Annexin V-FITC/PI Apoptosis Assay Kit

Flow Cytometry-compatible assay kit for the detection of phosphatidylserine (PS) at the outer surface of cell membrane upon the initiation of apoptosis

Catalog number: ARG80927

Package: 100 tests

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PRINCIPLE OF THE ASSAY

This is a flow cytometry compatible assay kit for the detection of phosphatidylserine (PS) at the outer surface of cell membrane upon the initiation of apoptosis. Soon after the initiation of apoptosis, the phosphatidylserine (PS) of cell membrane is translocated from the inner leaflet to the extracellular membrane in order to mark itself as a target for phagocytosis. At the cell surface, PS can be readily detected by staining with a FITC-conjugated Annexin V protein as it has a high affinity for PS. In addition, the kit can differentiate apoptosis from necrosis when used together with the Propidium Iodide (PI) staining included.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
rh Annexin V-FITC	0.5 ml (ready to use)	4°C.
4X Binding Buffer	20 ml	4°C.
Propidium Iodide (20 µg/ml)	1ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Flow Cytometer
- Pipettes and pipette tips
- Deionized or distilled water
- PBS
- FITC annexin V λex/em: 494/518 nm (FL1); Propidium iodide λex/em 535/617 nm (FL2 or FL3).

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times. Do not use the kit beyond expiration date.
- Do not mix or substitute reagents with reagents from other lots or other sources.
- Do not expose the kit and its components to strong lights during storage or incubation.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Change pipette tips between the addition of different reagent or samples.

REAGENT PREPARATION

- 1 X Binding Buffer: Dilute 4X Binding buffer into distilled water to yield 1X Binding buffer. (E.g. 1ml binding buffer + 3ml distilled water.)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use.

Standards and samples should be assayed in at least duplicates.

1. Cell preparation: Induce cells into apoptosis using proper method and including a mock-treated sample as a negative control.
2. Harvest cells:
   a. For suspension cells- Harvest by centrifugation at 1000-2000 rpm for 5-10 minutes to pellet the cells. Carefully remove the supernatant.
   b. For adherent cells

a) Collect the cultured media including death cells.
b) Rinse the attached cells with ice-cold PBS and collect the ice-cold PBS also.
c) Detached cells with EDTA free-trypsin (Since EDTA might induce apoptosis and EDTA might also interference Annexin V activity. So EDTA free trypsin is recommended).
d) Harvest the cells with media.
e) Combine all collected media, PBS and cells from a), b) and d). Centrifuge at 1000-2000 rpm for 5-10 minutes.

3. Wash cells: Remove the supernatant, wash the pallet with 1 ml of ice-cold PBS twice. After last centrifuge, discard the washing buffer (ice-cold PBS).
4. Resuspend cells in **1X Binding buffer**. Make sure that the cell density falls at **1-5 X 10^6 cells per ml**.
5. Add **100 µl** of **resuspended cells** from step 4 to a fresh 5 ml Flow Cytometry Tube.
6. Add **5 µl** of **Annexin V-FITC** mix well, incubate for **5 minutes at RT** in dark.
7. Add **10 µl** of **20 µg/ml PI** to the samples, mix gently. And then add **400 µl** of **PBS** into the tube.
8. Perform FACS analysis immediately. It is recommended assay the samples immediately and not over 30 min. (measuring the fluorescence emission at 530 nm (e.g., FL1) and >575 nm (e.g., FL3)

Note: The cells can also been observed by fluorescence microscopy using filters appropriate for fluorescein (FITC) and rhodamine (TRITC).