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Datasheet

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Annexin V-FITC/PI Apoptosis Detection kit

Cat# CC1052

Store at 2-8˚C and protected from light

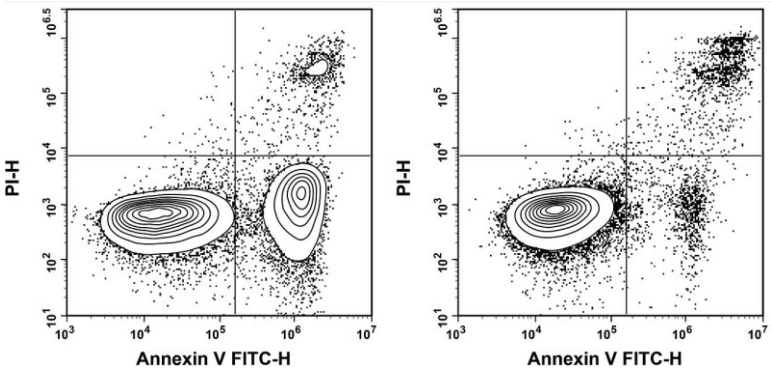
INTRODUCTION

Annexin V-FITC/PI Apoptosis Detection kit is developed to identify apoptotic and necrotic 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.

Propidium Iodide (PI) is a common DNA dye that is not permeable to cell membrane. Once binding to DNA, the flurescence of PI increases by nearly 20 fold. 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 μM Camptothecin and detected with this kit.



Jurkat cells were cultured with (Left) or without (Right) 5 μM 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.

COMPONENTS

Products	50 Tests	100 Tests	200 Tests
Annexin V-FITC Reagent	250 μL	500 μL	1 mL
Annexin V Binding Buffer (10 ×)	5.5 mL	11 mL	11 mL×2
Propidium Iodide (PI) Staining Solution	250 μL	500 μL	1 mL

The Annexin V Binding Buffer (10 ×) is a 10 × concentrated solution. Dilute with DI water to 1 × working solution before use.

For example: Take 1 mL Annexin V Binding Buffer (10 ×), dilute with DI water to 10 mL.

STAINING PROCEDURE

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 the cell counting.

   Tip: This product is only validated in suspension cells. Good cell viability is the key to the experiment. When the adherent cells are used for apoptotic detection, treatments like digestion may increase the ratio of necrotic or apoptotic cells and cause uncontrollable effects on the experimental results.
2. Split the cell suspension into tubes, 1~5 × 10^5 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-FITC and 5 µl of Propidium Iodide (PI) Staining Solution 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.

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 the cell counting.

   Tip: This product is only validated in suspension cells. Good cell viability is the key to the experiment. When the adherent cells are used for apoptotic detection, treatments like digestion may increase the ratio of necrotic or apoptotic cells and cause uncontrollable effects on the experimental results.
2. Split the cell suspension into tubes, 1~5 × 10^5 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 µl of Annexin V-FITC and 2.5 µl of Propidium Iodide (PI) Staining Solution 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.

CAUTIONS

1. For maximal assay performance, this kit should be used within 12 months. Avoid freeze / thaw cycles.

2. For FCM analysis, please set untreated cells stained with both Annexin V-FITC and PI as negative control. As for compensation controls, please use drug-treated cells stained with either Annexin V-FITC or PI.
3. Annexin V-FITC can be detected in FITC channel while PerCP/Cy5.5 channel is preferred to PE channel for PI detection due to the large compensation needed between FITC and PE channels.
4. Detect apoptosis as soon as possible after staining to avoid the increase number of apoptosis or necrosis
5. Avoid extended exposure of the samples to direct light to protect the fluorophores from quenching.
6. For your safety and health, please wear the lab coat and disposable gloves before the experiments.

PRODUCT USE LIMITATION

These products are intended for research use only.