HB220420

Annexin V-FITC/PI Apoptosis Detection Kit

Product Information

Product name	Cat#	Specification
Annexin V-FITC/PI Apoptosis Detection Kit	40302ES20	20 T
	40302ES50	50 T
	40302ES60	100 T

Product Description

Annexin V-FITC/PI Apoptosis Detection Kit uses FITC-labeled Annexin V as a probe to detect early apoptosis of cells.

The detection principle is that in normal living cells, phosphotidylserine (PS) is located on the inner side of the cell membrane, but in early apoptotic cells, PS reverses from the inner side to the surface of the cell membrane and is exposed to the extracellular environment. Annexin V is a Ca^{2+} -dependent phospholipid binding protein with a molecular weight of 35-36 kDa. Annexin V is a Ca^{2+} -dependent phospholipid binding protein with a high affinity for PS and binds to the membranes of early-apoptotic cells via externally exposed phosphatidylserine.

In addition, Propidium Iodide (PI) is provided in this kit to distinguish between surviving early cells and necrotic or late apoptotic cells. PI is a kind of nucleic acid dye, which can not penetrate the intact cell membrane of normal cells or early apoptotic cells, but can penetrate the cell membrane of late apoptotic and necrotic cells and make the nucleus 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^-). The apoptotic cells and necrotic cells were double positive by FITC and PI binding staining (Annexin V^+/PI^+).

This kit can be used for flow cytometry and fluorescence microscopy.

Product Components

Component		40302ES20（20T）	40302ES50（50T）	40302ES60（100T）
40302-A	Annexin V-FITC	100 μL	250 μL	500 μL
40302-B	PI Staining Solution	200 μL	500 μL	1.0 mL
40302-C	1×Binding Buffer	10 mL	25 mL	50 mL

Shipping and Storage

The components are shipped with ice pack and can be stored at -20°C for 1 year.

Cautions

1. As cell apoptosis is a rapid process, it is recommended that samples be analyzed within 1 hour after staining.
2. Digestion is a critical step for adherent cells. When adherent cells induce cell apoptosis, if there are floating cells, floating cells and adherent cells should be collected and combined with staining. When handling adherent cells, be careful to avoid artificial damage.
3. If cells need to be fixed in the experiment, such as cell cycle detection at the same time as apoptosis detection, Annexin V-FITC can only be used, but Annexin V-EGFP can not be used, because EGFP will denaturate and lose the ability to activate fluorescence in the process of fixation. Annexin V-FITC cells should be incubated with Annexin V-FITC before fixation, and the unbound Annexin V-FITC should be washed with Binding buffer. Due to increased cell permeability during immobilization, cell fragments can bind to Annexin V and interfere with the results.
4. If the sample comes from blood, be sure to remove platelets from the blood. Because platelets contain PS, Annexin V binds and interferes with the results. Platelets can be removed by buffering with EDTA and centrifugation at 200 g.
5. Please centrifuge the reagent briefly before opening the cover, and throw the liquid on the inner wall of the cover to the bottom of

the tube to avoid liquid spilling when opening the cover.

6. Annexin V-FITC and PI are photosensitive substances, please avoid light during operation.
7. For research use only!

Instructions

1. 1 Sample staining

1) Suspension cells: 300 g, centrifuged at 4℃ for 5 mins.

Adherent cells: After digestion with trypsin without EDTA, cells were collected by centrifugation at 300 g at 4℃ for 5 mins. Trypsin digestion time should not be too long to prevent false positive.

2) The cells were washed twice with pre-cooled PBS, 300 g each time, centrifuged at 4℃ for 5 mins.1 ~ 5×10^5 cells were collected.

3) PBS was discarded and 100 μL 1×Binding Buffer was added to resuscitate cells.

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

5) Avoid light and react at room temperature for 10-15 mins.

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

【Notes】: In order to avoid the loss of cells when washing cells, a small tip can be used to suck liquid.

1.2 Sample Analysis

A. Flow cytometry analysis:

The maximum excitation wavelength of FITC was 488 nm, and the maximum emission wavelength was 525 nm. The green fluorescence of FITC was detected in FL1 channel. The maximum excitation wavelength of pi-DNA complex was 535 nm, and the maximum emission wavelength was 615 nm. The red fluorescence of PI was detected in FL2 or FL3 channel. CellQuest and other software were used for analysis, and two-color dot plot was drawn, with FITC as abscissa and PI as ordinate. In typical experiments, cells can be divided into three subgroups, living cells only have very low intensity of background fluorescence, early apoptotic cells only have strong green fluorescence, and late apoptotic cells have double staining of green and red fluorescence.

B. Fluorescence microscope analysis:

1) One drop of Annexin V-FITC/PI double-stained cell suspension was placed on the slide and the cells were covered with a cover slide.

【Notes】: For adherent cells, cells can be directly cultured with cover glass and apoptosis can be induced.

2) Observation under fluorescence microscope with two-color filter. Annexin V-FITC fluorescence signal is green and PI fluorescence signal is red.