

Annexin V-FITC Assay Protocol for Annexin V-FITC Apoptosis Staining/Detection Kit

Introduction

This Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, cells translocate the membrane phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can be easily detected by staining with a fluoresce. The kit can differentiate apoptosis vs necrosis when performing both Annexin V-FITC and PI staining.

Materials

- Annexin V-FITC 500 µL
- 1X Binding Buffer 500 µL
- Propidium Iodide (PI) 500 µL
- Microcentrifuge
- Pipettes and pipette tips

Procedure

For adherent cells, gently trypsinise and wash cells once with serum-containing media before incubation with Annexin V-FITC (1.c-e).

• Incubation of Cells with Annexin V-FITC

1. Induce apoptosis by desired method.
2. Collect 1-5 x 10⁵ cells by centrifugation.
3. Re-suspend cells in 500 µL of 1X Binding Buffer.
4. Add 5 µL of Annexin V-FITC and 5 µL of propidium iodide (PI 50µg/ml, optional).
5. Incubate at room temperature for 5 minutes in the dark.

• Quantification by Flow Cytometry

1. Analyse Annexin V-FITC binding by flow cytometry (Ex = 488 nm; Em = 530 nm) using FITC signal detector and PI staining by the phycoerythrin emission signal detector.