Elite™ Mitochondrial Membrane Potential Detection Kit

CATALOG NUMBER: CA-M145, 10 plates

Description

Mitochondrial membrane potential is an important parameter of mitochondrial function used as an indicator of cell health. Elite™ Mitochondrial Membrane Potential Detection Kit uses a fluorescent cationic, lipophilic dye (**m-MPI)** to signal the loss of mitochondrial potential. In normal or healthy cells, **m-MPI** concentrates in the mitochondrial matrix where it forms red fluorescent aggregates. However, in apoptotic and necrotic cells, **m-MPI** diffuses out of mitochondria and changes to green fluorescent monomeric form. This kit provides all the necessary reagents for the analysis of mitochondrial integrity in cells. The aggregate red form has absorption/emission maxima of 585/590 nm. The green monomeric form has absorption/ emission maxima of 510/527 nm.

Features

- **Solubility:** Excellent water solubility, no precipitation unlike widely used JC-1 dye.
- **Specificity:** Selectively entering into mitochondria.
- **Application Platform:** Fluorescent microplate reader, flow cytometry, and fluorescence microscopy.
- **Robust and Highly Sensitive:** Excellent response to membrane potential change (Green/Red ratio). m-MPI signal changes proportionally to mitochondrial permeability defined by electrochemical gradient ($\Delta\Psi$) across the membrane.

Applications

- Cell apoptosis study.
- Cell toxicity.
- High Throughput Screening of apoptosis activator or inhibitors.

Kit Components

- **Component A:** Mitochondrial Membrane Potential Indicator (m-MPI) in DMSO — 100 µl (1000x)
- **Component B:** m-MPI Assay Buffer (10x) — 25 ml (10x)

Storage

Mitochondrial Membrane Potential Indicator (m-MPI) should be protected from light and stored at -20 ˚C.

Note: Aliquot Component A into 10 vials (10 µl each) and store at -20 °C when received.

m-MPI Assay Buffer should be stored at room temperature.

Materials Required (but not supplied)

- 96- or 384-well microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- Fluorescence microplate reader or flow cytometer.

ASSAY PROTOCOL

1. Cell Preparation

1.1 Harvest cells when they reach 80-90% confluence in flasks. Trypsinize cells, and count a portion of the cells with a hemocytometer.

Note: It is very important that the cells DO NOT reach >90% confluence.

1.2 Add 100 µl/well of cell suspension to 96-well plates the day before the experiment [BD Falcon imaging plate (BD Cat# 353219) is recommended if BD Pathway BioImager is used as the reader]. The cell number needs to be optimized for each assay. For assays performed on fluorescence plate reader, optimal assay conditions

Tel: 1-800-919-0755 • Fax: 1-240-683-5852 • info@eEnzyme.com • www.eEnzyme.com

require a confluent monolayer of cells prior to the assay (25K cells/well with HeLa cells and 32K cells/well with HepG2 cells). For assays performed on cell imagers, 6-12K cells/well is recommended.

1.3 Allow cells to attach by leaving the cell plates at room temperature for 30 minutes. Transfer the plates to a cell culture incubator and grow the cells overnight.

2. Preparation of 2X Dye-Loading Solution

2.1 Remove a vial of aliquoted m-MPI (**Component A**) from -20 ˚C, and allow it to thaw at room temperature.

2.2 Prepare 1X m-MPI assay buffer by adding 25 ml 10X m-MPI assay buffer (**Component B**) into 225 ml water.

2.3 Preparing 2X Dye-Loading Solution for one cell plate by transferring 10 µl of m-MPI Stock Solution (**Component A**) to 5 ml of 1x m-MPI Assay Buffer (from step 2.2). Mix well by vortexing for 10 seconds.

2.4 Aliquot the unused m-MPI, and store at -20 ^{o}C.

Note: Do not re-use 2x Dye-loading Solution. Avoid repetitive freeze-thaw of m-MPI DMSO stock.

