Mitochondrial membrane potential detection kit (JC-10)

Packing specification

Product Numbers: JC10-50, JC10-100, JC10-200,

Specifications: 50 times, 100 times, 2 * 100 times,

Storage conditions: Store at4 ℃ away from light, valid for one year.

product composition:

product name	JC10-50 packaging	JC10-100 packaging	JC10-200 packaging
JC-10 (200 ×)	50 μL / tube, 5 tubes in total	100 μL / tube, 5 tubes in total	2 * 100 μL / tube, 5 tubes in total
Ultra-pure water	45mL	90mL	2*90mL
JC-10 Staining buffer (5 ×)	40mL	80mL	2*80mL
CCCP (10mM)	10μL	20μL	2*20μL

Product introduction:

Mitochondrial membrane potential detection kit (JC-10) is a kit that uses JC-10 as a fluorescent probe to quickly and sensitively detect changes in cell, tissue or purified mitochondrial membrane potential, and can be used for early apoptosis detection .

JC-10 is an ideal fluorescent probe widely used to detect mitochondrial membrane potential △Ψm. Can detect cells, tissues or purified mitochondrial membrane potential. When the mitochondrial membrane potential is high, JC-10 gathers in the mitochondrial matrix to form a polymer, which can produce red fluorescence; when the mitochondrial membrane potential is low, JC-10 cannot gather in the mitochondrial matrix. 10 is a monomer, which can produce green fluorescence. In this way, it is very convenient to detect the change of mitochondrial membrane potential through the change of fluorescent color. The relative ratio of red and green fluorescence is commonly used to measure the ratio of mitochondrial depolarization.

The decrease of mitochondrial membrane potential is a landmark event in the early stage of apoptosis. The decrease in cell membrane potential can be easily detected by the transition of JC-10 from red fluorescence to green fluorescence. At the same time, the transition of JC-10 from red fluorescence to green fluorescence can also be used as a detection indicator in the early stage of apoptosis.

The maximum excitation wavelength of JC-10 monomer is 515nm and the maximum emission wavelength is 529nm; the maximum emission wavelength of JC-10 polymer is 590nm. For actual observation, use the conventional settings for observing red and green fluorescence.

This kit provides CCCP as a positive control for inducing a decrease in mitochondrial membrane potential.

Instructions:

1. Preparation of JC-10 dyeing working solution

The amount of JC-10 staining working solution required for each well of the six-well plate is 1mL, and the amount of JC-10 staining working solution for other culture vessels can be deduced by analogy. Dyeing working fluid. Take an appropriate amount of JC-10 (200 ×) and dilute JC-10 by adding 8 mL of ultrapure water per 50 μL of JC-10 (200 ×). Vigorously shake to fully dissolve and mix JC-10. Then add 2mL of JC-10 staining buffer (5 ×). After mixing, it is the JC-10 staining

working solution.

2. Setting of positive control:

The CCCP (10 mM) provided in the kit is recommended to be added to the cell culture solution at a ratio of 1: 1000, diluted to 10 μM, and treated for 20 minutes. Subsequently, JC-10 was loaded according to the following method to detect the mitochondrial membrane potential. For most cells, the membrane potential of the mitochondria will be completely lost after 10 μM CCCP treatment for 20 minutes, and the green fluorescence should be observed after JC-10 staining; normal cells should show red fluorescence after JC-10 staining. For specific cells, the concentration and duration of action of CCCP may be different, you need to refer to relevant literature to determine.

3. For suspended cells

Take 100,000 to 600,000 cells and resuspend in 0.5mL of cell culture fluid, which may contain serum and phenol red.

Add 0.5mL JC-10 staining working solution, mix upside down several times. Incubate in a cell incubator at 37 °C for 20 minutes.

During the incubation period, according to the ratio of adding 4 mL of distilled water per 1 mL of JC-10 staining buffer (5 ×), an appropriate amount of JC-10 staining buffer (1 ×) was prepared and placed in an ice bath.

After incubation at 37 °C, centrifuge at 600g at 4 °C for 3 to 4 minutes to pellet the cells. Discard the supernatant, taking care not to aspirate the cells.

Wash twice with JC-10 staining buffer (1×): add 1 mL of JC-10 staining buffer (1×) to resuspend the cells, centrifuge at 600g at 4 ℃ for 3-4 minutes, pellet the cells, and discard the supernatant.

