



TECHNICAL MANUAL

Annexin V-APC/PI Apoptosis Kit

- **SKU CODE:** AKES037
- **SIZE:** 20 Assays / 50 Assays
- **DETECTION PRINCIPLE:** Flow Cytometry
- **RUO:** Research-Use-Only

Annexin V-APC/PI Apoptosis Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Specification:

20 Assays / 50 Assays

Measuring instrument:

Flow cytometer or fluorescence microscope

Sample type:

Suspension cells and adherent cells

2. Storage & Expiry

This product should be stored at 2-8°C in dark conditions for up to 12 months. Avoid freeze/thaw cycles. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. Product Description

The Assay Genie Annexin V-APC/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-APC, the APC-conjugated format, binds specifically to the PS on the outer leaflet of the apoptotic cell membrane and can be detected with flow cytometry or fluorescence microscopy.

Due to the loss of membrane integrity, 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.

4. Kit Contents

No	Component Name	Size (20 Assays)	Size (50 Assays)	Storage
1	Annexin V-APC Reagent	125 µL	125 µL×2	2-8°C, protected from light
2	Annexin V Binding Buffer (10×)	1.4 mL×2	5.5 mL	2-8°C
3	PI Reagent (50 µg/mL)	125 µL	125 µL×2	2-8°C, protected from light

Note: All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.

Additional materials required:

1. Flow cytometer (or fluorescence microscope).
2. Centrifuge.
3. Precision pipettes and disposable pipette tips. Deionized water.
4. Disposable tubes.
5. PBS.

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.

3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
5. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.
6. Detect apoptosis as soon as possible after staining to avoid an increase in the number of apoptotic or necrotic cells. Avoid extended exposure of the samples to direct light to protect the fluorophores from quenching.

6. Reagent Preparation

1. **1× Annexin V Binding Buffer:** Dilute Annexin V Binding Buffer (10×) with deionized water to 1× Annexin V Binding Buffer before use.

7. Sample preparation

Adherent cells

1. When detecting adherent cells, the suspension cells generated after induction of apoptosis should be collected and detected together with the subsequently collected adherent cells.
2. Mechanical damage caused by digestion of adherent cells should be avoided as much as possible. At the same time, the trypsin digestion solution should preferably not contain EDTA, because EDTA will affect the binding of Annexin V to phosphatidylserine.

3. If trypsin containing EDTA is used, cells should be washed thoroughly after harvesting to ensure that EDTA is removed.

8. Assay Procedure

Control settings for Annexin V experiments are critical to experimental results.

One-step process

1. Induce apoptosis of suspension cells with reagents of interest. Collect cell cultures, centrifuge at 300 ×g for 5 min and discard the supernatant. Add PBS to wash the cells and resuspend the cells gently, followed by cell counting.
2. Split the cell suspension into tubes, 1~5×10⁵ cells for each, centrifuge at 300 ×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-APC 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-APC can be detected in the APC channel, while the ECD channel is preferred to the PE channel for PI detection; if the sample has autofluorescence in the FITC channel, the PerCP/Cy5.5 channel is selected for PI detection.

Two-step process

1. Induce apoptosis of suspension cells with reagents of interest. Collect cell cultures, centrifuge at 300 ×g for 5 min and discard the supernatant. Add PBS to wash the cells and resuspend the cells gently, followed by cell counting.
2. Split the cell suspension into tubes, 1~5×10⁵ cells for each, centrifuge at 300 ×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 μ L of Annexin V-APC and 2.5 μ L of PI to each tube. (Owing to the higher resolution of the two-step protocol, half the amount of the reagents can still guarantee a result of matched quality as in the one-step protocol. It is 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 μ L of 1 $\times$ 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-APC can be detected in the APC channel, while the ECD channel is preferred to the PE channel for PI detection; if the sample has autofluorescence in the FITC channel, the PerCP/Cy5.5 channel is selected for PI detection.*

9. Example Analysis

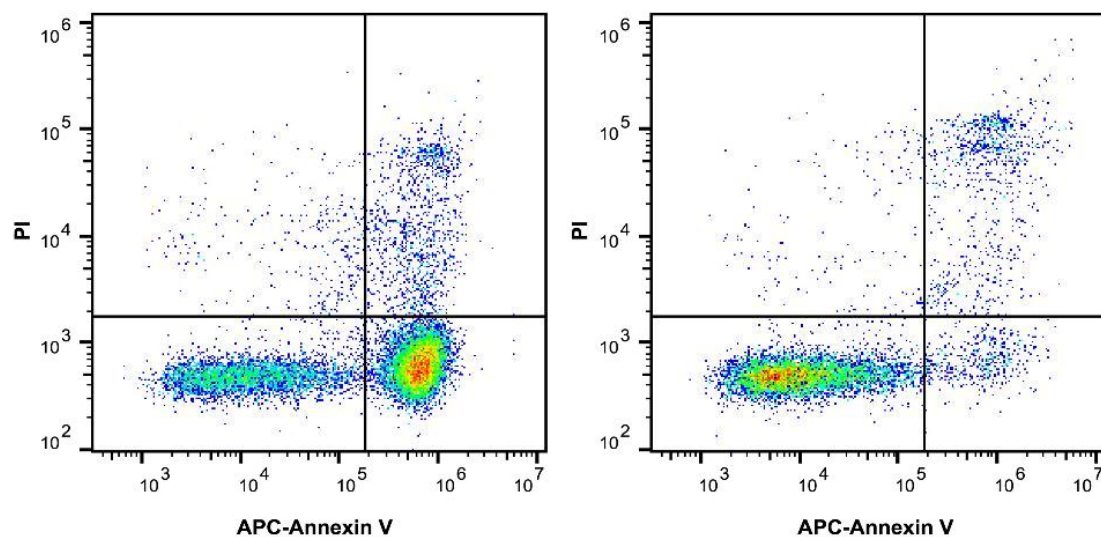
Jurkat cells were treated with 5 μ M Camptothecin and detected with this kit.

Sample Detection Results:

Jurkat cells were cultured with (Left) or without (Right) 5 μ M Camptothecin for 4 h:

- **Annexin V-APC single-positive cells:** early apoptotic cells.
- **Annexin V-APC and PI double-positive cells:** necrotic or late apoptotic cells.
- **PI single-positive cells:** naked nuclei.

The assay results for each sample are presented below:



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Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

