Annexin V-EGFP/PI Apoptosis Kit

Catalog No.: K018

Size: 20T/50T/100T

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

Applications- Detect early/middle stages of apoptosis; differentiate apoptosis from necrosis.

Kit components:

Reagents	20T	50T	100T
Annexin V-EGFP	100μL	250μL	500μL
1X Binding Buffer	10mL	25mL	50mL
Propidium Iodide (PI)	200μL	500μL	1mL

Storage:

2-8°C for 12 months. Annexin V-EGFP and propidium iodide need to be stored away from light.

Shipping Conditions: ice pack

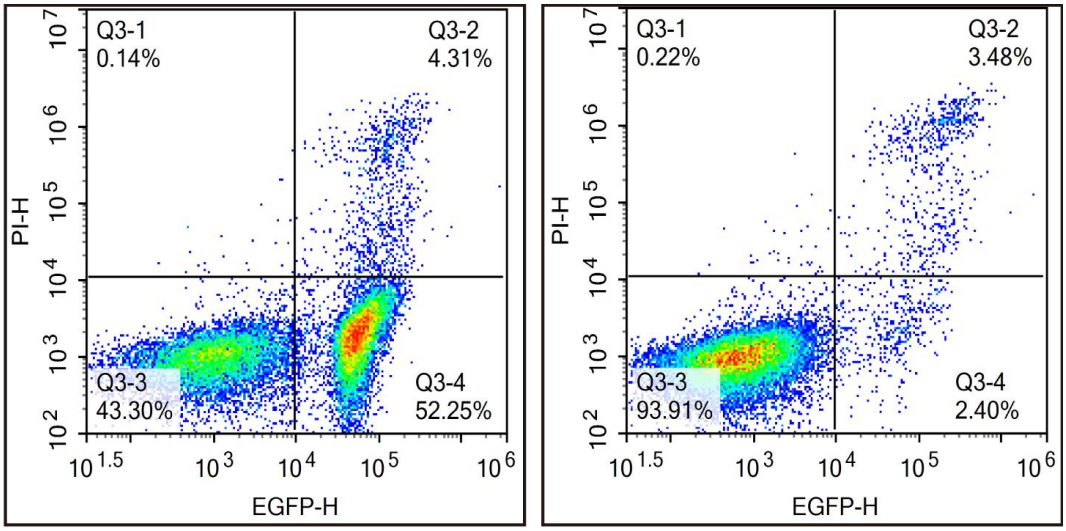
Description:

Annexin V-EGFP/PI apoptosis detection kit can be used to detect apoptosis in suspension cells and adherent cells.

Annexin V is a calcium-dependent phosphatidylserine binding protein with a high affinity for phosphatidylserine PS. Annexin V labelled EGFP can bind to the membrane of early apoptotic cells by means of the PS exposed outside the cells. Apoptosis can be detected by flow cytometry or fluorescence microscopy.

Propidium Iodide (PI) binds specifically to double-stranded DNA and produces strong fluorescence, which is normally unable to penetrate cell membranes. Due to late apoptotic or necrotic cell membrane loss of integrity, PI can enter cells to stain DNA and, when used in combination with Annexin V, distinguish cells at different stages of apoptosis.

The following figure shows the apoptosis effect of Jurkat cells induced by camptothecin detected by this kit.



Jurkat cells were treated with 5μM Camptothecin (left) or untreated (right) for 4 h. After staining with this kit, fluorescence detection was performed by flow cytometry. Annexin V-EGFP monopositive cells are early apoptotic cells, Annexin V-EGFP and PI double positive cells are necrotic or late apoptotic cells, and PI monopositive cells are nude cells.

Annexin V-EGFP/PI Assay Protocol:

A. Incubation of cells with Annexin V-EGFP

1. Induce apoptosis by desired method. Centrifuge at 300 g for 5 min, discard the supernatant, collect the cells, gently suspend the cells with PBS and count them.

2. Collect 1-5 x 10^5 cells by centrifugation, and the supernatant was discarded. The the cells were washed with PBS once, the supernatant was abandoned after 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. Incubate at room temperature for 15 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 EGFP 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 EGFP & 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).

3. It should be detected as soon as possible after staining. Too long a time may lead to an increase in the number of apoptotic or necrotic cells.

4.When detecting adherent cells, the suspension cells generated after inducing apoptosis should be collected and detected together with the adherent cells collected later.

5.Mechanical damage caused by digestive adherent cells should be avoided as much as possible. At the same time, the digestive juices of pancreatic enzymes should be as free of EDTA as possible, as EDTA affects Annexin V binding to phosphatidylserine.

6. If EDTA-containing pancreatic enzymes are used, the cells should be thoroughly washed after collection to ensure that EDTA is removed.

7.Fluorescent substances are prone to quenching, in the fluorescence observation, as far as possible to shorten the observation time, while in the operation and storage process also try to pay attention to the preservation of light.

8.For your safety and health, please wear a lab coat and disposable gloves.

9.This kit is for scientific research only.