Mitochondrial Superoxide Dye

Dyes and Stains

Sensitive fluorescent dye that detects superoxide production in mitochondria

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Catalog #100-0991

500 Tests

Product Description

Mitochondrial Superoxide is a fluorescent superoxide dye that selectively targets the mitochondria of living cells. This dye becomes oxidized when it reacts with superoxide, producing a red fluorescence. In the absence of the hydroxyl group, there is no absorption and negligible fluorescence. The high signal-to-noise ratio makes Mitochondrial Superoxide Dye a suitable reagent for superoxide detection in biological systems. Mitochondrial Superoxide Dye is resistant to oxidation by other reactive oxygen species (ROS) and reactive nitrogen species (RNS). In the absence of superoxide, there is no absorption and negligible fluorescence. The excess production of mitochondrial ROS is considered to be a novel indicator of cell proliferation, differentiation, and apoptosis (Phull et al.). Increased ROS production has been found to damage mitochondrial DNA and impair the electron transport chain (ETC) activity (Ikeda et al.). Mitochondrial Superoxide Dye is a valuable tool for the detection of superoxide production in the mitochondria and for modulating oxidative stress, and has been verified for detection using flow cytometry.

Excitation Wavelength:	510 nm
Emission Wavelength:	580 nm

Properties

Storage:	Store at -20°C.
Shelf life:	Product stable until expiry date (EXP) on bag label. Protect from prolonged exposure to light.
Format:	Orange solid

Directions for Use

Please read the entire protocol before proceeding. The following protocol is for staining cells in a 96-well plate. If using other cultureware, adjust volumes accordingly. Treat cells as desired before preparing the Mitochondrial Superoxide Dye working solution.

Preparation of Mitochondrial Superoxide Dye Stock and Working Solution

1. To prepare a 1000X stock solution, add 13 µL of dimethyl sulfoxide (DMSO) to the vial. Mix thoroughly.

   NOTE: If not used immediately, aliquot and store at -20°C. Do not exceed the expiry date as indicated on the label. Avoid repeated freeze-thaw cycles.
2. To prepare the 2X Mitochondrial Superoxide Dye working solution, add 2 µL of stock solution (prepared in step 1) to 998 µL of Hanks' Balanced Salt Solution with 20 mM HEPES (HHBS). Mix thoroughly. Use the working solution immediately; do not store.

   NOTE: The optimal concentration of the working solution should be determined for different cell types. To avoid cytotoxicity, the final concentration of Mitochondrial Superoxide Dye should not exceed 1X.

Staining Samples

1. Plate cells in a 96-well plate and prepare controls as follows:
   a. Superoxide positive control (e.g. 50 µM Antimycin A_1 [Cayman Catalog #19433])
   b. Untreated control
2. Incubate the cells with the superoxide positive control at 37°C and 5% CO_2 for an appropriate length of time.
   Example: 30 minutes is sufficient for Jurkat cells treated with 50 µM Antimycin A_1.
3. Add an equal volume of Mitochondrial Superoxide Dye working solution directly to the culture medium.
4. Incubate plate at 37°C and 5% CO_2 for 10 - 30 minutes.
5. Remove the Mitochondrial Superoxide Dye working solution and wash cells 3X with HHBS.
   NOTE: For non-adherent cells, centrifuge at 300 - 500 x *g* for 5 minutes before removing the working solution.

Fluorescence Detection

Monitor the fluorescence intensity using a fluorescence microscope equipped with an appropriate filter set or a flow cytometer at Ex/Em = 510/580 nm.

References

1. Ikeda M et al. (2015) Overexpression of TFAM or twinkle increases mtDNA copy number and facilitates cardioprotection associated with limited mitochondrial oxidative stress. PLoS One 10(3): 1–19.
2. Li X et al. (2018) Hsp70 suppresses mitochondrial reactive oxygen species and preserves pulmonary microvascular barrier integrity following exposure to bacterial toxins. Front Immunol 9: 1309.
3. Phull AR et al. (2018) Oxidative stress, consequences and ROS mediated cellular signaling in rheumatoid arthritis. Chem Biol Interact 281: 121–36.