FITC ANNEXIN V STAINING PROTOCOL

FITC Annexin V is used to quantitatively determine the percentage of cells within a population that are actively undergoing apoptosis. It relies on the property of cells to lose membrane asymmetry in the early phases of apoptosis. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner leaflet of the plasma membrane to the outer leaflet, thereby exposing PS to the external environment. Annexin V is a calcium-dependent phospholipid-binding protein that has a high affinity for PS, and is useful for identifying apoptotic cells with exposed PS. Propidium Iodide (PI) is a standard flow cytometric viability probe and is used to distinguish viable from nonviable cells. Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells are permeable to PI. Cells that stain positive for FITC Annexin V and negative for PI are undergoing apoptosis. Cells that stain positive for both FITC Annexin V and PI are either in the end stage of apoptosis, are undergoing

Reagents

1. FITC Annexin V: Included. Use 5 µl per test.

2. Propidium Iodide (PI): Not Included. PI (cat.no. 556463) is a convenient, ready-to-use nucleic acid dye. Use up to 10 µl per test of a 50 µg/ml solution.

3. 10× Binding Buffer :

- 0.1M HEPES-NaOH (pH7.4) → Stock sol at 1M
- 1.4M NaCl → Stock sol at 5M
- 25mM CaCl2 → Stock sol at 2M

Staining

1. Wash cells twice with cold PBS and then resuspend cells in 1× Binding Buffer at a concentration of 1 × 10^6 cells/ml.

2. Transfer 100 µl of the solution (1 × 10^5 cells) to a 5 ml culture tube.

3. Add 5 µl of FITC Annexin V, 20min at RT, dark. Add 300µl 1× Binding Buffer.

4. Add 10 µl PI. The optimal concentration of PI may vary among cell lines where 10 µl of a 50 µg/ml stock is most likely the maximum to be required.

Less may yield optimal results in some experimental systems.