Rhodamine 123 mitochondrial membrane potential assay kit

Introduction

Rhodamine 123 mitochondrial membrane potential assay kit supplies the fluorescent probe Rhodamine 123 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. Rhodamine 123 is a widely used mitochondrial membrane potential detection probe. Normally, Rhodamine 123 can enter mitochondria in a membrane potential-dependent manner and emit bright yellow-green fluorescence; when the mitochondrial membrane potential of cells decreases, Rhodamine 123 is released from the mitochondria, causing the yellow-green fluorescence in the mitochondria to weaken or disappear. The membrane potential change of mitochondria can be judged by the intensity of Rhodamine 123 fluorescence.

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 96-well plates, the kit can detect 1000 samples.

Components and Storage

Components	K2232-100 T
Rhodamine 123 (1000X)	100 μL
Dilution buffer	100 mL
CCCP (10 mM)	20 μL

This kit should be stored at -20°C, stable for 1 year. Rhodamine 123 (1000X) and CCCP (10 mM) should be stored at -20°C away from light, and avoid repeated freeze/thaw cycles.

Protocol

1. **Preparation of Rhodamine 123 working solution:** Dilute Rhodamine 123 (1000X) in Dilution buffer at a ratio of 1:1000 (1 μL Rhodamine 123 (1000X) +1 mL Dilution buffer) to make Rhodamine 123 working solution. The working solution is unstable, any unused working solution should be discarded after use.

***Note:** Please protect Rhodamine 123 from light when storage and use.

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 Rhodamine 123 probe is loaded as described below and mitochondrial membrane potential detection is performed. CCCP-treated cells exhibit weak yellow-green fluorescence or no fluorescence after Rhodamine 123 staining, while healthy cells exhibit bright yellow-green fluorescence after Rhodamine 123 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. 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) Seed cells in 6-well plates or other plates with designed treatment. After treatment, remove the medium, and wash cells in PBS one time if necessary.

2) Add Rhodamine 123 working solution to cover cells. Incubate at 37°C protected from light for 20-60 min. For 6-well plates, 1 mL/well Rhodamine 123 working solution is needed.

***Note:** The optimal time for incubation varies depending on the cell types.

3) After incubation, discard the working solution, and wash cells in a pre-warmed medium two times. Then add appropriate volume of medium and detect the fluorescence signal of Rhodamine 123 at Ex/Em: 507/529 nm or with the filter sets designed to detect FITC or GFP.

4. Staining of suspension cells:

1) Culture cells with designed treatment. After treatment, harvest cells and centrifuge at 800 rpm for 5 min. Then discard the supernatant.

2) Add Rhodamine 123 working solution to resuspend cells and adjust the cell density to 10^6 cells/mL. Incubate at 37°C protected from light for 20-60 min.

***Note:** The optimal time for incubation varies depending on the cell types.

3) After incubation, centrifuge at 800 rpm for 5 min to discard the supernatant, and wash cells in a pre-warmed medium two times. Then add appropriate volume of medium and detect the fluorescence signal of Rhodamine 123 at Ex/Em: 507/529 nm or with the filter sets designed to detect FITC or GFP.

5. Staining of Purified mitochondria:

1) Add 0.9 mL Rhodamine 123 working solution per 0.1 mL of purified mitochondria (total protein: 10-100 μg).

2) After mixing, detection can be performed directly by a fluorescence spectrophotometer with an excitation wavelength of 507 nm and an emission wavelength of 529 nm. Or observe under the microscope directly using the filter sets designed to detect FITC or GFP.

Note

1. Fluorescent probes are easy to quench, please protect them from light when storage and use.
2. CCCP is an inhibitor of the mitochondrial electron transport chain, which is toxic to the human, please pay attention to protection when using it.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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