Cell Meter™ Fluorimetric Intracellular Superoxide Detection Kit

**Red Fluorescence Optimized for Flow Cytometry **

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22970 (100 assays)	Frozen	Flow Cytometer

Introduction

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 Intracellular Superoxide Detection 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, and can be easily read at Ex/Em = 540/590 nm. 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.

Kit Components

Components	**Amount**
Component A: MitoROS™ 580	1 vial
Component B: Assay Buffer	1 bottle (10 mL)
Component C: DMSO	100 µL

Assay Protocol for Flow Cytometer

Brief Summary

Prepare cells at a density of 0.5 - 1 × 10^6 cells/mL → Treat the cells with test compounds to induce superoxide → Add 1 µL 500X MitoROS™ 580 into 0.5 mL cell suspension → Stain the cells at 37 ºC for 1 hour → Monitor the fluorescence intensity at the FL2 channel

Note: Thaw all the components at room temperature before use.

1. Prepare cells:

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. Prepare MitoROS™ 580 stock solution:

Add 100 µL of DMSO (Component C) into the vial of MitoROS™ 580 (Component A), and mix them well.

Note: 1 µL of reconstituted MitoROS™ 580 stock solution is for 0.5 mL cells. Unused portion can be aliquoted and stored at < -20 °C for more than one month if the tubes are sealed tightly and kept from light. Avoid repeated freeze-thaw cycles.

3. Run superoxide assay:

3.1 Teat 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.

3.2 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 ºC for 30 minutes to induce superoxide. See Figure 1 for details. Pyocyanin (50 µM) or H2O2 (1mM)can also be used to induce superoxide.

3.3 Add 1 µL of 500X MitoROS™ 580 (Component A) into 0.5 mL cell suspension, and incubate at 37 ºC for 1 hour.
Note 1: 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.
Note 2: The appropriate incubation time depends on the individual cell type and test compound used. Optimize the incubation time for each experiment.

3.4 Incubate the cells at 37 °C for 30 min to 1 hour. Monitor the fluorescence intensity at the FL2 channel (Ex/Em=488/590 nm) using a flow cytometer. Gate on the cells of interest, excluding debris.

Data Analysis

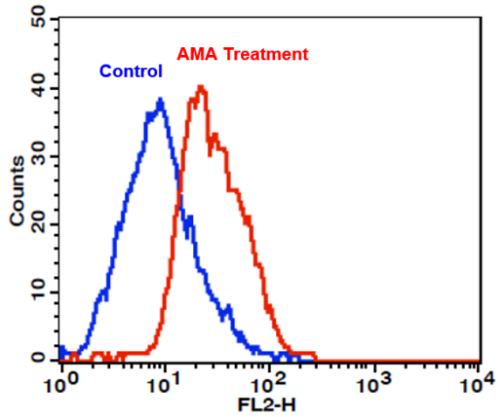

Figure 1. Detection of intracellular superoxide in Jurkat cells using Cell Meter™ Fluorimetric Intracellular Superoxide Detection Kit (Cat#22970). AMA Treatment (Red): Cells were treated with 50 µM Antimycin A (AMA) at 37 ºC for 30 minutes, then incubated with MitoROS™ 580 for 1 hour. Control (Blue): Cells were incubated with MitoROS™ 580 at 37 ºC for 1 hour without AMA treatment. The fluorescence signal was monitored at FL2 channel using a flow cytometer (BD FACSCalibur).

References

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2. Brand, M.D., C. Affourtit, T.C. Esteves, K. Green, A.J. Lambert, S. Miwa, J.L. Pakay, N. Parker. 2004. Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. Free Radic. Biol. Med. 37:755–767.
3. Mukhopadhyay P., Rajesh M, Hasko G, Hawkins BJ, Madesh M, Pacher P. 2007. Simultaneous detection of apoptosis and mitochondrial superoxide production in live cells by flow cytometry and confocal microscopy. Nat. Protoc. 2:2295–301.
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