Annexin V-FITC/7-AAD Apoptosis detection Kit

Cat#: orb1566753 (manual)

Size：10T/ 50T/ 100T

Product composition:

Reagents	orb1566753-10T	orb1566753-50T	orb1566753-100T
Annexin V-FITC	50μl	250μl	500μl
7-AAD	100μl	500μl	1ml
Binding Buffer(10×)	5ml	25ml	50ml
Manual	1 copy	1 copy	1 copy

Product Introduction :

This detection 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 the FITC-conjugated Annexin V (Annexin V- FITC), this important feature of apoptosis can be detected by flow cytometry or fluorescence microscopy.

7- Aminoactinin D (7-AAD) is a non-immersible dye that can be used to detect inactive cells. It does not pass through normal plasma membranes, and the permeability of plasma membranes to 7-AAD gradually increases with apoptosis and cell death. Once the dye enters the cell, it binds to the cellular DNA molecule and emits bright red fluorescence when excited by the appropriate wavelength of excitation light. 7-AAD has similar fluorescence characteristics with PIs, but its emission spectrum is narrower than PIs and less interference to other detection channels. It is the best substitute for PIs in polychromatic fluorescence analysis and can be used in combination with the Annexin V-FITC/PE. When the Annexin V-FITC was used in combination with 7-AAD, 7-AAD was excluded from viable cells (Annexin V-/7-AAD-) and early apoptotic cells (Annexin V+/7-AAD-), while late apoptotic cells and necrotic cells were both positive by FITC- and 7-AAD-binding staining (Annexin V+/7-AAD+).

The maximum absorption wavelength of FITC is 490nm and the excitation wavelength is 525nm. The 7-AAD can be excited by 488 nm argon ion laser with an emission wavelength of 647nm.

Storage condition:

Stored in 2-8°C protected from light, valid for one year. Annexin V-FITC should not be frozen.

Measurement steps:

1. Sample staining

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

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

cells, the cells should be digested with trypsin without EDTA. The digestion time of trypsin should not be too long or too short. It is better to add the cell culture medium when the adherent cells can be blown down by gentle blowing. The cells should be blown down and transferred to the centrifuge tube. The cells should be collected after centrifugation for 5 min at 500 -1000 g.

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) The cells were resuspended by adding 1×Binding buffer working solution to the cell precipitate to achieve a cell concentration of 1×10^6 cells/ml.

5) Pipette the 100 µl cell suspension (total number of cells 1×10^5 cells) into a new tube, add 5µl Annexin V- FITC and 5-10 µl 7-AAD, gently mixed and incubated at room temperature for 15 min in the dark.

Sample Detection:

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, 7-AAD single-staining and Annexin V-FITC single-staining. 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 analysed by Cell Quest or other software, and the dual dispersion plot (two-colour dot plot) is drawn. FITC is the abscissa and 7-AAD is the ordinate. When Annexin V- FITC and 7-AAD were used in combination, viable cells had only 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 cell-slides were stained and observed at the microscopy after incubation. FITCs and 7-AAD 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.

Precautions:

1. Centrifuge the reagent in the tube for a short time before opening the lid. Throw the liquid on the inner wall of the lid to the bottom of the tube to avoid spilling the liquid when opening the lid.
2. Annexin V-FITC and 7-AAD are photosensitive substances and should be protected from light during storage and operation.
3. In the final step of cell washing, try to discard the supernatant to avoid PBS residue affecting the test results.
4. To obtain the accurate test results, it is recommended that samples be analysed within 1 hour of staining.
5. For your safety and health, wear lab protective suit, gloves, masks and other necessary protective equipment.