

Annexin V-APC/PI Apoptosis Detection Kit

A1372287

Storage 2-8°C. 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.

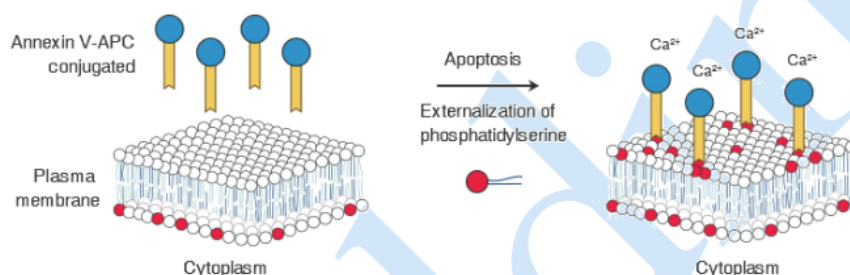


Fig. 1 Principal of Annexin-V Apoptosis Assay

In early-stage apoptosis, the plasma membrane excludes viability dyes such as 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

A1372287	Components	20 T	50 T	100 T	Storage	Quantity Per Test
A1372287A	10x Annexin V Binding Buffer	5 mL	10 mL	20 mL	2-8°C.	200 µL per 0.5– 1.0 × 10 ⁵ cells.
A1372287B	Annexin V (APC)	100 µL	250 µL	500 µL	2-8°C.Store in the dark.	5 µL per 0.5– 1.0 × 10 ⁵ cells.
A1372287C	Propidium iodide Staining Solution (PI)	100 µL	250 µL	500 µL	2-8°C.Store in the dark.	2 µL per 0.5– 1.0 × 10 ⁵ cells.

Note: The recommended number of cells to stain per test is 0.5–1.0 × 10⁵ cells.

Instruction for use

1. Dilute 10x Binding Buffer to 1x using distilled water (1 mL 10x Binding Buffer + 9 mL ddH₂O).
2. Wash cells twice with cold PBS and then resuspend the desired amount of cells in Annexin V Binding Buffer at a concentration of 0.5–1.0×10⁶/mL.
3. Add 5 µl of APC Annexin V and 2 µl PI to 100 µL of the cell suspension.
4. Gently vortex the cells and incubate for 10 min at RT (25°C) in the dark.
5. Add 100 µl of 1x Binding Buffer to each assay. Analyze by flow cytometry within 1 hr.

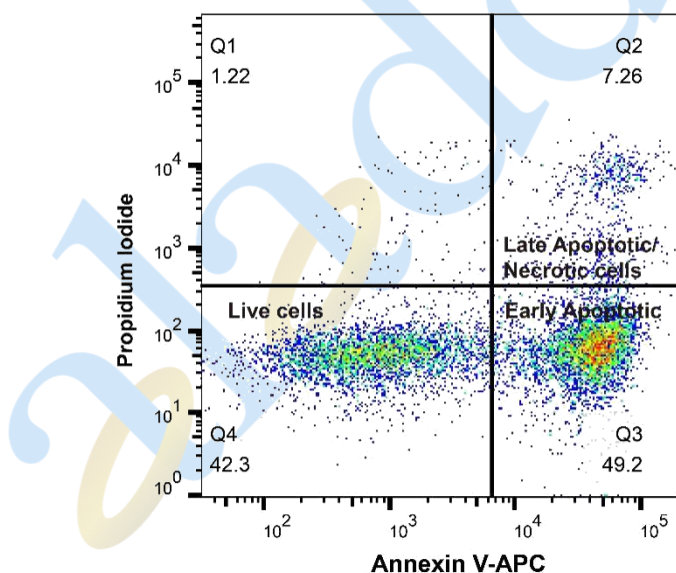


Fig. 2 Flow cytometry analysis of Jurkat cells treated with camptothecin using the Annexin V-APC/PI Apoptosis Detection Kit (A1372287). Q2: Late apoptotic or necrotic cells, Phosphatidylserine externalization (Annexin V+) with loss of membrane integrity (PI+). Q3:

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

Matters needing attention

1. Please try to avoid light when using to slow down the quenching of fluorescence.
2. Propidium Iodide 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.

