Cell Meter™ Mitochondrion Membrane Potential Assay Kit *Red Fluorescence Optimized for Flow Cytometry*

Catalog number: 22806
Unit size: 100 tests

Component	Storage	Amount
Component A: 500X MitoTell™ Red in DMSO	Freeze (<-15 °C), Minimize light exposure	Vial (100 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	Bottle (50 mL)

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cell viability. 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 MMP 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. Our Cell Meter™ Mitochondrial Membrane Potential Assay Kit provides all the essential components with an optimized assay method. This fluorimetric assay uses our proprietary cationic MitoTell™ Red for the detection of apoptosis in cells with the loss of MMP. In normal cells, the red fluorescence intensity is increased when MitoTell™ Red is accumulated in the mitochondria. However, in apoptotic cells, the fluorescence intensity of MitoTell™ Red decreases following the collapse of MMP. Cells stained with MitoTell™ Red can be visualized with a flow cytometer at APC or Cy5 channel. The kit is optimized for screening apoptosis activators and inhibitors with a flow cytometer.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds at the density of 5 × 10^5 to 1 × 10^6 cells/mL
2. Add 1 µL of 500X MitoTell™ Red into 0.5 mL of cell solution
3. Incubate the cells in a 37 °C, 5% CO_2 incubator for 15 - 30 minutes
4. Pellet the cells, and resuspend the cells in 0.5 mL of assay buffer
5. Analyze cells using flow cytometer with APC or Cy5 channel

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	640 nm laser
Emission:	660/20 nm filter
Instrument specification(s):	APC channel

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 of warm medium or buffer of your choice at the 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 apoptosis induction.

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

   Note We treated Jurkat cells with 20µM CCCP for 15 min at 37ºC to change the mitochondrial membrane potential. See Figure 1 for details. CCCP or FCCP can be added simultaneously with MitoTell™ Red. To get the best result, titration of the CCCP or FCCP may be required for each individual cell line.

3. Add 1 µL of 500X MitoTell™ Red (Component A) into the treated cells.

4. Incubate the cells in a 37 °C, 5% CO_2 incubator for 15 to 30 minutes.

   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 the incubation with MitoTell™ Red.

5. Centrifuge the cells at 800 rpm for 4 minutes, and then re-suspend cells in 0.5 mL of Assay Buffer (Component B) or buffer of your choice.

6. Monitor the fluorescence intensity using a flow cytometer wih APC or Cy5 channel. Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

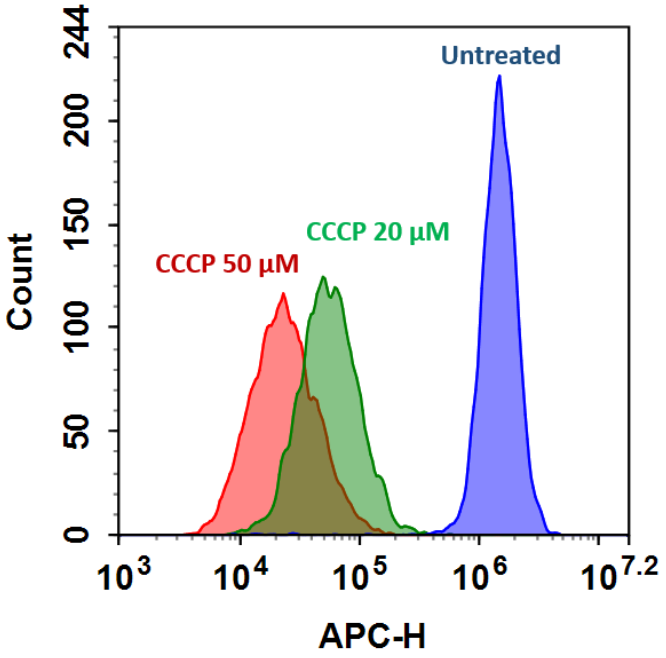

Figure 1.

The decrease in fluorescence intensity of MitoTell™ Red in response to CCCP treatment in Jurkat cells. Jurkat cells were loaded with MitoTell™ Red alone (Blue) or in the presence of 20 µM (Green) or 50 µM CCCP (Red) for 30 minutes. The fluorescence intensity of MitoTell™ Red was measured using ACEA NovoCyte flow cytometer at APC channel.

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