Product Name: JC-1 Mitochondrial Membrane Potential Assay Kit

Catalog No.: RA20023

Basic Information

Product name	JC-1 Mitochondrial Membrane Potential Assay Kit
Size	20T/100T
Storage	-20℃, away from light. Component B can also be stored at 4℃. To avoid repeated freezing and thawing, it is recommended to devide components A and C into small packages.
Shipping	Shipped with ice pack
Validity	12 months
Ex/Em(Low Membrane Potential)	485/535nm
Ex/Em(High Membrane Potential)	550/600nm

Product composition

Components	**20T**	**100T**
A :JC-1,100×in DMSO	100μL	500μL
B: 10×Assay Bu ff er	5mL	25mL
C: CCCP, 50mM	10μL	50μL

Product Introduction

A decrease in mitochondrial membrane potential is a sign of early apoptosis, which occurs before the externalization of phosphatidylserine on the cell membrane and the activation of caspase hydrolases. When the mitochondrial membrane permeability changes, the membrane potential decreases. This change in membrane potential is caused by the formation of Bax dimers and the activation of Bid, Bak, and Bad, which induce the formation of pores in the mitochondrial membrane. When these pro-apoptotic proteins are activated, mitochondria also release cytochrome C into the cytoplasm.

JC-1 is an ideal fluorescent probe widely used to detect mitochondrial membrane potential △Ψm. It can detect the mitochondrial membrane potential of cells, tissues or purified mitochondria. When the mitochondrial membrane potential is high, JC-1 aggregates in the matrix of mitochondria to form polymers and produce red fluorescence; when the mitochondrial membrane potential is low, JC-1 cannot aggregate in the matrix of mitochondria. At this time, JC-1 is a monomer and can produce green fluorescence. The decrease in membrane potential

can be easily detected by the transition of JC-1 from red fluorescence to green fluorescence. At the same time, the transition of JC-1 from red fluorescence to green fluorescence can also be used as an indicator for the early detection of cell apoptosis.

The maximum excitation wavelength of JC-1 monomer is 510nm, and the maximum emission wavelength is 527nm; the maximum excitation wavelength of JC-1 polymer is 585 nm, and the maximum emission wavelength is 590nm. This kit is simple and fast to operate, and can be detected by flow cytometry, fluorescence microscopy or fluorescence microplate reader. CCCP is also provided as a positive control for inducing a decrease in mitochondrial membrane potential.

Operation Steps

1. Reagent Preparation

Preparation of JC-1 working solution

Prepare 1mL of 1×JC-1 staining solution as follows: take 10 μL of 100×JC-1 staining solution, add it to 890 μL of sterile diH_2O, vortex to mix, add 100 μL of 10× Assay Buffer to the above mixture, vortex to mix, and you can get 1×JC-1 staining solution.

Note: ① The configuration volume can be enlarged or reduced in the same proportion.

② It is not recommended to dilute 100×JC-1 staining solution directly with 1×Assay Buffer, as precipitation may occur.

Preparation of 1× Assay Buffer

Prepare 1× assay buffer according to the ratio of 10× assay buffer: diH_2O = 1:9, such as 1mL 10× assay buffer + 9 mL diH_2O.

2. Cell Staining

Before starting the experiment, make sure that the JC-1 and CCCP solutions have returned to room temperature.

(1) Inoculate cells in a culture plate as required by the experiment (the number of suspended cells should not exceed 10^6 cells / mL).

(2) Positive control group: The provided CCCP (50 mM) in the kit is recommended to be added to the cell culture medium at a ratio of 1:1000 (e.g., to achieve a final concentration of 50 μM, add 1 μL of 50 mM CCCP solution to 1 mL of cell culture medium), and then incubate at 37℃ for 20 minutes. For specific cell types, the concentration and duration of CCCP treatment may vary and should be determined by referring to relevant literature.

For suspension cells:

(1) Take 5×10^5 cells and resuspend them in 0.5 mL of cell culture medium, which may contain

serum and phenol red.
(2) Add 0.5 mL of JC-1 staining working solution and invert several times to mix. Incubate in a cell culture incubator at 37°C for 20min.
(3) After incubation, centrifuge at 1000 rpm for 5 min and discard the supernatant (be careful not to remove the cells).
(4) Add 1 mL of pre-cooled 1× Assay Buffer to resuspend the cells. Centrifuge at 1000 rpm for 5 min. Remove the supernatant and repeat the process.
(5) Resuspend the sample in an appropriate amount of pre-cooled 1× Assay Buffer and observe under a fluorescence microscope or analyze using a flow cytometer.

For adherent cells:

Note: For adherent cells, if you want to use flow cytometry, you can collect the cells first, resuspend them and refer to the detection method of suspended cells. The following is the detection process of adherent cells for fluorescence microscope or fluorescence microplate reader.
(1) For one well of the six-well plate, remove the culture medium, wash the cells once with PBS or other appropriate solution if necessary according to the specific experiment, and add 1 mL of cell culture medium. The cell culture medium may contain serum and phenol red.
(2) Add 1 mL of JC-1 staining working solution and mix thoroughly. Incubate in a cell culture incubator at 37°C for 20 min.
(3) After incubation, remove the supernatant, add 1 mL of pre-cooled 1× Assay Buffer, remove the supernatant, and repeat once.
(4) Add 2 mL of cell culture medium, which may contain serum and phenol red. Observe under a fluorescence microscope or detect using a fluorescence microplate reader.

3. Results Analysis

(1) Flow cytometric analysis
For normal cells, red JC-1 aggregates in mitochondria can be detected in the PE or PI (FL2) channel; for apoptotic cells, green JC-1 monomers can be detected in the FITC (FL1) channel.
(2) Fluorescence microscopy analysis
a. Observe cells using a fluorescence microscope with a dual-channel filter that can detect fluorescein and rhodamine, or fluorescein and Texas Red simultaneously.
b. For normal cells, the mitochondria have intact mitochondrial membrane potential and emit red fluorescence at 590nm; for apoptotic or necrotic cells , the dye exists in monomeric form and emits green fluorescence at 530 nm.

(3) Fluorescence microplate reader analysis

a . Red fluorescence: Ex/Em =550/600 nm; green fluorescence Ex/ Em =485/535 nm.

b. Calculate the ratio of red to green fluorescence.

c. Compared with normal cells, the red-green fluorescence ratio is reduced in apoptotic or necrotic cells.

Note

1. All fluorescent dyes have quenching problems. Please try to avoid light to slow down fluorescence quenching.
2. To avoid repeated freezing and thawing, this product can be divided into small quantities.
3. Before use, please centrifuge the product to the bottom of the tube and then proceed with subsequent experiments.
4. JC-1 (100× in DMSO) will solidify and stick to the bottom, wall or lid of the centrifuge tube at a lower temperature. It can be incubated in a 20-25℃ water bath for a while until it is completely melted before use.
5. When preparing the JC-1 staining working solution, you must first fully dissolve the JC-1 (100× in DMSO) provided in the kit with sterile diH2O and then add the 10× Assay Buffer. Do not prepare the 1× Assay Buffer first and then add JC-1 (100× in DMSO), as this will make it difficult for JC-1 to fully dissolve, which will seriously affect subsequent detection.
6. When washing with 1× Assay Buffer after JC-1 staining, keep the 1× Assay Buffer at around 4°C. This will achieve better washing results.
7. After JC-1 staining and washing, try to complete the subsequent test within 30 minutes. Keep in ice bath before testing.
8. This product is For Research Use Only, Not for Diagnostic Use.