Cell Meter™ Mitochondrial Membrane Potential Assay Kit

Red Fluorescence Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22806 (100 assays)	Keep in freezer and avoid exposure to light	Flow cytometer

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 visualized with a flow cytometer at APC or Cy5 channel. The kit is optimized for screening apoptosis activators and inhibitors with a flow cytometer.

Kit Components

Components	**Amount**
Component A: 500X MitoTell™ Red in DMSO	1 vial (100 µL)
Component B: Assay Buffer	1 bottle (50 mL)

Assay Protocol for Flow Cytometer

Brief Summary

Prepare cells with test compounds at the density of 5×10^5 to 1×10^6 cells/mL → Add 1 µL of 500X MitoTell™ Red into 0.5 mL of cell suspension → Incubate at room temperature for 15 - 30 minutes→ Pellet the cells and resuspend the cells in 0.5 mL of assay buffer → Analyze cells by using a flow cytometer

Note: Thaw all the kit components at room temperature before use.

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 1: We treated Jurkat cells with 20µM CCCP for 15 min at 37ºC to change the mitochondrial membrane potential. See Figure 1 for details.
   Note 2: CCCP or FCCP can be added simultaneously with MitoTell™ Red (See Step 3). 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 (from Step 2), and incubate the cells in a 37 ºC, 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.

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

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

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

Data Analysis

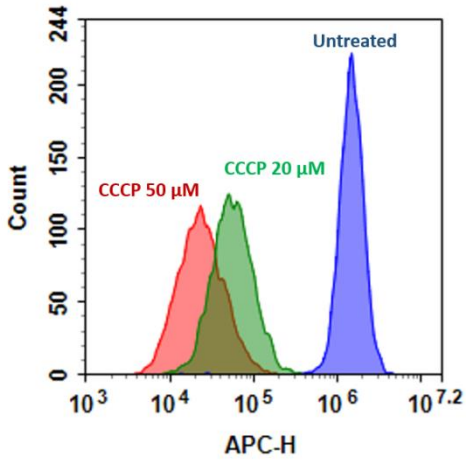

Figure 1. The decrease in fluorescence intensity of MitoTell™ Red is in response to CCCP treatment in Jurkat cells. Jurkat cells were loaded with MitoTell™ Red alone (Blue) or in the presence of 20 µM (Green) or 50 µM CCCP (Red) for 30 minutes. The fluorescence intensity of MitoTell™ Red was measured using ACEA NovoCyte flow cytometer in APC channel.

Warning: This kit is only sold to to our authorized distributors and end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at info@aatbio.com if you have any questions.