ANNEXIN V

Protocol modified from
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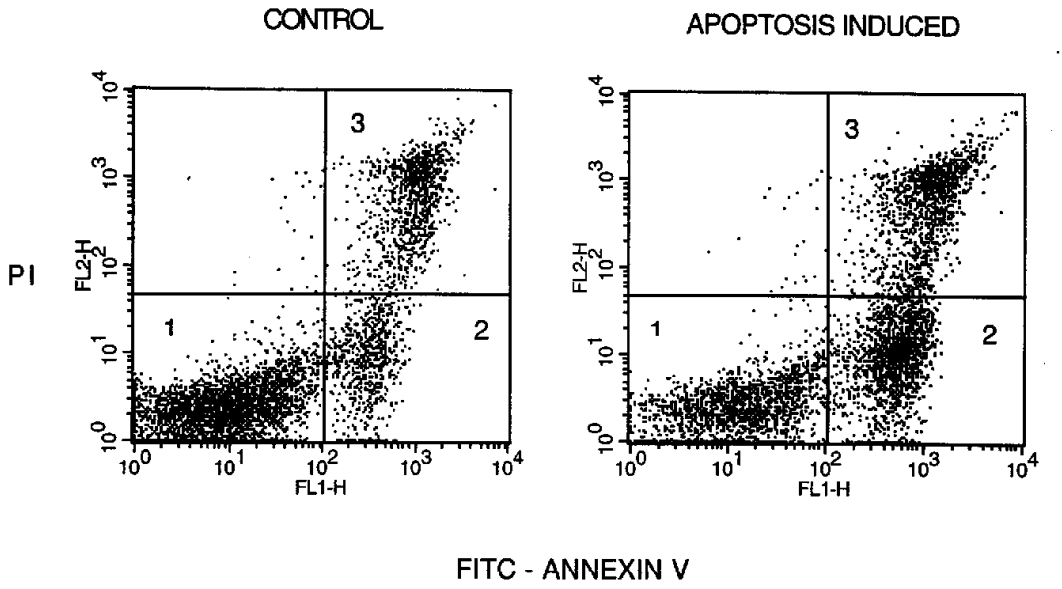
Basic protocol/Methodology

1. Wash 2x 1x10^6 cells with PBS
2. Dilute FITC-Annexin V at a concentration of 1mg/ml in binding buffer and resuspend cells in 1 ml of this solution (prepare fresh each time).
3. Incubate 10 min in the dark at RT.
4. Add to the cell suspension 0.1ml of PI solution prior to analysis to give final concentration of 1mg/ml.
5. Analyze cells by flow cytometry

- Collect 10,000 events/sample.
- Exclude debris by FW vs. SS gating
- Display data as two-color dot plot with FITC-Annexin V (green fluorescence, X-axis) vs. PI (red fluorescence, y-axis).



1. Live cells
2. Early apoptotic cells
3. Late apoptotic or necrotic cells

Other Staining Methods

Annexin V-PE is typically used in conjunction with a vital staining dye such as 7-Amino-actinomycin to allow investigators to detect early apoptotic cells (Annexin V-PE positive, 7AAD negative). For example cells that are viable are Annexin V-PE and 7AAD negative; cells that are in early apoptosis are Annexin V-PE positive and 7AAD negative and cells that are in late apoptosis or already dead are both Annexin V-PE positive and 7AAD positive. The movement of cells through these stages suggests apoptosis.