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

Catalog number: 22802
Unit size: 100 Tests

Component	Storage	Amount
Component A: 200X MitoLite™ NIR in DMSO	Freeze (<-15 °C), Minimize light exposure	1 vial (500 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (100 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 for monitoring cell viability. This particular kit is designed to monitor cell apoptosis through 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. Our Cell Meter™ NIR Membrane Potential Detection Kit provides all the essential components with an optimized assay method for the detection of apoptosis in cells with the loss of mitochondrial membrane potential. This fluorometric assay is based on the detection of the mitochondrial membrane potential in cells by our proprietary cationic MitoLite NIR™ dye. In normal cells, MitoLite NIR™ accumulates primarily in mitochondria, however, in apoptotic cells, MitoLite NIR™ staining intensity decreases. Cells stained with MitoLite NIR™ can be visualized by flow cytometry with red excitation and far red emission (FL4 channel). The kit can be paired with other reagents, such as blue-excited propidium iodide and Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (#22803) for multi-parametric study of cell vitality and apoptosis. The kit is optimized for screening of apoptosis activators and inhibitors by flow cytometry.

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 5 µL of 200X MitoLite™ NIR into 1 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 1 mL of growth medium
5. Analyze cells using flow cytometer with FL4 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 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 oC, 5% CO_2 incubator for 15 to 30 minutes.

   Note CCCP or FCCP can be added simultaneously with MitoLite™ NIR. 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.

4. Incubate the cells in a 37 °C, 5% CO2 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.

5. 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.

6. Monitor the fluorescence intensity using a flow cytometer wih FL4 channel (Ex/Em = 635/660 nm). Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

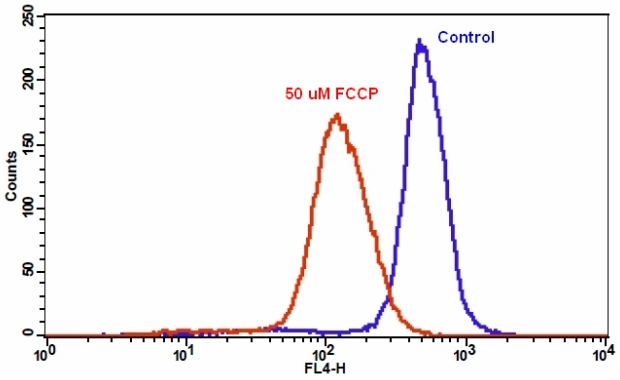

Figure 1. The decrease in fluorescence intensity of MitoLite™ NIR with the addition of FCCP in Jurkat cells. Jurkat cells were loaded with MitoLite™ NIR alone (blue line) or in the presence of 50 µM FCCP (red line) for 10 minutes. The fluorescence intensity of MitoLite™ NIR was measured with a FACSCalibur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

DISCLAIMER