Product Information

Catalog number: REK0006

Size: 100T

Storage : Please store at -20℃ away from light, valid for 12 months. Avoid freeze / thaw cycles.

Product components:

Components	Size
JC-1(200×)	100μL×5
Ultrapure water	90mL
JC-1 staining buffer (5×)	80mL
CCCP(10mM)	20μL

Product Description

The Mitochondrial Membrane Potential Detection Kit (JC-1) is a kit that uses JC-1 as a fluorescent probe to quickly and sensitively detect the mitochondrial membrane potential in variety of cell types, as well as intact tissues and isolated mitochondria. It can be used for early apoptosis detection. When the mitochondrial membrane potential is high, JC-1 aggregates in the matrix of mitochondria to form polymers, which can 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. In this way, it is very convenient to detect changes in mitochondrial membrane potential by changing the color of fluorescence. The relative ratio of red and green fluorescence is often used to measure the ratio of mitochondrial depolarization.

The decrease in mitochondrial membrane potential is a marker event in the early stages of apoptosis. The change in cell membrane potential can be easily detected by the change of JC-1 from red fluorescence to green fluorescence. At the same time, the change of JC-1 from red fluorescence to green fluorescence can also be used as a detection indicator for early apoptosis.

The maximum excitation wavelength of JC-1 monomer is 515nm, and the maximum emission wavelength is 529nm; the maximum excitation wavelength of JC-1 polymer is 585nm, and the maximum emission wavelength is 590nm. In actual observation, the conventional settings for observing red fluorescence and green fluorescence can be used.

This kit provides CCCP as a positive control for inducing a decrease in mitochondrial membrane potential. For samples in a 6-well plate, this kit can detect a total of about 100 samples.

Operation Steps

Preparation of JC-1 staining working solution

The amount of JC-1 staining working solution required for each well of a 6-well plate is 1 mL. The amount of JC-1 staining working solution for other culture vessels is similar: 0.5 mL of JC-1 staining working solution is required for every 0.5-1×10^6 cells in the cell suspension. Take an appropriate amount of JC-1 (200×) and dilute JC-1 at a ratio of 8 mL of ultrapure water per 50 μL of JC-1 (200×). Vigorously shake to fully dissolve and mix JC-1. Then add 2 mL of JC-1 staining buffer (5×), and mix to obtain JC-1 staining working solution.

Setting of positive control

Add CCCP (10 mM) provided in the kit to the cell culture medium at a ratio of 1:1000, dilute to 10 μM, and treat the cells for 20 minutes. For most cells, the mitochondrial membrane potential will be completely lost after 20 minutes of treatment with 10μM CCCP, and green fluorescence should be observed after JC-1 staining; while normal cells should show red fluorescence after JC-1 staining. For specific cells, the concentration and duration of CCCP may vary, and please refer to relevant literature.

For suspended cells

1. Take 1-6×10^5 cells and resuspend them in 0.5mL cell culture medium, which can contain serum and phenol red.
2. Add 0.5mL JC-1 staining working solution and mix by inversion several times. Incubate in a cell culture incubator at 37℃ for 20 minutes.
3. During the incubation period, prepare an appropriate amount of JC-1 staining buffer (1×) according to the ratio of adding 4mL distilled water to every 1mL JC-1 staining buffer (5×), and place it in an ice bath.
4. After the 37℃ incubation, centrifuge at 600g at 4℃ for 3-4 minutes to precipitate the cells. Discard the supernatant and be careful not to aspirate the cells as much as possible.
5. Wash twice with JC-1 staining buffer (1×): add 1mL JC-1 staining buffer (1×) to resuspend the cells, centrifuge at 600g at 4℃ for 3~4 minutes, precipitate the cells, and discard the supernatant. Add 1 mL of JC-1 staining buffer (1×) to resuspend the cells, centrifuge at 600g at 4℃ for 3 to 4 minutes, precipitate the cells, and discard the supernatant.
6. Resuspend with an appropriate amount of JC-1 staining buffer (1×) and observe with a fluorescence microscope or laser confocal microscope. You can also use a fluorescence spectrophotometer or flow cytometer for analysis.

