Annexin V-FITC Apoptosis Kit

Product No.: K019

Description:

Annexin V Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, cells translocate the membrane phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can be easily detected by staining with a fluorescent conjugate of Annexin V, a protein that has a high affinity for PS. The one-step staining procedure takes only 10 minutes. Detection can be analyzed by flow cytometry or by fluorescence microscopy. The kit can differentiate apoptosis vs necrosis when performing both Annexin V-FITC and PI staining.

Kit Summary:

• Detection method- Flow cytometry (Ex=488 nm; Em=530 nm) and fluorescence microscopy
• Sample type- Living cells (suspension and adherant)
• Species reactivity- Mammalian
• Kit size- 20 assays, 50 assays
• Applications- Detect early/middle stages of apoptosis; differentiate apoptosis from necrosis.

Features & Benefits:

• Simple one step staining procedure in 10 minutes
• Fast and convenient
• Kit can differentiate apoptosis vs necrosis when performing both Annexin V-FITC and PI staining

Kit components:

• Annexin V-FITC
• 1X Binding Buffer
• Propidium Iodide (PI)

Storage Conditions:

+4°C for six mouths.

Shipping Conditions:

Ice pack

Annexin V-FITC Assay Protocol:

A. Incubation of cells with Annexin V-FITC

1. Induce apoptosis by desired method. For adherent cells, gently trypsinize and wash cells with serum-containing media.
2. Collect cells by centrifugation, suspend with PBS and count.
3. Collect 1-5 x 10^5 cells, resuspend cells in 195 µl of 1X Binding Buffer.
4. Add 5 µl of Annexin V-FITC and 10 µl of propidium iodide, mix gently.
5. Incubate at room temperature(20-25℃) for 10min in the dark, then put on the ice.

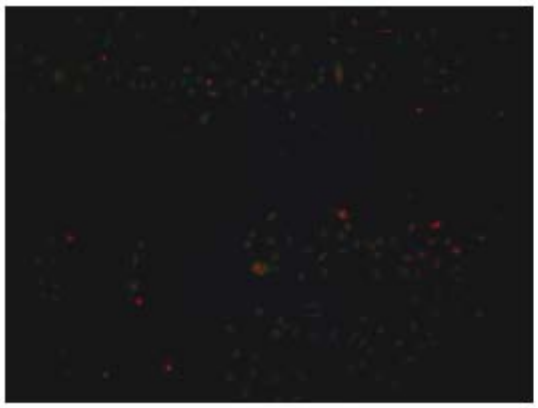
Proceed to B or C below depending on method of analysis.

B. Quantification by Flow Cytometry

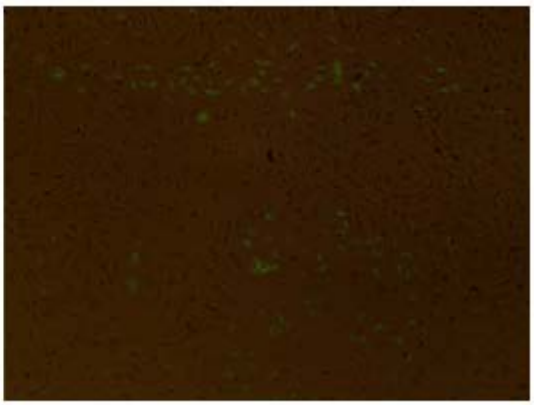
Analyze Annexin V-FITC binding by flow cytometry (Ex = 488 nm; Em = 530 nm) using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2).

C. Detection by Fluorescence Microscopy

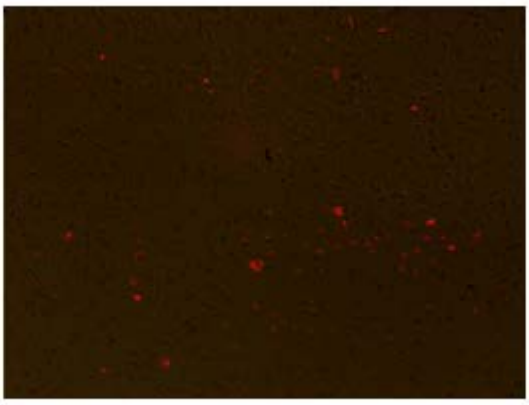
1. Centrifuge the cell suspension from Step A.5 to collect cells, gently suspend with 50-100µl 1X Binding Buffer. Place the cell suspension on a glass slide. Cover the cells with a glass coverslip and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization. (Cells must be incubated with Annexin V-FITC before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane.)
2. Observe the cells under a fluorescence microscope using a dual filter set for FITC & rhodamine. Cells that have bound Annexin V-FITC will show green staining in the plasma membrane. Cells that have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (FITC) on the cell surface (plasma membrane).



Annexin V-FITC-PI staining



Annexin V-FITC staining



PI staining

Attention:

1. If there is bacterial or fungal infection, it will seriously affect the results.
2. Should detect after dyeing, long time may lead to an increased number of apoptotic or necrotic cells
3. If the cells were collected using trypsin, should remove the residual trypsin. Residual trypsin will degrade Annexin V-FITC.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety, please wear the experimental clothes and disposable gloves.