Annexin V-Phycoerythrin

Catalog Number: NXPE
Lot Number: LKK02
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated human Annexin V: 1 mL of Annexin V-PE-labeled at a concentration of 10 μg/mL.

Reagents Not Provided

Annexin V Binding buffer:
10 mM HEPES/NaOH (pH 7.4) + 140 mM NaCl + 2.5 mM $CaCl_2$

Storage

Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Intended Use

Designed to quantitatively determine the percentage of cells that bind Annexin V.

Sample Staining

1. Cells to be used for staining should be washed with cold PBS twice and then resuspended in an Annexin V binding buffer at a concentration 1 x 10^6 cells/mL.
2. Transfer 100 μL of the cells (1 x 10^5 cells) to a 5 mL culture tube.
3. Add 10 μL of PE-conjugated Annexin V reagent.
4. Gently vortex the cell mixture and then incubate for 15 minutes at 20° - 25° C (room temperature) in the dark.

Following the incubation and without washing the cells of excess reagents, add 400 μL of an Annexin V binding buffer to each tube. Analyze on a flow cytometer as soon as possible (within 1 hour of completion of cell staining).

We recommend also setting up an additional control tube that will aid in instrument set-up: a) unstained cells; b) cells stained with Annexin V-PE only. Positive staining with Annexin V can only be demonstrated in populations of cells that contain a fraction of cells that are dying. Example of a cells that can serve as a positive staining control for Annexin V are the U-937 human cell line cultured in RPMI + 10% FCS + 2 - 4 ng/mL of TNF-α for 2 - 3 hours or murine thymocytes left in culture media for 1 - 2 hours.

Analyzing the Stained Cells by Flow Cytometry

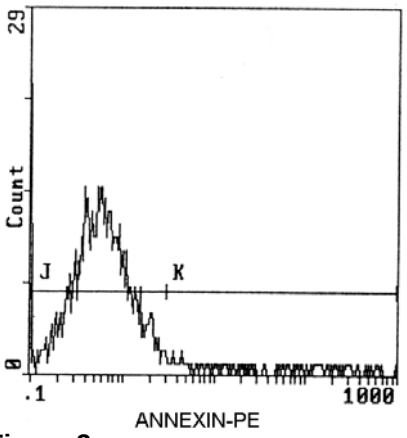
Proper flow cytometric analysis of double stained cells with Annexin V-PE and any other cell marker requires that electronic compensation of the instrument be set to exclude any overlap of the two emission spectra. Since this is directly related to the voltage settings on the PMTs, electronic compensation settings will vary between instruments. We recommend analyzing single stained cells at the beginning of the experiment and correcting for any spectral overlap by making instrument adjustments under the signal compensation menu for your specific cytometer.

R&D Systems, Inc.
1-800-343-7475

Technical Notes

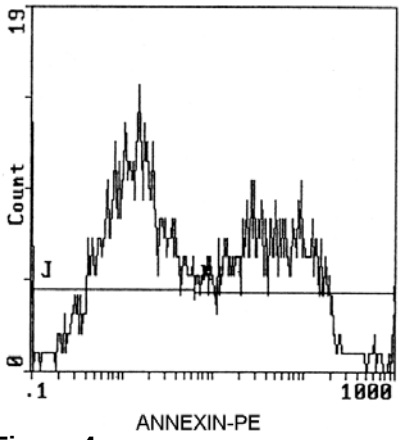
It is advisable that the staining and subsequent analysis of cells be performed as rapidly as possible. Although fixation of cells with 1.0% formaldehyde is not recommended in this procedure, it may be possible with certain cell types to treat Annexin V stained cells with such a fixative following a single washing step of the cells. Cells are then resupended in 1X binding buffer that contains the fixative. This latter procedure however normally results in decreased staining intensity for Annexin V.

Figure 1



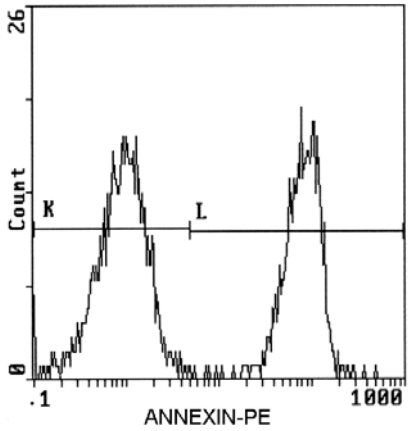
10.6%

Figure 2



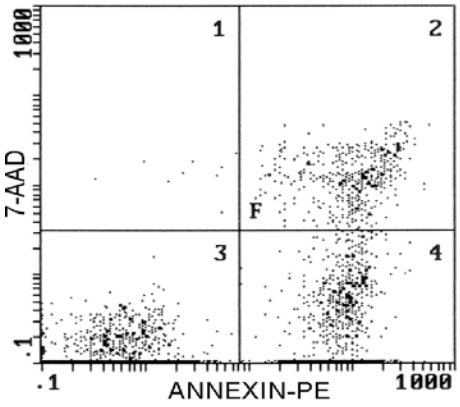
47.4%

Figure 3



43.1%

Figure 4



14.6%

39.6%

Figure 1 - Log fluorescence histogram of untreated THP-1 cells stained with Annexin V-PE.

Figure 2 - Log fluorescence histogram of THP-1 cells stained with Annexin V-PE following exposure to 0.15 μM camptothecin for 4 hours at 37° C prior to being stained.

Figure 3 - Log fluorescence histogram of Annexin V-PE stained Jurkat cells following exposure to 100 ng/mL of anti-Fas monoclonal for 3 hours at 37° C.

Figure 4 - Log fluorescence dot plot of Annexin V-PE and 7-AAD stained Jurkat cells following exposure to 100 ng/mL of anti-Fas monoclonal for 4 hours 37° C. Cells were first stained with 5 μg/mL of 7-AAD for 15 minutes at room temperature, then washed one time with Annexin V binding buffer and then stained with Annexin V-PE as per manufacturer's instructions.