



Mitochondrial Membrane Potential Detection Kit

(Cat/No.:BC157 Size:50T,20T,10T JC-1 method)

1. Product Description

A large number of studies have shown that mitochondria are closely related to apoptosis, in which the disruption of the mitochondrial transmembrane potential ($\Delta\psi$), which is considered to be one of the earliest events in the apoptotic cascade reaction process, occurs before the appearance of nuclear apoptotic features (chromatin condensation, DNA breaks), and apoptosis is irreversible once the mitochondrial transmembrane potential collapses.

This kit utilizes JC-1(5,5',6,6-tetrachloro-1,1',3,3-tetraethylbenzimidazolcarbocyanine iodide), which is a cationic lipid fluorescent dye used as an indicator of mitochondrial transmembrane potential. JC-1 exists in both monomeric and multimeric states, in the form of monomers at low concentrations and multimers at high concentrations. The emission spectra of the two are different, but both can be detected in the green (FL-1) channel of the flow cytometer with green fluorescence, JC-1 can be aggregated intracellularly in a monomeric state through the normal cell membrane, the membrane potential ($\Delta\psi$) of normal healthy mitochondria has polarity, and JC-1 is rapidly ingested into mitochondria relying on the polarity of the $\Delta\psi$, and due to the concentration of the polymorphism is formed within the mitochondria, and the emission of the polymorphism is red fluorescence. The light emitted by the multimer is red fluorescence; it can be detected by the red (FL-2) channel of the flow cytometer; when the cell undergoes apoptosis, the mitochondrial transmembrane potential is depolarized, JC-1 is released from the mitochondria, and the intensity of the red light decreases, and it exists as a monomer in the cytoplasm and emits green fluorescence. According to this feature to detect the mitochondrial membrane potential changes.

This kit can be applied to cell, tissue or purified mitochondrial membrane potential detection.

2. Composition

Composition	10 assays	20 assays	50 assays
JC-1	10 μ l	20 μ l	50 μ l
10 \times Incubation Buffer	2.0ml	4.0ml	10.0ml

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E:



3. Instruments and Reagents are self-prepared

Flow cytometer or fluorescence microscope, high-speed centrifuge, CO₂ incubator, micropipettes 1.5mL Microtube, slides, coverslips (for fluorescence microscopy), PBS, sterilized deionized water.

4. Precautions

1. Centrifuge the liquid before using the micro reagent.
2. JC-1 Store and use away from light.
3. The number of cells cultured should not exceed 1×10^6 , otherwise the cells will naturally apoptose and affect the detection.
4. Cells that are too sensitive to pH changes are recommended to incubate with fetal bovine serum instead of Buffer for staining and washing, or to extend the observation time.
5. Mitochondrial membrane potential changes detected by flow cytometry are affected by a variety of factors, including different fluorescence intensity ratios depending on the inducer, cell line type, and time of day, so there is no universal standard guideline for compensating and setting gates, and therefore negative and positive controls should be set up for fluorescence compensation and gating for each test.
6. Tissues need to be prepared in single-cell suspension or extracted and purified mitochondria before testing, which can be done with the Cell Suspension Preparation Kit or Mitochondrial Extraction Kit.

5. Procedures

1. Apoptosis was induced by appropriate methods, and a negative control group and a positive control group were set up [with appropriate apoptosis inducers (e.g., staurosporine), and apoptosis was confirmed by other assays (e.g., AnnexinV or Caspase 3 activity) after the appropriate time of induction], and the cells were collected;
2. Cells were washed twice with PBS (centrifuged at 2000 rpm for 5 min) and no more than 1×10^6 cells were collected;
3. Take 100 μ L of 10 \times Incubation Buffer and add 900 μ L of sterilized deionized water to dilute it into 1 \times Incubation Buffer, mix well and preheat to 37 $^{\circ}$ C;
4. Aspirate 500 μ L of 1 \times Incubation Buffer, add 1 μ L of JC-1, and vortex to make JC-1 working solution. Since JC-1 has a low solubility in water, the insoluble particles can be removed by centrifugation (10,000 rpm, 1 min), and the supernatant of the centrifuged solution can be used to eliminate interference;
5. Take 500 μ L of JC-1 working solution to suspend the cells uniformly, and incubate them for 15~20 min in an incubator at 37 $^{\circ}$ C with 5% CO₂;
6. Cells were collected by centrifugation (2000 rpm, 5 min) at room temperature and washed twice with 1 \times Incubation Buffer;
7. Pipette 500 μ L of 1 \times Incubation Buffer to resuspend the cells;
8. Fluorescence microscopy observation or flow cytometry analysis.



6. Fluorescence microscopy observation

1. Place a drop of the above cell suspension on a slide, cover with a coverslip and observe under a fluorescence microscope;
2. For adherent cells, coverslips can also be used directly to culture cells and induce apoptosis; cells were washed twice with PBS; 100 μ L of JC-1 working solution was added dropwise, coverslips were added, and the cells were incubated for 15-20 min at 37°C in an incubator with 5% CO₂; the cells were washed for 1-2 times with 1 \times Incubation Buffer; the coverslips were inverted onto slides, and the cells were visualized under the fluorescence microscope; the cells were then incubated for 15-20 min at 37°C in an incubator with 5% CO₂; the coverslips were inverted onto slides and observed under the fluorescence microscope.

Normal cells: green ++ red ++ (high green and high red) when viewed through a two-color filter, or yellow-green when viewed through the same filter.

Apoptotic cells: green ++ red - (high green, low red) when viewed through a two-color filter, green when viewed through the same filter.

B. Flow cytometry analysis

Apoptosis was detected by flow cytometry (Ex=488 nm; Em=530 nm). Green fluorescence was detected by FITC channel usually FL1; red fluorescence was detected by PI channel usually FL2. Normal cells {FL-1 bright, FL-2 bright; R1}, apoptotic cells {FL-1 bright, FL-2 dark; R2}, the position of the gate varies according to the cell type, experimental

conditions, etc., the test should be set up for the untreated normal cells as a negative control group and a positive control group, according to the two-parameter scatter plots of the negative and positive control groups to set up the gate position.

7. Experiment Example

P388 cells were induced to apoptosis with apoptosis inducer, incubated at 37°C, 5% CO₂ incubator for 4~6h, and detected using Apoptosis Mitochondrial Membrane Potential Detection Kit (JC-1), and the results were analyzed and analyzed by flow cytometry as follows.