Add 1 mL of JC-10 staining buffer (1×) to resuspend the cells, centrifuge at 600g at 4 °C for 3 to 4 minutes, pellet the cells, and discard the supernatant.

After resuspending with an appropriate amount of JC-10 staining buffer (1×), observe with a fluorescence microscope or laser confocal microscope. It can also be detected with a fluorescence spectrophotometer or analyzed by flow cytometry.

4. For adherent cells

Note: For adherent cells, if using a fluorescence spectrophotometer or flow cytometer to detect, first collect the cells, resuspend and refer to the detection method of suspended cells.

For one well of a six-well plate, aspirate the culture solution, and if necessary, wash the cells with PBS or other appropriate solution once, and add 1 mL of cell culture solution. The cell culture fluid may contain serum and phenol red.

Add 1mL JC-10 staining working solution and mix well. Incubate in a cell incubator at 37 °C for 20 minutes.

During the incubation period, an appropriate amount of JC-10 staining buffer (1×) was prepared according to the proportion of adding 4 mL of distilled water per 1 mL of JC-10 staining buffer (5 ×), and placed in an ice bath.

After incubation at 37 °C, the supernatant was aspirated and washed twice with JC-10 staining buffer (1×).

Add 2mL of cell culture fluid, which can contain serum and phenol red.

Observe under a fluorescence microscope or a laser confocal microscope.

5. For purified mitochondria

Dilute the prepared JC-10 staining working solution with JC-10 staining buffer (1 ×) 5 times.

0.9mL 5-fold diluted JC-10 staining working solution was added with 0.1mL total protein amount of 10 ~ 100 μg purified mitochondria.

Detection with a fluorescence spectrophotometer or a fluorescence microplate reader: After mixing, directly perform a time scan with a fluorescence spectrophotometer, the excitation wavelength is 485nm, and the emission wavelength is 590nm. If you use a fluorescence microplate reader, the excitation wavelength can not be set to 485nm, you can set the excitation wavelength in the range of 475 ~ 520nm. In addition, you can also refer to the wavelength setting in step 6 below for fluorescence detection.

Observe with a fluorescence microscope or laser confocal microscope: the method is the same as step 6 below.

6. Fluorescence observation and result analysis

When detecting JC-10 monomer, the excitation light can be set to 490nm and the emission light is set to 530nm; when detecting JC-10 polymer, the excitation light can be set to 525nm and the emission light is set to 590nm. Note: It is not necessary to set the excitation light and emission light at the maximum excitation wavelength and maximum emission wavelength when measuring fluorescence here. For example, when using a fluorescence microscope, you can refer to the settings for observing other green fluorescence when detecting JC-10 monomer, such as the settings for GFP or FITC; for other red fluorescence, such as propylene iodide, for JC-10 polymer. Settings for pyridine or Cy3. The presence 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 presence of red fluorescence indicates that the mitochondrial membrane potential is normal and the state of the cells is relatively normal.

Precautions:

JC-10 (200×) will solidify at low temperature such as 4 ℃ and ice bath and stick to the bottom, wall or cap of the centrifuge tube. It can be incubated in a 20 ～ 25 ℃ water bath for a while until it is completely melted. .

JC-10 (200×) must be fully dissolved and mixed with the ultrapure water provided in the kit before adding JC-10 staining buffer (5×). Do not prepare JC-10 staining buffer (1×) before adding JC-10 (200×), as this will make it difficult for JC-10 to dissolve sufficiently, which will seriously affect the subsequent detection.

After washing with JC-10 staining buffer (1×) after loading JC-10, keep the JC-10 staining buffer (1×) at about 4 ° C. The washing effect at this time is better.

After loading and washing the JC-10 probe, try to complete the follow-up test within 30 minutes. Store in an ice bath before testing.

Do not prepare all of JC-10 staining buffer (5×) as JC-10 staining buffer (1×). JC-10 staining buffer (5 ×) should be used directly during the use of this kit.

If it is found that there is a precipitate in the JC-10 staining buffer (5×), it must be dissolved before it can be used. To promote dissolution, it can be heated at 37 ° C.

CCCP is a mitochondrial electron transport chain inhibitor, it is toxic, please pay attention to protection.

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

For scientific research use only, it is forbidden to use for other purposes.