Cell Meter™ NIR Mitochondrial Membrane Potential Assay Kit

Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22802 (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. 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 fluorometric assay uses our proprietary cationic MitoLite™ NIR for the detection of apoptosis in cells with the loss of mitochondrial membrane potential. In normal cells, the red fluorescence intensity is increased when MitoLite™ NIR is accumulated in the mitochondria. However, in apoptotic cells, MitoLite™ NIR stain intensity is decreased following the collapse of MMP. Cells stained with MitoLite™ NIR can be visualized with a flow cytometer at red excitation and far red emission (FL4 channel). Our Cell Meter™ NIR Mitochondrial Membrane Potential Assay Kit provides all the essential components. The kit can be used together with other reagents, such as blue laser excited propidium iodide and Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (22790) for studying cell vitality and apoptosis. The kit is optimized for screening apoptosis activators and inhibitors with a flow cytometer.

Kit Components

Components	**Amount**
Component A: 200X MitoLite™ NIR in DMSO	1 vial (500 µL)
Component B: Assay Buffer	1 bottle (100 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 5 µL of 200X MitoLite™ NIR into 1 mL of cell solution → Incubate at room temperature for 15-30 minutes → Pellet the cells, and resuspend the cells in 1 mL of growth medium → Analyze cells by using a flow cytometer (FL4 channel)

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

1. For each sample, prepare cells in 1 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.
   For Negative Control: Treat cells with vehicle only.
   For Positive Control: Treat cells with FCCP or CCCP at 5-50 µM in a 37 °C, 5% CO_2 incubator for 15 to 30 minutes.
   Note: CCCP or FCCP can be added simultaneously with MitoLite™ NIR (See Step 3). To get the best result, titration of the CCCP or FCCP may be required for each individual cell line.

3. Add 5 µL of 200X MitoLite™ NIR (Component A) into the treated cells (from Step 2), and 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 MitoLite™ NIR dye-loading solution.

4. Centrifuge the cells at 1000 rpm for 4 minutes, and then re-suspend cells in 1 mL of Assay Buffer (Component B) or buffer of your choice.

5. Monitor the fluorescence intensity by using a flow cytometer in the FL 4 channel (Ex/Em = 635/660 nm). Gate on the cells of interest, excluding debris.

Data Analysis

In live non-apoptotic cells, the red fluorescence intensity is increased when MitoLite™ NIR is accumulated in the mitochondria. In apoptotic and dead cells, MitoLite™ NIR stain intensity is decreased following the collapse of MMP.

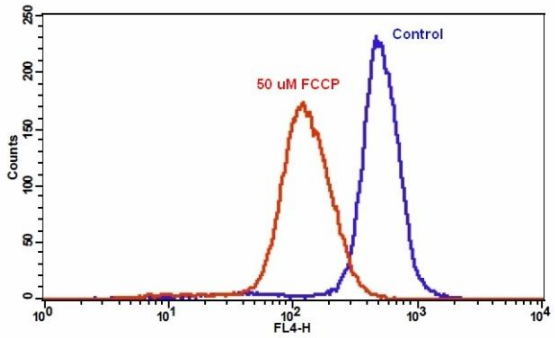

Figure 1 The decrease in fluorescence intensity of MitoLite™ NIR with the addition of FCCP in Jurkat cells. Jurkat cells were loaded with MitoLite™ NIR alone (blue line) or in the presence of 50 µM FCCP (red line) for 10 minutes. The fluorescence intensity of MitoLite™ NIR was measured with a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using FL4 channel.

Warning: This kit is only sold to 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.