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| Apoptosis Assay using Annexin V-FITC and PI | Date: 06-17-2009 | - 1 - |
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Apoptosis Assay using Annexin V-FITC and Propidium Iodide

1. Test principle

This assay is intended for detection of early apoptotic cells by flow cytometry. During the early stages of apoptosis, phosphatidylserine (PS) on the inner leaflet of the plasma membrane is translocated to the outer layer. PS is exposed on the external surface of the cell. During apoptosis, the cell membrane remains intact; whereas, during necrosis, the cell becomes leaky and loses its integrity. Annexin V is a sensitive probe for cell surface exposure of PS for quantification of apoptotic cells; Propidium iodide is a probe for discriminating between apoptotic and necrotic cells.

2. Specimen

Cells in suspension, from whole blood, bone marrow or cell culture

3. Materials and reagents

- 5ml Falcon polypropylene Round-Bottom test tubes (12x75 mm)
- cooling centrifuge
- sterile Phosphate-buffered saline (PBS) with 10 % FBS
- FITC- conjugated anti-Annexin V : it can be obtained from different company (Invitrogen ApoDETECT Cat No. 33-1200, Clontech, or Boehringer Mannheim, etc.)
- Propidium iodide
- Annexin V Binding Buffer: 10 mM HEPES, pH 7.4
150 mM NaCl
5 mM KCl
1 mM MgCl₂
1.8 mM CaCl₂

4. Controls:

- Negative control (unstained cells)
- Positive single staining control: Annexin V-FITC only stained unhealthy cells
Propidium iodide only stained unhealthy cells
- Untreated cells stained with Annexin V-FITC and PI

5. Procedure

1. Prepare approximately 10^6 cells in 1 ml of PBS with 10% FBS in each test tube.
 2. Centrifuge 5 min at 200 xg, 4 °C and discard the supernatant.
 3. Resuspend cells in 100 ul Annexin V Binding Buffer (ice -cold).
 4. Add 5 ul of Annexin V and 10 ul of PI to each tubes except single stained control.
 5. Incubate 15 minute in the dark at room temperature.
 6. Add 400 ul ice-cold Annexin V Binding Buffer.
 7. Keep the samples on ice, under the foil and analyze on the flow cytometry.
- PI is a nucleic acid-specific, red-fluorescent dye. It is also a suspected carcinogen, so employ appropriate safety precautions when working with this dye. This dye will bind to both DNA and RNA, however, treatment of the ethanol-fixed cells with RNase A, as described above, will digest all of the RNA so that the PI will fluorescently label the DNA, specifically.
 - Many vendors sell kits with pre-prepared buffers and reagents. Alternatively, individual reagents may be purchased separately. The above protocol is a general guide and individual kits may vary in the amounts of reagents used and the procedure of staining.
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