

Cell Meter™ Mitochondrial Membrane Potential Assay Kit

Orange Fluorescence Optimized for Microplate Reader

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 22805 (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.

This fluorimetric assay uses our proprietary cationic MitoLite™ Orange for the detection of the mitochondrial membrane potential change in cells. In normal cells, the orange fluorescence intensity is increased when MitoLite™ Orange is accumulated in the mitochondria. However, in apoptotic cells, the fluorescence intensity of MitoLite™ Orange is decreased following the collapse of MMP. Cells stained with MitoLite™ Orange can be fluorometrically monitored at Ex/Em = 540/590 nm. Our Cell Meter™ Mitochondrial Membrane Potential Assay Kit provides all the essential components with an optimized assay method. The kit can be used for screening activators and inhibitors of apoptosis. And the assay can be performed in a convenient 96-well and 384-well fluorescence microtiter-plate format without a wash step.

Kit Components

Components	Amount
Component A: 200X MitoLite™ Orange	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™ Orange dye-loading solution (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Incubate at room temperature for 1 hour
→ Add Assay Buffer B (50 µL/well/96-well plate or 12.5 µL/well/384-well plate)
→ Monitor the fluorescence increase at Ex/Em = 540/590 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 plates 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™ Orange dye-loading solution:

- 2.1 Thaw all the kit components at room temperature before use.
- 2.2 Add 50 µL of 200X MitoLite™ Orange (Component A) into 10 mL of Assay Buffer A (Component B), and mix them well.

Note: Aliquot and store the unused 200X MitoLite™ Orange (Component A) at -20°C. Avoid repeated freeze/thaw cycles.

3. Run MitoLite™ Orange 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 5% CO_2 , 37 °C incubator for 15 to 30 minutes.

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

Note: It is important to remove the cell medium before adding MitoLite™ Orange dye-loading solution.

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

3.4 Incubate the dye-loading plate in a 5% CO_2 , 37 °C incubator for 15-30 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).

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 = 540/590 nm (bottom read) 10 to 30 minutes after Step 3.5 either using the endpoint mode or using the kinetic mode.

Data Analysis

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

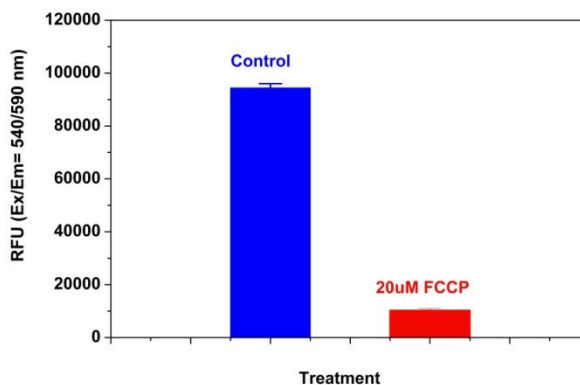


Figure 1. The decrease in MitoLite™ Orange fluorescence with the addition of FCCP in HeLa cells. HeLa cells were dye loaded with MitoLite™ Orange alone or in the presence of 20 μM FCCP for 15 minutes. The fluorescence intensity of MitoLite™ Orange was measured 30 minutes after adding Assay Buffer B (Component C) with a FlexStation™ microplate reader (Molecular Devices) at Ex/Em = 540/590 nm (cut off 570 nm, bottom read).

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.