

Annexin V-AF647 / PI Apoptosis Detection Kit

Catalog No: E-CK-A213

Sizes: 20 T / 50 T / 100 T / 200 T

Cat.	Products	20 T	50 T	100 T	200 T	Storage
E-CK-A113	Annexin V-AF647	100 µL	250 µL	500 µL	1 mL	2-8°C
E-CK-A151	Annexin V Binding Buffer (10 ×)	1.4 mL×2	5.5 mL	11 mL	11mL×2	2-8°C
E-CK-A161	Propidium Iodide (PI) Staining Solution	100 µL	250 µL	500 µL	1 mL	2-8°C
Manual				One Copy		

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

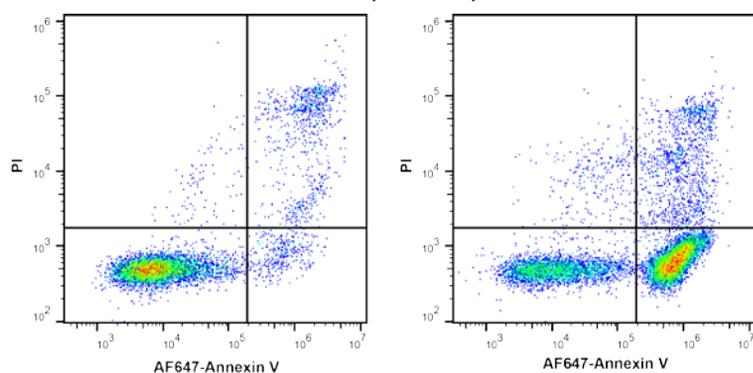
Introduction

Elabscience® Annexin V-AF647/PI Cell 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. The fluorescent format of this intracellular protein, Annexin V-AF647, binds specifically to the PS on outer leaflet of cell membrane by 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 fluorescence 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 1 μ M Camptothecin and detected by this kit:



Jurkat cells were treated with 1 μ M Camptothecin (**Right**) or not (**Left**) for 4 h. Annexin V-AF647 single-positive cells were early apoptotic cells, Annexin V-AF647 and PI double-positive cells were necrotic or late apoptotic cells, and PI single-positive cells were nude nuclear cells.

Instructions

The Annexin V Binding Buffer (10 \times)[Cat:E-CK-A151] is a 10 \times concentrated solution. Dilute with DI water to 1 \times working solution before use.

For example: Take 1 mL Annexin V Binding Buffer (10 \times), 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 and centrifuge at 300 g for 5 min, discard the supernatant. Add appropriate PBS to wash the cells, resuspend gently and count the cells.

Note: 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. Please be aware!

2. Split the cell suspension into tubes, 1-5 \times 10⁵ cells for each. Centrifuge at 300 g for 5 min, discard the supernatant. Add appropriate PBS to wash the cells and discard the supernatant. Add 500 μ L of 1 \times Annexin V Binding Buffer to resuspend the cells.
3. Add 5 μ L of Annexin V-AF647 and 5 μ L 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 and centrifuge at 300 g for 5 min, discard the supernatant. Add appropriate PBS to wash the cells, resuspend gently and count the cells.

Note: 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. Please be aware !

2. Split the cell suspension into tubes, $1-5 \times 10^5$ cells for each. Centrifuge at 300 g for 5 min, discard the supernatant. Add appropriate PBS to wash the cells and discard the supernatant. Add 100 μ L of $1 \times$ Annexin V Binding Buffer to resuspend the cells.
3. Add 2.5 μ L of Annexin V-AF647 and 2.5 μ L 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 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.

Storage

Store at 4°C for up to one year. Annexin V-AF647 and PI Staining Solution should be stored in dark.

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-AF647 and PI as negative control. As for compensation controls, please use drug-treated cells stained with either Annexin V-AF647 or PI.
3. Annexin V-AF647 can be detected in APC channel while PE channel is preferred to PerCP/Cy5.5 channel for PI detection due to the large compensation needed between APC and PerCP/Cy5.5 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.