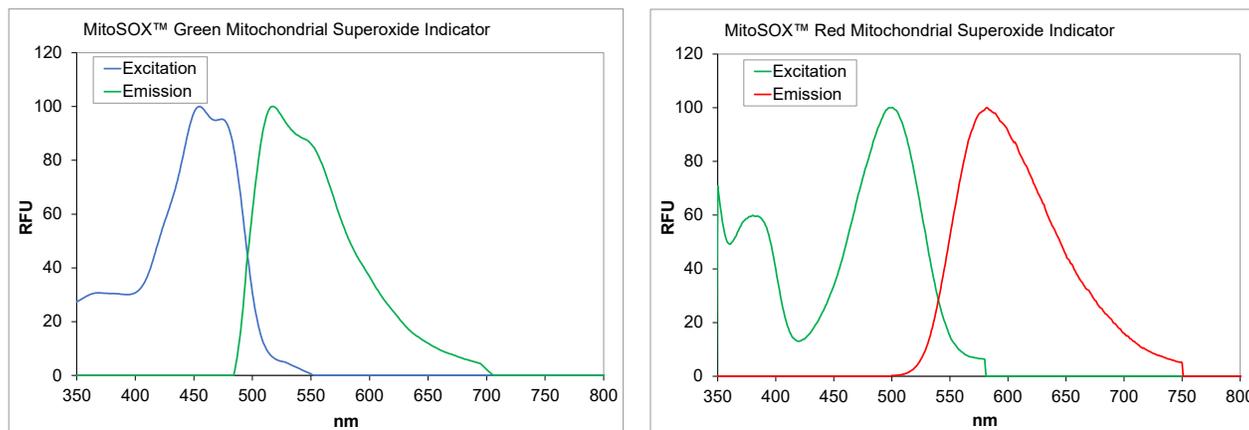


Figure 2 MitoSOX™ Green and MitoSOX™ Red Mitochondrial Superoxide Indicators excitation and emission spectra.

Contents and storage

These reagents are 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. Allow the vials to warm to room temperature before opening.

Product	Cat. No.	Amount	Storage ^[1]
MitoSOX™ Green Mitochondrial Superoxide Indicator	M36005	1 vial (9 µg)	Store at –5°C to –25°C. Store upright, desiccated, and protected from light.
	M36006	5 vials (9 µg each)	
MitoSOX™ Red Mitochondrial Superoxide Indicator	M36007	1 vial (50 µg)	
	M36008	10 vials (50 µg each)	
MitoSOX™ Variety pack	M36009	1 vial each of MitoSOX™ Green reagent and MitoSOX™ Red reagent	

^[1] When stored as directed, kit components are stable for at least 6 months.

Required materials not supplied

Unless otherwise indicated, all materials are available through thermofisher.com. "MLS" indicates that the material is available from fisherscientific.com or another major laboratory supplier.

Catalog numbers that appear as links open the web pages for those products.

Item	Source
HBSS, calcium, magnesium, no phenol red	14025-092
DMSO, Anhydrous (MitoSOX™ Red reagent)	D12345
N,N-Dimethylformamide (DMF), Anhydrous (MitoSOX™ Green reagent)	610941000

Procedural guidelines

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

Use these protocols as a starting point. Determine optimal labeling conditions empirically.

Prepare MitoSOX™ Green reagent stock and working solution

Dissolve MSG reagent in anhydrous DMF. Do not dissolve in DMSO or other solvents.

- Make a 1 mM stock solution of MSG reagent by dissolving the contents of the vial in 10 μ L of DMF.

Note: This stock solution is stable for one day.

- To make a working solution with the MSG reagent, add 10 μ L of 1 mM stock solution to 10 mL of HBSS with Calcium and Magnesium or other buffer.

Note: While the recommended concentration of working solution is 1 μ M, this should be optimized between 0.5 μ M to 5 μ M with different cell types to maximize signal-to-noise ratio and minimize cellular toxicity.

Prepare MitoSOX™ Red reagent stock and working solution

- Make a 5 mM stock solution of MSR reagent by dissolving the contents of the vial in 13 μ L of anhydrous DMSO.

Note: This stock solution is stable for one day.

