Technical Data Sheet

Codex Homogeneous Mitochondrial Membrane Potential Assay Kit

Components

1000X Mitochondrial Membrane Potential Indicator (m-MPI) in DMSO, 200 μl. **Fluorescence excitation/emission maxima: 514/529 nm for monomer form; 585/590 nm for J-aggregate form.**
200X signal enhancer, 1 ml
10X m-MPI Assay Buffer, 15 ml

Storage

1000X Mitochondrial Membrane Potential Indicator (m-MPI) should be protected from light and stored at -20˚C.

200X signal enhancer, should be stored at room temp.

10X m-MPI Assay Buffer should be stored at 4˚C.

ASSAY PROTOCOL

CELL PREPARATION

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.

2. Add 20 μl/well of cell suspension to 384-well plates (black/clear) the day before the experiment. The cell number needs to be optimized for each assay. For assays performed on fluorescence plate reader, optimal assay conditions require a confluent monolayer of cells prior to the assay (6K cells/well with HeLa cells and 8K cells/well with HepG2 cells).
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.

PREPARATION of 2X DYE-LOADING SOLUTION

1. Remove a vial of m-MPI from -20˚C, and allow it to thaw at room temperature.
2. Prepare 1X m-MPI assay buffer by adding 135 ml water into 15 ml of 10X m-MPI assay buffer.
3. Preparing 2X Dye-Loading Solution for one cell plate by transferring 20 μl of m-MPI Stock Solution (1000X) and 100 μl signal enhancer (200X) to 10 ml of 1X m-MPI Assay Buffer. Mix well by votexing for 10 seconds.
4. Aliquot the unused m-MPI, and store at -20ºC.

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

DYE LOADING and MITOCHONDRIAL MEMBRANE POTENTIAL ASSAY

- **Kinetic assay with on-line compound addition**

a) Dilute testing compounds in 1X HBSS buffer at 5x final concentrations.
b) Remove cell plates from incubator. Add 20 μl of 2X Dye-Loading Solution to each well and incubate at 37ºC for 30 min.
c) 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 10 μl of testing compounds to the cell plates

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.

Codex BioSolutions, **Inc**. 401 Professional Drive, Suite 160, Gaithersburg, MD 20879
Tel: (240)-632-8810; Fax: (240)-632-8820 E-Mail: orders@codexbiosolutions.com
http://www.codexbiosolutions.com/

- **Endpoint assay**

a) Prepare 5X testing compound solutions in 1X HBSS.
b) Remove cell plates from incubator, add 5 µl of 5X compound solution. Incubate at 37°C for proper amount of time (For FCCP and oligomycin, 60 min incubation is recommended).
c) Add 25 µl 2X dye-loading solution to each well and incubate at 37°C for 30 min.
d) 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.

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

1) Dye-loading procedure is the same as described above.
2) Image the plates on a BD Pathway™ Bioimager using appropriate filters (table 1).

Table 1. Pathway 855 Bioimager Filter Sets

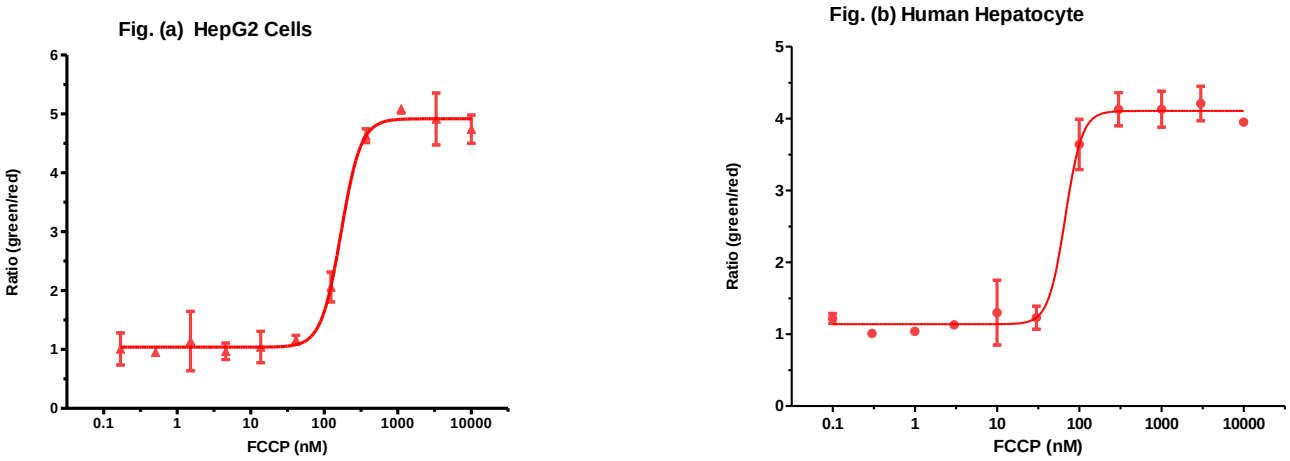
Channels	Excitation	Emission	Dichroic
Green (Monomer)	488/10	Fura/Fitc	Fura/Fitc
Red (J-aggregates)	548/20	Fura/Tritc	570LP

MITOCHONDRIAL MEMBRANE POTENTIAL QUANTIFICATION

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

Appendix

Dose Response Curves of FCCP in HepG2 and Hela Cells



End point assays performed on FlexStation (MDS). **(a)** HepG2 Cells treated with FCCP for 60 min. **(b)** Human Hepatocytes treated with FCCP for 60 min. Fluorescence signals were recorded by a FlexStation. Dose response curves were plotted by Prism (GraphPad).

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