3. Dye Loading and Mitochondrial Membrane Potential Assay

3.1 Kinetic assay with on-line compound addition

a) Dilute testing compounds in HBSS buffer at 5x final concentrations.

b) Remove cell plates from incubator; replace culture medium with 50 µl of fresh culture medium. Add 50 µl of 2X Dye-Loading Solution to each well and incubate at 37^{o}C for 30 min.

c) After dye-loading, wash the cells once with 80 µl of 1x m-MPI Assay Buffer per well.

d) Add 80 µl of 1x m-MPI Assay Buffer into each well.

e) Place the cell plates on a fluorescence plate reader with on-line liquid handling system (e.g. FlexStation or FDSS), and perform the mitochondrial membrane potential assay with on-line addition of test compounds to the cell plates.

f) For assays performed on a FlexStation (MDS), use the following wavelength parameters. Green channel (Monomer form): Excitation: 485 nm; Emission: 530 nm; Cutoff 515 nm; Red channel (J-aggregates): Excitation: 485 nm; Emission: 590 nm; Cutoff 570 nm.

Note: Dispense speed and height for compound additions need to be optimized for each instrument.

3.2 Endpoint assay

a) Prepared 1X testing compounds in 1x m-MPI Assay Buffer.

b) Remove cell plates from incubator; replace culture medium with 50 µl of compound solution. Incubate at 37 ^{o}C for proper amount of time (For FCCP and oligomycin, 30 min incubation is recommended).

c) Add 50 µl 2X dye-loading solution to each well and incubate at 37 ^{o}C for 30 min.

d) Wash the cell plate once with 80 µl of 1x m-MPI Assay Buffer per well.

e) Add 80 µl of 1x m-MPI Assay Buffer into each well

f) Read on a fluorescence plate reader with bottom read-head. Test filter settings to optimize fluorescence light collection and eliminate bleedthrough of excitation light to emission filters.

3.3 Assay on BD Pathway 855 (Cat No. 341036) and BD Pathway 435 (Cat No. 641250) Bioimagers

a) Dye-loading procedure is the same as described above.

b) Image the plates on a BD Pathway^{TM} Bioimager using appropriate filters (table 1).

Table 1. Pathway 855 Bioimager Filter Sets

Channels	Excitation	Emission	Dichroic
Green (Monomer)	488/10	Fura/Fitc	Fura/Fitc
Red (J-aggregates)	548/20	Fura/Tritc	570LP

Mitochondrial Membrane Potential Quantitation: The ratio of green (monomer) to red (J-aggregates) fluorescence is used to quantify mitochondrial membrane potential changes.

Data Analysis

Comparison of JC-1 and m-MPI in HeLa Cells Treated with Different Compounds

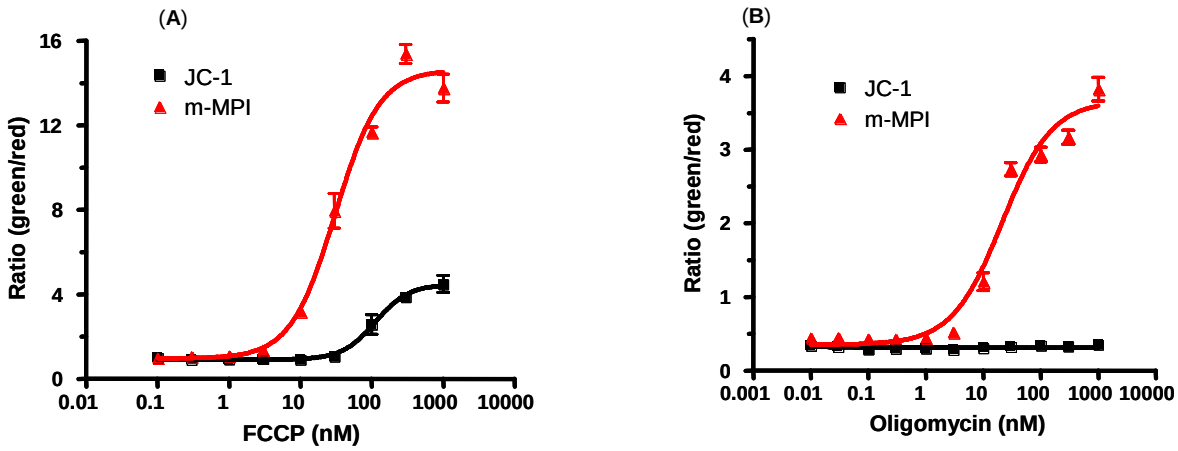

Figure 1. End point assays performed on FlexStation (MDS). **(A)** HeLa Cells treated with FCCP for 30 min. **(B)** HeLa Cells treated with oligomycin for 30 min. Fluorescence signals were recorded by a FlexStation. Dose response curves were plotted by Prism (GraphPad).