Protocol for Apoptosis Assay by Flow Cytometry Using Annexin V Staining Method

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[Abstract] This assay is used to count the number of cells that have undergone apoptosis. Apoptosis will be detected by initially staining the cells with Annexin V and propidium Iodide solution followed by flow cytometry analysis. It is based on the principle that normal cells are hydrophobic in nature as they express phosphatidyl serine in the inner membrane (side facing the cytoplasm) and when the cells undergo apoptosis, the inner membrane flips to become the outer membrane, thus exposing phosphatidyl serine. The exposed phosphatidyl serine is detected by Annexin V, and propidium iodide stains the necrotic cells, which have leaky DNA content that help to differentiate the apoptotic and necrotic cells.

Materials and Reagents

1. Annexin V FLUOS staining kit (F. Hoffmann-La Roche, catalog number: 11858777001)
2. The kit contains ready-to-use Annexin-V-FLUOS solution, propidium iodide solution, and incubation buffer
3. Trypsin
4. NaCl
5. KCl
6. Na_2HPO_4
7. KH_2PO_4
8. PBS buffer (pH 7.4) (see Recipes)

Equipment

1. Flow cytometer
2. Centrifuge
3. T25 culture flask

Procedure

1. Seed cells (1 x 10^6 cells) in a T25 culture flask (in triplicate for experiments) and three T25 culture flask for control (unstained, Annexin only, and propidium iodide only).
2. After 48 h incubation, collect the supernatant (floating apoptotic cells) and trypsinize the adherent cells (~2 x 10^6 cells) from each T25 flask (combine respective floating and trypsinized cells resulting in six tubes).
3. Wash the collected cells twice with PBS and centrifuge (670 *x g*, 5 min, RT).
4. Re-suspend each pellet (~2 x 10^6 cells) in PBS (400 µl).
   For experimental cells (Triplicate) - (400 µl of cells + 100 µl of incubation buffer with 2 µl of Annexin [1 mg/ml] and 2µl of propidium iodide [1 mg/ml]).
   For control cells
   Control 1: (unstained) - (without any stain (400 µl of cells + 100 µl of incubation buffer)
   Control 2: (Annexin V only) - (400 µl of cells + 100 µl of incubation buffer with 2 µl of Annexin (1 mg/ml))
   Control 3: (propidium iodide only) - (400 µl of cells + 100 µl of incubation buffer with 2 µl of propidium iodide (1 mg/ml))
5. Analyze the cells using a flow cytometry without washing the cells
   Cells that were propidium iodide (PI) negative and Annexin V negative are considered healthy, cells, PI negative and Annexin V positive cells are considered apoptotic, and cells that are positive to both PI and Annexin V considered necrotic (Figure 1).

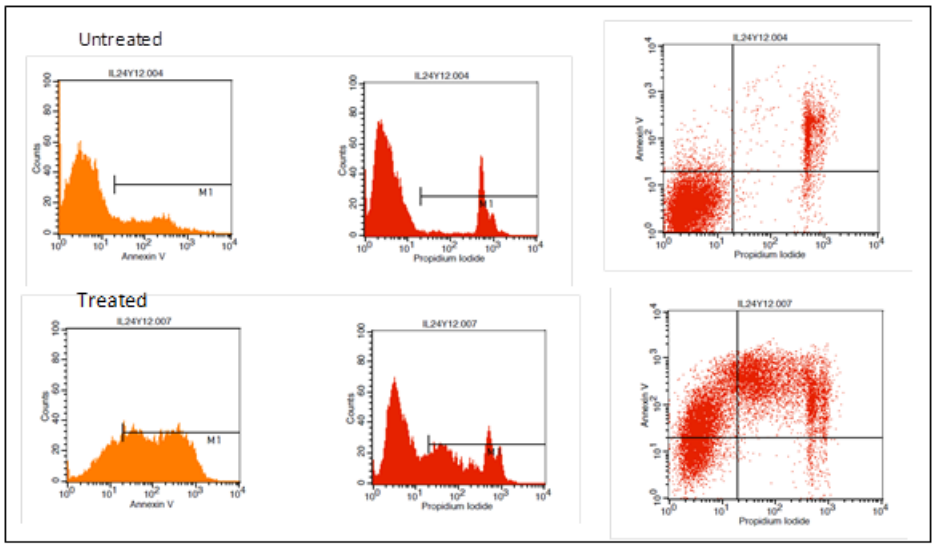

Figure 1.

Recipes

1. PBS buffer (pH 7.4)
   8 g NaCl
   0.2 g KCl
   2.3 g Na_2HPO_4
   0.22 g KH_2PO_4
   In 800 ml of distilled H_2O adjust the pH to 7.4.

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References

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