

MitoROS™ 580 *Optimized for Detecting Reactive Oxygen Species (ROS) in Mitochondria*

Catalog number: 16052
Unit size: 500 Tests

Component	Storage	Amount
MitoROS™ 580 *Optimized for Detecting Reactive Oxygen Species (ROS) in Mitochondria*	Freeze (<-15 °C), Minimize light exposure	5 vials (100 tests/vial)

OVERVIEW

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen. Examples include superoxide, hydroxyl radical, singlet oxygen and peroxides. ROS is highly reactive due to the presence of unpaired valence shell electrons. ROS forms as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically. This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. MitoROS™ 580 is a superoxide-sensitive dye that is localized in mitochondria upon loading into live cells. Oxidation of MitoROS™ 580 by superoxide generates red fluorescence. MitoROS™ 580 can be used for monitoring superoxide in mitochondria either with a fluorescence microscope or a fluorescence flow cytometer. MitoROS™ 580 reagent permeates live cells where it selectively targets mitochondria. It is rapidly oxidized by superoxide. It is less likely to be oxidized by other reactive oxygen species (ROS) and reactive nitrogen species (RNS). The oxidized product is highly fluorescent in cells. MitoROS™ 580 provides a valuable tool for investigating oxidative stress in various pathologies.

AT A GLANCE

Important This protocol only provides a guideline, and should be modified according to your specific needs. Treat cells as desired before making the MitoROS™ 580 working solution.

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.

1. MitoROS™ 580 Stock Solution (1000X)

Add 13 µL of DMSO to the MitoROS™ 580 vial and mix well.

Note The unused stock solution can be stored at -20 °C. Protect from light.

PREPARATION OF WORKING SOLUTION

MitoROS™ 580 working solution(2X):

Dilute the DMSO stock solution into Hanks solution with 20 mM Hepes buffer (HHBS) to make 2X working solution.

Note The 2X MitoROS™ 580 working solution is not stable, use it promptly.

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells as desired.
2. Incubate the cells (such as 100 µL/well in 96-well plate) with equal volume of 2X MitoROS™ 580 working solution for 10-30 minutes at 37 °C, protected from light.

Note The final in cell concentration of the MitoROS™ 580 should not exceed 1 X. Concentrations exceeding 1 X can produce cytotoxic effects, including altered mitochondrial morphology and redistribution of fluorescence to nuclei and the cytosol.

Note

Different cells react to MitoROS™ 580 differently, adjust the working concentration accordingly.

3. Wash cells gently three times and replace it with HHBS buffer.
4. Analyze the cells with a proper fluorescence instrument (e.g., a fluorescence microscope, flow cytometer) with Ex/Em = 510/580 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

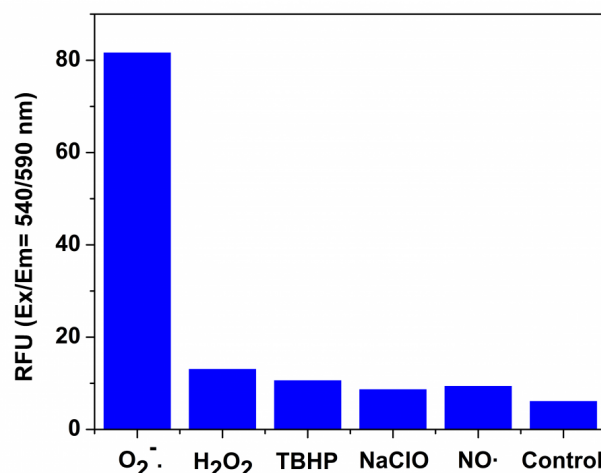


Figure 1. Fluorescence response of MitoROS™ 580 (10 µM) to different reactive oxygen species (ROS) and reactive nitrogen species (RNS). The fluorescence intensities were monitored at Ex/Em = 540/590 nm.

DISCLAIMER

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