Mitochondrial membrane potential assay kit with JC-1

Cat#: orb1566776 (User Manual)

Size: 100T

Product composition:

JC-1 (200 x)	100µl/tube x 5 tubes
Ultrapure water	90ml
JC-1 staining buffer (5 x)	80ml
CCCP (10mM)	50µl
Manual	1

Product introduction:

JC -1 is the kit used as a fluorescence probe to detect the membrane potential changes of cell, tissue or purified mitochondrial, it can be used to detect the early occurrence of apoptosis.

JC-1 is an ideal fluorescent probe widely used to detect mitochondrial membrane potential (△ψm). The loss of mitochondrial membrane potential is considered as an early event of apoptosis. The uptake of JC -1 by mitochondria can be used as an effective differentiation between apoptotic cells and normal cells. When the mitochondrial membrane potential is high, JC-1 appears in the mitochondrial matrix as J-aggregates and produces a red fluorescence. At low mitochondrial membrane potential, JC-1 can not aggregate in mitochondria, but diffuses throughout the cell in the form of monomer, producing green fluorescence. Therefore, fluorescence and color change reflect the change of mitochondrial membrane potential. The proportion of mitochondrial depolarization is usually measured by the relative proportion of red-green fluorescence.

JC-1 monomer has a maximum excitation wavelength of 514 nm and a maximum emission wavelength of 529 nm, and JC-1 polymer (J-aggregates) has a maximum excitation wavelength of 585 nm and a maximum emission wavelength of 590 nm.

This kit provides CCCP (10mM) as a positive control for induced mitochondrial membrane potential decline. The kit can detect 100 samples in six-well plates and 200 samples in 12-well plates.

Operation Steps:

1. Preparation of JC-1 staining solution:

The amount of JC-1 staining working solution required per well in six-well plates is 1 ml, and the amount of JC-1 staining working solution used in other culture dishes was similar; for cell suspensions, 0.5 ml of JC-1 staining working solution is required per 0.5~1 million cells. JC-1(200 x) is diluted with 8 ml of ultrapure water per 50 µl of JC-1(200 x) . Fully dissolve and mix with JC-1, which can be left at room temperature for 1-2 minutes to ensure complete dissolution of JC-1. Then, add 2 ml JC-1 staining buffer (5x) , mix and get the JC-1 staining working solution.

2. The setting of positive controls:

According to the different cells, CCCP (10mM) provided in the kit is added to the cell culture medium at a ratio of 1:1000-1:100, and the cells are treated for 20 minutes. Load the JC-1 as the following manner and detect the mitochondrial membrane potential. Normally, for most cells mitochondrial membrane potential will completely lost after 20 min of 10µM CCCP treatment, with green fluorescence can be observed after JC-1 staining. Whereas normal cells should show red fluorescence after JC-1 staining. CCCP can be added at the same time as JC-1, but the concentration and time of CCCP may be different for specific cells.

3. For suspended cells:

1) Take 100,000~600,000 cells and resuspend them in 0.5 ml cell culture medium, which can contain serum and phenol red.
2) Add the 0.5 ml JC-1 staining solution and mix it upside down several times. The cells are incubated with 37°C for 20 min.
3) During incubation, add 4 ml of ultrapure water per 1 ml JC-1 of staining buffer (5×) to prepare an appropriate amount of JC-1 staining buffer (1×) and place in the ice bath.
4) At the end of 37°C incubation, centrifuge at 600g, 4°C for 3~4 minutes to precipitate cells. Discard the supernatant and take care not to remove cells.
5) Wash twice with JC-1 staining buffer (1×) from the ice bath: Add 1 ml JC-1 staining buffer 1(×) to resuspend cells, centrifuge for 3-4 minutes at 600 g 4°C, precipitate cells, and discard supernatant. Repeat once.
6) After two washes, resuspend with an appropriate amount of JC-1 staining buffer, analyze by flow cytometry, or observe by fluorescence microscope or laser confocal microscope.

4. For adherent cells:

Note: For adherent cells, if flow cytometry is desired, the cells can be collected first and then suspended again.

