Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit*Optimized for Microplate Reader*

Catalog number: 22971
Unit size: 200 Tests

Component	Storage	Amount
Component A: MitoROS™ 580	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (20 mL)
Component C: DMSO	Freeze (<-15 °C)	100 µL

OVERVIEW

Mitochondria are major producers of cellular superoxide. The production of low to moderate levels of superoxide is critical for the proper regulation of many essential cellular processes including gene expression, signal transduction, and muscle adaptation to endurance exercise training. Uncontrolled mitochondrial superoxide production can trigger cellular oxidative damage that contributes to the pathogenesis of a wide variety of disorders including cancer, cardiovascular diseases, neurodegenerative diseases and aging. The detection of intracellular mitochondrial superoxide is of central importance to understanding proper cellular redox regulation and the impact of its dysregulation on various pathologies. Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit uses our unique Superoxide Indicator to quantify superoxide level in live cells. MitoROS™ 580 is live-cell permeant and can rapidly and selectively target superoxide in mitochondria. It generates red fluorescence when it reacts with superoxide. The Cell Meter™ Fluorimetric Intracellular Superoxide Detection Kit provides a sensitive, one-step fluorimetric assay to detect mitochondrial superoxide in live cells with one hour incubation. This kit can be used for fluorescence microplate readers and fluorescence microscopy applications.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Treat the cells with test compounds to induce superoxide
3. Add MitoROS™ 580 working solution
4. Stain the cells at 37°C for 30 - 60 minutes
5. Monitor the fluorescence increase (bottom read mode) at Ex/Em= 540/590 nm (Cutoff = 570 nm) or fluorescence microscope with TRITC filter set

Important Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode

Instrument:	Fluorescence microscope
Excitation:	TRITC filter
Emission:	TRITC filter
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.

1. MitoROS™ 580 stock solution (500X):
Add 50 µL of DMSO (Component C) into the vial of MitoROS™ 580 (Component A) and mix well to make 500X MitoROS™ 580 stock solution. Protect from light.

Note 25 µL of 500X MitoROS™ 580 stock solution is enough for 1 plate. For storage, seal tubes tightly.

PREPARATION OF WORKING SOLUTION

Add 25 µL of 500X MitoROS™ 580 stock solution into 10 mL of Assay Buffer (Component B) and mix well to make MitoROS™ 580 working solution.

Note This MitoROS™ 580 working solution is stable for at least 2 hours at room temperature.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells with 10 µL of 10X test compounds (96-well plate) or 5 µL of 5X test compounds (384-well plate) in your desired buffer (such as PBS or HHBS). For control wells (untreated cells), add the corresponding amount of compound buffer.

2. To induce superoxide, incubate the cell plate at 37°C for a desired period of time, protect from light.

   Note We treated HeLa cells with 50 µM Antimycin A (AMA) at 37°C for 30 minutes to induce superoxide. See Figure 1 for details.

3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of MitoROS™ 580 working solution into the cell plate.

4. Incubate the cells at 37°C for 30 to 60 minutes.

5. Monitor the fluorescence increase with a fluorescence microplate reader (bottom read mode) at Ex/Em = 540/590 nm (Cutoff = 570 m) or observe cells using a fluorescence microscope with TRITC filter.

EXAMPLE DATA ANALYSIS AND FIGURES

AMA Treatment **Untreated Control**

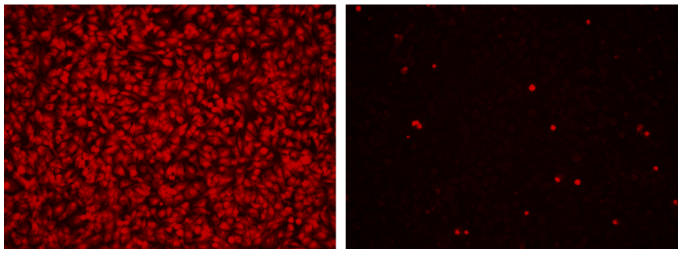

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Figure 1. Fluorescence images of superoxide measurement in HeLa cells using Cell Meter™ Fluorimetric Intracellular Superoxide Detection Kit (Cat#22971). HeLa cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. AMA Treatment: Cells were treated with 50 µM Antimycin A (AMA) at 37 °C for 30 minutes, then incubated with MitoROS™ 580 for 1 hour. Untreated Control: HeLa cells were incubated with MitoROS™ 580 at 37 °C for 1 hour without AMA treatment. The fluorescence signal was measured using fluorescence microscope with a TRITC filter.

DISCLAIMER