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MitoSOX Red

Catalog Number	Packaging Size
C259-1	50 μg
C259-2	10×50 μg

Storage upon receipt: -20°C, protected from light

Introduction

The MitoSOX Red Indicator is a fluorogenic dye for the highly selective detection of superoxide in the mitochondria of live cells. The MitoSOX Red reagent is live-cell permeant which rapidly and selectively target the mitochondria. Once in the mitochondria, the MitoSOX Red reagent is oxidized by superoxide and exhibit bright red fluorescence. The production of superoxide by mitochondria can be visualized in fluorescence microscopy. The MitoSOX Red reagent is readily oxidized by superoxide but not by other reactive oxygen species (ROS) and reactive nitrogen species (RNS).

Prepare MitoSOX Red reagent stock and working solution

- Make a 5 mM stock solution of MitoSOX Red reagent by dissolving the contents of the vial in 13 μL of anhydrous DMSO.

  Note: This stock solution is stable for one day.

- To make the working solution with the MitoSOX Red reagent, add 5 μL 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 μM to maximize signal-to-noise ratio and minimize cellular toxicity.

Prepare controls (optional)

- **Positive control:** To induce the superoxide, cells can be incubated with 30 μM MitoPQ (Abcam, Cat. No. ab146819) in low-glucose media. Incubate cells with 30 μM MitoPQ for 18 hours, wash the cells.
- **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% CO_2.

Label live eukaryotic cells

1. Prepare a stock solution and working solution of MitoSOX Red reagent (see “Prepare MitoSOX Red reagent stock and working solution”).
2. Apply 1-2 mL of the MitoSOX Red reagent working solution to cover cells adhering to coverslip(s) in a well of 35 mm dish, or 100 μL 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% CO_2 and protect from light.
4. Wash cells gently 3 times with warm buffer (HBSS with Calcium and Magnesium or suitable buffer).
5. View cells using a 396-nm excitation filter within 2 hours of staining.