Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit*Optimized for Flow Cytometry*

Catalog number: 22970
Unit size: 100 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 (10 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. Therefore, 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 is optimized for flow cytometry applications.

AT A GLANCE

Protocol summary

1. Prepare cells at a density of 0.5 - 1 × 10^6 cells/mL
2. Treat the cells with test compounds to induce superoxide
3. Add 1 µL 500X MitoROS™ 580 into 0.5 mL cell suspension
4. Stain the cells at 37°C for 1 hour
5. Monitor the fluorescence intensity using flow cytometer with FL2 channel

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	575/26 nm filter
Instrument specification(s):	PE channel

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 100 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 For storage, seal tubes tightly.

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. For each sample, prepare cells in 0.5 mL growth medium or buffer of your choice at a density of 5×10^5 to 1×10^6 cells/mL.

   Note Each cell line should be evaluated on an individual basis to determine the optimal cell density for superoxide induction.

2. Treat cells with 25 µL of 20X test compounds in Assay Buffer (Component B) or your desired buffer (such as PBS) to induce superoxide. For control cells (untreated cells), add the corresponding amount of compound buffer.

3. Incubate the cells at 37°C for at least 30 minutes or a desired period of time, protected from light.

   Note Jurkat cells were treated with 50 µM Antimycin A (AMA) at 37°C for 30 minutes to induce superoxide. See Figure 1 for details. Pyocyanin (50 µM) or H_2O_2 (1 mM) can also be used to induce superoxide.

4. Add 1 µL of 500X MitoROS™ 580 stock solution into 0.5 mL cell suspension.

5. Incubate at 37°C for 1 hour.

   Note For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact, and wash the cells once with serum-containing media prior to incubation with MitoROS™ 580. The appropriate incubation time depends on the individual cell type and test compound used. Optimize the incubation time for each experiment.

6. Monitor the fluorescence intensity using a flow cytometer with FL2 channel (Ex/Em=488/590 nm). Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

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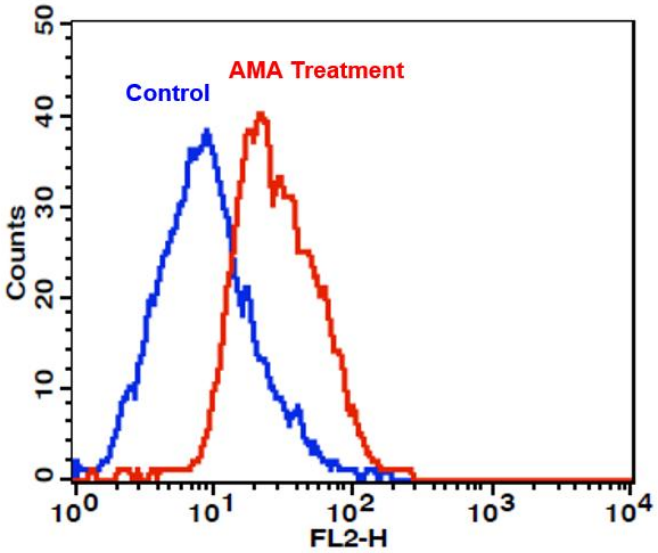

Figure 1. Detection of intracellular superoxide in Jurkat cells using Cell Meter™ Fluorimetric Intracellular Superoxide Detection Kit. AMA Treatment (Red): Cells were treated with 50 µM Antimycin A (AMA) at 37 °C for 30 minutes, then incubated with MitoROS™ 580 for 1 hour. Control (Blue): Cells were incubated with MitoROS™ 580 at 37 °C for 1 hour without AMA treatment. The fluorescence signal was monitored at FL2 channel using a flow cytometer (BD FACSCalibur).

DISCLAIMER

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