Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

Caspase 3/7 Activity Apoptosis Assay kit (Green Fluorescence)

Cat #: KTA3027 Size: 50 T/100 T

	Caspase 3/7 Activity Apoptosis Assay kit (Green Fluorescence)		
REF	**Cat #:** KTA3027	LOT	**Lot #:** Refer to product label
	Applications: Flow cytometry, fluorescence microscopy and fluorescence microplate reader detect for cell samples		
	Fluorescence Excitation/ Emission: Caspase 3/7 Green: E_x/E_m=488/525 nm		
	Note: Stored at -20℃ for 12 months, protected from light		

Assay Principle

The caspase 3/7 Active Apoptosis Detection Kit (Green fluorescence) is used for apoptosis detection by measuring the activity of caspase 3/7. Because 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).

In this kit, caspase 3/7 Green is conjugated to a high-affinity DNA fluorescent dye using the caspase 3/7 recognition sequence (DEVD), which has cell membrane permeability and can penetrate the cell membrane to enter the cytoplasm. 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 high-affinity DNA dyes. After binding to DNA, it generates strong fluorescence (Ex /Em: 488nm / 525 nm, flow cytometry recommended FITC channel), which is used for the detection of caspase-3/7 activity and apoptosis. This kit can be used to detect the activity and apoptotic status of caspase 3/7 in cells by flow cytometry, fluorescence microplate reader and fluorescence microscope.

Materials Supplied and Storage Conditions

Kit components	Size		storage condition
	50 T	100 T	
Caspase 3/7 Green	50 μL	100 μL	-20℃, protected from light
Z-VAD (OMe)-FMK	10 μL	20 μL	-20℃, protected from light

Materials Required but Not Supplied

·Centrifuge, fluorescence microscopy, flow cytometer, Fluorescence microplate reader
·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.

Z-VAD (OMe)-FMK: Ready to use as supplied, the concentration is 10 mM. 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, and at the same time set up a control group without induction. This kit provides a certain amount of the inhibitor Z-VAD (OMe)-FMK, and the inhibitor group can be added simultaneously with induction as needed. Although Z-VAD(OMe)-FMK is a widely recognized Caspase inhibitor, in the specific experimental process, the inhibitory effect of Z-VAD (OMe)-FMK on different apoptosis inducers and apoptosis of different cells varies. Researchers need to explore the drug concentration and experimental methods by themselves according to the experimental purpose. During the development of this reagent, camptothecin and raptinal were used to induce apoptosis in hela and jurkat cells, and the addition of inhibitors showed significant inhibitory effects. In addition to Z-VAD (OMe)-FMK, other Caspase inhibitors such as Ac-DEVD-CHO can also be selected, and screening should be conducted based on the experimental purpose.

1. Flow cytometry analysis and microplate reader detection

(1) Collect cells, centrifuge at 3 00g for 5 min at 4℃, collect 1-5×10^5 cells, and wash twice with ice-cooled PBS.

Note: Adherent cells should be digested with trypsin and then collected by centrifugation. If the digestion time is too long, it may cause damage to the cell membrane structure and lead to false positives of cell necrosis. Therefore, the trypsin digestion time should be controlled (it is recommended to use mild digestion solution).

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

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

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

(5) Place on ice after incubation. Within 30 min after staining, the staining was detected by flow cytometry (FITC channel) or inoculated into a 96-well full black plate and detected by fluorescence microplate reader (Ex/Em: 488nm/525 nm).

2. Fluorescence microscopy analysis

A. For suspension cells:

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

(2) Suspend the stained cells on a glass slide and cover them with a coverslip. Analyze the cells through a fluorescence microscope as soon as possible using an appropriate filter.

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

(1) Inoculate the cells onto a slide or chamber slide and culture the cells.

(2) Induce apoptosis of cells by the required method, and at the same time 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 for every 200 μL of PBS and gently mix well.

(5) Add 200 μL of the prepared working solution to each well.

