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siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Elabscience®

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

Annexin V-EGFP / PI Apoptosis Detection Kit

Catalog No: E-CK-A219

Sizes： 20 Assays / 50 Assays / 100 Assays / 200 Assays

Cat.	Products	20 Assays	50 Assays	100 Assays	200 Assays	Storage
E-CK-A119	Annexin V-EGFP Reagent	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-A161	Propidium Iodide (PI) Staining Solution	100 μL	250 μL	500 μL	1 mL	2~8 ℃
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.

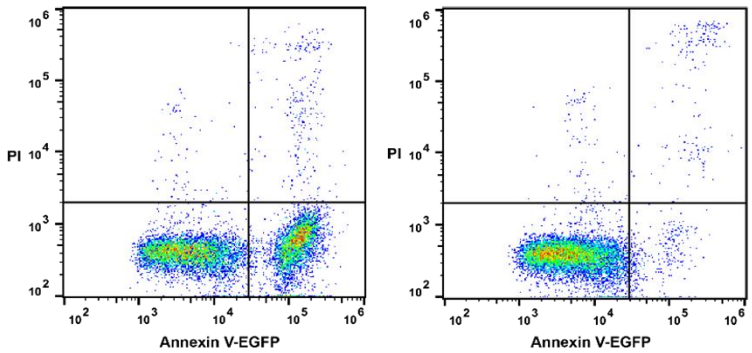
Introduction

Elabscience® Annexin V-EGFP / 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. Annexin V-EGFP, the EGFP-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-EGFP single-positive cells were early apoptotic cells, Annexin V-EGFP and PI double-positive cells were necrotic or late apoptotic cells, and PI single-positive cells were naked nuclei.

Instructions

Dilute Annexin V Binding Buffer (10 ×) with DI water to 1 ×Annexin V Binding working solution before use. For example, take 1 mL Annexin V Binding Buffer (10 ×) [E-CK-A151] and add it to 9 mL DI water to get 10 mL Annexin V Binding working solution.

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. Please be aware！
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 working solution to resuspend the cells.

3. Add 5 μL of Annexin V-EGFP 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.

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. Please be aware !
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 working solution to resuspend the cells.
3. Add 2.5 μL of Annexin V-EGFP and 2.5 μL of PI 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 working solution 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-EGFP Reagent and Propidium Iodide (PI) Staining Solution should be stored in dark. All reagents are stored at 2~8 ℃ for 12 months.

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-EGFP and PI as negative control. As for compensation controls, please use drug-treated cells stained with either Annexin V-EGFP or PI.
3. Annexin V-EGFP can be detected in FITC channel while PerCP/Cy5.5 channel is preferred to ECD channel for PI detection.
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.