Cell Meter™ NIR Mitochondrial Membrane Potential Assay Kit

Optimized for Microplate Reader Assays

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22803 (5 plates)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

Introduction

Our Cell Meter™ assay kits are a set of tools for monitoring cellular functions. 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 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 Assay Kit provides all the essential components with an optimized assay method. This fluorometric assay uses our proprietary cationic MitoLite™ NIR for the detection of the mitochondrial membrane potential change in cells. 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 monitored fluorimetrically at 660-680 nm with excitation at 620-640 nm. The kit can be used for screening apoptosis activators and inhibitors. The assay can be performed in a convenient 96-well and 384-well fluorescence microtiter-plate format.

Kit Components

Components	**Amount**
Component A: 200X MitoLite™ NIR in DMSO	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 MitoLite™ NIR dye-loading solution (100 µL/well/ 96-well plate or 25 µL/well/384-well plate) → Incubate at 37 °C, 5% CO_2 incubator for 30-60 minutes → Add Assay Buffe B (50 µL/well/96-well plate or 12.5 µL/well/384-well plate) → Monitor fluorescence intensity at Ex/Em = 640/680 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 plate 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 MitoLite™ NIR dye-loading solution:

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

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

2.2 Add 50µL of 200X MitoLite™ NIR (Component A) into 10 mL of Assay Buffer A (Component B), and mix them well.
Note: Aliquot and store the unused 200X MitoLite™ NIR (Component A) at -20°C. Avoid repeated freeze/thaw cycles.

3. Run MitoLite™ NIR Assay:

3.1 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.

3.2 Remove the cell medium before adding MitoLite™ NIR dye-loading solution (See Step 3.3).
Note: It is important to remove the cell medium before adding MitoLite™ NIR dye-loading solution.

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

3.4 Incubate the dye-loading 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.

3.5 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 3.4) before monitoring the fluorescence signal.
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).

3.6 Monitor the fluorescence intensity at Ex/Em = 640/680 nm (bottom read) either using the endpoint mode or using the kinetic mode 10 to 30 minutes after Step 3.5.

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.

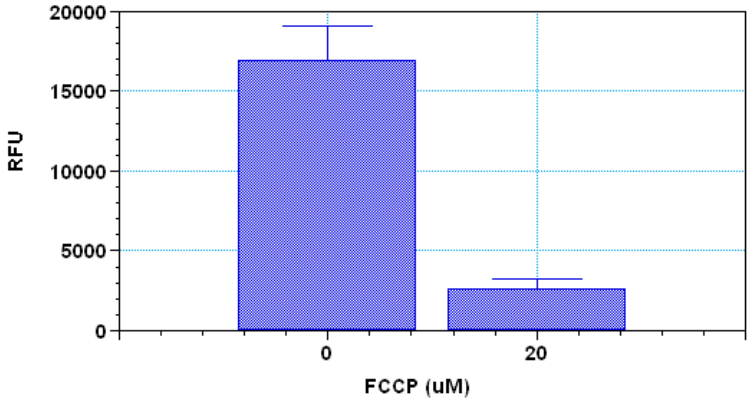

Figure 1. The decrease in MitoLite™ NIR fluorescence with the addition of FCCP in Hela cells. Hela cells were dye loaded with MitoLite™ NIR alone or in the presence of 20 µM FCCP for 15 minutes. The fluorescence intensity of MitoLite™ NIR was measured 30 minutes after adding Assay buffer B (Component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