MitoROS Brite™ NIR 780 *Optimized for Detecting Reactive Oxygen Species (ROS) in Mitochondria*

Catalog number: 16049
Unit size: 1 mg

Component	Storage	Amount (Cat No. 16049)
MitoROS™ NIR 780	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Mitochondrial reactive oxygen species (mtROS or mROS) are generated within mitochondria, primarily during oxidative phosphorylation at the electron transport chain (ETC) on the inner mitochondrial membrane. Electron leakage from complexes I and III partially reduces oxygen to superoxide, which is dismutated to hydrogen peroxide by SOD2 in the mitochondrial matrix and SOD1 in the intermembrane space. Low levels of mtROS are essential for metabolic adaptation and inflammatory response regulation, while elevated mtROS can induce apoptosis, autophagy, cellular senescence, and aging. Recent findings implicate mtROS production in lung monocytes and macrophages in COVID-19 pathogenesis, highlighting mtROS as a potential therapeutic target for novel coronavirus treatments. MitoROS Brite™ NIR 780 is a hydrogen peroxide-sensitive fluorescent probe specifically designed for targeting mitochondria in live cells. Upon oxidation by hydrogen peroxide anions, it emits near-infrared fluorescence, allowing precise quantification of mitochondrial hydrogen peroxide levels via fluorescence microscopy or flow cytometry. Its high cell permeability and mitochondrial selectivity ensure specific detection of hydrogen peroxide with minimal cross-reactivity to other ROS or reactive nitrogen species (RNS). The retention of the oxidized fluorophore within cells makes MitoROS Brite™ NIR 780 invaluable for investigating oxidative stress-related pathologies. Furthermore, its compatibility with multiplexing techniques facilitates concurrent use with other fluorescent mitochondrial probes, enabling comprehensive analysis of multiple mitochondrial parameters within the same cell population. For instance, it can be combined with MitoLite™ Green FM to assess ROS levels and mitochondrial mass or morphology, or with TMRE or TMRM to measure both mitochondrial membrane potential and ROS levels, advancing research into mitochondrial-associated diseases.

AT A GLANCE

Important Note

Before using MitoROS Brite™ NIR 780 for the first time, allow it to thaw at room temperature. Then, briefly centrifuge it to collect the dried pellet.

Protocol Summary

1. Prepare the cells in a growth medium.
2. Stain cells with MitoROS Brite™ NIR 780 working solution.
3. Treat cells with test compound.
4. Monitor the fluorescence intensity using a fluorescence microscope equipped with a Cy7 filter set.

KEY PARAMETERS

Fluorescence microscope

Emission	Cy7 filter set
Excitation	Cy7 filter set
Recommended plate	Black wall/clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

MitoROS Brite™ NIR 780 Stock Solution

1. Prepare a 5 to 10 mM MitoROS Brite™ NIR 780 stock solution in DMSO. For example, to make a 10 mM stock solution, add 141 µL of DMSO to the MitoROS Brite™ NIR 780 vial.

   Note: Any unused stock solution can be stored at -20 °C, protected from light. Avoid repeated freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

MitoROS Brite™ NIR 780 Working Solution

1. Prepare a working solution of 0.5 to 5 µM by diluting the MitoROS Brite™ NIR 780 stock solution in Hanks solution with 20 mM Hepes buffer (HHBS).

   Note: For optimal results, use this solution within a few hours of preparation.

   Note: Cover the working solution with foil or store it in a dark place to protect it from light.

SAMPLE EXPERIMENTAL PROTOCOL

Sample Protocol

1. Plate the cells as desired in a 96-well black wall-clear bottom plate.
2. Add 100 µL of the MitoROS Brite™ NIR 780 working solution directly to the cells.
3. Incubate the cells at 37°C for 15-30 minutes, protected from light.

   Note: The concentration and incubation time of MitoROS Brite™ NIR 780 may vary depending on the cell line. Test different concentrations to determine the optimal dose.

4. Remove the dye working solution and wash the cells twice with HHBS buffer.
5. Treat cells as desired.
6. Remove the treatment and was the cells twice with HHBS buffer.
7. Add HHBS buffer and analyze the cells using a fluorescence microscope equipped with a Cy7 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

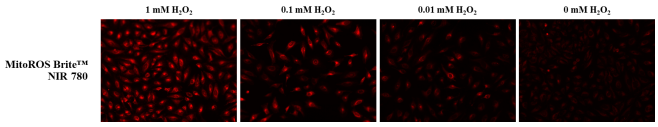

Figure 1. The fluorescence response of MitoROS Brite™ NIR 780 (0.5 μM) to varying concentrations of H2O2 in HeLa cells was assessed. Fluorescence intensities were monitored using a fluorescence microscope equipped with a Cy7 filter.

DISCLAIMER

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