Mitochondrial Permeability Transition Pore Assay Kit

Catalogue No.: K125

Size: 100T

Kit component:

Item	Size（100T）	Storage
Assay buffer	100 mL*2	-20℃
Calcein AM(1000x)	100 µL	-20℃, shading light
$Cocl_2$(100x)	1 mL	-20℃
Ionomycin(200x)	500 µL	-20℃, shading light

Storage:

The kit should be stored at -20℃ shading light for 12 months.

Principle of the Assay:

Mitochondrial Permeability Transition Pore Assay Kit or MPTP Assay Kit is a kit that uses the membrane-permeable fluorescent probe Calcein AM to detect the opening degree of the mitochondrial permeability transition pore. It is also often used in the study of cell death such as apoptosis and necrosis. The kit is a more direct detection method for detecting the opening of MPTP than only based on mitochondrial membrane potential analysis.

Firstly, loading Calcein AM through passive transportation, which is a kind of cell staining reagent for fluorescent labeling of living cells. It can easily penetrate the living cell membrane and is cleaved by intracellular esterase to form the membrane-impermeable polar molecule Calcein, which is then trapped in the cell and causes the cytoplasm including mitochondria to emit strong green fluorescence. After adding $CoCl_2$, the fluorescence from the cytoplasm is quenched by $CoCl_2$, only left fluorescence in the mitochondria. As a control, cells can be loaded with Calcein AM and $CoCl_2$, and treated with Ionomycin, so as to make cells load more Ca^{2+}, which causes the activation of MPTP and quenching of mitochondrial fluorescence.

Additional Materials Required:

Cell culture plate, Precision Pipettes, Disposable Pipette Tips, Centrifuge, Fluorescence Microscopy or Flow Cytometer

Reagent preparation:

Calcein AM staining solution: Mix 1 µL Calcein AM (1000×) in each 1 mL Assay Buffer.

Note: The final concentration of Calcein AM needs to be optimized through pre-experiments based on different cells and experiments. The recommended working concentration for Calcein AM is 1×, which can be adjusted between 0.5×-5×.

Fluorescence quenching solution: Mix 10 μL $CoCl_2$ (100×) in each 1 mL Calcein AM staining solution.

Note: The final concentration of $CoCl_2$ is recommended to be 1×,which usually provides better quenching effect. The final concentration of $CoCl_2$ can also be optimized according to the type of cells used in the experiment to find the best quenching effect, and can be adjusted between 0.1×-1×.

Ionomycin control: Mix 5 μL Ionomycin (200×) in each 1 mL Fluorescence quenching solution.

Note: The final concentration of Ionomycin is recommended to be 1×, which can also be adjusted between 0.5×-5×.

Assay Procedure (For reference):

Note: This kit (100 T) can detect 1,000 T with 100 μL of detection system per well in 96-well plate.

A. Analysis by Flow Cytometry

1. Treat cells with the desired method.

2. For non-adherent cells, Collect 1-5×10^5 cells by centrifugation (300 g, 5 min), wash with PBS twice and discard the PBS. For adherent cells, using Trypsin (EDTA free) to digest cells firstly and collect cells by centrifugation (300 g, 5 min), wash with PBS twice and discard the PBS.

Note: We recommend keeping unstained control cells (i.e. without staining) suspended in 1×Assay Buffer for both treated and untreated samples to set up the flow cytometer instrument.

3. Add appropriate volumes of Calcein AM staining solution, Fluorescence queuing solution, and Ionomycin control respectively, the cells were resuspended to a cell density of approximately 1×10^6 /mL. Incubate at 37℃ for 30-45 min, protected from light, different cells have different optimum incubation times.

4. Cells were collected by centrifugation at 300 g for 5 min. Add 1mL Assay Buffer, then gently resuspend the cells, centrifugate at 300 g for 5 min to collect cells.

5. Add 400 μL Assay Buffer to resuspend the cells, then analyze the cells by flow cytometry.

B. Analysis by Fluorescence Microscopy

1. For adherent cells

(1) Grow cells directly on a coverslip in cell culture plate. Incubate in a CO_2 Incubator at 37℃ for at least 24 h before treatment.

(2) Treat cells with the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

(3) Wash cells with PBS twice.

(4) Add appropriate volumes of Calcein AM staining solution, Fluorescence queuing solution, and Ionomycin control to the cells respectively. Generally, 100μL was added to 96-well plate per well, 250 μL to 24-well plate per well, 500 μL to 12-well plate per well, and 1 mL to 6-well plate per well. Then incubate at 37℃ for 30-45 min, protected from light, different cells have different optimum incubation times.

(5) After incubation, replace staining solution with fresh culture medium preheated at 37℃, and incubated at 37℃ for 30min, protected from light, to ensure that Calcein AM was fully hydrolyzed by lactonase into Calcein with green fluorescence.

(6) Wash cells with PBS 2-3 times, add Assay Buffer to cover the cells, then observe the samples under the fluorescence microscope (Calcein AM is green fluorescence, Ex/Em=494/517nm).

2. For non-adherent cells

(1) Treat cells with the desired method, counting cells.

(2) Centrifuge 300 g for 5 min to collect appropriate cells, discard the supernatant, wash the cells with PBS twice, and discard the PBS.

(3) Add appropriate volumes of Calcein AM staining solution, Fluorescence queuing solution, and Ionomycin control to the cells respectively, the cells were resuspended to a cell density of approximately 1×10^6/mL. Incubate at 37℃ for 30-45 min, protected from light, different cells have different optimum incubation times.

(4) Centrifuge 300 g for 5 min, discard the supernatant, slowly add 1 mL of 37℃ preheated culture medium to resuspend cells, and incubated at 37℃ for 30min, protected from light, to ensure that Calcein AM was fully hydrolyzed by lactonase into Calcein with green fluorescence.

(5) Centrifuge 300 g for 5 min, discard most of the culture medium, resuspend the cells and drop on the glass slide, then observe the samples under the fluorescence microscope.

Notes:

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.

2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.

3. Protection methods must be taken by wearing a lab coat and latex gloves during the experiment.

4. Fluorescent dyes are all prone to quenching. Please try to avoid light as much as possible to slow down fluorescence quenching.

5. Calcein AM (1000X) should avoid repeated freezing and thawing as much as possible. If used multiple times, please aliquot appropriately.

6. $CoCl_2$ is corrosive, harmful or irritating to the human body. It has specific organ toxicity to the respiratory and reproductive systems, or teratogenic and carcinogenic toxicity. Please handle it with care and ensure effective protection to avoid direct contact with the skin or inhalation. Also, be cautious to prevent corrosion of other items. $CoCl_2$ is toxic or harmful to aquatic organisms and must not be directly discharged into the environment.