Annexin V-APC/DAPI Apoptosis Detection Kit

A1456534

Storage: 2-8℃. Protect from light. Do not freeze

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.

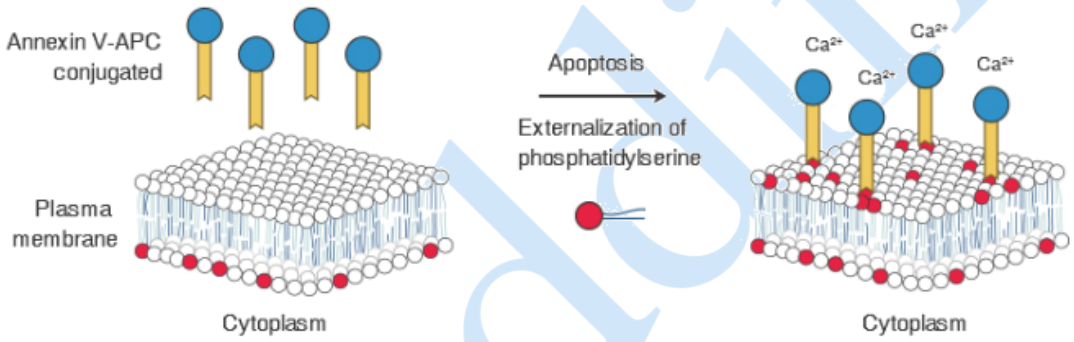

Fig. 1 Principal of Annexin-V Apoptosis Assay

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

A1456534	Components	20 T	50 T	100 T	Storage	Quantity Per Test
A1456534A	10× Annexin V Binding Buffer	5 mL	10 mL	20 mL	2-8℃	200 μL per 0.5-1.0x10^5 cells
A1456534B	Annexin V -APC	100 μL	250 μL	500 μL	2-8℃. Store in the dark.	5 μL per 0.5-1.0x10^5 cells
A1456534C	DAPI Staining Solution (25 μg/mL)	20 μL	50 μL	100 μL	2-8℃. Store in the dark.	1 μL per 0.5-1.0x10^5 cells

Note: The recommended number of cells for use is 0.5-1.0x10^5 cells/test.

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Instruction for use

1. Dilute 10X Binding Buffer to 1x using distilled water (1 mL 10x Binding Buffer + 9 mL ddH_2O).
2. Wash cells twice with cold PBS and then resuspend the desired amount of cells in Annexin V Binding Buffer at a concentration of 0.5-1.0x10^6 cells /mL.
3. Add 5 μL of Annexin V-APC and 1 μL of DAPI staining solution to 100 μL of cell suspension.
4. Add 100 μL of 1x Binding Buffer to each assay. Gently vortex the cells and incubate for 10 min at RT (25°C) in the dark.
5. Analyze by flow cytometry within 1 hr.

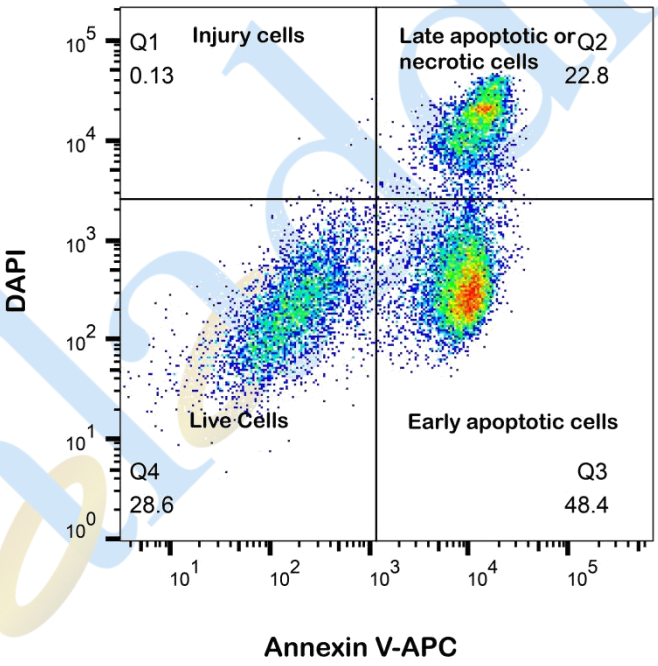

Fig. 2 Flow cytometry analysis of Jurkat cells treated with camptothecin using the Annexin V-APC/DAPI Apoptosis Detection Kit (A1456534). Q1: Injury cells, Loss of membrane integrity (Annexin V-) allows DAPI to enter the nucleus and bind DNA (DAPI+). Q2: Late apoptotic or necrotic cells, Phosphatidylserine externalization (Annexin V+) with loss of

membrane integrity (DAPI+). Q3: Live cells, Intact membrane without phosphatidylserine exposure (Annexin V-/DAPI-). Q4: Early apoptotic cells, Phosphatidylserine externalization (Annexin V+) with intact membrane (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.