



Technical Manual

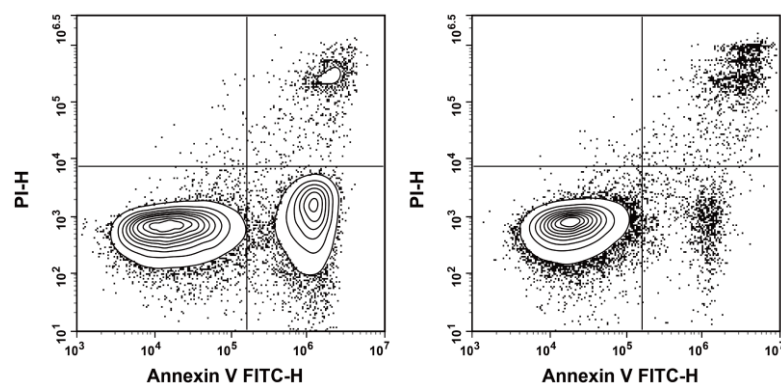
Annexin V-FITC/PI Apoptosis Kit

- **Catalogue Code: AKES032**
- **Research Use Only**
- **Size: 20/50/100/200 Assays**

1. Introduction

The Assay Genie Annexin V-FITC/PI Apoptosis Kit is developed to detect the apoptosis of suspension cells and adherent cells. Annexin V is a member of the annexin family, which binds to phosphatidylserine (PS) in a calcium-dependent manner. Annexin V-FITC, the FITC-conjugated format, binds specifically to the PS on the outer leaflet of apoptotic cell membrane and can be detected with flow cytometry or fluorescence microscopy. Due to the loss of integrity of membrane, PI can enter late apoptotic or necrotic cells to stain DNA. Cells at different apoptotic stages can be distinguished by using Annexin V and PI.

Jurkat cells were treated with 5 μ M Camptothecin and detected with this kit.



Jurkat cells were cultured with (Left) or without (Right) 5 μ M Camptothecin for 4 h. Annexin V-FITC single-positive cells were early apoptotic cells, Annexin V-FITC and PI double-positive cells were necrotic or late apoptotic cells, and PI single-positive cells were naked nuclei.

2. Kit Components

SKU	Product	20 Assays	50 Assays	100 Assays	200 Assays	Storage
AKES007	Annexin V-FITC Reagent	100 μ L	250 μ L	500 μ L	1 mL	2~8°C, avoid direct sunlight
AKES028	Annexin V Binding Buffer (10 \times)	1.4 mL \times 2	5.5 mL	11 mL	11 mL \times 2	2~8°C
AKES029	PI Staining Solution (50 μ g/mL)	100 μ L	250 μ L	500 μ L	1 mL	2~8°C, avoid direct sunlight

3. Storage

Store at 2~8°C for one year. Avoid freeze / thaw cycles.

4. Instructions

1X Annexin V Binding Buffer: Dilute Annexin V Binding Buffer (10 \times) with deionized water to 1X Annexin V Binding Buffer before use.

5. Staining Procedure

One-Step Process

1. Induce apoptosis of suspension cells with reagents of interest. Collect cell cultures, centrifuge at 300 x g for 5 min and discard the supernatant. Add PBS to wash the cells and resuspend the cells gently followed by the cell counting.
2. Split the cell suspension into tubes, 1~5×10⁵ cells for each, centrifuge at 300 x g for 5 min and discard the supernatant. Add PBS to wash the cells and discard the supernatant. Add 500 µL of 1×Annexin V Binding Buffer to resuspend the cells.
3. Add 5 µL of Annexin V-FITC and 5 µL of PI to each tube.
4. Gently vortex the cells and incubate at room temperature for 15~20 min in the dark.
5. Analyze the cells immediately with proper machine settings. Otherwise, place the cells on ice in the dark and analyze within 1 h.
Note: Annexin V-FITC can be detected in FITC channel while PerCP/Cy5.5 channel is preferred to ECD channel for PI detection.

Two-Step Process

1. Induce apoptosis of suspension cells with reagents of interest. Collect cell cultures, centrifuge at 300 x g for 5 min and discard the supernatant. Add PBS to wash the cells and resuspend the cells gently followed by the cell counting.
2. Split the cell suspension into tubes, 1~5×10⁵ cells for each, centrifuge at 300 x g for 5 min and discard the supernatant. Add PBS to wash the cells and discard the supernatant. Add 100 µL of 1×Annexin V Binding Buffer to resuspend the cells.
3. Add 2.5 µL of Annexin V-FITC and 2.5 µL of PI to each tube. (Attributed to the higher resolution of two-step protocol, half the amount of the reagents can still guarantee a result of matched quality as in the one-step protocol. It's also recommended that users titrate the reagents for optimal performance in specific models).
4. Gently vortex the cells and incubate at room temperature for 15~20 min in the dark.
5. Add 400 µL of 1×Annexin V Binding Buffer to the tube, and mix gently.
6. Analyze the cells immediately with proper machine settings. Otherwise, place the cells on ice in the dark and analyze within 1 h.
Note: Annexin V-FITC can be detected in FITC channel while PerCP/Cy5.5 channel is preferred to ECD channel for PI detection.

6. Notes

1. This kit is for research use only.
2. When detecting adherent cells, the suspension cells generated after induction of apoptosis should be collected and detected together with the subsequently collected adherent cells.
3. Mechanical damage caused by digestion of adherent cells should be avoided as much as possible. At the same time, trypsin digestion solution should not contain EDTA, because EDTA will affect the binding of Annexin V to phosphatidylserine.
4. If trypsin containing EDTA is used, cells should be washed thoroughly after harvesting to ensure that EDTA is removed.
5. Detect apoptosis as soon as possible after staining to avoid the increase number of apoptosis or necrosis. Avoid extended exposure of the samples to direct light to protect the fluorophores from quenching.
6. For your safety and health, please wear a lab coat and disposable gloves before the experiment.