Annexin V-FITC/PI Apoptosis Detection Kit

Product description

The Annexin V-FITC/PI Apoptosis Detection Kit uses FITC-labeled Annexin V as a probe to detect the onset of early apoptosis. The detection principle is as follows: in normal living cells, phosphatidylserine (PS) is located on the inner side of the cell membrane, but in early apoptotic cells, PS flips from the inner side of the cell membrane to the surface of the cell membrane and is exposed to the extracellular environment. Annexin V, a calcium-dependent phospholipid-binding protein with a molecular weight of 35-36 kDa, has high affinity for PS and can bind to the membrane of early apoptotic cells through the exposed PS on the outer side of the cell membrane.In addition, Propidium Iodide (PI) is provided in this kit to distinguish surviving early cells from necrotic or late apoptotic cells.PI is a nucleic acid dye that does not penetrate through the intact cell membranes of normal or early apoptotic cells, but can penetrate through the cell membranes of late apoptotic and necrotic cells and stain the nuclei red. Therefore, when Annexin V was used in combination with PI, PI was excluded from living cells (Annexin V-/PI-) and early apoptotic cells (Annexin V+/PI-), while late apoptotic and necrotic cells stained double-positively (Annexin V+/PI+) with both FITC and PI.
This kit can be used for detection with flow cytometry or fluorescence microscopy.

Specifications

Components No.	C331406E	C331406S	C331406M
Size	20 T	50 T	100 T

Shipping and Storage

Transportation of ice packs: Store at -20°C in the dark to avoid repeated freezing and thawing, effective for one year.
[Note]If multiple reuses are required within a short period, store in the dark at 4°C, effective for six months.

Notes

1. Since apoptosis is a rapid process, it is recommended that samples be analyzed within 1 hour of staining.
2. For adherent cells, digestion is a critical step. If there are floating cells during apoptosis induced by adherent cells, the floating cells and adherent cells should be collected and combined for staining. Handle the adherent cells with care and avoid artificial damage. If the digestion time of trypsin is too short, the cells need to be blown hard to be dislodged, which is easy to cause damage

to the cell membrane;excessive PI intake or prolonged digestion time can also cause damage to the cell membrane and may affect the binding of phosphatidylserine to Annexin V-FITC.After spreading trypsin all over the bottom of the well plate during digestion, shake gently to make trypsin fully contact with the cells, then pour out most of the trypsin, and use the remaining small amount of trypsin to digest for a period of time, to be terminated when the inter-cellular space increases, and the bottom of the vials show a blossom shape. Avoid using EDTA in the digestion solution as it may will affect the binding of Annexin V to PS.

3. If the sample is derived from blood, be sure to remove the platelets from the blood. This is because platelets contain PS, which binds to Annexin V and thus interferes with the results of the experiment. Platelets can be removed by using a buffer containing EDTA and centrifuging at 200 g.

4. Centrifuge the reagents briefly before uncapping and shake the liquid from the inside of the cap to the bottom of the tube to avoid spilling the liquid when uncapping.

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

6. For research use only!

Instructions

1. Sample staining

1) Suspension cells: collect cells by centrifugation at 300 g, 4°C for 5 min.

2) Adherent cells: after digestion with EDTA-free trypsin, collect the cells by centrifugation at 300 g, 4°C for 5 min. Trypsin digestion should not be too long to prevent false positives.

3) Wash the cells twice with pre-cooled PBS, each time 300 g, centrifuge at 4°C for 5 min. 1×10^5-5×10^5 cells were collected.

4) PBS was aspirated and discarded, and 100 μL of 1×Binding Buffer was added to resuspend the cells.

5) Add 5 μL Annexin V-FITC and 10 μL PI Staining Solution, gently mix.

6) React for 10-15 min at room temperature, protected from light.

7) Add 400 μL 1×Binding Buffer, mix well and place on ice, samples were detected by flow cytometry or fluorescence microscope within 1 hour.

【Note】To avoid losing cells when washing the cells, you can use a large Tip head over a small Tip head to pipette the liquid when pipetting.

2. Sample Analysis

1) Flow cytometry analysis

The maximum excitation wavelength of FITC is 488 nm, the maximum emission wavelength is 525 nm, and the green fluorescence of FITC is detected in the FL1 channel; the maximum excitation wavelength of PI-DNA complex is 535 nm, the maximum emission wavelength is 615 nm, and the red fluorescence of PI is detected in the FL2 or FL3 channel. The cells were analyzed with software

such as CellQuest, and a two-color scatter plot (two-color dot plot) was plotted with FITC as the horizontal coordinate and PI as the vertical coordinate. In typical experiments, cells can be divided into three subpopulations, live cells with only very low intensity background fluorescence, early apoptotic cells with only strong green fluorescence, and late apoptotic cells with double staining of green and red fluorescence.

2) Fluorescence microscopy analysis

a. Place a drop of cell suspension stained with Annexin V-FITC/PI dual staining on a glass slide and cover with a coverslip.

[Note]For adherent cells, the cells can be directly incubated with coverslips and apoptosis can be induced.

b. Observed under a fluorescence microscope with a two-color filter. annexin V-FITC fluorescence signal is green, and PI fluorescence signal is red.