1) For one-well of a six-well plate, aspirate the cell culture medium and wash if necessary with PBS or other suitable solution once, add 1 ml of cell culture medium. The cell culture medium can contain serum and phenol red.

2) Add 1 ml JC-1 staining solution and mix well. The cells are incubated with 37°C for 20 min.

3) During incubation, appropriate amounts of JC-1 staining buffer (1×) and placed in an ice bath.

4) After 37°C incubation, aspirate supernatant and wash twice with JC-1 staining buffer (1×) (after the ice bath).

5) Add 2 ml of cell culture medium, which can contain serum and phenol red.

6) Observation with fluorescence microscope or laser confocal microscope.

5. For purified mitochondria:

1) Dilute the prepared JC-1 staining working solution five times with JC-1 staining buffer (1×).

2) 0.1 ml of purified mitochondria with a total protein amount of 10~100μg are added to the 0.9 ml 5-times diluted JC-1 staining working solution.

3) Use fluorescence spectrophotometer or fluorescence microplate reader for detection: After mixing, directly use fluorescence spectrophotometer for timescan, with excitation wavelength of 485nm and emission wavelength of 590nm. If the excitation wavelength cannot be set to 485nm with the fluorescence microplate reader used, the excitation wavelength can be set within the range of 475 -520nm. In addition, refer to the wavelength setting in step 6 below for fluorescence detection.

4) Observation with fluorescence microscope or laser confocal microscope: The method is the same as step 6 below.

6. Fluorescence observations and analysis of results:

When detecting JC-1 monomer, set excitation light to 490nm and emission light to 530nm. When detecting JC-1 polymer, set the excitation light to 525nm and the emission light to 590nm. Note: It is unnecessary to set the excitation light and emission light at the maximum excitation wavelength and maximum emission wavelength when measuring fluorescence.

If the fluorescence microscope is used for observation, you can refer to the settings for observing other green fluorescence such as GFP or FITC when detecting JC-1 monomer. For the detection of JC-1 polymer, you can refer to the setting when observing other red fluorescence, such as propidium iodide or Cy3. The presence of green fluorescence indicates a decrease in mitochondrial membrane potential, and the cell is likely to be in the early stages of apoptosis. The appearance of red fluorescence indicates that the mitochondrial membrane potential is normal and the cell status is normal.

Storage conditions

-20°C Save. JC-1 (200×) should be protected from light and should avoid repeated freezing and thawing. Ultrapure water and JC-1 staining buffer (5×) can be saved at 4°C.

Precautions

1. JC-1 (200×) can be used after incubation in a 20-25℃ water bath for a short period of time until it is completely melted. Centrifuge briefly before opening the lid, throw the liquid on the inner wall of the lid to the bottom of the tube, and avoid spilling the liquid when opening the lid.

2. JC-1 is a photosensitive substance, be careful to protect it from light when saving and handling.

3. When preparing the JC-1 staining working solution, the JC-1 staining buffer 5× can be added only after the 200× is fully dissolved and vortexed with the ultrapure water provided by testing kit. If JC-1 staining buffer 1× is prepared first and then JC-1 200× is added, JC-1 will be difficult to dissolve sufficiently, which will seriously affect subsequent tests.

4. Samples should be kept on ice and analyzed as soon as possible, preferably not more than 30 minutes after staining.

5. Do not prepare the JC-1 staining buffer 5× into the JC-1 staining buffer 1×. Use the JC-1 staining buffer 5× directly during the testing kit.

6. If a precipitate is found in the JC-1 staining buffer, 5X, it must be completely dissolved before use and heated at 37℃ to promote dissolution.

7. CCCP is a mitochondrial electron transfer chain inhibitor, which is harmful to human body. Please be careful during operation and pay attention to effective protection to avoid direct contact with human body or inhalation.

8. This product is limited to the scientific research of professionals, shall not be used for clinical diagnosis or treatment, shall not be used for food or medicine, and shall not be stored in ordinary residence.

9. For your safety and health, wear a lab coat and disposable gloves.