Annexin V Protocol

Annexin V conjugates allow the identification of cell surface changes that occur early during the apoptotic process using flow cytometry. Early in the apoptotic process, phosphatidylserine becomes exposed on the cell surface by flipping from the inner to outer leaflet of the cytoplasmic membrane. This event is thought to be important for macrophage recognition of cells undergoing apoptosis. The binding of Annexin V to phosphatidylserine is calcium dependent, reversible, and very tight with a Kd of approximately 5×10^{-10} M.

General Comments:

Different cell types vary in their phosphatidylserine (PS) content, along with the amount of PS exposure on the cell surface after cell death. The following protocol is a guideline for getting started, however it may be necessary to adjust the concentration of the Annexin V-FITC. Typically a 1 to 100 dilution of the Annexin V-conjugate is appropriate, however dilutions of 1:10 up to 1:1000 may be needed.

When setting up an experiment, it is necessary to calibrate the flow cytometer to avoid spectral overlap between the two PMT channels. For each experiment, a set of treated cells should be processed unstained and stained separately with Annexin V-FITC and propidium iodide to define the boundaries of each population.

Protocol for Flow Cytometry:

1. Collect cells (approximately 5×10^5 to 1×10^6 cells per tube) by gently centrifugation at room temperature in 6-ml polystyrene Falcon tubes (#2058). **Remember when using adherent cells that both the supernatant and attached cells should be collected and examined together.**

2. Wash the cells once in 500 ul of cold 1X PBS buffer gently resuspending the cells then pelleting by centrifugation as in step 1.

3. Per each sample of cells, prepare 100 ul of Annexin V incubation reagent by combining:

   10 ul 10X Binding Buffer
   10 ul Propidium Iodide
   1 ul Annexin V-FITC
   79 ul dH_2O
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   100 ul Total

   Keep this cocktail in the dark and on ice.

4. Prepare 400 ul of 1X Binding Buffer per each sample for diluting the cells after incubation. 1:10 dilution in dH_2O of 10X Binding Buffer.

5. Gently resuspend the washed cells in 100 ul of the Annexin V incubation reagent prepared in step 3.

6. Incubate in the dark for 15 minutes at room temperature.

7. Add 400 ul of 1X Binding Buffer to each 100 ul reaction sample.

8. Examine by flow cytometry within 1 hour for maximal signal.