Annexin V-FITC/PI Apoptosis Detection Kit

A1372286

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.

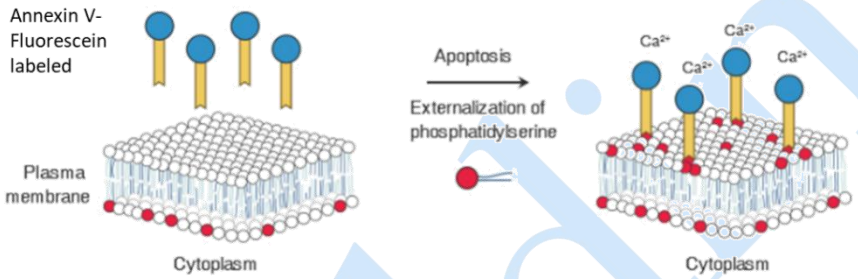

Fig. 1 Principal of Annexin-V Apoptosis Assay

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

Component	20 Test	50 Test	100 Test	Storage
10x Annexin V Binding Buffer	5 mL	10 mL	20 mL	2-8℃.
Annexin-FITC	100 μL	250 μL	500 μL	2-8℃. Store in the dark.
Propidium iodide Staining Solution (PI)	100 μL	250 μL	500 μL	2-8℃. Store in the dark.

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 1.0-5.0 x 10^6 cells/mL.
3. Add 5 µl of FITC 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.

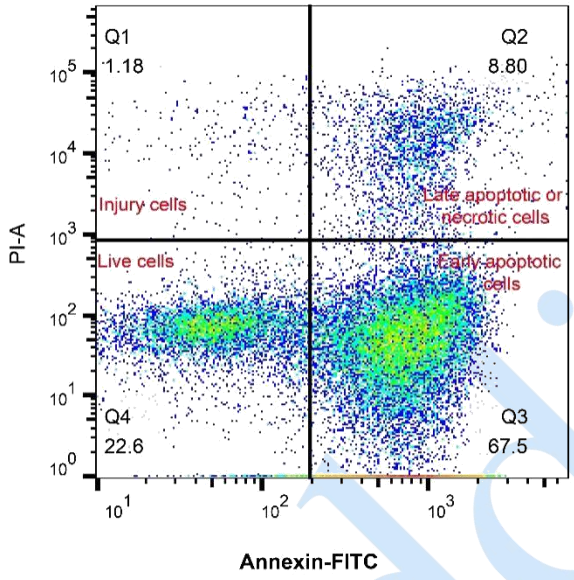

Fig. 2 Annexin-V Apoptosis Assay

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.