

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

Annexin V-APC / 7-AAD Apoptosis Detection Kit

Catalog No: E-CK-A218

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

Cat.	Products	20 T	50 T	100 T	200 T	Storage
E-CK-A117	Annexin V-APC	100 µL	250 µL	500 µL	1 mL	2-8°C
E-CK-A151	Annexin V Binding Buffer (10 ×)	1.4 mL	1.4 mL×2	5.5 mL	11 mL	2-8°C
E-CK-A162	7-AAD Viability Staining Solution	100 µL	250 µL	500 µL	1 mL	-20°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.

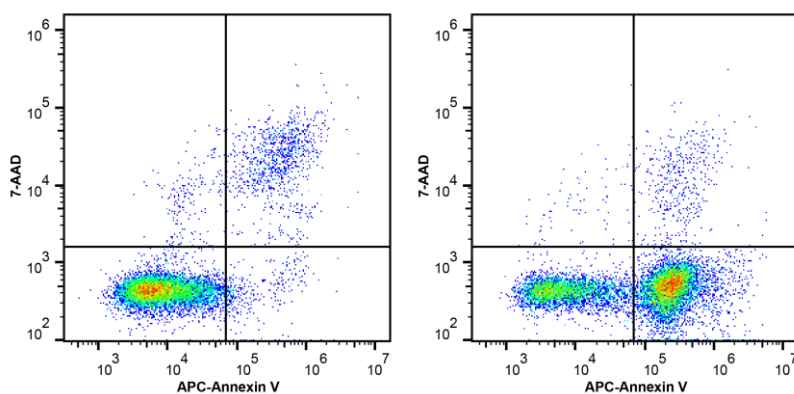
Introduction

Elabscience® Annexin V-APC/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-APC, 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 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-APC single-positive cells were early apoptotic cells, Annexin V-APC 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 \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

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 500 μ L of 1 \times Annexin V Binding Buffer to resuspend the cells.
3. Add 5 μ L of Annexin V-APC and 5 μ L 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.

Storage

Annexin V-APC 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-APC and 7-AAD as negative control. As for compensation controls, please use drug-treated cells stained with either Annexin V-APC or 7-AAD.
3. Annexin V-APC can be detected in APC 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.