Annexin V-EGFP/7-AAD Apoptosis Kit

Cat. No: E-CK-A220 **Size: 20 Assays/100 Assays**

Cat.	Products	20 Assays	100 Assays	Storage
E-CK-A119	Annexin V-EGFP Reagent	125 μL	500 μL	2-8°C, shading light
E-CK-A151	Annexin V Binding Buffer(10×)	1.4 mL×2	5.5 mL×2	2-8°C
E-CK-A162	7-AAD Reagent (100μg/mL)	100 μL	500 μL	2-8°C, shading light
Manual		One Copy		

Storage

Store at 2-8°C for one year. Avoid freeze / thaw cycles.

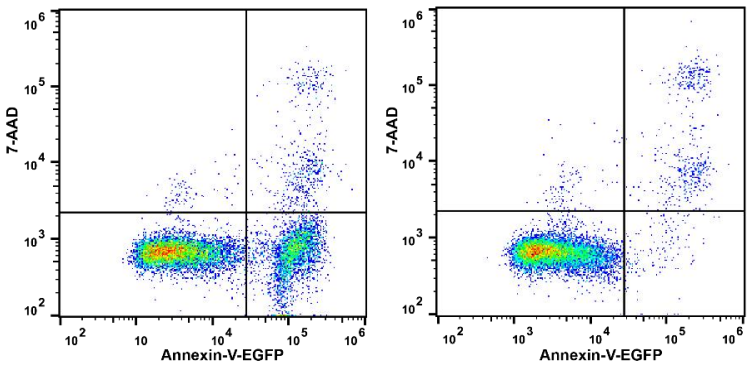
Introduction

Elabscience® Annexin V-EGFP/7-AAD Apoptosis Kit is developed to detect the apoptosis of suspension cells and adherent 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.

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 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 7-AAD double-positive cells were necrotic or late apoptotic cells, and 7-AAD single-positive cells were naked nuclei.

Reagent Preparation

1×Annexin V Binding Buffer： Dilute Annexin V Binding Buffer (10×) with deionized water to 1×Annexin V Binding Buffer before use.

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Staining Procedure

Control settings for Annexin V experiments are critical to experimental results. It is strongly recommended to read this article before the experiment: https://www.elabscience.com/resources/cell-function-technical-topics/984

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.
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-EGFP and 5 μL of 7-AAD 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.

   Note: Annexin V-EGFP can be detected in FITC channel while 7-AAD can be detected in PerCP/Cy5.5 channel.

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.
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-EGFP and 2.5 μL of 7-AAD 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.

   Note: Annexin V-EGFP can be detected in FITC channel while 7-AAD can be detected in PerCP/Cy5.5 channel.

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Cautions

1. This kit is for reseach use only.
2. When detecting adherent cells, the suspension cells generated after induction of apoptosis should be collected and detected together with the subsequently collected adherent cells.
3. Mechanical damage caused by digestion of adherent cells should be avoided as much as possible. At the same time, trypsin digestion solution should not contain EDTA as much as possible, because EDTA will affect the binding of Annexin V to phosphatidylserine.
4. If trypsin containing EDTA is used, cells should be washed thoroughly after harvesting to ensure that EDTA is removed.
5. Detect apoptosis as soon as possible after staining to avoid the increase number of apoptosis or necrosis. 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.

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