JC-10

Flow Cytometery Mitochondrial Membrane Potential Detection Kit.

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Introduction

The loss of mitochondrial membrane potential ($\Delta\Psi$) is a hallmark for apoptosis. The APO LOGIX JC-10 Assay Kit measures the mitochondrial membrane potential in cells. In non-apoptotic cells, JC-10 exists as a monomer in the cytosol (green) and also accumulates as aggregates in the mitochondria which stain greenish orange. Whereas, in apoptotic and necrotic cells, JC-10 exists in monomeric form and stains the cytosol green. JC-10 was developed to increase solubality in aqueous buffers as compaired to its conterpart JC-1.

Background

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).

JC-1 has been used extensively to detect mitochondrial membrane potential ($\Delta\Psi$) , however due to its poor solubility in aquose buffers it proves difficult to work with particularly when high concentrations are needed.

The JC-10 Assay Kit uses a unique cationic dye to signal the loss of the mitochondrial membrane potential. In healthy cells, the dye stains the mitochondria bright red. The negative charge established by the intact mitochondrial membrane potential allows the lipophilic dye, bearing a delocalized positive charge, to enter the mitochondrial matrix where it accumulates. When the critical concentration is exceeded, J-aggregates form which become fluorescent grennish orange where as it exists in the cytoplasm as monomers and fluoresces green. In apoptotic cells, the mitochondrial membrane potential collapses, and the JC-10 cannot accumulate within the mitochondria.

In these cells JC-10 remains in the cytoplasm in its monomeric green fluorescent form. Apoptotic cells, showing primarily green fluorescence, are easily differentiated from healthy cells which show grennish orange and green fluorescence. Both apoptotic and healthy cells can be visualized simultaneously by flow cytometery using 490 excitation and emission measured in FL1 (green) and FL2 (grennish orange). The JC-10 reagent is easy to use, simply dilute the reagent in the buffer provided or cell culture media and add it to the cells. After a 30-60- minute incubation analyze the samples via flow cytometery.

Warnings and Precautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. We are not aware of any toxicity data for JC-10. Gloves, protective clothing and eyewear should be worn and safe laboratory practices followed.

Storage and Shelf Life

1. Multiple storage conditions see individual component lable.
2. The JC-10 reagent should be aliquoted in small amounts sufficient for one day of experimental work and stored at or below -20°C, protected from light and moisture.

Kit Components

1. JC-10 Dye ready to use in DMSO buffer at 2mg/mL (appox 3mM) 100µL Part# 4027.
2. 10X Assay Buffer. Part# 3002.
3. FCCP Positive control 1 vial Part# 7025.

Materials Required But Not Supplied

1. Solutions
a) Cell culture media as required.
b) Absolute Ethanol
2. Equipment
a) Flow Cytometer
b) FACS tubes

Preparation and Setup

A. Dilution of JC-10 Reagent

1. Ready to use.
2. Thaw the vial and mix by inverting the vial several times at room temperature until contents are completely dissolved. Spin down vial gently as reagent maybe trapped in the cap.
3. Aliquot the resuspended JC-10 reagent in small amounts sufficient for one day of experimental work and store the remainder at -20°C.
4. Immediately prior to use, dilute the JC-10 dye reagent 1:10- 1:20 in 1X assay buffer. Vortex the solution.
   Protect reagent from light at all times.Each researcher should determine the optimal dilution of the JC-10 dye depending on their cell line.

B. Dilution of 10X Assay Buffer.

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

C. Positive Control

Reconstitute the vial with 100µL of ethanol. This will make a 10mM solution. Store reconstituted reagent at -20°C.

Controls

1. Negative control: Treat cells with vehicle only.
2. Postive control: Treat cells with FCCP at 2-10µM final concentration.
   FCCP can be added simultanously with JC-10 in Step B #4 below.

Staining Protocol For Suspension Cell Lines

1. Cells should be cultured to a density not to exceed 1 x 10^6 cells/mL. ***Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction***.
2. Induce apoptosis according to your specific protocol.
3. Transfer 0.3 mL cell suspension into a sterile FACS tubes.
4. Without washing add 10 µL of the diluted JC - 10 reagent (from Step A) to the samples.
5. Incubate the samples at 37^{0}C in a cell culture incubator (5-10% CO2) for 15-60 minutes. ***Each researcher should determine the optimal incubation time depending on their cell line.***
6. Next bring up the volume of your samples to 0.5mL by adding 0.2mL 1X Assay Buffer.
7. Cells are ready for flow cytometery analysis.

Staining of Monolayer Cells

1. Plate cells in 24 well tissue culture plates over night to allow cells to adhere. Next induce apoptosis according to your specific protocol.
   Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.
2. Using standard techniques harvest adherent cells after your experimental protocol. Keep in mind that apoptotic adherent cells may detach and float in the media, therefore retain the media plus washes and pool the samples in their respective tubes. Next wash the pooled samples in serum media to quench any remaining effects of the detachement media used
3. Re suspend the samples in media to a density of 1 x 10^6 cells/mL. If there are clumps present allow them to settle and transfer 300µL of sample to FACS tubes.
4. Add 10 µL of the diluted JC - 10 dye (from Step A) to each sample.
5. Incubate the cells at 37°C in a 5-10% CO2 incubator for 15-60 minutes.
6. Next bring up the volume of your samples to 0.5mL by adding 0.2mL 1X Assay Buffer.
7. Cells are ready for flow cytometery analysis.

Flow Cytometry Analysis

1. Gate cells using FSC vs SSC.
2. Excitation: 488nm.
3. Emission: Set up dot plot from gated cells FL1 (X axis green) vs FL2 (Y axis greenish orange). Set up quadrants as showen below in Figure 1.
4. Run Negative control tube to adjust PMT voltage to register a dual positive population fluorescence (healthy cells) falling within the second and third log decade scale of FL1and FL2 (upper right quadrant).
5. Next run Positive control to adjust Compensation: FL1 – % FL2 and FL2 – % FL1 as shown in figure 2.
6. Next run the experimantal samples, using the PMT settings established above. One should see a population of cells that appears in the lower right quadrant. This reflects a loss of red emission on the FL2 axis.
7. If the induced sample exhibits only a minimal decrease in grennish orange emission, increase the FL2-%FL1 compensation.

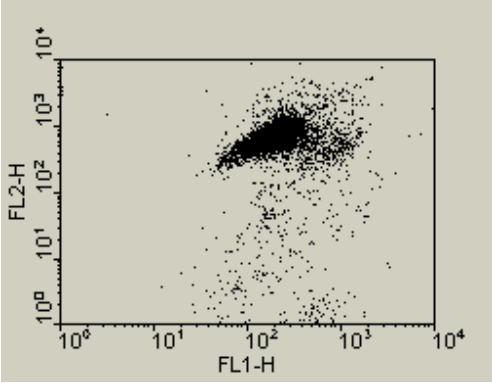

Figure 1.

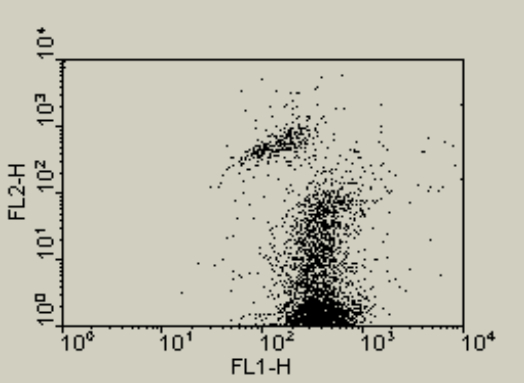

Figure 2.

References Cited in Manual

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