

## Annexin V-AF 488/PI Apoptosis Detection kit

Cat #: D-AKE0002

Size: 50T

Storage: Stored at -20°C 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  $\text{Ca}^{2+}$  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-AF 488/PI Apoptosis Detection kit provides a rapid and convenient assay for apoptosis. After staining a cell population with Annexin V-AF 488/PI Apoptosis Detection kit 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 (50T)	Storage conditions
Annexin V Binding Buffer (5×)	5 mL	-20°C
Annexin V-AF 488	250 µL	-20°C, protected from light
Propidium Iodide (PI)	100 µL	-20°C, protected from light
Apoptosis Inducer A	5 µL	-20°C
Apoptosis Inducer B	5 µL	-20°C

## 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°C for 6 months.

**Annexin V-AF 488:** Ready to use as supplied. Equilibrate to room temperature before use. The unused reagents are sub-packaged and stored at -20°C, 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°C, protected from light. Avoid repeated freezing and thawing.

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

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

## Assay Procedure

### I Positive cell apoptosis induction

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-AF 488/PI

#### 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  $1-2 \times 10^5$  cells by centrifugation ( $4^\circ\text{C}$ , 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  $\mu\text{L}$  1 $\times$ Annexin V Binding Buffer.
4. Add 5  $\mu\text{L}$  Annexin V- AbFluor™ 488 and 2  $\mu\text{L}$  PI to each 100  $\mu\text{L}$  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  $\mu\text{L}$  1 $\times$ Annexin V Binding Buffer, mix gently, and keep the samples on ice. 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 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 (Annexin V- AbFluor™ 488 can be imaged using FITC settings, while PI can be imaged using Cy®3 or Texas Red® settings).

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, either preheated or at room temperature.

4) Prepare working solution: add 5 µL Annexin V- AbFluor™ 488 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 (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.