CELL TECHNOLOGY

Mito Flow

A Flow Cytometry Assay for: Mitochondrial Membrane Potential Detection.

Notes:

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Introduction

Background

The loss of mitochondrial membrane potential ($\Delta\Psi$) is a hallmark for apoptosis. The mitochondrial permeability transition is an important step in the induction of cellular apoptosis. During this process, the electrochemical gradient (referred to as $\Delta\Psi$) across the mitochondrial membrane collapses. The collapse is thought to occur through the formation of pores in the mitochondria by dimerized Bax or activated Bid, Bak, or Bad proteins. Activation of these pro-apoptotic proteins is accompanied by the release of cytochrome c into the cytoplasm (1-4).

Assay Principle

Cell Technology's Mito Flow assay utilizes cationic dye to visualize mitochondrial membrane potential (5-7). The Mito Flow reagent is a cell permeable cationic dye that has a strong fluorescent signal in the red region and exhibits low membrane potential independent (non specific) binding and toxicity. In healthy cells Mito Flow reagent is accumulated by the mitochondria in proportion to the DeltaPsi (membrane potential). In most cell lines, accumulation of Mito Flow reagent in the mitochondria results in a higher fluorescence intensity. In apoptotic cells, where the mitochondrial membrane potential is compromised, Mito Flow reagent does not accumulated in the mitochondria and these cells exhibit a lower fluorescence signal. .

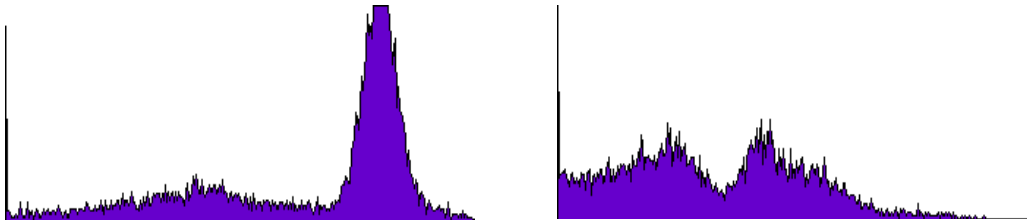

Figure 1. Jurkat cells were stimulated with Staurosporine for 3 hours (B) or DMSO (A). Mito Flow reagent was added according to the protocol, incubated for 30 minutes and analyzed by flow cytometry: Ex:488nm Em: FL2 channel.

A. Warnings and Precautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. We are not aware of any toxicity data for Mito Flow Reagent. Gloves, protective clothing and eyewear should be worn and safe laboratory practices followed.
3. Some cells over expressing multidrug resistance proteins may show a low mitochondrial membrane potential. See Technical note #1 below.

A. Storage and Shelf Life

1. Store the kit at -20°C until first use. The performance of this product is guaranteed for six months from the date of purchase if stored and handled properly.
2. Reconstituted Mito Flow reagent should be aliquoted in small amounts sufficient for one day of experimental work and stored at -20°C. Protect from light.
3. Avoid multiple freeze-thaw cycles.

C. Kit Components

1. Mitochondria Membrane Potential Dye (Mito Flow Reagent).....Part number: 4004
2. 10X Dilution Buffer.....Part number: 3004

D. Materials Required But Not Supplied

1. Solutions

a. Dimethyl Sulfoxide (DMSO)

2. Equipment

a.Flow Cytometer with 488nm laser for excitation and emission in FL2 channel.
b. FACS tubes

E. Preparation and Setup

1. Dilution of Mitochondria Membrane Potential Dye Reagent (Mito Flow Reagent).

A. Reconstitute the Mito Flow Reagent by adding 50 µL DMSO to the vial to obtain a 300X stock solution.
B. Mix by vortexing the vial several times at room temperature until contents are completely dissolved.
C. Aliquot the Mito Flow reagent in small amounts sufficient for one day of experimental work and store the remainder at -20°C in amber vials.
D. Immediately prior to use, dilute the 300X Mito Flow reagent to 20X. This can be accomplished by diluting the Reagent 1:15 in 1X dilution buffer. Vortex the solution until completely dissloved.
Add 5uL of 20X Mito Flow Reagent per 100 uL sample.
Protect reagent from light at all times. Each investigator should titrate out the Mito Flow Reagent to optimize staining.

2. Dilution of 10X Dilution Buffer.

A. If necessary warm the 10X Assay Buffer until any salt crystals are completely dissolved.
B. Dilute the Assay Buffer 1:10 with DI water (e.g. 1ml 10X assay buffer + 9ml DI water).

