Annexin V-EGFP/PI Apoptosis Detection Kit

Cat #: KTA0005 Size: 20 T/50 T/100 T

	Annexin V-EGFP/PI Apoptosis Detection Kit		
REF	**Cat #:** KTA0005	LOT	**Lot #:** Refer to product label
	Applications: Flow cytometry and fluorescence detection for cell samples		
	Fluorescence Excitation/ Emission: Annexin V-EGFP: E_x/E_m=491/517 nm, PI: E_x/E_m=535/617 nm		
	Note: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Apoptosis is a form of programmed cell death to remove unwanted, damaged, or senescent cells from tissues. In normal cells, the negative phospholipids reside on the inner side of the cellular membrane while the outer surface of the membrane is occupied by uncharged phospholipids (PS). After a cell has entered apoptosis, the negatively charged PS are transported from the inner to the outer leaflet of the plasma membrane, thus exposing PS to the external cellular environment. The human anticoagulant, Annexin V, is a 35-36 kDa Ca^{2+} dependent phospholipid-binding protein that has a high affinity for PS. Annexin V labeled with a fluorophore or biotin can identify apoptotic cells by binding to PS exposed on the outer leaflet. Propidium iodide (PI) is a fluorescent nucleus dye, impermeant to live cells and apoptotic cells, but stains dead cells with red fluorescence, binding tightly to the nucleic acids in the cell. Annexin V-EGFP/PI Apoptosis Detection kit provides a rapid and convenient assay for apoptosis. After staining a cell population with Annexin V-EGFP/PI in the provided binding buffer, early apoptotic cells show green fluorescence of the cellular membrane, dead cells show red fluorescence of the nucleus and green fluorescence of the cellular membrane, and live cells show little or no fluorescence. In addition, to address the non-standardization of apoptosis detection and results, the kit also provides a patented positive reference substance for apoptosis. Detection can be analyzed by flow cytometry or by fluorescence microscopy.

Materials Supplied and Storage Conditions

Kit Components	Size			Storage Condition
	20 T	50 T	100 T	
Annexin V Binding Buffer (5×)	2 mL	5 mL	10 mL	-20℃
Annexin V-EGFP	100 μL	250 μL	500 μL	-20℃, protected from light
Propidium Iodide (PI)	40 μL	100 μL	200 μL	-20℃, protected from light
Apoptosis Inducer A	5 μL	5 μL	10 μL	-20℃
Apoptosis Inducer B	5 μL	5 μL	10 μL	-20℃

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.

1×Annexin V Binding Buffer: Prepared before use. Dilute Annexin V Binding Buffer (5×) to 1×Annexin V Binding Buffer with deionized water. Store the surplus reagent at 4℃ for 6 months.

Annexin V-EGFP: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃ for 6 months, protected from light. If long-term preservation, please pack it separately and keep it away from light at -20℃ to 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.

Apoptosis Inducer A: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Apoptosis Inducer B: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20℃.

Assay Procedure

Ⅰ Positive induction of apoptosis (selected)

1. For cultured cells to be induced apoptosis, the Apoptosis Inducer A or Apoptosis Inducer B were added into the culture medium according to the volume ratio of 1:1,000-1:3,000 (Apoptosis Inducer A and Apoptosis Inducer B can be added into the culture medium together).
2. Cell apoptosis was observed after 4, 8, 12, 16 or 24 h. For the most part, after 16-24 h, obvious changes in cell morphology can be seen under the light microscope, which can be used to observe apoptosis staining (It is recommended to adjust the induction time and concentration for different cells).

Ⅱ Detection cell apoptosis by Annexin V-EGFP/PI

A. Quantification by Flow Cytometry

1. Apoptosis was induced by the required methods, and uninduced cells were cultured as control.
2. After the treatment, 300g 5 min was centrifuged, the supernatant was discarded, resuspension cells with PBS and counted.

Note: For adherent cells, using trypsin (EDTA free) to digest cells firstly and then centrifugation. The time of trypsinization should not be too long, because trypsin could destroy the membrane structure.

3. Take 1-2×10^5 cells, 300g 5 min was centrifuged, the supernatant was discarded, resuspension cells with 100 μL 1×Annexin V Binding Buffer.
4. Add 5 μL Annexin V-EGFP and 2 μL PI and mix gently.
5. Incubate the cells at room temperature for 15 min in the dark.
6. After the incubation period, add 400 μL 1×Annexin V Binding Buffer, mix gently, and keep the samples on ice.

Note: Analyze the cells by flow cytometry within 30 min of staining. Use 491 nm and 535 nm excitation and measure fluorescence emission near 517 nm (FITC channel) and 617 nm (PE or PI channel).

B. Detection by Fluorescence Microscopy

1. For suspension cells:

(1) Follow the protocol for flow cytometry from step A.1 to step A.6.

(2) Place the cells in step A.6 on the slide, cover the cells with a cover slide, and analyze the cells through a fluorescence microscope as soon as possible using an appropriate filter.

