# Annexin V-Elab Fluor® Violet 500 Reagent

**Cat. No: E-CK-A135** **Size: 50 Tests/200 Tests**

| Cat. | Products | 50 Tests | 200 Tests | Storage |
|---|---|---|---|---|
| E-CK-A135 | Annexin V-Elab Fluor® Violet 500 Reagent | 125 μL×2 | 500 μL×2 | 2~8°C, shading light |
| | Manual | | One Copy | |

## Storage

Store at 2~8°C for one year in the dark. Avoid freeze / thaw cycles.

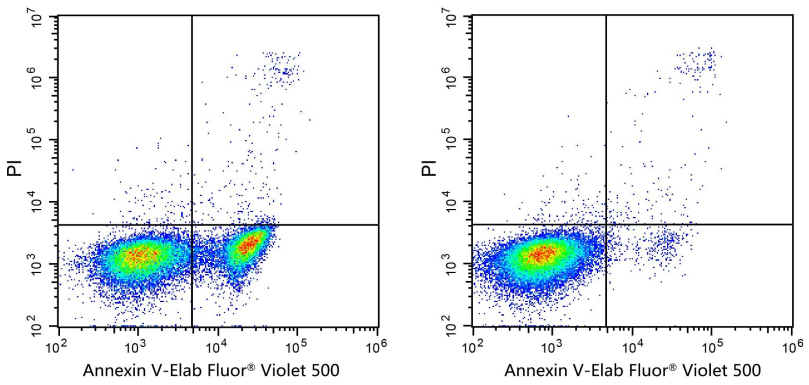
## Introduction

Elabscience® Annexin V-Elab Fluor® Violet 500 Reagent is developed to identify apoptotic cells.

Annexin V is a member of the annexin family, which binds to phosphatidylserine (PS) in a calcium-dependent manner. Annexin V-Elab Fluor® Violet 500, the Elab Fluor® Violet 500-conjugated format, binds specifically to the PS on the outer leaflet apoptotic cell membrane and can be detected with flow cytometry or fluorescence microscopy.

Cells at different apoptotic stages can be distinguished by using Annexin V and membrane impermeable DNA dyes like Propidium Iodide (PI), 7 7-Amino Actinomycin D (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-Elab Fluor® Violet 500 single-positive cells were early apoptotic cells, Annexin V-Elab Fluor® Violet 500 and PI double-positive cells were necrotic or late apoptotic cells, and PI single-positive cells were naked nuclei.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## Staining Procedure

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 [E-CK-A151] to resuspend the cells.
3. Add 5 µL of Annexin V-Elab Fluor® Violet 500 Reagent and 5 µL of DNA dye (PI [E-CK-A161] or 7-AAD [E-CK-A162])) 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.

## 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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