Annexin V-EGFP Apoptosis Kit

Product No.: K018

Description:

The Annexin V-EGFP Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, most cell types translocate the membrane phospholipid 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 an enhanced green fluorescent protein (EGFP) fusion of annexin V, a protein that has a strong natural affinity for PS. The one-step staining procedure takes only 10 minutes. In addition, the assay can be directly performed on live cells. Detection can be analyzed by flow cytometry or by fluorescence microscopy with a FITC filter. EGFP is brighter and more photo-stable than other fluorescent reagents. The kit can differentiate apoptosis vs necrosis when performing both annexin V-EGFP 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
• EGFP is a bright and photo-stable green fluorescent protein and the kit can differentiate apoptosis vs necrosis when performing both annexin V-EGFP and PI staining

Kit components:

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

Storage Conditions: +4°C

Shipping Conditions: gel pack

Annexin V-EGFP Assay Protocol:

A. Incubation of cells with Annexin V-EGFP

1. Induce apoptosis by desired method.
2. Collect 1-5 x 10^5 cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Binding Buffer.
4. Add 5 µl of Annexin V-EGFP and 5 µl of propidium iodide (PI 50µg/ml, optional.)
5. 5. Incubate at room temperature for 5 min in the dark.
   Proceed to B or C below depending on method of analysis.

B. Quantification by Flow Cytometry

Analyze Annexin V-EGFP 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. For adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-EGFP (A.3-5).

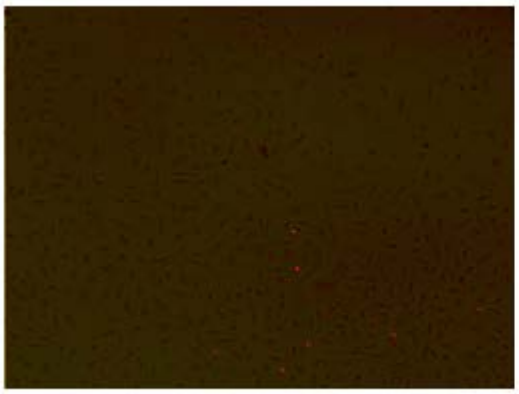
C. Detection by Fluorescence Microscopy

1. Place the cell suspension from Step A.5 on a glass slide. Cover the cells with a glass coverslip.

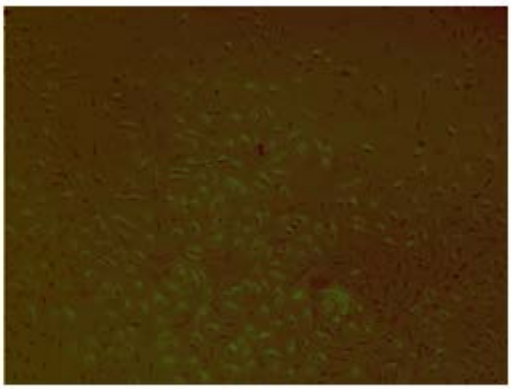
For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.5), invert coverslip on glass slide and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization

Note:

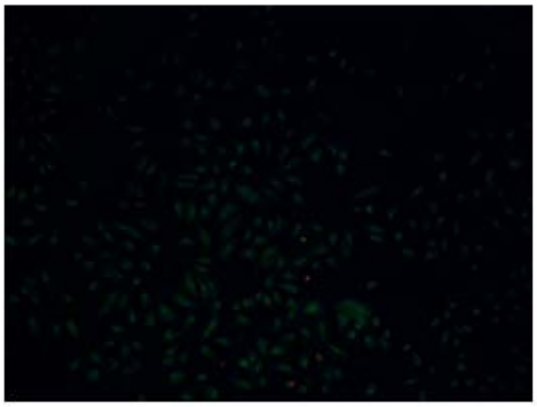
1. Cells must be incubated with Annexin V-EGFP before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane
2. Observe the cells under a fluorescence microscope using a dual filter set for FITC & rhodamine. Cells which have bound Annexin V-EGFP will show green staining in the plasma membrane. Cells which have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (EGFP) on the cell surface (plasma membrane).



PI staining



Annexin V-EGFP staining



Annexin V-EGFP-PI staining