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

Note: Fluorescence is prone to quenching when taking pictures with a fluorescence microscope. It is recommended to dilute the dye at a ratio of 1:200 when the dye concentration is high during staining. Additionally, when taking pictures, first use white light to find the field of view and then immediately take pictures with fluorescence for preservation

Typical Data

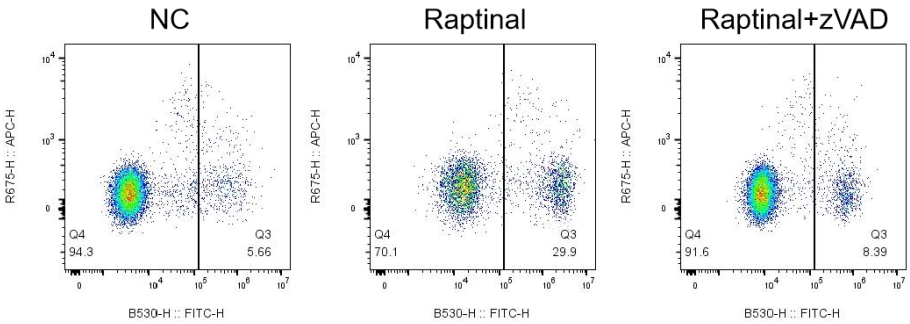

Figure 1. Jurkat cells were induced with Raptinal (5 μM) for 4 hours and with or without zVAD (10 μM), then stained with Caspase 3/7 Activity Apoptosis Assay kit (Green Fluorescence), and detected by flow cytometry (FITC channel). It can be seen that the proportion of apoptotic cell subsets (Q3) in the Raptinal induction group alone increased significantly (from 5.66% to 29.9%), while the proportion of apoptosis decreased significantly (8.39%) after the addition of inhibitors.

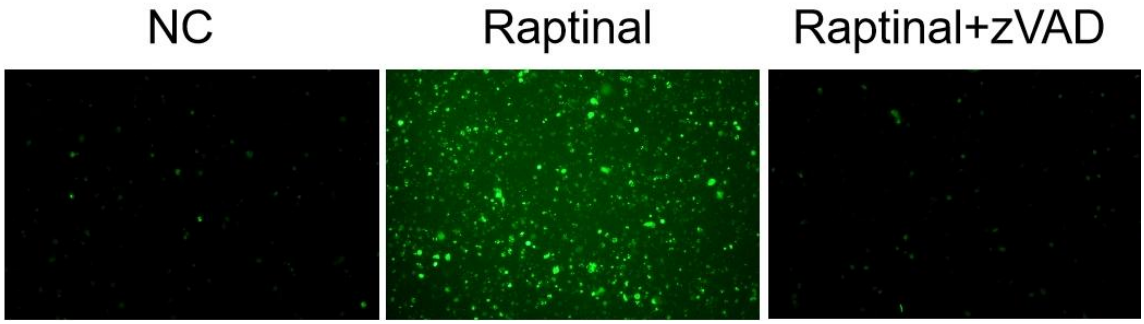

Figure 2. Jurkat cells were induced with Raptinal (5 μM) for 4 h and with or without zVAD(10 μM), and then stained with Caspase 3/7 Activity Apoptosis Assay kit (Green Fluorescence) and detected by fluorescence microscope. It can be seen that the fluorescence intensity of the Raptinal induction group alone was bright, while the fluorescence signal decreased significantly after the addition of the inhibitor.

FAQ

1．Why can the dye concentration be reduced and the time be shortened during flow dyeing?

The detection sensitivity of flow cytometry is relatively high, and the detection is instantaneous without fluorescence quenching. However, for fluorescence enzyme microscopy, the fluorescence signal is too low, and the exposure time needs to be extended, which is prone to fluorescence quenching and results in poor detection effect.

2．Can this kit be stained together with other reagents of membrane impermeability such as PI for apoptosis detection?

Sure, but it should be noted that clustering is not the same as the Annexin V /PI combination. Caspase 3/7 Green essentially detects the activity of Caspase 3/7, so its combination with PI differs from the detection clustering of Annexin V /PI.

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.