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

Catalog number: 16052
Unit size: 500 Tests

| Component | Storage | Amount (Cat No. 16052) |
|---|---|---|
| MitoROS™ 580 *Optimized for Detecting Reactive Oxygen Species (ROS) in Mitochnodria* | 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 has 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 to monitor superoxide in mitochondria either with a fluorescence microscope or 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. MitoROS™ 580 is equivalent to the MitoSOX™ Mitochondrial Superoxide Indicator (#M36008) that is often used for live-cell imaging (MitoSOX™ is the trademark of ThermoFisher).

## 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™ 580 Stock Solution (1000X)**

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

   **Note:** Any unused stock solution can be stored at -20 °C, protected from light.

## PREPARATION OF WORKING SOLUTION

**MitoROS™ 580 Working Solution(2X)**

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

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

## SAMPLE EXPERIMENTAL PROTOCOL

**Important Note**

This protocol is a mere guideline and should be optimized to suit your specific requirements. Prior to making the MitoROS™ 580 working solution, treat cells as desired.

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 1X. Higher concentrations can lead to cytotoxic effects, such as altered mitochondrial morphology and fluorescence redistribution to nuclei and 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

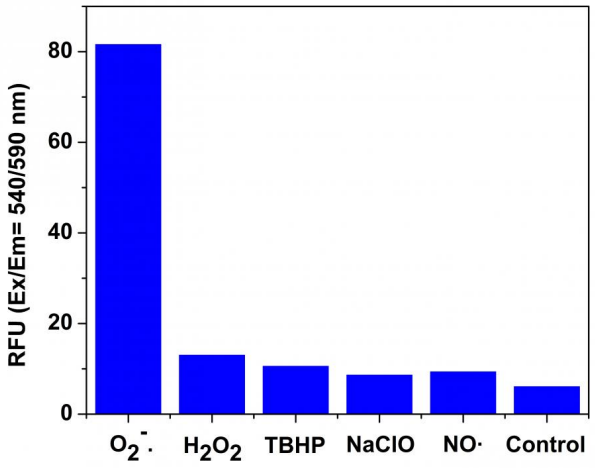


**Figure 1.** Fluorescence response of MitoROS™ 580 (10 µM)to different reactive oxygen species (ROS) and reactive nitrogen species (RNS). The fluorescence intensities were monitored at Ex/Em = 540/590 nm.

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