Product Information

Mitochondrial Permeability Transition Pore Assay Kit

I. Kit Contents:

Components	K2061-100 100 assays	Cap Color	Part Number
MPTP Staining Dye	5 x 50 µg	Green	K2061-C-1
$CoCl_2$	1 ml	Red	K2061-C-2
Ionomycin free acid	1 ml	Blue	B6947
MPTP Wash Buffer	2 x 100 ml	NM	K2061-C-3

II. Introduction:

Mitochondria is the cell power centrals and have a significant role in the energy production. Apoptosis is induced upon damages of mitochondria. MPT pore or MPTP (mitochondrial permeability transition pore) is a nonspecific channel. It formed by inner and out mitochondrial membranes components and mediates in the releases of mitochondrial components in cell death. MPTP's switch between open and closed states in healthy cells, but during cell death, it significantly change the mitochondria permeability. Cytochrome c release and loss of mitochondrial membrane potential are subsequent to continuous pore activation. Mitochondrial Permeability Transition Pore Assay Kit gives a direct method of measuring cell death by measuring MPTP opening rather than relying on mitochondrial membrane potential alone.

III. Reagents Preparation:

Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

MPTP Staining Dye: Dissolve one-vial content in 50 µl DMSO. Store at -20℃, protected from light. Stable for one year as provided (powder). Once dissolved in DMSO, use within a short time for one series of experiments. (Dye degrades slowly over time, can be used for up to a week but best results obtained when used same day.)

MPTP Wash Buffer: Store at -20℃. Bring to 37℃ before use.

IV. Mitochondrial Permeability Transition Pore Assay Protocol:

1. Sample Preparation: Grow cells of interest in desired medium and culture conditions preferably as a single-cell suspension. Resuspend cells in pre-warmed MPTP Wash Buffer at a final concentration of 10^6 cells/ml. For each sample, prepare 1 ml aliquots of cell suspension in four separate tubes using MPTP Wash Buffer - one tube without any treatment (tube 1), one tube with MPTP Staining Dye only (tube 2), one tube with MPTP Staining Dye and $CoCl_2$ (tube 3) and one tube with MPTP Staining Dye, $CoCl_2$ and Ionomycin (tube 4).

2. Cell Labeling: Dilute MPTP Staining Dye 1:500 in MPTP Wash Buffer (i.e. mix 5 µl of MPTP Staining Dye Stock with 2.495 ml of MPTP Wash Buffer) and mix well. To tube 2, 3 and 4, add 5 µl of diluted MPTP Staining Dye and mix. To tube 3 and 4, add 5 µl of $CoCl_2$ supplied with the kit and mix well. To tube 4, add 5 µl of Ionomycin and mix well. Incubate cells at 37℃ for 15 min., protected from light. After incubation, centrifuge cells at 1,000 X g for 5 min. to pellet cells. Re-suspend in 1 ml of MPTP Wash Buffer to remove excess staining and quenching reagents. After staining, keep cells on ice and analyze within 1 hr.

Note: This protocol is optimized for Jurkat cells. If using different cell type, optimize the final concentration of Ionomycin.

3. Data analysis: Analyze the samples using a flow cytometer with 488 nm excitation filter. Use untreated sample (tube 1) to set up the instrument (Figure 1, tube 1). Samples stained with MPTP Staining Dye only shows high fluorescence signal from both cytoplasm and mitochondria (Figure1, tube 2). Samples stained with MPTP Staining Dye and treated with $CoCl_2$ shows only mitochondrial fluorescence as treatment with $CoCl_2$ quenches the cytosolic signal and shows intermediate fluorescence (Figure1, tube 3). Samples having all reagents show the lowest fluorescence as signal gets quenched from both cytoplasm and mitochondria (Figure 1, tube 4). The difference in fluorescence intensity between tubes 3 and 4 indicates the degree of MPTP activation and subsequent depolarization of the mitochondrial membrane. Complete depolarization, as is achieved with ionomycin, results in a complete abolishment of the fluorescence signal (essentially identical to tube 1) and giving the greatest difference between tube 3 and 4. Completely ineffective treatment would cause no depolarization and a fluorescence signal in treated tube (tube 4) identical to tube 3.

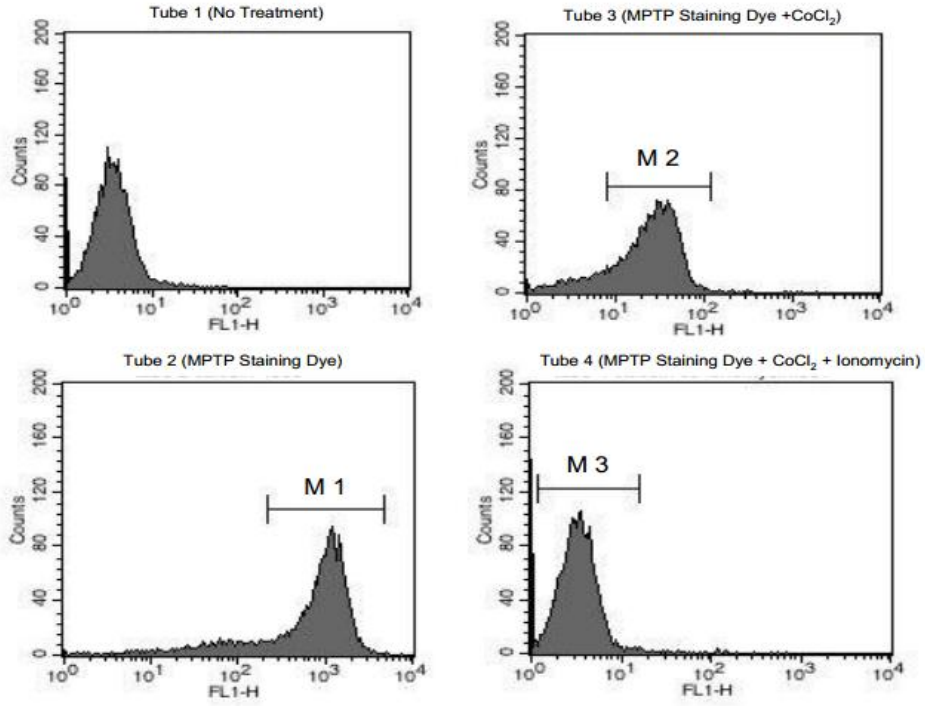

Figure 1: Jurkat cells were incubated with the reagents of the Mitochondrial Permeability Transition Pore Assay Kit and analyzed by flow cytometer. Tube 1: sample without treatment - used for instrument setup; Tube 2: sample stained with MPTP Staining Dye showing cumulative fluorescence signal from both cytoplasm and mitochondria; Tube 3: sample stained with MPTP Staining Dye and treated with $CoCl_2$ showing mitochondrial fluorescence only; Tube 4: samples with all reagents showing the lowest fluorescence. The difference in fluorescence between tubes 3 and 4 indicates the degree of MPTP activation and subsequent depolarization of the mitochondrial membrane.

V. Storage Conditions:

Store kit at -20℃, protected from light.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com