Mitochondrial Membrane Potential Assay Kit(JC-1 Assay)

Cat : M8650

Size: 100T

Storage: storage at -20℃ for 1 year, in shadow, avoid refrozen as soon as possible, ultrapure water and JC-1 Dye buffer(5×)can be storage at 4℃.

Component:

JC-1(200×)	100μL/ tube, 5 tubes total.
ultrapure water	90mL
JC-1 Dyeing buffer(5×)	80mL
CCCP(10mM)	20μL

Introduction

JC-1 are cationic dyes that exhibit potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green (~525nm) to red (~590nm). Consequently, mitochondrial depolarization is indicated by a decrease in the red/ green fluorescence intensity ratio. The potential-sensitive color shift is due to concentration dependent formation of red fluorescent J-aggregates. JC-1 can be used as an indicator of mitochondrial potential in a variety of cell types, including myocytes 3 and neurons, 4 as well as in intact tissues 5 and isolated mitochondria. Use of fluorescence ratio detection therefore allows researchers to make comparative measurements of membrane potential and determine the percentage of mitochondria within a population that respond to an applied stimulus. Subtle heterogeneity in cellular responses can be discerned in this way. The most widely implemented application of JC-1 is for detection of mitochondrial depolarization occurring in the early stages of apoptosis.

This kit provides CCCP as a positive control for inducing a decrease in mitochondrial membrane potential. For samples in a six-well plate, the kit can detect a total of 100 samples; for samples in 12 wells, the kit can detect a total of 200 samples.

Protocol

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

2. **Set positive control:** Add CCCP(10mM) to cell medium in a ratio of 1:1000, diluted to 10μM, and the cells are treated for 20 minutes, install JC-1 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-1 staining, while normal cells should show red fluorescence after staining with JC-1. For a specific cell, the concentration and duration of action of CCCP may vary, and it is up to the relevant literature to determine.

3. For suspense cell:

1) Take 0.1~0.6 million cells suspense in cell culture medium(can include serum and phenol red).
2) Add 0.5mL JC-1 dyeing working solution, mix thoroughly, incubate at 37℃ for 20min in cell incubator box.
3) Prepare JC-1 dyeing buffer(1×) as the ratio of adding 1ml JC-1 dyeing buffer(5×) to 4ml ultrapure water in ice bath.
4) After the incubation at 37℃,centrifuge at 600g for 3-4min at 4℃, precipatate cell and discard the supernatant .
5) Wash twice with JC-1 dyeing buffer(1×), add 1ml JC-1 dyeing buffer(1×), centrifuge at 600g for3~4min at 4℃, add 1ml JC-1 dyeing buffer(1×),centrifuge at 600g for 3~4min at 4℃. Re-suspense with JC-1 dyeing buffer(1×), then observe with fluorescence microscope or laser confocal microscope, detect with fluorescent spectrophotometer or flow analysis of cytometey.

4. For Adherent cells

1) Remove cell culture medium in each well, wash with PBS or other solution, add 1ml cell culture medium (can include serum and phenol red).
2) Add 1ml TC-10 dyeing working solution, mix thoroughly, incubate at 37℃ for 20min in cell incubator box.
3) Prepare JC-1 dyeing buffer(1×)as the ratio of adding 1ml JC-1 dyeing buffer(5×)to 4ml ultrapure water in ice bath.
4) Discard the supernatant after 37℃ incubation. Wash twice with JC-1 dyeing buffer.
5) Add 2ml cell culture medium (can include serum and phenol red).
6) Observe with fluorescence microscope or laser confocal microscope.

5. For purify mitochondria:

1) Dilute JC-1 dyeing working solution 5 times with JC-1 dyeing buffer(1×).
2) Add 0.1mL purify mitochondria which including 10-100μg protein to 0.9ml diluted JC-1 dyeing working solution.
3) Detect with fluorescent spectrophotometer or microplate reader: Time scan with fluorescent spectrophotometer after mix thoroughly, excitation wavelength is 485nm, emission wavelength is 590nm. Excitation wavelength couldn't set to 485nm, but excitation wavelength can set to 475-520nm. Detect fluorescent can reference step 6.
4) Observe with fluorescence microscope or laser confocal microscope.

6. Fluorescence observation and result analysis:

Detect JC-1 monomer, set the excitation wavelength to 490nm, emission wavelength to 530nm; Detect JC-1 polymer , set the excitation wavelength to 525nm, emission wavelength to 590nm.

Note:

1) JC-1 (200×)will be solidification and stick on the tube in low temperature like 4℃, ice bath, use after 20~25℃ water bath to dissolve thoroughly.

2) JC-1 (200×) must be thoroughly dissolved and mixed with the ultrapure water provided in the kit firstly, then add JC-1 dyeing buffer(5×).
3) Keep the temperature 4℃ when wash with JC-1 dyeing buffer(1×).
4) Finish detection in 30min after washing as soon as possible, storage in ice bath before detection.
5) Do not dissolve all JC-1 dyeing buffer(5×)to JC-1 dyeing buffer(1×).this kit use JC-1 dyeing buffer(1×)directly.
6) Must dissolve all solidification if find solidification in JC-1 dyeing buffer(5×), heat for accumulating dissolve.
7) CCCP is mitochondrial electron transport chain inhibitor, toxic, please be careful.
8) Please wear the lab coat and wear a disposable glove for your safety and health.