Annexin V- AbFlour™ 647 Apoptosis Detection Kit (Red Fluorescence)

Cat #: KTA0004 Size: 50 T/100 T

	Annexin V-AbFlour™ 647 Apoptosis Detection Kit (Red Fluorescence)		
REF	**Cat #:** KTA0004	LOT	**Lot #:** Refer to product label
	Applicable samples: Flow cytometry and fluorescence detection of cell		
	Fluorescence excitation/emission: Annexin V-AbFlour™ 647: 650 nm/665 nm, PI: 535 nm/617 nm		
	Storage: Stored at 4℃ for 6 months		

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-AbFlour™ 647 Apoptosis Detection Kit (Red Fluorescence) provides a rapid and convenient assay for apoptosis. After staining a cell population with Annexin V-AbFlour™ 647 and PI in the provided binding buffer, early apoptotic cells show crimson fluorescence of the cellular membrane, dead cells show red fluorescence of nucleus and crimson fluorescence of the cellular membrane, and live cells show little or no fluorescence. Detection can be analyzed by flow cytometry or by fluorescence microscopy.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	50 T	100 T	
Annexin V Binding Buffer (5×)	5 mL	10 mL	4℃
Annexin V- AbFluor™ 647	250 μL	500 μL	4℃, protected from light
Propidium Iodide (PI)	100 μL	200 μL	4℃, protected from light

Materials Required but Not Supplied

- Centrifuge

- Pipettes and pipette tips
- Deionized water (ddH_2O)
- Glass slides
- Fluorescence Microscopy or Flow Cytometer
- 96-well plate for cell culture

Reagent Preparation

1×Annexin V Binding Buffer: Prepare 1×Annexin V Binding Buffer by dilute Annexin V Binding Buffer (5×) with deionized water.
Annexin V-AbFlour™ 647: Ready to use. Equilibrate to room temperature before use.
Propidium Iodide (PI): Ready to use. Equilibrate to room temperature before use.

Assay Procedure

A. Quantification by Flow Cytometry

1. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.
2. Collect 1-2×10^5 cells by centrifugation (4℃, 300 g, 5 min) and wash with ice-cold PBS twice.

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. Resuspend the cells in 100 µL 1×Annexin V Binding Buffer.
4. Add 4-5 µL Annexin V-AbFlour™ 647 and 1-2 µL PI to each 100 µL of cell suspension 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. Analyze the cells by flow cytometry within 30 min of staining. Use 650 nm and 535 nm excitation and measure fluorescence emission near 665 nm and 617 nm.

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 cell suspension from Step A.6 on a glass slide. Cover the cells with a glass coverslip. Analyze cells by fluorescence microscopy using the appropriate filters as soon as possible.

2. For adherent cells: the suggested protocol is as below:

(1) Grow cells on coverslips or chamber slides.

(2) Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

(3) Wash cells with PBS twice.

(4) Prepare working solution: add 4-5 µL Annexin V-AbFlour™ 647 and 1-2 µL PI to each 100 µL 1×Annexin V Binding Buffer and mix gently.

Note: The optimal concentration may need to be determined by specific experimental requirement.

(5) Add appropriate amounts of working solution to cells and incubate at room temperature for 15-30 min in the dark. (Incubation can be carried out on ice to slow down the apoptotic process, but the incubation time is extended to at least 30 min).

(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 c hamber 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.

Recommended Products

Catalog No.	Product Name
KTA0001	Annexin V-AbFluor™ 405 Apoptosis Detection Kit (Blue Fluorescence)
KTA0002	Annexin V-AbFluor™ 488 Apoptosis Detection Kit (Green Fluorescence)
KTA0003	Annexin V-AbFluor™ 555 Apoptosis Detection Kit (Orange Fluorescence)
KTA2010	TUNEL Apoptosis Detection Kit (Green Fluorescence)
KTA2011	TUNEL Apoptosis Detection Kit (Orange Fluorescence)
KTA4001	Mitochondrial Membrane Potential Assay Kit (JC-1)
KTD102-EN	Apoptosis Assay Cocktail
BMU104-EN	SuperKine™ Enhanced Antifade Mounting Medium

Disclaimer

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