Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay Kit *Green Fluorescence*

Catalog number: 16060
Unit size: 200 Tests

Component	Storage	Amount
Component A: MitoROS™ 520	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)	1 vial (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 great importance to understanding proper cellular redox regulation and the impact of its dysregulation on various pathologies. Cell Meter™ Fluorimetric Intracellular Superoxide Detection Kit uses MitoROS™ 520, our unique superoxide indicator, to quantify superoxide level in live cells. MitoROS™ 520 is cell permeant and can rapidly and selectively detect superoxide in mitochondria. It generates green fluorescence upon reacting with superoxide. The Cell Meter™ Fluorimetric Mitochondrial Superoxide Activity Assay 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 flow cytometry and fluorescence microscopy applications.

AT A GLANCE

Protocol summary (Fluorescence Microscope)

1. Prepare cells in growth medium
2. Treat the cells with test compounds to induce superoxide
3. Add MitoROS™ 520 working solution
4. Incubate the cells at 37°C for 1 hour
5. Monitor the fluorescence using FITC fliter set

Protocol summary (Flow Cytometry)

1. Prepare cells in growth medium
2. Treat the cells with test compounds to induce superoxide
3. Add MitoROS™ 520 stock solution and incubate the cells at 37°C for 1 hour
4. Monitor the fluorescence intensity with a flow cytometer using 530/30 nm filter (FITC channel)

Important Thaw all the components at room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	FITC filter set
Emission:	FITC filter set
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	FITC filter set

Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	530/30 nm filter
Instrument specification(s):	FITC 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™ 520 stock solution (500X):
Add 50 µL of DMSO (Component C) into the vial of MitoROS™ 520 (Component A) and mix well.

Note 25 µL of reconstituted MitoROS™ 520 stock solution is enough for 1 plate.

Note 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.

PREPARATION OF WORKING SOLUTION

Only for Fluorescence Microscope
Add 5 µL of 500X DMSO reconstituted MitoROS™ 520 stock solution into 2 mL of Assay Buffer (Component B) and mix well.

Note This working solution is not stable and needs to be prepared freshly before use.

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

For Fluorescence Microscopes/96-Well Microplates:

1. Treat cells with 10 µL of 10X test compounds (96-well plate) or 5 µL of 5X test compounds (384-well plate) in medium or 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 at 37°C for a desired period of time, protected from light.

   Note We treated RAW 264.7 macrophage cells with 5 µM Antimycin A (AMA) at 37°C for 2 hours 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™ 520 working solution into the cell plate.

4. Incubate the cells at 37°C for 1 hour, and take images using fluorescence microscope with a FITC filter set.

For Flow Cytometers:

1. Treat cells as desired.

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

   Note We treated Jurkat cells with 50 µM Antimycin A (AMA) at 37°C for 2 hours to induce superoxide.

3. Add 1 µL/0.5 mL cells of MitoROS™ 520 stock solution (500X) into the cells.

4. Incubate the cells in a 5% CO_2, 37°C incubator for 1 hour, and monitor the fluorescence intensity using a flow cytometry with 530/30 nm filter (FITC channel).

EXAMPLE DATA ANALYSIS AND FIGURES

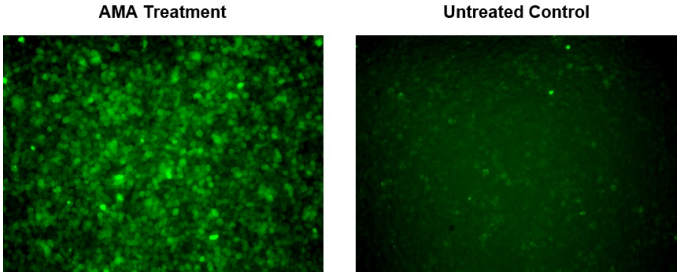

Figure 1. Fluorescence images of superoxide measurement in macrophage cells using cat#16060. RAW 264.7 cells at 100,000 cells/well/100 µL were seeded overnight in a 96-well black wall/clear bottom plate. AMA Treatment: Cells were treated with 5 µM Antimycin A (AMA) at 37 °C for 2 hours, then incubated with MitoROS™ 520 for 1 hour. Untreated Control: RAW 264.7 cells were incubated with MitoROS™ 520 at 37 °C for 1 hour without AMA treatment. The fluorescence signal was measured using fluorescence microscope with a FITC filter

DISCLAIMER