Description of Mitochondrial Membrane Potential Detection Kit (JC-10)

Cat: CA1310
Size: 100T
Product content:

Ingredients	Specifications
JC-10 (200×)	100μL/ tube for a total of 5 tubes
Ultra-pure water	90mL
JC-10 Stain buffer (5×)	80mL
CCCP (10mM)	20μL

Storage conditions:

-20℃ store away from light, try to avoid repeated freezing and thawing, valid for one year. Ultra-pure water and JC-10 dyeing buffer (5×) can also be stored at 4℃.

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 the changes of mitochondrial membrane potential in cells, tissues or purified mitochondria, and can be used for early apoptosis detection.

JC-10 is an ideal fluorescent probe widely used to detect mitochondrial membrane potential △Ψm. It can detect cell, tissue or purified mitochondrial membrane potential. When the mitochondrial membrane potential is high, JC-10 gathers in the matrix of the mitochondria and forms a polymer that can produce red fluorescence; When the mitochondrial membrane potential is low, JC-10 cannot gather in the matrix of mitochondria, and at this time JC-10 is a monomer and can produce green fluorescence. In this way, it is very convenient to detect the change of mitochondrial membrane potential through the change of fluorescence color. The proportion of mitochondrial depolarization is often measured by the relative ratio of red-green fluorescence.

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

The maximum excitation wavelength and emission wavelength of JC-10 monomer were 515nm and 529nm respectively. The maximum excitation wavelength of JC-10 polymer is 585nm and the maximum emission wavelength is 590nm. For actual observation, use the conventional Settings for observing red and green fluorescence.

This kit provides CCCP as a positive control for induced mitochondrial membrane potential decline. For samples in six-well plates, this kit can detect a total of 100 samples; For 12 well samples, the kit can test a total of 200 samples.

Protocols: (only for reference):

1. Preparation of JC-10 dyeing working liquid:

The amount of JC-10 dyeing working liquid required for each hole of the six-hole plate is 1mL, and the amount of JC-10 dyeing working liquid for other culture vessels is similar; For cell suspension, 0.5mL of JC-10 dyeing working solution is required for every 500,000 to 1 million cells. Take an appropriate amount of JC-10 (200×) and dilute JC-10 by adding 8mL of ultra-pure water per 50μL JC-10 (200×). Shake violently to dissolve JC-10 thoroughly and mix it well. Then add 2mL JC-10 dyeing buffer (5×), and mix it into JC-10 dyeing working liquid.

2. Setting of positive control:

The kit provided in the CCCP (10mm) recommended according to the proportion of 1:1000, added to the cell cultures of dilution to 10 microns, handle cells for 20 minutes. Then JC-10 was loaded in the following way for the detection of mitochondrial membrane potential. For most cells, the mitochondrial membrane potential will be completely lost after 20 minutes of 10μM CCCP treatment, and JC-10 should be observed as green fluorescence after staining. However, normal cells should show red fluorescence after JC-10 staining. For specific cells, the action concentration and time of CCCP may be different, which should be determined by referring to the relevant literature.

3. For suspension cells:

(1) Take 100,000 to 600,000 cells and re-suspend them in 0.5mL cell culture solution, which can contain serum and phenol red.

(2) Add 0.5mL JC-10 staining solution, reverse and mix well several times. Incubate at 37℃ in the cell incubator for 20 minutes.

(3) During incubation, an appropriate amount of JC-10 stain buffer (1×) was prepared by adding 4mL of distilled water to every 1mL of JC-10 stain buffer (5×) and placed in an ice bath.

(4) After incubation at 37°C, centrifuge 600g at 4°C for 3 to 4 minutes to precipitate the cells. Discard the supernatant and take care not to suck the cells as much as possible.

(5) Wash with JC-10 staining buffer (1×) twice: Add 1mL JC-10 staining buffer (1×) to the suspension cells, centrifuge 600g at 4℃ for 3~4 minutes, precipitate the cells, discard the supernatant. Then 1mL JC-10 staining buffer (1×) was added to the suspended cells, 600g was centrifuged at 4℃ for 3~4 minutes, the cells were precipitated, and the supernatant was discarded.

(6) After re-suspension with appropriate amount of JC-10 staining buffer (1×), the cells were observed by fluorescence microscope or laser confocal microscope, and also detected by fluorescence spectrophotometer or flow cytometry.

