MitoROS Brite™ 670 *Optimized for Detecting Reactive Oxygen Species (ROS) in Mitochondria*

Catalog number: 15999
Unit size: 1 mg

Component	Storage	Amount (Cat No. 15999)
MitoROS Brite™ 670 *Optimized for Detecting Reactive Oxygen Species (ROS) in Mitochondria*	Freeze (< -15 °C), Minimize light exposure	1 mg

OVERVIEW

Mitochondrial ROS (mtROS or mROS) are reactive oxygen species (ROS) produced in mitochondria. Selective detection of mtROS is a critical task to investigate the cellular functions of mitochondria. The cell-permeant MitoROS Brite™ 670 reagent is cell-permeable and selectively located in mitochondria. It is nonfluorescent and produces bright red fluorescence upon ROS oxidation in mitochondria. The resulting fluorescence can be measured using fluorescence imaging, high-content imaging, microplate fluorometry, or flow cytometry. mtROS was considered to be the by-products of cellular metabolism. However, they are now recognized as important signaling molecules. mtROS is primarily formed during oxidative phosphorylation at the electron transport chain (ETC) on the inner mitochondrial membrane. Electrons leak from complexes I and III, partially reducing oxygen to form superoxide. Superoxide is rapidly converted to hydrogen peroxide by two dismutases: SOD2 in the mitochondrial matrix and SOD1 in intermembrane space. At low levels, mtROS are essential for metabolic adaptation (e.g., in hypoxia). They regulate inflammatory responses triggered by danger signals. High mtROS levels activate apoptosis and autophagy pathways, potentially inducing cell death. Mitochondrial dysfunction leads to increased ROS levels, contributing to aging. mtROS induces cellular senescence, a stress response. Recently it has been reported that monocytes/macrophages in the lungs produce mtROS in COVID-19 patients, affecting disease pathogenicity, thus targeting mtROS could be a therapeutic strategy for novel drugs against coronavirus.

AT A GLANCE

Important Note

Before use, thaw MitoROS Brite™ 670 at room temperature. Once thawed, briefly centrifuge to collect the dried pellet.

Subsection title

1. Prepare the cells in a growth medium.
2. Stain the cells using the MitoROS Brite™ 670 working solution.
3. Treat the cells with your desired test compounds.
4. Monitor fluorescence intensity with a Cy5 filter set or Ex/Em = 650/670 nm.

KEY PARAMETERS

Fluorescence microscope

Emission	Cy5 Filter Set
Excitation	Cy5 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™ 670 Stock Solution

1. Prepare a 5 to 10 mM MitoROS Brite™ 670 stock solution in DMSO.

   Note: Prepare single-use aliquots of the MitoROS Brite™ 670 stock solution and store at ≤ -20°C, protected from light. Avoid freeze-thaw cycles.

PREPARATION OF WORKING SOLUTION

MitoROS Brite™ 670 Working Solution

1. Prepare a 5 to 10 µM MitoROS Brite™ 670 working solution by diluting the MitoROS Brite™ 670 stock solution into Hanks solution with 20 mM Hepes buffer (HHBS).

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

   Note: Protect the working solution from light by covering it with foil or storing it in a dark place.

SAMPLE EXPERIMENTAL PROTOCOL

1. Plate the cells as desired in a 96-well black wall-clear bottom plate.
2. Add 100 µL of the MitoROS Brite™ 670 working solution to the cells.
3. Incubate the cells at 37°C for 30 to 60 minutes, protected from light.

   Note: The optimal concentration and incubation time for MitoROS Brite™ 670 may vary between different cell lines. You may need to test different concentrations to determine the best conditions.

4. Remove the dye working solution and wash the cells twice with HHBS buffer.

5. Treat cells as desired.

6. Remove the treatment and wash cells twice with HHBS buffer.

7. Add HHBS buffer and examine the cells using a fluorescence microscope with a Cy5 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

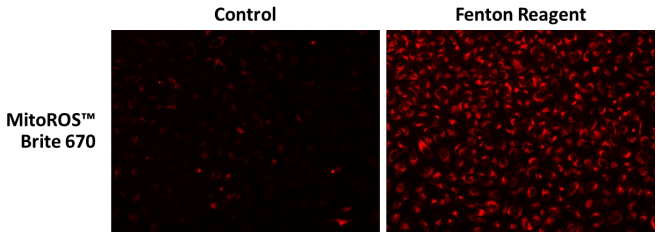

Figure 1. The fluorescence response of MitoROS™ Brite 670 (10 μM) to Fenton Reagent (10 μM CuCl2 and 100 μM H2O2 in HH buffer) was assessed in HeLa cells. Fluorescence intensities were measured using a fluorescence microscope equipped with a Cy5 filter.

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