2. For adherent cells:

Note: The advantage of this method is that apoptosis can be observed in situ, but the disadvantage is that some apoptotic cells can not be detected because they are not attached to the wall, and wash the cells as gently as possible.

(1) The corresponding cell climbing tablets were placed in the orifice plate, and an appropriate amount of cells were inoculated on the cell climbing plates to culture cells for at least 24 hours to ensure that the cells completely adhered to the wall.

(2) Apoptosis was induced by the required methods, and uninduced cells were cultured as control.

It is suggested that if conditions permit, 1,000g centrifuge 5 min can be used to centrifuge the porous plate after the end of apoptosis induction.

(3) Wash cells with PBS twice.

(4) Prepare working solution: add 5 μL Annexin V-EGFP and 2 μL PI to each 100 μL 1×Annexin V Binding Buffer and mix gently.

(5) Add appropriate amounts of working solution to cells and incubate at room temperature for 15 min in the dark (to ensure that the working solution completely covers the cells).

(6) Wash cells with 1×Annexin V Binding Buffer twice.

Note: Do not use PBS to wash cells during this step.

(7) Mount coverslips onto slides with a drop of 1×Annexin V Binding Buffer. For cells on chamber slides, add enough 1×Annexin V Binding Buffer to completely cover cells.

Note: Anti-fluorescence quenching agent can also be used.

(8) Analyze cells by fluorescence microscopy using the appropriate filters as soon as possible.

Highlight moment: In addition to detecting apoptosis based on changes in membrane, changes in cytoplasm and mitochondria can also reflect apoptosis. Cytoplasmic changes include Caspase (KTA3020, KTA3023) expression differences. Besides, decrease in mitochondrial membrane potential occurs in early apoptosis (KTA4001). And Tunel is a classic method for detecting apoptosis based on nucleus rupture (KTA2010, KTA2011). Scan the QR code on the right and follow the official account of Abbkine to learn more about Abbkine products.

Typical Data

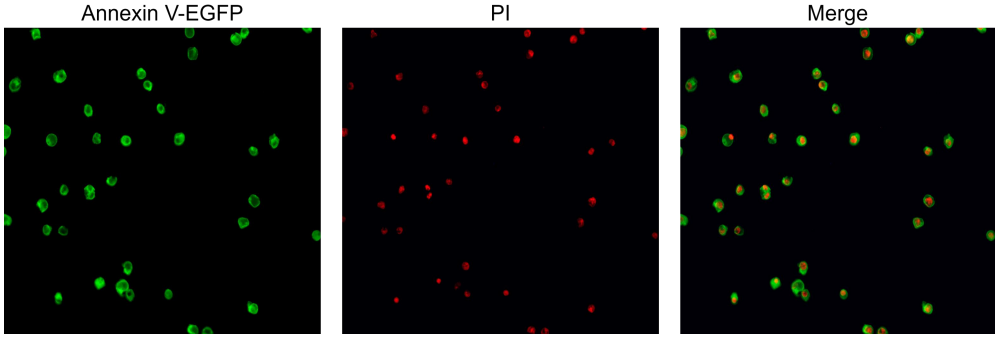

Figure 1. Jurkat cells were induced by this kit and were stained with Annexin V-EGFP/PI Apoptosis Detection Kit to detect the apoptotic effect.

FAQ

1．After incubation, can we do the Flow cytometry (FC) without adding 1×Annexin V Binding Buffer?

Yes, the volume of Annexin V Binding Buffer added was determined according to the loading volume required by flow cytometry. If the volume of the cell incubated suspension is sufficient, it's unnecessary to add the buffer.

2．How to set the positive control of the kit?

According to the datasheet, use the positive reagent provided in the kit for positive induction test. If the induction effect is not obvious, it is recommended to use other inducers (such as camptothecin) for induction.

3．After adding 100 μL Annexin V binding buffer, can you wait for 60 min or so to do the next step?

Because this experiment is a live cell stain. If the experimental pause time is too long, the cell vitality will be affected.

Recommended Products

Catalog No	Product Name	Recommended Reason
KTA2010	One-step TUNEL Apoptosis Assay Kit (Green Fluorescence)	Detection of cells and tissues apoptosis
KTA2011	One-step TUNEL Apoptosis Assay Kit (Orange Fluorescence)	Detection of cells and tissues apoptosis
KTA4001	Mitochondrial Membrane Potential Assay Kit (JC-1)	Apoptosis was reflected by the change of membrane potential
KTA3022	Caspase-3 Assay Kit (Colorimetric)	Caspase-3 activity reflects apoptosis
KTA3026	Caspase-9 Assay Kit (Colorimetric)	Caspase-9 activity reflects apoptosis
KTA3020	Caspase-1 Assay Kit (Colorimetric)	Caspase-1 activity reflects apoptosis

Disclaimer

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