

# Annexin V-FITC Apoptosis Kit

**Product No.:** K019

**Size:** 50 tests

## Description:

Annexin V Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, cells translocate the membrane phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can be easily detected by staining with a fluorescent conjugate of Annexin V, a protein that has a high affinity for PS. The one-step staining procedure takes only 10 minutes. Detection can be analyzed by flow cytometry or by fluorescence microscopy. The kit can differentiate apoptosis vs necrosis when performing both Annexin V-FITC and PI staining.

## Results of flow cytometry, refer to figure 1 and 2:

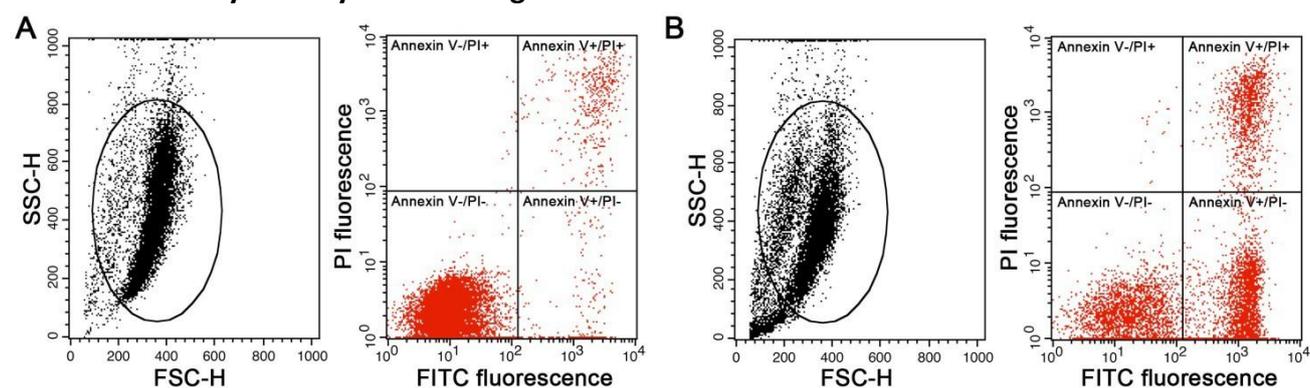


Figure 1: The cells were stained with this kit and the effect of apoptosis was detected by flow cytometry. Untreated Jurkat cells (A) or 10 M Camptothecin treated for 4 hours(B) were stained with this kit. Cells scattering and fluorescence were detected by flow cytometry. It can be seen from the figure, for the apoptosis inducing agent camptothecin treated cells, Annexin V-FITC staining positive and PI staining negative cells, namely apoptotic cells, increased significantly (Figure B in the lower right quadrant), double positive cells of Annexin V-FITC and PI staining, namely the necrotic cells, also increased (Figure B, right upper quadrant). The measured data may be different due to cell type, cell apoptosis, testing equipment and so on, the data in the figure is for reference only.

