

Annexin V Staining

Supplies and Reagents

- Annexin V-FITC Apoptosis Detection Kit
 - 10X Annexin V Binding Buffer
 - make 15mL of fresh 1X buffer with H₂O
 - FITC Annexin V
 - Propidium iodide Staining Solution (PI)
- PBS (phosphate buffered saline)

I. Wash Cells

1. Transfer 1×10^6 cells to a 1.5mL Eppendorf tube containing 1mL media.
2. Pellet cells at 1,500 rcf for 4 minutes.
3. Aspirate supernatant and resuspend the cell pellet in 1mL cold PBS.
4. Pellet cells at 1,500 rcf for 4 minutes.
5. Repeat steps 3 and 4 one more time.
6. Aspirate supernatant, and resuspend cells in 1mL 1X buffer.

II. Label Cells

1. Transfer 100 μ L of cells to a FACS tube.
2. Add 5 μ L Annexin V-FITC and 5 μ L PI to the 100 μ L of cells.
3. Gently Vortex the tube and incubate 15 minutes at room temperature in the dark.
4. Add 400 μ L of 1X binding buffer to the FACS tube, and run through a FACS machine within one hour.

III. Analyze

1. Acquire at least 20,000 live events.
2. Analyze the data with FlowJo or Cell Quest.
3. A dot plot should be used to view the FL1 channel (FITC) and FL3 channel (PI).
 - a. The end result should have either Annexin V-FITC on one axis and propidium iodide on the other axis.
4. Place a 4-quadrant gate on the plot, using it to dissect the three populations.
 - a. Separate populations within the Annexin V-FITC labeling are not always easy to identify, but propidium iodide should be visibly separate.
 - b. If there is no clear population separation on the Annexin V axis, set the gate using the propidium iodide positive population.
5. Analyze data in quadrants.
 - a. Cells negative for Annexin V and negative for propidium iodide are non-apoptotic.

- b. Cells positive for Annexin V and negative for propidium iodide are viable, but undergoing apoptosis.
- c. Cells positive for Annexin V and positive for propidium iodide are dead.
- d. There should be very little, if any, cells negative for Annexin V and positive for propidium iodide.

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