Elabscience®

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（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Annexin V-PE / 7-AAD Apoptosis Detection Kit

Catalog No: E-CK-A216

Sizes： 20 T / 50 T / 100 T / 200 T

Cat.	Products	20 T	50 T	100 T	200 T	Storage
E-CK-A115	Annexin V-PE	100 μL	250 μL	500 μL	1 mL	2-8℃
E-CK-A151	Annexin V Binding Buffer (10 ×)	1.4 mL×2	5.5 mL	11 mL	11 mL×2	2-8℃
E-CK-A162	7-AAD Viability Staining Solution	100 μL	250 μL	500 μL	1 mL	-20℃
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.

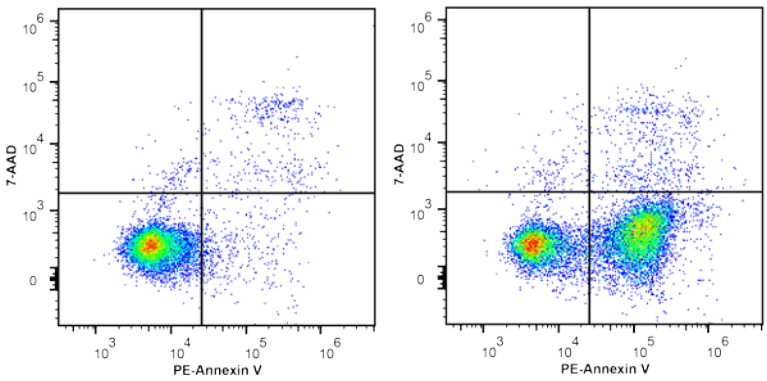
Introduction

Elabscience® Annexin V-PE/7-AAD 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-PE, binds specifically to the PS on outer leaflet of cell membrane by flow cytometry or fluorescence microscopy.

7-AAD (7-amino-actinomycin D) has a high DNA binding constant and is efficiently excluded by intact cells. It is useful for DNA analysis and dead cell discrimination during flow cytometric analysis. Due to the loss of integrity of membrane, 7-AAD can enter late apoptotic or necrotic cells to stain DNA. Cells at different apoptotic stages can be distinguished by using Annexin V and 7-AAD.

Jurkat cells were treated with 1μM Camptothecin and detected by this kit.



Jurkat cells were treated with 1μM Camptothecin (**Right**) or not (**Left**) for 4 h. Annexin V-PE single-positive cells were early apoptotic cells, Annexin V-PE and 7-AAD double-positive cells were necrotic or late apoptotic cells, and 7-AAD single-positive cells were nude nuclear cells.

Instructions

The Annexin V Binding Buffer (10 ×)[Cat:E-CK-A151] 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 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 × 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 500 μL of 1 × Annexin V Binding Buffer to resuspend the cells.
3. Add 5 μl of Annexin V-PE and 5 μl of 7-AAD Viability 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 × 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 µL of 1 × Annexin V Binding Buffer to resuspend the cells.
3. Add2. 5 µl of Annexin V-PE and 2.5 µl of 7-AAD Viability 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.

Storage

Annexin V-PE and 7-AAD Viability Staining Solution should be stored in dark.

7-AAD Viability Staining Solution should be split into small tubes and storage at -20°C.

Other reagents should be stored at 4 °C.

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-PE and 7-AAD as negative control. As for compensation controls, plesase use drug-treated cells stained with either Annexin V-PE or 7-AAD.
3. Annexin V-PE can be detected in PE channel while 7-AAD can be detected in PerCP/Cy5.5 channel.
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.