JC-1 Mitochondrial membrane potential assay kit

Introduction

JC-1 Mitochondrial membrane potential assay kit supplies the fluorescent probe JC-1 for the detection of mitochondrial membrane potential. This kit can be used for membrane potential detection of cellular mitochondria, tissue mitochondria, or purified mitochondria.

Mitochondrial membrane potential, also called ΔΨm, is an important parameter of mitochondrial function and has been used as an important marker of cell apoptosis. JC-1 is a widely used mitochondrial membrane potential detection probe. When the mitochondrial membrane potential is high, JC-1 aggregates in the mitochondrial matrix, emitting bright red fluorescence; When the mitochondrial membrane potential is low, JC-1 cannot aggregate in the mitochondria and transfer to a monomer with green fluorescence. The relative ratio of red/green fluorescence can be used to determine the change in mitochondrial membrane potential.

This kit provides CCCP as a positive control. For most cells, the mitochondrial membrane potential almost disappears completely after CCCP treatment. For 6-well plates, this kit can detect 100 samples; For 12-well plates, the kit can detect 200 samples.

Components and Storage

Components	K2002-100 T
JC-1 (200X)	5 x 100 μL
ddH_2O	90 mL
Dilution buffer (5X)	80 mL
CCCP (10 mM)	20 μL

This kit should be stored at -20°C, stable for 1 year. JC-1 (200X) and CCCP (10 mM) should be stored at -20°C away from light, and avoid repeated freeze/thaw cycles.

Protocol

1. **Preparation of JC-1 working solution:** Add 50 μL JC-1 (200X) per 8 mL ddH_2O to dilute JC-1. Vortex vigorously to dissolve if necessary. Then add 2 mL dilution buffer (5X) and mix to form the JC-1 working solution. The working solution is unstable, any unused working solution should be discarded after use.

***Note:** For JC-1 (200X) dilution, it must be done according to the instructions. Dissolve and mix well with ddH_2O prior to adding

staining buffer (5X). Because JC-1 has a low solubility in water, it is hard to dissolve JC-1 (200X) directly with dilution buffer (1X).

2. **Positive control group:** CCCP (10 mM) is diluted in culture medium at a ratio of 1:1000 to make a CCCP working solution (10 μM). Cells are treated with a CCCP working solution for 20 min. Subsequently, the JC-1 probe is loaded as described below and mitochondrial membrane potential detection is performed. CCCP-treated cells exhibit green fluorescence after JC-1 staining, while healthy cells exhibit red fluorescence after JC-1 staining.

***Note:** For most cells, the mitochondrial membrane potential is completely lost after CCCP treatment (10 μM, 20 min). However, for special cells, the concentration and time of CCCP treatment can be adjusted according to the experiment.

3. **JC-1 staining of adherent cells:**

If adherent cells need to be detected with a fluorescence spectrophotometer or flow cytometry, the cells can be harvested and resuspended according to the suspension cells procedure.

1) Dilute appropriate dilution buffer (5X) in ddH_2O to make a dilution buffer (1X). Immerse the dilution buffer (1X) in an ice bath, and always use chilled dilution buffer (1X).

2) For 6-well plates, remove the medium and wash cells in chilled dilution buffer (1X) one time if necessary. For CCCP-treated cells, remove the medium and wash cells in chilled dilution buffer (1X) one time. Mix JC-1 working solution and culture medium at a ratio of 1:1. Culture media can contain serum and phenol red. Then add the mixture to cover cells. Incubate at 37°C protected from light for 20 min.

***Note:** For 6-well plates, 2 mL of JC-1 working solution and cell culture medium mixture can be added per well. The mixture can be prepared 2-5 minutes in advance.

3) After incubation, wash cells in chilled dilution buffer (1X) two times.

4. **JC-1 staining of suspension cells:**

1) Dilute appropriate dilution buffer (5X) in ddH_2O to make a dilution buffer (1X). Immerse the dilution buffer (1X) in an ice bath, and always use chilled dilution buffer (1X).

2) Mix JC-1 working solution and culture medium at a ratio of 1:1. Culture media can contain serum and phenol red. Harvest the cells and resuspend them in the JC-1/medium mixture, adjusting the cell density to 1×10^5-1×10^6 cells/mL. Then incubate at 37°C protected from light for 20 min.

***Note:** The mixture can be prepared 2-5 minutes in advance.

3) After incubation, centrifuge at 800 rpm, 4 °C for 5 min. Discard the supernatant, and wash cells in chilled dilution buffer (1X) two times.

5. **Detection:** Measure the fluorescence of JC-1 monomers at Ex/Em: 490/530 nm or with the filter sets designed to detect FITC or GFP; and measure the fluorescence of JC-1 aggregates at Ex/Em: 525/590 nm or with the filter sets designed to detect PI or Cy3.

Note

1. The JC-1 (200X) may coagulate or precipitate at low temperatures. Please heat it at 20-25°C bathing until completely thawed. To avoid repeated freeze/thaw cycles, it is better prepared in single-use aliquots.
2. For JC-1 (200X) dilution, it must be done according to the instructions. Dissolve and mix well with ddH_2O prior to adding staining buffer (5X). Because JC-1 has a low solubility in water, it is hard to dissolve JC-1 (200X) directly with dilution buffer (1X).
3. After JC-1 staining, detection needs to be performed as much as possible (≤ 30 min), and the samples need to be stored in an ice bath before detection.
4. Do not dispense all dilution buffer (5X) into dilution buffer (1X), as the dilution buffer (5X) needs to be used directly in the experiment.
5. If precipitation is found in the dilution buffer (5X), it can be completely dissolved by heating at 37°C before use. Do not directly use dilution buffer (5X) containing precipitation.
6. CCCP is an inhibitor of the mitochondrial electron transport chain, which is toxic to the human, please pay attention to protection when using it.
7. For your safety and health, please wear lab coats and gloves during the experiment.
8. For research use only. Not to be used in clinical diagnostic or clinical trials.

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