

## Annexin V-PE-Cy7/DAPI Apoptosis Detection Kit

### A1456541

**Storage** 2-8°C. Protect from light. Do not freeze.

**Shipping** Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

### Introduction

Annexins are a family of calcium-dependent phospholipid-binding proteins that preferentially bind phosphatidylserine (PS). Under normal physiologic conditions, PS is predominantly located in the inner leaflet of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution across the phospholipid bilayer and is translocated to the extracellular membrane leaflet marking cells as targets of phagocytosis. Once on the outer surface of the membrane, PS can be detected by fluorescently labeled Annexin V in a calcium-dependent manner.

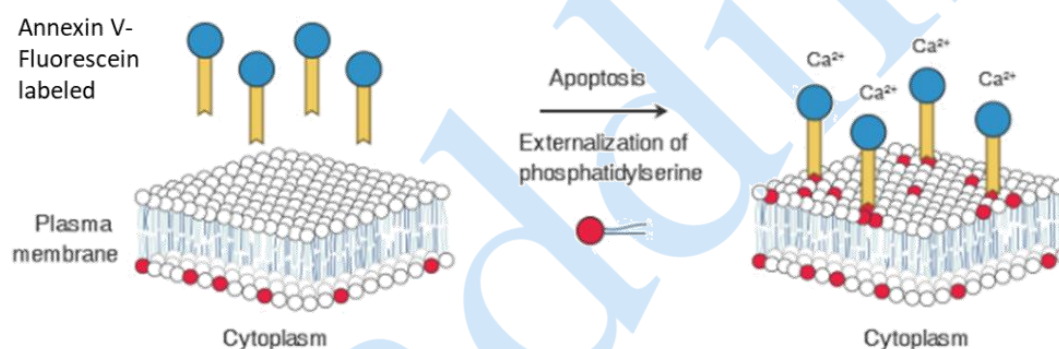


Fig. 1 Principal of Annexin-V Apoptosis Assay

In early-stage apoptosis, the plasma membrane excludes viability dyes such as 4',6-diamidino-2-phenylindole (DAPI), propidium iodide (PI), 7-AAD. These cells will stain with Annexin V but not a viability dye, thus distinguishing cells in early apoptosis. However, in late stage apoptosis, the cell membrane loses integrity thereby allowing Annexin V to also access PS in the interior of the cell. A viability dye can be used to resolve these late-stage apoptotic and necrotic cells (Annexin V, viability dye-positive) from the early-stage apoptotic cells (Annexin V positive, viability dye-negative).

This kit is suitable for the identification and enumeration of dead cells, such as apoptotic or necrotic cells, by flow cytometry.

## Kit Contents

| A1456541  | Components                   | 20 T   | 50 T   | 100 T   | Storage                  | Quantity Per Test                        |
|-----------|------------------------------|--------|--------|---------|--------------------------|------------------------------------------|
| A1456541A | 10× Annexin V Binding Buffer | 5 mL   | 10 mL  | 20 mL   | 2-8°C                    | 200 µL per 0.5-1.0×10 <sup>5</sup> cells |
| A1456541B | Annexin V-PE-Cy7             | 200 µL | 500 µL | 1000 µL | 2-8°C. Store in the dark | 10 µL per 0.5-1.0×10 <sup>5</sup> cells  |
| A1456541C | DAPI Staining Solution       | 40 µL  | 100 µL | 200 µL  | 2-8°C. Store in the dark | 2 µL per 0.5-1.0×10 <sup>5</sup> cells   |

Note: The recommended number of cells for use is 0.5-1.0×10<sup>5</sup> cells/test.

## Instruction for use

1. Dilute 10× Binding Buffer to 1× using distilled water (1 mL 10x Binding Buffer + 9 mL ddH<sub>2</sub>O).
2. Wash cells twice with cold PBS and then resuspend the desired amount of cells in Annexin V Binding Buffer at a concentration of 0.5-1.0×10<sup>6</sup> cells /mL .
3. Add 10 µL of Annexin V-PE-Cy7 and 2 µL of DAPI staining solution to 100 µL of cell suspension.
4. Add 100 µL of 1× Binding Buffer to each assay. Gently vortex the cells and incubate for 10 min at RT (25°C) in the dark.
5. Perform flow cytometry analysis within 1 hr.

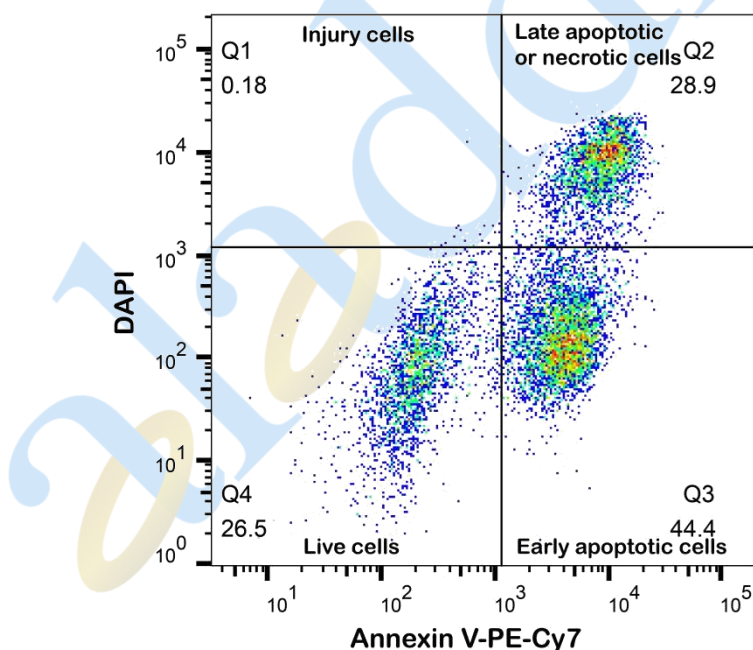


Fig. 2 Flow cytometry analysis of Jurkat cells treated with camptothecin using the Annexin V-PE-Cy7/DAPI Apoptosis Detection Kit (A1456541). Q1: Injury cells, Loss of membrane

integrity (Annexin V-) allows DAPI to enter the nucleus and bind DNA (DAPI+). Q2: Late apoptotic or necrotic cells, Phosphatidylserine externalization (Annexin V+) with loss of membrane integrity (DAPI+). Q3: Early apoptotic cells, Phosphatidylserine externalization (Annexin V+) with intact membrane (DAPI-). Q4: Live cells, Intact membrane without phosphatidylserine exposure (Annexin V-/DAPI-).

### **Matters needing attention**

1. Please try to avoid light when using to slow down the quenching of fluorescence.
2. DAPI Solution is toxigenic and mutagenic; handle with care.
3. Due to the calcium dependence of the Annexin V:PS interaction, it is critical to avoid buffers containing EDTA or other calcium chelators during Annexin V experiments.