For adherent cells

1. For one well of a 6-well plate, remove the culture medium. If necessary, wash the cells once with PBS or other appropriate solution according to the specific experiment, and add 1mL of cell culture medium. The cell culture medium may contain serum and phenol red.
2. Add 1mL of JC-1 staining working solution and mix thoroughly. Incubate in a cell culture incubator at 37℃ for

20 minutes.

3. During the incubation period, prepare an appropriate amount of JC-1 staining buffer (1×) according to the ratio of adding 4mL of distilled water to every 1mL of JC-1 staining buffer (5×), and place it in an ice bath.
4. After the incubation at 37℃, remove the supernatant and wash twice with JC-1 staining buffer (1×): add 1mL of JC-1 staining buffer (1×) to resuspend the cells, centrifuge at 600g at 4℃ for 3-4 minutes, precipitate the cells, and discard the supernatant. Add 1mL JC-1 staining buffer (1×) to resuspend the cells, centrifuge at 600g at 4℃ for 3-4 minutes, precipitate the cells, and discard the supernatant.
5. Add 2mL cell culture medium, which can contain serum and phenol red.
6. Observe under a fluorescence microscope or laser confocal microscope. Note: For adherent cells, if using a fluorescence spectrophotometer or flow cytometer for detection, first collect the cells, resuspend and refer to step （**Fluorescence observation and result analysis**）.

For isolated mitochondria

1. Dilute the prepared JC-1 staining working solution 5 times with JC-1 staining buffer (1×).
2. Add 0.1 mL of isolated mitochondria with a total protein of 10-100 μg to 0.9 mL of the 5-fold diluted JC-1 staining working solution.
3. Detect with a fluorescence spectrophotometer or fluorescence microplate reader: After mixing, directly use a fluorescence spectrophotometer for time scanning (time scan), with an excitation wavelength of 485 nm and an emission wavelength of 590 nm. If a fluorescence microplate reader is used, if the excitation wavelength cannot be set to 485 nm, the excitation wavelength can be set within the range of 475-520 nm. Alternatively, you can also refer to step（**Fluorescence observation and result analysis**）.

Fluorescence observation and result analysis

When detecting JC-1 monomers, the excitation light can be set to 490nm and the emission light can be set to 530nm; when detecting JC-1 polymers, the excitation light can be set to 525nm and the emission light can be set to 590nm. Note: When measuring fluorescence here, it is not necessary to set the excitation light and emission light at the maximum excitation wavelength and the maximum emission wavelength. If using a fluorescence microscope for observation, when detecting JC-1 monomers, you can refer to the settings when observing other green fluorescence, such as the settings when observing GFP or FITC; when detecting JC-1 polymers, you can refer to the settings when observing other red fluorescence, such as propidium iodide or Cy3. The appearance of green fluorescence indicates that the mitochondrial membrane potential has decreased, and the cell is likely to be in the early stage of apoptosis. The appearance of red fluorescence indicates that the mitochondrial membrane potential and the cell state are relatively normal.

Protocol

1. JC-1 (200×) will solidify and stick to the bottom, wall or cap of the centrifuge tube at a lower temperature such as 4℃ or ice bath. It can be used after incubation in a 20-25℃ water bath for a while until it is completely melted.
2. JC-1 (200×) must be fully dissolved and mixed with the ultrapure water provided by the kit before adding JC-1 staining buffer (5×). Do not prepare JC-1 staining buffer (1×) first and then add JC-1 (200×), otherwise JC-1 will be difficult to fully dissolve, which will seriously affect subsequent testing.
3. When washing with JC-1 staining buffer (1×) after adding JC-1 staining working solution, keep JC-1 staining buffer (1×) at about 4℃, and the washing effect is better at this time.
4. After adding JC-1 staining working solution and washing, try to complete subsequent testing within 30 minutes; it needs to be stored in an ice bath before testing.
5. Do not use all of JC-1 staining buffer (5×) to make JC-1 staining buffer (1×). JC-1 staining buffer (5×) is required when preparing JC-1 staining working solution.
6. If precipitation is found in JC-1 staining buffer (5×), it must be completely dissolved before use. To promote dissolution, it can be heated at 37℃.
7. CCCP is a mitochondrial electron transport chain inhibitor and is toxic. Please be careful.
8. For your safety and health, please wear a lab coat and disposable gloves when operating.

Notes

This product is for research use only.