EzWay™ Annexin V-FITC Apoptosis Detection Kit

1. Catalog No. K29100

2. No. of Applications 100 Tests

3. Storage Store at 4°C and protected from prolonged exposure to light. Do not freeze.

4. Contents

Component	Volume
Annexin V-FITC	125ul (200ug/ml)
5X Binding buffer	12.5ml x 2
Propidium Iodide	1.5ml (30ug/ml)

5. Description

EzWay™ Annexin V-FITC Apoptosis Kit can identify apoptosis at an earlier stage than DNA-based assays such as TUNEL. Annexin V is a calcium-dependant phospholipid binding protein that has a high affinity for phosphatidylserine (PS), a plasma membrane phsopholipid. In non-apoptotic cells, most PS are localized at the inner layer of the plasma membrane, but after inducing apoptosis, PS are exposed to the extracellular environment, and then Annexin V binds to PS.
Cells that stain positive for Annexin V-FITC (green) and negative for PI are undergoing apoptosis. Cells that stain positive for both Annexin V-FITC (green) and PI (red) are either in the end stage of apoptosis, are undergoing necrosis, or are already dead. Cells that stain negative for both Annexin V-FITC and PI are alive and not undergoing measurable apoptosis.

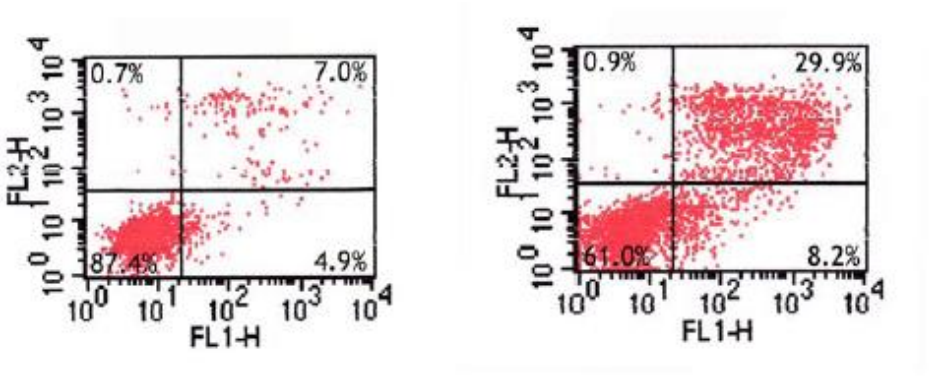
- Utilizes FITC-conjugated Annexin V protein for detection of cells undergoing apoptosis
- Annexin V-FITC is detected as a green fluorescence and propidium iodide is detected as a red fluorescence.
- Detection by flow cytometry or fluorescence microscopy
  (FITC : ex 488nm/em 518nm, PI : ex 488-540nm/em 617-620nm)
- Non-apoptotic cells: Annexin-V negative and PI negative
- Early apoptotic cells: Annexin-V positive and PI negative
- Necrotic cells or late apoptotic cells: Annexin-V positive and PI positive

6. Procedure

1. Induce apoptosis in a 1 x 10^6 cells/ml.
2. Transfer 0.5ml cell suspension (5 x 10^5 cells/ml) to a microtube.
3. Remove medium by centrifugation at 1000 x g for 5 minutes at RT.
4. Wash cells with 0.5ml cold PBS.
5. Remove PBS by centrifugation at 1000 x g for 5 minutes at RT.
6. Wash cells with 0.5ml cold 1x Binding buffer (Dilute in D.W.).
7. Add 1.25ul of Annexin V-FITC and incubate for 15 minutes at RT in the dark.
8. Remove the supernatant by centrifugation at 1000 x g for 5 minutes at RT.
9. Wash cells with 0.5ml cold 1x Binding buffer (Dilute in D.W.).
10. Add 10ul PI to cell suspension.
11. Analyze by flow cytometry or fluorescent microscopy immediately.

