Cell Meter™ Mitochondrial Membrane Potential Assay Kit

Red Fluorescence Optimized for Microplate Reader

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22807 (500 Tests)	Keep in freezer Avoid exposure to light	Fluorescence microplate reader Fluorescence microscope

Introduction

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 monitored at Ex/Em = 610/650 nm 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.

Kit Components

Components	**Amount**
Component A: 200X MitoTell™ Red	1 vial (250 µL)
Component B: Assay Buffer A	1 bottle (50 mL)
Component C: Assay Buffer B	1 bottle (25 mL)

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells → Add test compounds → Add MitoTell™ Red dye-loading solution (100 µL/well/ 96-well plate or 25 µL/well/384-well plate) → Incubate at room temperature for 30 minutes → Add Assay Buffer B (50 µL/well/96-well plate or 12.5 µL/well/384-well plate) → Monitor the fluorescence increase at Ex/Em = 610/650 nm

1. Prepare cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 20,000 to 80,000 cells/well/100 µL for a 96-well plate or 5,000 to 20,000 cells/well/25 µL for a 384-well plate.

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellets in culture medium at 100,000-200,000 cells/well/90 µL for a 96-well poly-D lysine plate or 25,000-50,000 cells/well/20 µL for a 384-well poly-D lysine plate. Centrifuge the plates at 800 rpm for 2 minutes with brake off prior to the experiments.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction

2. Prepare MitoTell™ Red dye-loading solution:

2.1 Thaw all the kit components at room temperature before use.

2.2 Add 25 µL of 200X MitoTell™ Red (Component A) into 10 mL of Assay Buffer A (Component B), and mix them well.
Note: Aliquot and store the unused 200X MitoTell™ Red (Component A) at -20°C. Avoid repeated freeze/thaw cycles.

3. Run MitoTell™ Red Assay:

3.1 Treat cells with test compounds for a desired period of time to induce apoptosis, and set up parallel control experiments.
Note 1: We treated HeLa cells with 20 µM CCCP for 15 minutes to change the mitochondrial membrane potential. See Figure 1 for details.
Note 2: 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.

3.2 Add 100 µL/well/96-well plate or 25 µL/well/384-well plate of MitoTell™ Red dye-loading solution (from Step 2.2) into the cell plate.

3.3 Incubate the dye-loading 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.

3.4 Add 50 µL/well/96-well plate or 12.5 µL/well/384-well plate of Assay Buffer B (Component C) into the dye-loaded cell plate (from Step 3.3).
Note 1: DO NOT wash the cells after loading.
Note 2: 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).

3.5 Monitor the fluorescence intensity at Ex/Em = 610/650 nm (bottom read) 10-30 minutes after adding Assay Buffer B using a fluorescence microplate reader, or using a fluorescence microscope with a Cy5 filter set.

Data Analysis

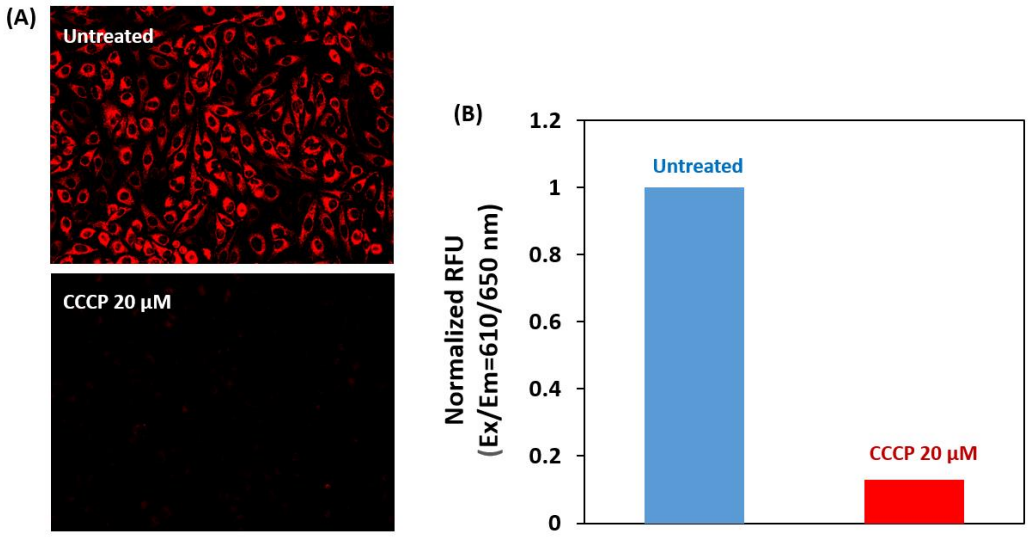

Figure 1. Hela cells were dye-loaded with MitoTell™ Red alone or in the presence of 20 µM CCCP for 30 minutes. The fluorescence intensity of MitoTell™ Red was measured 5 minutes after adding Assay Buffer B (Component C) using (A) a fluorescence microscope with Cy5 filter set or (B) FlexStation microplate reader at Ex/Em = 610/650 nm (cut off 630 nm, bottom read).