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Caspase 3/7 and PI Apoptosis Double Staining kit

Cat #: KTA3029 Size: 20 T/100 T

	Caspase 3/7 and PI Apoptosis Double Staining kit		
REF	**Cat #:** KTA3029	LOT	**Lot #:** Refer to product label
	Applications: Flow cytometry and fluorescence detection for cell samples		
	Fluorescence Excitation/ Emission: Caspase 3/7 Green: E_x/E_m=488/525 nm, PI: E_x/E_m=535/617 nm		
	Note: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Caspase 3 (CPP32/ Apopain) plays a significant role in the initiation of apoptosis, it has been widely accepted as an indicator of apoptosis. Caspase 3 has a specific substrate polypeptide recognition sequence, namely aspartic acid-glutamate-valine-aspartic acid (DEVD).

The Caspase 3/7 and PI Apoptosis Double Staining kit is used for apoptosis detection by measuring the activity of Caspase 3/7 and the integrity of the cell membrane. Caspase 3/7 Green uses the Caspase 3/7 recognition sequence (DEVD) coupled to a high-affinity DNA fluorescent dye, which has cell membrane permeability and can penetrate the cell membrane into the cytoplasm. The Caspase 3/7 Green reagent itself is non-fluorescent and has a charge repulsion effect with DNA. When cells undergo apoptosis, Caspase 3/7 Green, as a substrate, is cleaved by activated Caspase 3/7 and releases a high-affinity DNA dye. After binding to DNA, it generates strong fluorescence (Ex/Em: 488 nm/525 nm, flow cytometry recommended FITC channel). When cells enter the late stage of apoptosis or necrosis, the integrity of the cell membrane is lost. Propidium Iodide (PI) can enter the cells of late apoptotic and necrotic cells, specifically bind to double-stranded DNA and produce intense red fluorescence (Ex/Em:535 nm/617 nm). When used in combination with Caspase 3/7 Green, it can simultaneously distinguish the activity of Caspase 3/7 enzymes in necrotic cells and apoptotic cells.

Materials Supplied and Storage Conditions

Kit components	Size		storage condition
	50 T	100 T	
Caspase 3/7 Green	20 μL	100 μL	-20℃, protected from light
PI	40 μL	200 μL	-20℃, protected from light

Materials Required but Not Supplied

·Centrifuge, fluorescence microscopy or flow cytometer
·Precision pipettes, disposable pipette tips, deionized water, glass slides
·Cell culture plate

Reagent Preparation

Note: Briefly centrifuge small vials at low speed prior to opening.

Caspase 3/7 Green: Ready to use as supplied. Equilibrate to room temperature before use. The unused reagents are sub-packaged and stored at -20℃, protected from light. Avoid repeated freezing and thawing.

Propidium Iodide (PI): Ready to use as supplied. Equilibrate to room temperature before use. The unused reagents are sub-packaged and stored at -20℃, protected from light. Avoid repeated freezing and thawing.

Assay Procedure

Experimental Design

Select an appropriate method to induce apoptosis in cells and set up a control group without induction. It is highly recommended to prepare positive single-staining tubes and negative controls when performing flow cytometry. Adjust the voltage appropriately during detection, which is very helpful for later cell population classification.

1. Flow Cytometry Analysis

(1) Collect cells, centrifuge at 300 g for 5 min at 4℃ to obtain 1-5×10^5 cells, and wash twice with ice-cold PBS.

Note: For adherent cells, use trypsin digestion to collect cells. If the digestion time is too long, it may cause cell membrane damage and false positive results of cell necrosis. Therefore, control the trypsin digestion time (it is recommended to use a mild digestion solution).

(2) Resuspend the cells in 500 μL of PBS.

(3) Add 1 μL of Caspase 3/7 Green staining solution and 1 μL of PI to each 500 μL of cell suspension, and gently mix.

Note: The amount of PI can be adjusted according to the actual situation. It is highly recommended to prepare positive single-staining tubes.

(4) Incubate at room temperature in the dark for 20-30 min.

(5) After incubation, place on ice. Analyze the cells using a flow cytometer within 30 min after staining.

2. Fluorescence Microscopy Analysis

A. For suspension cells:

(1) Prepare samples according to the flow cytometry analysis steps.

(2) Place the stained cell suspension on a glass slide and cover with a coverslip. Analyze the cells using a fluorescence microscope with appropriate filters as soon as possible.

B. For adherent cells, follow the following steps (using a 24-well plate as an example):

(1) Seed cells on coverslips or chamber slides and culture the cells.

(2) Induce apoptosis in cells using the desired method and set up a control group without induction.