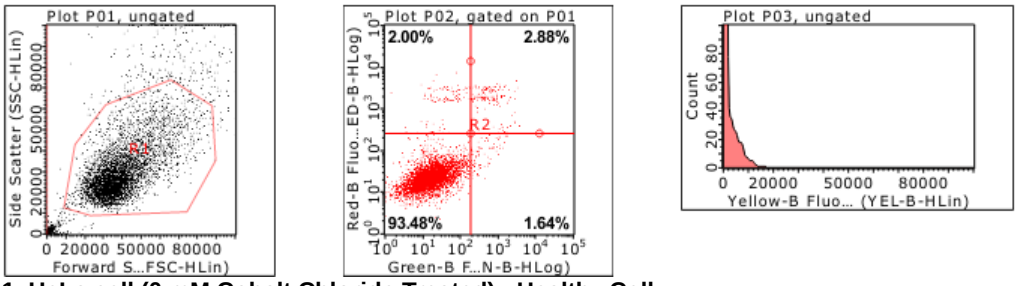
Note:

1) For adherent cells, trypsinize cells. Wash cells with PBS and centrifuge at 1000 x g for 5 minutes at RT. Resuspend the cell pellet and wash with serum-containing media. Start from the procedure 1.
2) Analyze cells by flow cytometry using FL1 channel for detecting FITC (518nm) and FL2 channel for detecting PI (620nm).

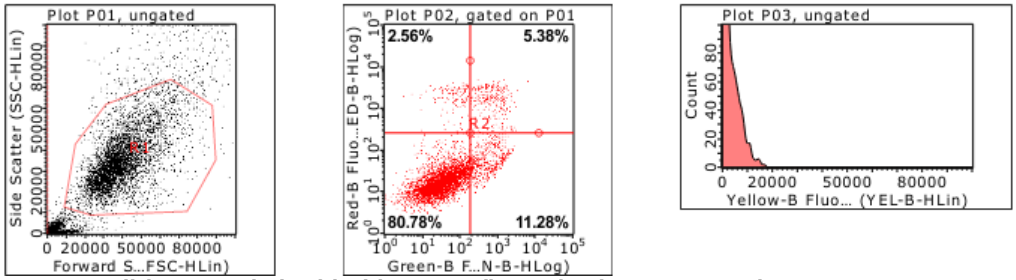


Staining of healthy and apoptotic Jurkat cells using flow cytometry

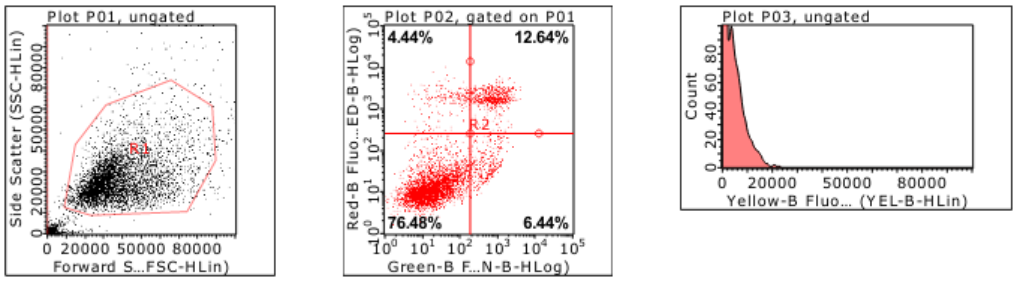
Jurkat cells were incubated with 0.5ug/ml actinomycin D for 20 hours.
Untreated cells are shown in the left panel, apoptotic induced cells are shown in the right panel.



1. HeLa cell (0 mM Cobalt Chloride Treated) : Healthy Cell



2. HeLa cell (1 mM Cobalt Chloride Treated) : Early phase Apoptosis



3. HeLa cell (1.5 mM Cobalt Chloride Treated) : Late phase Apoptosis

Staining of healthy and apoptotic HeLa cells using flow cytometry

HeLa cells were incubated with 0, 1, 1.5 mM Cobalt Chloride for 20 hours.
Untreated cells are shown in the left panel, apoptotic induced cells are shown in the right panel.