METHODS OF USING ANNEXIN V FOR FLOW CYTOMETRY

Materials: preparation of positive control

1. Prepare Camptothecin stock solution (Sigma-Aldrich Cat. No. C-9911): 1 mM in DMSO.
2. Jurkat T cells (ATCC TIB-152).

Procedure

1. Add Camptothecin (final conc. 4-6 µM) to 1 x 10^6 Jurkat cells.
2. Incubate the cells for 4-6 hr at 37°C.
3. Proceed with the FITC Annexin V Staining Protocol to measure apoptosis.

Reagents

1. FITC Annexin V (cat. no. 556420, 556419). Use 5 µl per test.
2. Propidium Iodide (PI) (cat. no. 556463). is a convenient, ready-to-use nucleic acid dye. Use 5 µl per test.
3. 10X Annexin V Binding Buffer (cat. no. 51-66121E): 0.1 M Hepes/NaOH (pH 7.4), 1.4 M NaCl, 25 mM CaCl2. For a 1X working solution, dilute 1 part of the 10X Annexin V Binding Buffer to 9 parts of distilled water.
4. Purified Recombinant Annexin V (cat. no. 554781). Use 5-15 µg per test. Researchers are encouraged to titrate the reagent for optimal results.

Staining

1. Wash cells twice with cold PBS and then resuspend cells in 1X Binding Buffer at a concentration of 1 x 10^6 cells/ml.
2. Transfer 100 µl of the solution (1 x 10^5 cells) to a 5 ml culture tube.
3. Add 5 µl of FITC Annexin V and 5 µl PI.
4. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.
5. Add 400 µl of 1X Binding Buffer to each tube. Analyze by flow cytometry within 1 hr.

Blocking

1. Wash cells twice with cold PBS and then resuspend cells in 1X Binding Buffer at a concentration of 1 x 10^6 cells/ml.
2. Transfer 100 µl of the solution (1 x 10^5 cells) to a 5 ml culture tube.
3. Add 5-15 µg of purified recombinant Annexin V. The amount of purified recombinant Annexin V required to saturate binding sites may vary according to cell type and stage of apoptosis. In some cases, investigators may need to reduce the number of cells to 0.5 x 10^5 and still add 5-15 µg of recombinant Annexin V to obtain optimal results. Titration is strongly recommended.
4. Gently vortex the cells and incubate for 15 min at room temperature.
5. Add 5 µl FITC Annexin V and 5 µl PI.
6. Gently vortex the cells and incubate for 15 min at room temperature in the dark.
7. Add 400 µl of 1X Binding Buffer to each tube. Analyze by flow cytometry as soon as possible (within 1hr).