Ver.251201

Mitochondrial Membrane Potential (JC-10Assay) Assay Kit

CA1310 (100Test)

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Product Description

The Mitochondrial Membrane Potential Detection Kit (JC-10) is a kit that uses JC-10 as a fluorescent probe to rapidly and sensitively detect changes in the mitochondrial membrane potential of cells, tissues, or purified mitochondria. It can be used for the detection of early apoptosis. JC-10 is an ideal fluorescent probe widely used for detecting mitochondrial membrane potential. When the mitochondrial membrane potential is high, JC-10 aggregates in the mitochondrial matrix to form polymers that emit red fluorescence. When the mitochondrial membrane potential is low, JC-10 cannot aggregate in the mitochondrial matrix and exists as a monomer, which emits green fluorescence. This allows for the convenient detection of changes in mitochondrial membrane potential through the shift in fluorescence color. The ratio of red to green fluorescence is commonly used to measure the degree of mitochondrial depolarization.

A decrease in mitochondrial membrane potential is a hallmark event in the early stages of apoptosis. The shift of JC-10 fluorescence from red to green can easily detect the decrease in mitochondrial membrane potential and can also be used as an indicator for early apoptosis detection. The maximum excitation wavelength of JC-10 monomer is 515 nm, and the maximum emission wavelength is 529 nm. The maximum excitation wavelength of JC-10 polymer is 585 nm, and the maximum emission wavelength is 590 nm. For observation, the standard settings for detecting red 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 up to 100 samples; for samples in a 12-well plate, this kit can detect up.

Kit components

Reagent	Volume	Storage
JC-10 (200×)	100μL/ tube, 5 tubes total.	-20℃
Ultrapure water	90mL	-20℃
JC-10 Dyeing buffer (5×)	80mL	-20℃
CCCP (10mM)	20μL	-20℃

Operation Procedures

Sample Preparation

I. **Prepare working solution**: For six well plate, add 1ml JC-10 dyeing working solution in each well, for cell suspension, 0.5~1 million cell need 0.5ml JC-10 dyeing working solution. Take appropriate amount of JC-10 (200×) and dilute JC-10 according to the ratio of adding 8mL of ultrapure water per 50μL of JC-(200×). Shake violently and mix thoroughly, add 2ml JC-10 Dye buffer to get JC-10 dyeing working solution.

II. **Set positive control**: Add CCCP (10mM) to cell medium in a ratio of 1:1000, dilutedto10μM, and the cells are treated for 20 minutes, install JC-10 after handling cell for 20min, detect membrane potential. For most cells, the membrane potential of mitochondria is completely lost after 20 minutes of treatment with 10μM CCCP, and green fluorescence should be observed after JC-10 staining ,while normal cells should show red fluorescence after staining with JC-10. For a specific cell, the concentration and duration of action of CCCP may vary, and it is up to the relevant literature to determine.

III. **For suspense cell:**

- Take 0.1-0.6 million cells suspense in cell culture medium (can include serum and phenol red).
- Add 0.5ml JC-10 dyeing working solution, mix thoroughly, incubate at 37℃ for 20min in cell incubator box.
- Prepare JC-10 dyeing buffer (1×) as the ratio of adding 1ml JC-10 dyeing buffer (5×) to 4ml ultrapure water in ice bath.

- After the incubation at 37°C, centrifuge at 600g for 3-4min at 4°C, precipatate cell and discard the supernatant .
- Wash twice with JC-10 dyeing buffer (1×): add 1ml JC-10 dyeing buffer (1×) to Resuspend the cells, Centrifuge the cells at 600g for 3 to 4 minutes at 4°Cto pellet the cells, and discard the supernatant. Repeat once.
- Re-suspense with JC-10 dyeing buffer (1×), then observe with fluorescence microscope or laser confocal microscope, detect with fluorescent spectrophotometer or flow analysis of cytometey.

IV. For Adherent cells

For adherent cells, if detection using a fluorescence spectrophotometer or flow cytometer is desired, the cells can first be collected and resuspended, and then the detection method for suspension cells can be referred to.

- Remove cell culture medium in each well, wash with PBS or other solution, add1ml cell culture medium (can include serum and phenol red).
- Add 1ml TC-10 dyeing working solution, mix thoroughly, incubate at 37°Cfor 20minincellincubator box.
- Prepare JC-10 dyeing buffer (1×) as the ratio of adding 1ml JC-10 dyeing buffer (5×) to4ml ultrapure water in ice bath.
- Discard the supernatant after 37°C incubation. Wash twice with JC-10 dyeing buffer.
- Add 2ml cell culture medium (can include serum and phenol red).
- Observe with fluorescence microscope or laser confocal microscope.

V. For purify mitochondria:

- Dilute JC-10 dyeing working solution 5 times with JC-10 dyeing buffer (1×).
- Add 0.1ml purify mitochondria which including 10-100μg protein to 0.9ml diluted JC-10 dyeing working solution.
- Detect with fluorescent spectrophotometer or microplate reader: Time scan with fluorescent spectrophotometer after mix thoroughly, excitation wavelength is 485nm, emission wavelength is 590 nm. If the excitation wavelength of the fluorescence microplate reader cannot be set to485nm, it can be set within the range of 475–520 nm. Detect fluorescent can reference step 6.
- Observe with fluorescence microscope or laser confocal microscope.

VI. Fluorescence observation and result analysis:

Detect JC-10 monomer, set the excitation wavelength to 490 nm, emission wavelengthto530nm;
Detect JC-10 polymer , set the excitation wavelength to 525 nm, emission wavelengthto590nm.

Note: When measuring fluorescence here, it is not necessary to set the excitation and emission wavelengths to the maximum excitation and emission wavelengths. If using a fluorescence microscope for observation, when detecting JC-10 monomers, you can refer to the settings used for observing other green fluorescence, such as those used for GFP or FITC. When detectingJC-10aggregates, you can refer to the settings used for observing other red fluorescence, such as propidium iodide or Cy3. The appearance of green fluorescence indicates a decrease in mitochondrial membrane potential, and the cell is likely in the early stages of apoptosis. The appearance of red fluorescence indicates that the mitochondrial membrane potential is relatively normal, and the cell status is also normal.