

Annexin V-AbFluor 647 Apoptosis Detection kit (Red Fluorescence)

Cat #: orb1147903 (manual)

Size: 50 T/100 T

Product name: Annexin V-AbFluor 647 Apoptosis Detection kit (Red Fluorescence)

Catalog number: orb1147903

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°C 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 Ca²⁺-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°C
Annexin V- AbFluor 647	250 µL	500 µL	4°C, protected from light
Propidium Iodide (PI)	100 µL	200 µL	4°C, protected from light

Materials Required but Not Supplied

- Centrifuge
- Pipettes and pipette tips
- Deionized water (ddH₂O)
- 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 1-2×10⁵ cells by centrifugation (4°C, 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 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.

Disclaimer

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