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

Catalog number: 22807
Unit size: 500 tests

Component	Storage	Amount
Component A: 200X MitoTell™ Red	Freeze (<-15 °C), Minimize light exposure	Vial (250 µL)
Component B: Assay Buffer A	Freeze (<-15 °C), Minimize light exposure	Bottle (50 mL)
Component C: Assay Buffer B	Freeze (<-15 °C), Minimize light exposure	Bottle (25 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 either visualized with a fluorescence microscope Cy5 channel, or with a fluorescence microplate reader. The kit is optimized for screening apoptosis activators and inhibitors with a fluorescence microplate reader. 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™ Red 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 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 = 610/650 nm (Cutoff = 630 nm) or fluorescence microscope with Cy5 filter

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	610 nm
Emission:	650 nm
Cutoff:	630 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode
Instrument:	Fluorescence microscope
Excitation:	Cy5 filter
Emission:	Cy5 filter
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

Add 50 µL of 200X MitoTell™ Red (Component A) into 10 mL of Assay Buffer A (Component B) and mix well to make MitoTell™ Red 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.

   Note We treated HeLa cells with 20 µM CCCP for 15 minutes 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.

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

3. Incubate the plate at 37ºC 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.

4. 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).

5. Monitor the fluorescence intensity with a fluorescence microplate reader (bottom read mode) at Ex/Em = 610/650 nm (Cutoff = 630 nm) 10 to 30 minutes after adding Assay Buffer B (Component C) or observe the fluorescence signal under a fluorescence microscope with Cy5 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

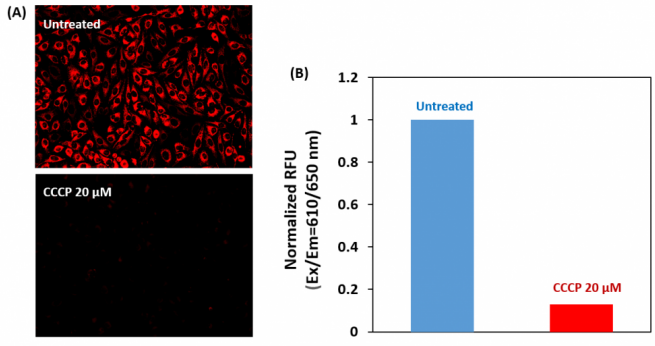

Figure 1. Hela cells were dye-loaded with MitoTell™ Red alone or in the presence of 20 µM CCCP for 30 minutes. The fluorescence intensity of MitoTell™ Red was

measured 5 minutes after adding Assay Buffer B (Component C) using (A) a fluorescence microscope with Cy5 filter set or (B) FlexStation microplate reader at Ex/Em = 610/650 nm (Cutoff = 630 nm, bottom read mode).

DISCLAIMER