- To make the working solution with the MSR reagent, add 5 μ L of the 5 mM stock solution to 50 mL of HBSS with Calcium and Magnesium, or other buffer, to make 500 nM working solution.

Note: For different cell types, optimize the staining solution concentration between 100 nM to 1 μ M to maximize signal-to-noise ratio and minimize cellular toxicity.

Prepare controls

1. (*Optional positive control*) To induce the superoxide, cells can be incubated with 30 μ M MitoPQ (Abcam, Cat. No. ab146819) in low-glucose media. Incubate cells with 30 μ M MitoPQ for 18 hours, wash the cells, then proceed to step 1 (see Figure 3).

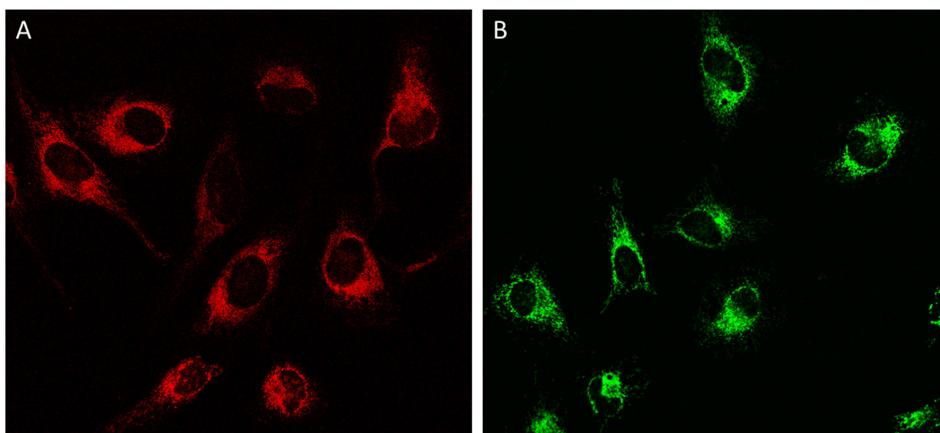


Figure 3 Positive control.

U2OS cells treated with 30 μ M MitoPQ overnight to induce mitochondrial superoxide production. Cells were stained with **(A)** 500 nM MSR reagent or **(B)** 1 μ M MSG reagent for 30 minutes and then imaged in HBSS with Calcium and Magnesium.

2. (*Optional negative control*) To inhibit formation of superoxide, treat cells with 2 mg/mL of DETA NONOate (Cayman, Cat. No. 82100), or Spermine NONOate (Cayman, Cat. No. 82150) in HBSS with Calcium and Magnesium. Make this solution fresh, then add to cells within 1 minute of preparation. Incubate cells for 30 minutes at 37°C in 5% CO₂.
3. (*Optional control*) To assess relative mitochondrial membrane potential as a factor in mitochondrial accumulation of MitoSOX™ sensors, load cells with MitoTracker™ Deep Red (Cat. No. M22426) per instructions in the manual.

Label live eukaryotic cells

1. Prepare a stock solution and working solution of MSR reagent or MSG reagent (see “Prepare MitoSOX™ Green reagent stock and working solution” on page 3 and “Prepare MitoSOX™ Red reagent stock and working solution” on page 3).
2. Apply 1–2 mL of the MSR reagent or MSG reagent working solution to cover cells adhering to coverslip(s) in a well of 35 mm dish, or 100 μ L per well of 96 well plate.

Note: Use sufficient volume to fully cover the cells.

3. Incubate cells for 30 minutes at 37°C and 5% CO₂.

IMPORTANT! Protect from light.

4. Wash cells gently 3 times with warm buffer (HBSS with Calcium and Magnesium or suitable buffer).
5. View cells using spectral properties within 2 hours of staining (see “Spectral properties” on page 2).

Limited product warranty

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Life Technologies Corporation | 29851 Willow Creek Road | Eugene, Oregon 97402 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0028459

Revision	Date	Description
B.0	10 November 2022	The volume of DMSO that is used to prepare a 5-mM stock solution of MitoSOX™ Red reagent was changed to 13 µL.
A.0	12 October 2022	New document for MitoSOX™ Green and MitoSOX™ Red Mitochondrial Superoxide Indicators.

The information in this guide is subject to change without notice.

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