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

Catalog number: 22803
Unit size: 500 Tests

Component	Storage	Amount
Component A: 200X MitoLite™ NIR in DMSO	Freeze (<-15 °C), Minimize light exposure	1 vial (250 µL)
Component B: Assay Buffer A	Freeze (<-15 °C), Minimize light exposure	1 bottle (50 mL)
Component C: Assay Buffer B	Freeze (<-15 °C), Minimize light exposure	1 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 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 Mitochondria 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 monitored fluorimetrically at 660-680 nm with excitation of 620-640 nm. The kit can be used for screening of apoptosis activators and inhibitors. The assay can be performed in a convenient 96-well and 384-well fluorescence microtiter-plate format.

AT A GLANCE

Protocol summary

1. Prepare cells
2. Add test compounds
3. Add MitoLite™ NIR working solution (100 µL/well/ 96-well plate or 25 µL/well/384-well plate)
4. Incubate at 37°C, 5% CO_2 incubator for 30 - 60 minutes
5. Add Assay Buffer B (50 µL/well/96-well plate or 12.5 µL/well/384-well plate)
6. Monitor fluorescence intensity (Bottom read mode) at Ex/Em = 640/680 nm (Cutoff = 665 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	640 nm
Emission:	680 nm
Cutoff:	665 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode

PREPARATION OF WORKING SOLUTION

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

   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. To get the best result, titration of the CCCP or FCCP may be required for each individual cell line.

2. Remove the cell medium before adding MitoLite™ NIR working solution.

   Note It is important to remove the cell medium before adding MitoLite™ NIR working solution.

3. Add 100 µL/well/96-well plate or 25 µL/well/384-well plate of MitoLite™ NIR working solution into the cell plate.

4. Incubate the plate in a 37°C, 5% CO_2 incubator for 30 - 60 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.

5. 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 before monitoring the fluorescence signal.

   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).

6. Monitor the fluorescence intensity with a fluorescence microplate reader (bottom read mode) at Ex/Em = 640/680 nm (Cutoff = 665 nm) either using the endpoint mode or using the kinetic mode 10 to 30 minutes after adding Assay Buffer B (Component C).

EXAMPLE DATA ANALYSIS AND FIGURES

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

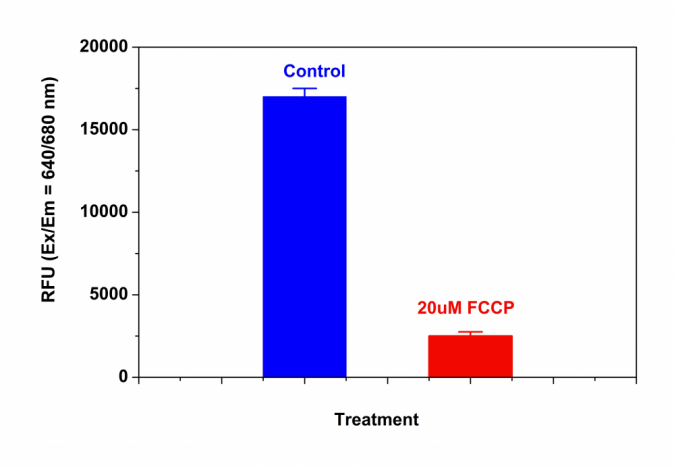

Figure 1. The decrease in MitoLite™ NIR fluorescence with the addition of FCCP in HeLa cells. HeLa cells were loaded with MitoLite™ NIR alone or in the presence of 20 μM FCCP for 15 minutes. The fluorescence intensity of MitoLite™ NIR was measured 30 minutes after adding assay buffer with a FlexStation™ microplate reader (Molecular Devices) at Ex/Em = 640/680 nm (Cutoff = 665 nm, bottom read mode).

DISCLAIMER