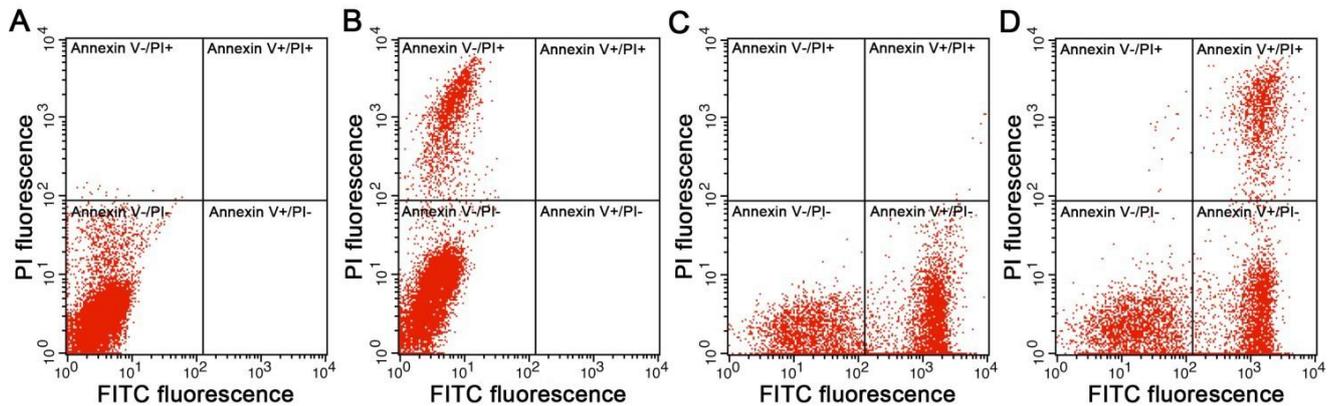


Figure 2: The cells were stained with this kit and detection results were verified by flow cytometry. Jurkat cells were stained with 10 M camptothecin for 4 hours, then without staining (A), staining only with PI of the kit (B), staining only with Annexin V-FITC of the kit (C), double staining with Annexin V-FITC and PI of the kit (D). It can be seen from the figure, undyed cells were double negative cells (A), only with PI staining appeared positive cells expected for PI staining, only with Annexin V-FITC staining appeared positive cells expected for Annexin V-FITC staining, double staining with Annexin V-FITC and PI, there appeared positive apoptotic cells stained by Annexin V-FITC and double positive necrotic cells. The measured data may be different due to cell type, cell apoptosis, testing equipment and so on, the data in the figure is for reference only.

**Kit Summary:**

Detection method- Flow cytometry (Ex = 488 nm; Em = 530 nm) and fluorescence microscopy

Sample type- Living cells (suspension and adherent)

Species reactivity- Mammalian

Kit size- 20 assays, 50 assays

Applications- Detect early/middle stages of apoptosis; differentiate apoptosis from necrosis.

**Features :**

Simple one step staining procedure in 10 minutes

Fast and convenient

Kit can differentiate apoptosis vs necrosis when performing both Annexin V-FITC and PI staining

**Kit components:**

Annexin V-FITC

1X Binding Buffer

Propidium Iodide (PI)

**Storage:**

4°C for 12 months.

**Annexin V-FITC Assay Protocol:**

**A. Incubation of cells with Annexin V-FITC**

1. Induce apoptosis by desired method. For adherent cells, gently trypsinize and wash cells with serum-containing media.
  2. Collect cells by centrifugation, suspend with PBS and count.
  3. Collect  $1-5 \times 10^5$  cells, resuspend cells in 195  $\mu$ l of 1X Binding Buffer.
  4. Add 5  $\mu$ l of Annexin V-FITC and 10  $\mu$ l of propidium iodide, mix gently.
  5. Incubate at room temperature (20-25°C) for 10min in the dark, then put on the ice.
- Proceed to B or C below depending on method of analysis.

**B. Quantification by Flow Cytometry**

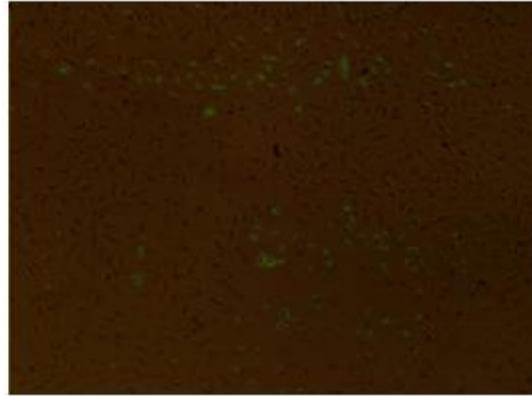
Analyze Annexin V-FITC binding by flow cytometry (Ex = 488 nm; Em = 530 nm) using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2).

**C. Detection by Fluorescence Microscopy**

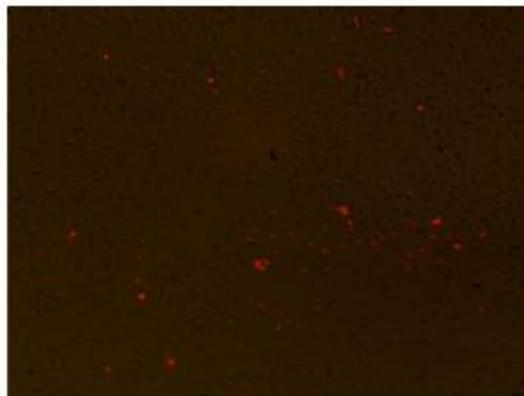
1. Centrifuge the cell suspension from Step A.5 to collect cells, gently suspend with 50-100 $\mu$ l 1X Binding Buffer. Place the cell suspension on a glass slide. Cover the cells with a glass coverslip and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization. (Cells must be incubated with Annexin V-FITC before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane.)
2. Observe the cells under a fluorescence microscope using a dual filter set for FITC & rhodamine. Cells that have bound Annexin V-FITC will show green staining in the plasma membrane. Cells that have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (FITC) on the cell surface (plasma membrane).



**Annexin V-FITC-PI staining**



**Annexin V-FITC staining**



**PI staining**

**Attention:**

1. If there is bacterial or fungal infection, it will seriously affect the results.
2. Should detect after dyeing, long time may lead to an increased number of apoptotic or necrotic cells
3. If the cells were collected using trypsin, should remove the residual trypsin. Residual trypsin will degrade Annexin V-FITC.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety, please wear the experimental clothes and disposable gloves.