

B24081-25



Annexin V-FITC / PI

Apoptosis Detection Kit (25 Tests)

Overview

The Annexin V-FITC / PI Apoptosis Detection Kit (B24081) is designed to detect apoptosis in a one-step 15-minute staining procedure. The kit uses Annexin V fluorescent conjugate to stain phosphatidylserine (PS) molecules that have moved from the inner face of the plasma membrane to the cell surface, indicating apoptosis. Since the translocation of membrane PS molecules occurs shortly after initiating apoptosis, Annexin V staining can identify apoptosis at early stages. The protein Annexin V has a high affinity for PS in a calcium-dependent manner, which is detected on the cell surface by the fluorescent conjugate. The detection can be performed using flow cytometry or fluorescence microscopy with a FITC filter. This kit can differentiate between apoptosis and necrosis through simultaneous staining with Annexin V and propidium iodide (PI). Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells are permeable to PI.

Sample Type: Living cells (suspension and adherent)

Species Reactivity: Mammalian Applications

Kit Components Item Quantity

| Item | Quantity |
|--------------------|----------|
| Annexin V-FITC | 130 µL |
| Propidium Iodide | 70 µL |
| 10X Binding Buffer | 8 mL |

Store at +4°C. Do not freeze.

Remarks

For a 1X Annexin V binding buffer, Dilute 10X Binding Buffer by 10-fold with distilled water.

FITC and PI are light sensitive. All staining procedures must be performed without direct exposure to intense light.

Assay Protocol

Briefly spin Annexin V-FITC and PI vials at low speed prior to opening.

1. Wash cells twice with cold PBS and then resuspend cells in 1X Binding Buffer at a concentration of $1-2 \times 10^6$ cells/ml.

2. Transfer 100 µl of the solution ($1-2 \times 10^5$ cells) to a 5 ml flow cytometry tube.

3. Add 5 µl of Annexin V-FITC and 2.5 µl PI.

4. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.

5. Add 400 µl of 1X Binding Buffer to each tube. Analyze by flow cytometry within 1 hr.

Data Interpretation: Annexin V and PI-negative cells are considered viable; Cells that stain positive for Annexin V and negative for PI are in early-stage apoptosis. Cells that stain positive for both Annexin V and PI are in end-stage of apoptosis or already dead.