F. Staining Protocol For Suspension Cells

1. Cells should be cultured to a density not to exceed 1x 10^6 cells/mL.
Each cell line should be evaluated on an individual basis to determine optimal density for cell culture and apoptosis induction.
2. Use100uL cells for each sample.
3.Induce apoptosis according to your specific protocol and or add test compounds.
A negative control should also be set up at this time point.
4. After the required activation time add 5uL of the 20X Mito Flow Reagent to each tube.
5. Incubate the samples for 30 to 60 minutes at the same culture conditions as required in the experimental protocol (e.g. 37^0C at 10% CO_2).
6. Add 400-500 uL of 1X Dilution Buffer to each sample and analyze via flow cytometry.
Excitation: 488nm emission: FL2.
Do not fix samples. Fixing samples will lead in mitochondrial membrane potential collapse and erroneous results.

G. Staining Protocol For Monolayer Adherent Cells

1. Culture cells in 96 well plates at 100uL per well or by any other suitable culture dish.
2. Induce apoptosis according to your specific protocol and or add test compounds. A negative control should also be set up at this time point.
3. After the required activation time add 5uL of the 20X Mito Flow Reagent to each well.
4. Incubate the samples for 30 to 60 minutes at the same culture conditions as required in the experimental protocol (e.g. 37^0C at 10% CO_2).
5 Detach cells from the wells or tissue culture dish.
6. The cells maybe transferred to a FACS tube and analyzed via flow cytometry.

Do not fix samples. Fixing samples will lead in mitochondrial membrane potential collapse and erroneous results.

H. Analyzing Samples: Flow Cytometer

1. Suspension Cells. See Technical note #2

Excitation: 488nm and measure emission on the FL2 channel.

1. First run the Non-induced healthy cells. Adjust the PMT voltage so that the cells fall with the 2^{nd} - 3^{rd} decade on the log scale. Also adjust the markers.
2. Collect the desired number of events.
3. Next run the induced apoptotic cells.

J. Technical Notes

1. Cell lines over expressing Multidrug Resistance proteins and p-glycoproteins.

Over expression of Multidrug Resistance proteins and p-glycoproteins is associated with a decrease in intracellular accumulation of certain compounds including the Mito Flow dye. This will result in falsely low readings of mitochondrial membrane potential (8). Pre treatment of cells with inhibitors of Multidrug Resistance proteins and p-glycoproteins for 24 hours prior to staining with the Mito Flow dye may solve this (8).

2. Analyzing Cell via Flow Cytometry

Non apoptotic healthy cells will have a strong red fluorescence when excited with a 488nm laser and emission detected in the FL2 channel. Apoptotic cells will show a reduced red fluorescence thus a peak shit to the left. Optimal Ex: 547 Em: 574

References Cited in Manual :

1. Desagher, S., Osen-Sand, A., Nichols, A., Eskes, R., Montessuit, S., Lauper, S., Maundrell, K., Antonsson, B., and Martinou, J.C. Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. J. Cell Biol. 144 (5): 891-901 (1999).
2. Narita, M., Shimizu, S., Ito, T., Chittenden, T., Lutz, R. J., Matsuda, H., and Tsujimoto, Y. Bax interacts with the permeability transition pore to induce permeability transition and cytochrome c release in isolated mitochondria. Proc. Natl. Acad. Sci. USA 95: 14681-14686 (1998).
3. Basanez, G., Nechushtan, A., Drozhinin, O., Chanturiya, A., Choe, E., Tutt, S., Wood, K. A., Hsu, Y. T., Zimmerberg, J., and Youle, R. J. Bax , but not Bcl-XL decreases the lifetime of planar phospholipid bilayer membranes at subnanomolar concentrations. Proc. Natl. Acad. Sci. USA 96: 5492-5497 (1999).
4. Luo, X., Budihardio, I., Zou, H., Slaughter, C., and Wang, X. Bid, a Bcl-2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 94: 481-490 (1998).
5. Ehrenberg B, Montana V, Wei MD, Wuskell JP, Loew LM. Membrane potential can be determined in individual cells from the nernstian distribution of cationic dyes. Biophys J. 1988 May;53(5):785-94.
6. Farkas DL, Wei MD, Febbroriello P, Carson JH, Loew LM. Simultaneous imaging of cell and mitochondrial membrane potentials. : Biophys J. 1989 Dec;56(6):1053-69. Erratum in: Biophys J 1990 Mar;57(3):following 684.
7. Russell C. Scaduto, Jr. and Lee W. Grotyohann. Measurement of mitochondrial membrane potential using fluorescent rhodamine derivatives. Biophys J. 1999 Jan;76(1 Pt 1):469-77.