4. For adherent cells:

Note: For adherent cells, if you want to use fluorescence spectrophotometer or flow cytometry detection, you can collect cells first, and then refer to the detection method of suspended cells after resuspension.

(1) For one hole of the six-well plate, the culture solution is removed, and if necessary, the cells can be washed once with PBS or other appropriate solution, and 1mL of the cell culture solution is added. The cell culture solution may contain serum and phenol red.

(2) Add 1mL JC-10 stain working solution and mix well. Incubate at 37℃ in the cell incubator for 20 minutes.

(3) During incubation, an appropriate amount of JC-10 stain buffer (1×) was prepared by adding 4mL of distilled water to every 1mL of JC-10 stain buffer (5×) and placed in an ice bath.

(4) 37 ℃ after the incubation, gettering supernatant, using JC-10 buffer (1×) washing twice.

(5) Add 2mL cell culture solution, which can contain serum and phenol red.

(6) Observe under fluorescence microscope or laser confocal microscope.

5. For purified mitochondria:

(1) The prepared JC-10 dyeing working solution is diluted 5 times with JC-10 dyeing buffer (1×).

(2) 0.1mL purified mitochondria with a total protein content of 10~100μg were added into 0.9mL JC-10 working solution with 5 times dilution.

(3) Detection by fluorescence spectrophotometer or fluorescent enzyme spectrometer: after mixing, time scan was performed by fluorescence spectrophotometer directly. The excitation wavelength was 485nm and the emission wavelength was 590nm. If the fluorescence enzyme spectrometer is used, when the excitation wavelength cannot be set to 485nm, the excitation wavelength can be set in the range of 475~ 520nm. In addition, you can also refer to the wavelength setting in step 6 below for fluorescence detection.

(4) 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 can be set to 530nm. When detecting JC-10 polymer, the excitation light can be set to 525nm and the emission light can be set to 590nm.

Note: It is not necessary to set the excitation and emission light to the maximum excitation wavelength and the maximum emission wavelength when measuring fluorescence here. If using fluorescence microscopy, the detection of JC-10 monomer can refer to the setting when observing other green fluorescence, such as GFP or FITC; The detection of JC-10 polymers can refer to the observation of other red fluorescence Settings, such as propyl iodide or Cy3 Settings. The presence of green fluorescence indicates a decreased mitochondrial membrane potential and that the cell is

most likely in the early stages of apoptosis. The presence of red fluorescence indicates that the mitochondrial membrane potential is normal and the cell's state is normal.

Note:

1. JC-10 (200×) at 4℃, ice bath and other low temperature will solidify and stick to the centrifugal tube bottom, tube wall or tube cover, can be 20~25℃ water bath temperature for a while to all melted after use.
2. JC-10 (200×) must be fully dissolved and mixed with ultra-pure water provided by the kit before JC-10 dyeing buffer (5×) can be added. It is not possible to prepare JC-10 dyeing buffer (1×) and then add JC-10 (200×), so that JC-10 will be difficult to dissolve fully, which will seriously affect the subsequent detection.
3. After loading JC-10, when washing with JC-10 dyeing buffer (1×), keep JC-10 dyeing buffer (1×) at about 4℃, and the washing effect is better at this time.
4. JC-10 probe after loading and washing as far as possible within 30 minutes to complete the follow-up test. Ice bath should be stored before testing.
5. Please do not put the JC - 10 dyeing buffer mixture JC - 10 (5×) all dyeing buffer (1×), and this kit in use process need dyeing directly to use JC - 10 buffer (5×).
6. If there is precipitation in JC-10 dye buffer (5×), it must be completely dissolved before use. In order to promote dissolution, it can be heated at 37℃.
7. CCCP is a mitochondrial electron transport chain inhibitor, toxic, please pay attention to careful protection.
8. For your safety and health, please wear a lab coat and disposable gloves.

Related literature:

[1] Qiu li, Ou Yang, Nengguo Tao, et al. A Damaged Oxidative Phosphorylation Mechanism Is Involved in the Antifungal Activity of Citral against Penicillium digitatum. Frontiers in Microbiology. February 2018. (IF 4.019)

[2] YingJuan Liu, Zhenzhen Deng, Lihua Geng, et al. In vitro evaluation of the neuroprotective effect of oligo-porphyran from Porphyra yezoensis in PC12 cells. January 2019. (IF 2.635)

Note: For more information about this product, please refer to the Solarbio website.