Mitochondrial Membrane Potential (JC-1) Assay Kit

User Manual

Catalog # CAK2008

(Version 1.1A)

Detection mitochondrial membrane potential by fluorescence microplate reader

For research use only. Not for diagnostic or therapeutic procedures.

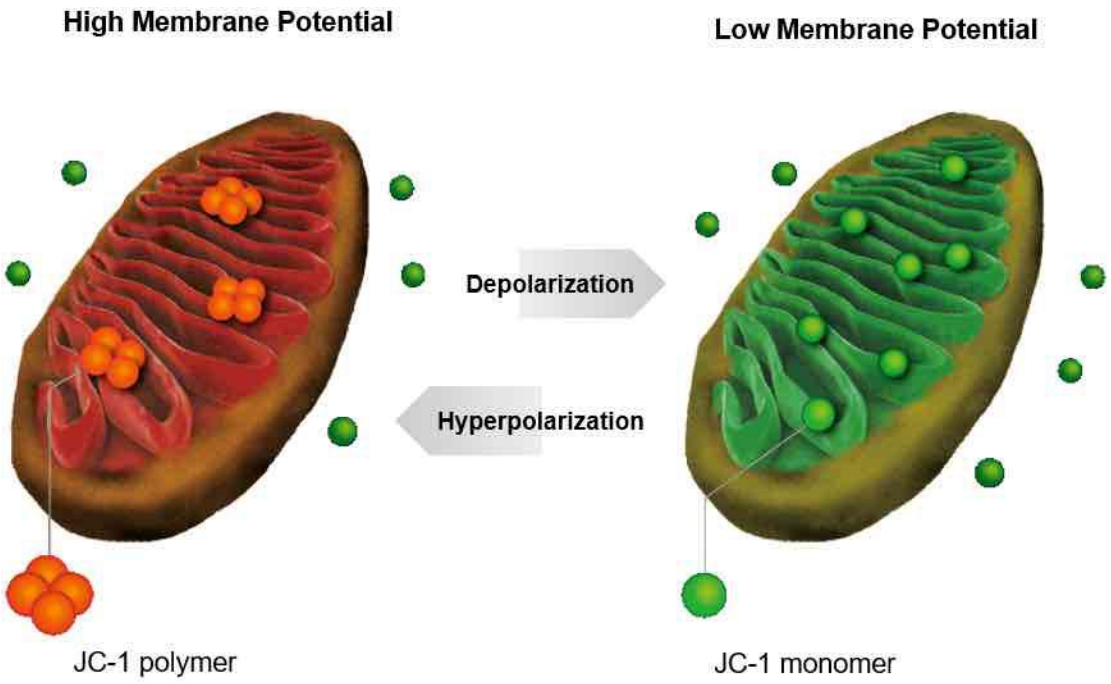
I. INTRODUCTION

The mitochondrial permeability transition is an important step in the induction of cellular apoptosis. During this process, the electrochemical gradient 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.

Mitochondrial Membrane Potential Assay Kit uses a unique cationic dye. 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, aggregates form which become fluorescent red. In apoptotic cells, the mitochondrial membrane potential collapses, and the JC-1 cannot accumulate within the mitochondria. In these cells JC-1 remains in the cytoplasm in a green fluorescent monomeric form. Apoptotic cells, showing primarily green fluorescence, are easily differentiated from healthy cells which show red and green fluorescence. The aggregate red form has absorption/emission maxima of 585/590 nm. The green monomeric form has absorption/ emission maxima of 510/527 nm. Both apoptotic and healthy cells can be visualized simultaneously by fluorescence microscopy using a wide band-pass filter suitable for detection of fluorescein and rhodamine emission spectra. The kit is easy to use. Simply dilute the reagent in cell culture medium and add to the cells. After 15 minutes incubation, wash the cells and analyze by flow cytometry or fluorescence microscopy or fluorescence plate reader.

II. PRINCIPLES

The loss of mitochondrial membrane potential is a hallmark for apoptosis. In non-apoptotic cells, JC-1 exists as a monomer in the cytosol (green) and also accumulates as aggregates in the mitochondria which stain red. Whereas, in apoptotic and necrotic cells, JC-1 exists in monomeric form and stains the cytosol green.



III. KIT COMPONENTS

Component	100 Assays	Storage
JC-1 (200X)	50 µl x 1	-20 °C
Staining Buffer (5X)	8 ml x 1	4 °C
CCCP (1 mM)	20 µl x 1	-20 °C
Distilled water	8 ml x 1	4 °C
Manual	1	

IV. MATERIALS REQUIRED BUT NOT PROVIDED

1. Fluorescence plate reader
2. General tissue culture supplies
3. PBS
4. Fetal Bovine Serum (FBS)
5. Sterile, tissue culture treated, clear bottom, dark sided 96-well microplates
6. Multichannel pipette (50 - 300 µl)

V. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use. The sample volumes below are sufficient for 96-wells 100 µl tests; adjust volumes as needed for the number of wells used in your experiment.

1X Staining Buffer: Prepare 1X Staining Buffer by adding 1 ml Staining Buffer (5X) into 4 ml distilled water. Mix gently and thoroughly.

JC-1 Working Solution: To prepare the JC-1 Working Solution, add 25 µl JC-1 (200X) to previously warmed 5 ml 1X Staining Buffer.

Positive Control: Recommend CCCP (1 mM) 1:100 add into the cell culture medium, the final concentration is 10 µmol/L, incubate for 20 mins.

VI. ASSAY PROCEDURE

A. For suspension cells

1. Resuspend 10 x 10^4 cells/ml cells in 0.5ml cell culture medium, which may contain serum and phenol red.

2. Add 0.1 ml JC-1 Working Solution and mix it upside down several times. Incubate the cells at 37°C for 20 mins.

3. After incubation, centrifuge 600g at 4°C for 3-4 mins to precipitate cells, discard the supernatant.

4. Add 0.5 ml PBS to resuspend the cells, centrifuge 600g at 4°C for 3-4 min, precipitate the cells, discard the supernatant. Repeat wash the cells twice.

5. Resuspend with an appropriate amount of 1X Staining Buffer, observation was made by fluorescence microscope or laser confocal microscope, or fluorescence spectrophotometer or flow cytometry.

B. For adherent cells (24-well Plate)

1. Remove the cell culture medium, wash the cells with PBS twice, then add 0.5 ml cell culture medium, the cell culture medium can contain serum and phenol red.

2. Add 0.1 ml JC-1 Working Solution and mix thoroughly. Incubate the cells at 37°C for 20 mins.

4. After incubation, remove the supernatant and wash the cells with PBS twice.

5. Add 0.5 ml cell culture medium.

6. Observation under fluorescence microscope or laser confocal microscope.

Note: For adherent cells, if need to use fluorescence spectrophotometer or flow cytometry for detection, you may collect cells first and refer to the detection method of suspension cells.

VII. STORAGE/STABILITY

The kit ships on wet ice and storage at 2-8 °C is recommended. Stored at -20°C for 1 year, avoid multiple freeze-thaw cycles.

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES