Cell Meter™ Mitochondrion Membrane Potential Assay Kit *Orange Fluorescence Optimized for Microplate Reader*

Catalog number: 22805
Unit size: 500 Tests

Component	Storage	Amount
Component A: 200X MitoTell™ Orange	Freeze (<-15 °C), Minimize light exposure	1 vial (250 µL)
Component B: Assay Buffer A	Freeze (<-15 °C), Minimize light exposure	1 bottle (50 mL)
Component C: Assay Buffer B	Freeze (<-15 °C), Minimize light exposure	1 bottle (25 mL)

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cellular functions. There are a variety of parameters that can be used. This particular kit is designed to detect cell apoptosis by measuring the loss of the mitochondrial membrane potential (MMP). The collapse of mitochondrial membrane potential coincides with the opening of the mitochondrial permeability transition pores, leading to the release of cytochrome C into the cytosol, which in turn triggers other downstream events in the apoptotic cascade. This fluorimetric assay uses our proprietary cationic MitoLite™ Orange for the detection of the mitochondrial membrane potential change in cells. In normal cells, the orange fluorescence intensity is increased when MitoLite™ Orange is accumulated in the mitochondria. However, in apoptotic cells, the fluorescence intensity of MitoLite™ Orange is decreased following the collapse of MMP. Cells stained with MitoLite™ Orange can be fluorometrically monitored. Our Cell Meter™ Orange Mitochondrial Membrane Potential Assay Kit provides all the essential components with an optimized assay method. The kit can be used for screening activators and inhibitors of apoptosis. And the assay can be performed in a convenient 96-well and 384-well fluorescence microtiter-plate format without a wash step.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Add test compounds
3. Add MitoTell™ Orange working solution (100 µL/well/ 96-well plate or 25 µL/well/384-well plate)
4. Incubate the plate in a 5% CO_2, 37°C incubator for 15 - 30 minutes
5. Add Assay Buffer B (50 µL/well/96-well plate or 12.5 µL/well/384-well plate)
6. Monitor the fluorescence increase (bottom read mode) at Ex/Em = 540/590 nm (Cutoff = 570 nm)

Important Thaw all the kit 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

PREPARATION OF WORKING SOLUTION

Add 50 µL of 200X MitoTell™ Orange (Component A) into 10 mL of Assay Buffer A (Component B) and mix well to make MitoTell™ Orange working solution. Protect from light.

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 test compounds for a desired period of time to induce apoptosis, and set up parallel control experiments.

   For Negative Control: Treat cells with vehicle only.

   For Positive Control: Treat cells with FCCP or CCCP at 5 - 50 µM in a 5% CO_2, 37°C incubator for 15 to 30 minutes.

   Note CCCP or FCCP can be added simultaneously with MitoTell™ Orange. To get the best result, titration of the CCCP or FCCP may be required for each individual cell line.

2. Remove the cell medium.

   Note It is important to remove the cell medium before adding MitoTell™ Orange working solution.

3. Add 100 µL/well/96-well plate or 25 µL/well/384-well plate of MitoTell™ Orange working solution into the cell plate.

4. Incubate the plate in a 5% CO_2, 37°C incubator for 15 - 30 minutes, protected from light.

   Note The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

5. Add 50 µL/well/96-well plate or 12.5 µL/well/384-well plate of Assay Buffer B (Component C) into the cell plate.

   Note DO NOT wash the cells after loading. For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after adding Assay Buffer B (Component C).

6. Monitor the fluorescence intensity with a fluorescence microplate reader (bottom read mode) at Ex/Em = 540/590 nm (Cutoff = 570 nm) either using the endpoint mode or using the kinetic mode 10 to 30 minutes after adding Assay Buffer B (Component C).

EXAMPLE DATA ANALYSIS AND FIGURES

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

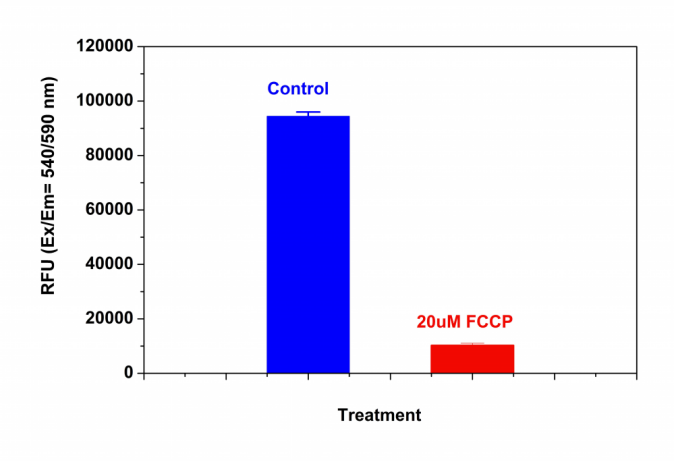

Figure 1. The decrease in the fluorescence intensity of MitoTell™ Orange with the addition of FCCP in HeLa cells. HeLa cells were dye loaded with MitoTell™ Orange alone or in the presence of 20 µM FCCP for 15 minutes. The fluorescence intensity of MitoTell™ Orange was measured 30 minutes after adding assay buffer with a FlexStation™ microplate reader (Molecular Devices) at Ex/Em = 540/590 nm (Cutoff = 570 nm, bottom read mode).

DISCLAIMER