Annexin V-FITC/PI Apoptosis Detection Kit

Cat #: orb1566781 (manual)

Size: 10T/ 50T/ 100T/50T×5/100T×5

Product Content

Product Composition	orb1566781 10T/Kit	orb1566781 50T/Kit	orb1566781 100T/Kit	orb1566781 50T×5/Kit	orb1566781 100T×5/Kit
Annexin V-FITC	50μL	250μL	500μL	250μL×5	500μL×5
Propidium iodide	100μL	500μL	1mL	500μL×5	1mL×5
Binding Buffer (10×)	5mL	25mL	50mL	25mL×5	50mL×5
Manual	1 copy	1 copy	1 copy	5 copies	5 copies

Product Introduction

This testing kit is used to detect early apoptosis in which Annexin V is a member of the intracellular protein annexin family and selectively binds to phosphatidyl serine (PS) in a calcium-dependent manner.

PS is normally distributed inside the cell membrane, i.e., on the side adjacent to the cytoplasm. At the early stage of cell apoptosis, different types of cells everted PS to the outside of the cell membrane. With FITC-conjugated Annexin V, or Annexin V-FITC, this important feature of apoptosis can be detected by flow cytometry or fluorescence microscopy.

Propidium iodide (PI) is a nuclear staining for reagent that embeds red fluorescence. PI does not penetrate intact cell membranes, but can penetrate necrotic cells or cells that lose cell membrane integrity in late stages of apoptosis. Therefore, when Annexin V was used in combination with PIs, PIs were excluded from viable cells (Annexin V-/PI-) and early apoptotic cells (Annexin V+/PI-), while late apoptotic and necrotic cells were both positive by FITC and PI binding staining (Annexin V+/PI+).

The maximum absorption wavelength of FITC is 490nm, the emission wavelength is 525nm, and the maximum absorption and emission wavelengths of PI-DNA complex are 535nm and 615nm, respectively.

Method of use

(I) Sample staining

1. Dilute Binding Buffer (10×) to 1×Binding Buffer working solution for standby (add 9 ml sterile deionized water for 1 ml Binding Buffer (10×)).

2. For suspended cells, the cells were collected by centrifugation at 500 -1000 g for 5 min.

For adherent cells, the cells shall be digested with trypsin without EDTA. The digestion time of trypsin should not be too long or too short. It is better to add cell culture medium when the adherent cells can be

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blown down by gentle blowing, and the cells shall be blown down and transferred to the centrifuge tube, 500 -1000 g. Cells were collected by centrifugation for 5 minutes.

3. After collecting cells, add pre-cooled PBS solution to shake gently or gently blow with pipette to wash, centrifuge to collect cells, and wash twice in total.

4. 1×Binding Buffer working solution was added to the cell precipitate and the cells were resuspended to a cell concentration of 1× 106 cell/ml.

5. Pipette 100 µl cell suspension (total number of cells 1× 105 cell) into a new tube and add 5µl Annexin V-FITC and 5-10µl PI, gently mix and incubate for 15 min at room temperature, protected from light.

(II) Sample test

1. Flow Cytometry:

After staining incubation, add 400 µl 1×Binding Buffer of working solution to each tube, mix well, and test with flow cytometer (within one hour).

It is recommended to set three control groups: Normal cells, single-staining PI and single-staining Annexin V-FITC. The normal cell group can be used as fluorescence compensation adjustment to remove spectral overlap and set the position of the cross gate. If the position of the cross gate is not easy to set, cells induced by apoptosis can be used for setting. The results can be analyzed by CellQuest and other software, and the dual-dispersion plot (two-color dot plot) is drawn. FITC is the abscissa, and PI is the ordinate. When Annexin V-FITC and PIs were used together, viable cells had only very low intensity background fluorescence, early apoptotic cells had only strong green fluorescence, and late apoptotic cells had both green and red fluorescence.

2. Fluorescence microscopy:

The smears were stained and observed microscopically after incubation. FITC and PI were observed using blue and green light channels on a fluorescence microscope, respectively. Cells bound by Annexin V-FITC showed a green halo on the serosa. Cells that lose membrane integrity have red nuclei and a green halo on the membrane.

Storage conditions

2~8℃ Protected from light, valid for one year from the date of production. Annexin V-FITC can not be frozen.

Precautions

1. Vertical centrifuge tube reagent Please centrifuge briefly before opening the lid, throw the liquid on the inner wall of the lid to the bottom of the tube to avoid liquid spilling during opening the lid.
2. Annexin V-FITC and propidium iodide (PIs) are photosensitive substances and should be protected from light when stored and handled.
3. In the final step of cell washing, try to discard the supernatant to avoid PBS residue affecting the test results.
4. To obtain accurate test results, it is recommended that samples be analyzed within 1 hour of staining.
5. For your safety and health, wear lab protective suit, gloves, masks and other necessary protective equipment.

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