Flow Cytometry Protocol

1. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

2. Harvest the cells after the incubation period and re-suspend in 1ml cold phosphate-buffered saline (PBS 1X).

3. Prepare 1X annexin-binding buffer. For example, for ~10 assays, add 1 mL 5X annexin binding buffer (Component C) to 4 mL deionized water.

4. Prepare a 100 µg/mL working solution of PI by diluting 5 µL of the 1 mg/mL PI stock solution (Component B in 45 µL 1X annexin-binding buffer. Store the unused portion of this working solution for future experiments.

5. Re-centrifuge the washed cells (from step 2), discard the supernatant and resuspend the cells in 1X annexin-binding buffer. Determine the cell density and dilute in 1X annexin-binding buffer to ~1 × 10^6 cells/mL, preparing a sufficient volume to have 100 µL per assay.

6. Add 5 µL Alexa Fluor® 488 annexin V (Component A) and 1 µL 100 µg/mL PI working solution (prepared in step 4) to each 100 µL of cell suspension

7. Incubate the cells at room temperature for 15 minutes.

8. After the incubation period, add 400 µL 1X annexin-binding buffer, mix gently and keep the samples on ice.

9. As soon as possible, analyze the stained cells by flow cytometry, measuring the fluorescence emission at 530 nm (e.g., FL1) and >575 nm (e.g., FL3).