Mitochondrial Permeability Transition Pore Assay Kit

Cat #: KTA4002 Size: 50 T/100 T

	Mitochondrial Permeability Transition Pore Assay Kit		
REF	**Cat #:** KTA4002	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells		
	Fluorescence Excitation/ Emission: Calcein-AM: Ex/Em=494/517 nm		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Mitochondrial permeablity transition pore (MPTP), also known as magachannel, is a non selective high conductivity channel that exists between the inner and outer membranes of mitochondria and is composed of multiple protein complexes. MPTP may be involved in the release of mitochondrial components during cell death. The normal mitochondrial inner membrane of a cell can maintain a normal mitochondrial potential gradient to ensure cellular respiration and energy supply. With the intake and release of Ca^{2+}, a low conductivity permeability transition pore switches back and forth between opening and closing. During apoptosis and pathological death of cells, the permeability of mitochondrial membrane potential transfer pore changes, Ca^{2+} overload, mitochondrial glutathione oxidation, increase of reactive oxygen species, including subsequent release of cytochrome C, and mitochondrial membrane potential decline all lead to the activation of MPTP. Mitochondrial Permeability Transition Pore Assay Kit s a more direct detection method for detecting the opening of MPTP than only based on mitochondrial membrane potential analysis. The principle is: 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 CoCl2, and treated with Ionomycin, so as to make cells load more Ca^{2+}, which causes the activation of MPTP and quenching of mitochondrial fluorescence.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	100 T	
Calcein AM (1000×)	50 μL	100 μL	-20℃, protected from light
$CoCl_2$ (100×)	0.5 mL	1 mL	-20℃
Ionomycin (200×)	250 μL	500 μL	-20℃, protected from light

Assay Buffer	100 mL	100 mL×2	-20℃

Materials Required but Not Supplied

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

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

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 then centrifugation.

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 lactase 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 lactase 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.

Strawberry moment: In addition to In addition to Mitochondrial Permeability Transition Pore Assay, Abbkine also offers JC-1 Mitochondrial Membrane Potential Assay Kit (KTA4001) and other cell state assay kits, such as Apoptosis Detection kit (KTA0002), One-step TUNEL Apoptosis Assay Kit (KTA2010/KTA2011), etc. Scan the QR code on the right and follow the Abbkine official account to learn more about Abbkine products.

Typical Data

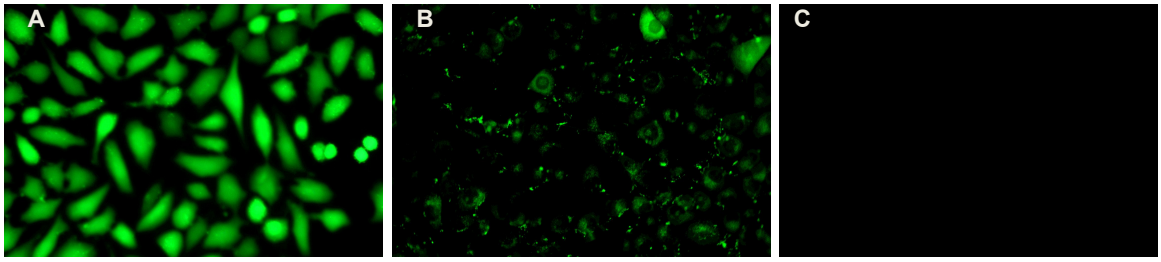

Figure 1. L929 cells were tested for MPTP opening using Mitochondrial Permeability Transition Pore Assay Kit

A-Calcein AM staining solution group: The mitochondria and cytoplasm have green fluorescence and strong fluorescence signal (refer to Figure 1A).

B-Fluorescence quenching solution group: only mitochondria have green fluorescence, with moderate fluorescence intensity (refer to Figure 1B).

C-Ionomycin control group: Both mitochondrial and cytoplasmic green fluorescence were quenched, with almost no fluorescence signal ratio.

The changes in the green fluorescence signals of B and C indicate that the MPTP is opened, and part of Calcein is released from mitochondria, and Co^{2+} enters mitochondria, which causes the quenching of the green fluorescence of Calcein.

Recommended Products

Catalog No.	Product Name
KTA4001	Mitochondrial Membrane Potential Assay Kit (JC-1)
KTA2010	One-step TUNEL Apoptosis Assay Kit (Green Fluorescence)
KTA0002	Annexin V-AbFluor™ 488/PI Apoptosis Detection kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.