(3) Remove the culture medium and wash the cells twice with PBS.

(4) Prepare the working solution: Add 1 μL of Caspase 3/7 Green and 1 μL of PI to 200 μL of PBS, and gently mix.

(5) Add 200 μL of the prepared working solution to each well and incubate at room temperature in the dark for 30 min.

(6) Analyze the cells under a fluorescence microscope with appropriate filters.

Note: Green fluorescence is prone to quenching during fluorescence microscopy photography. It is recommended to use a higher dye concentration and dilute it at a ratio of 1:200 during staining. Also, when taking pictures, first find the field of view with white light and then immediately take pictures with fluorescence to save.

Typical Data

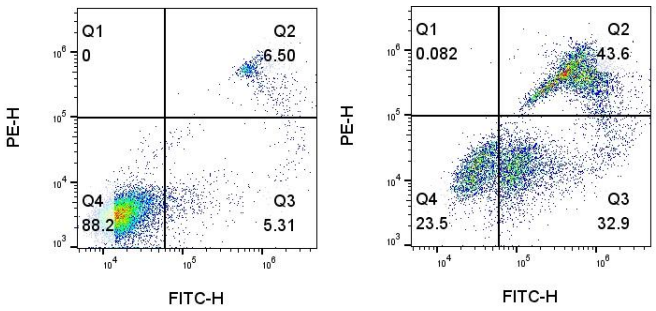

Figure 1. Hela cells were treated with DMSO(left) or A+B (1:2,000) (right) for 16 h and then stained with Caspase 3/7 and PI Apoptosis Double Staining kit. The stained samples were detected by flow cytometry, and PI was detected by PE channel. Caspase 3/7 Green was tested with FITC. Cell populations are mainly divided into living cells (Q4), apoptotic cells (Q3), and necrotic cells (Q2). After induction, apoptotic and necrotic cells increase significantly.

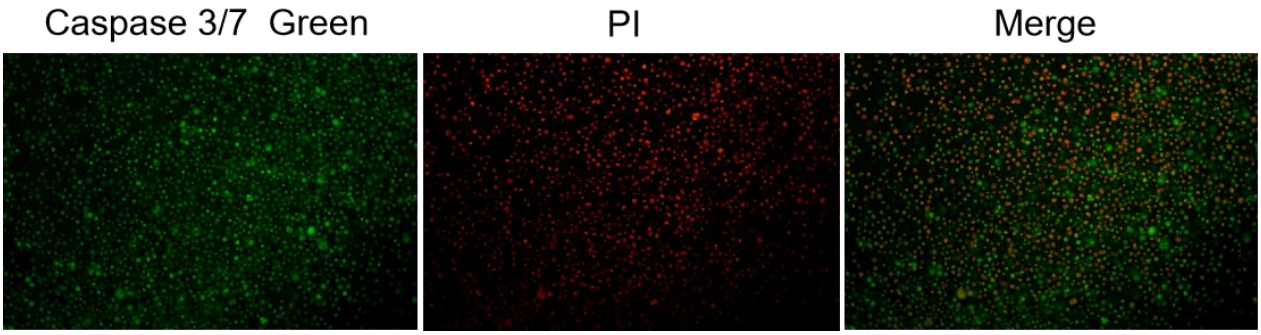

Figure 2. Jurkat cells were induced with inducer A+B (1:2,000) for 16 h, then stained with Caspase 3/7 and PI Apoptosis Double Staining kit, and detected by fluorescence microscope.

FAQ

1．What are the reasons for the overly strong signal of PI during flow cytometry detection?

PI is cytotoxic. If the staining concentration is too high or the staining time is too long, it will cause the signal to be too high. Generally, about 5 minutes of PI staining is sufficient. After staining, the sample should not be stored for too long. It is recommended to put it on the machine in time and set up a single anode tube for voltage and compensation adjustment.

2．The flow cytometry clustering is not obvious and cannot be closed?

It is recommended to set up strict controls, such as negative and single positive tubes. When conducting the test, adjust the appropriate voltage. It is suggested that people who are familiar with flow cytometry experiments perform the operation. Additionally, we have also tried some cell lines and found that flow cytometry grouping was not obvious (but fluorescence microscopy could). This might be related to the characteristics of the cells.

Recommended Products

Catalog No.	Product Name
KTA0002	Annexin V-AbFluor™ 488/PI Apoptosis Detection kit
KTA2011	One-step TUNEL Apoptosis Assay Kit (Red Fluorescence)
KTA4001	Mitochondrial Membrane Potential Assay Kit (JC-1)

Disclaimer

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