ELK Biotechnology

For research use only.

Annexin V-FITC Apoptosis Kit Instructions

(Item number: BC148)

1. Meaning of determination:

Annexin is a type of calcium ion-dependent phospholipid-binding protein widely distributed in the cytoplasm of eukaryotic cells and participates in intracellular signal transduction. Annexin V selectively binds to phosphatidylserine (PS). Phosphatidylserine is mainly distributed on the inside of the cell membrane, that is, on the side adjacent to the cytoplasm. In the early stages of cell apoptosis, different types of cells will evert phosphatidylserine to the cell surface, that is, to the outside of the cell membrane. Phosphatidylserine promotes coagulation and inflammation when exposed to cell surfaces. Annexin V binds to phosphatidylserine that is extrapolated to the cell surface and blocks the procoagulant and proinflammatory response activities of phosphatidylserine. Therefore, Annexin V is used as one of the sensitive indicators for detecting early apoptosis of cells. Using Annexin V labeled with FITC, a fluorescent probe with green fluorescence, namely Annexin V-FITC, the evagination of phosphatidylserine, a component of apoptosis, can be detected very simply and directly using flow cytometry or fluorescence microscopy. important characteristics.

Annexin V-FITC Apoptosis Detection Kit is a cell apoptosis detection that uses FITC-labeled recombinant human Annexin V to detect phosphatidylserine that appears on the cell membrane surface during apoptosis. Reagent test kit. Detection can be performed using flow cytometry, fluorescence microscopy or other fluorescence detection equipment.

This kit also provides a propidium iodide staining solution. Propidium iodide can stain necrotic cells or cells that have lost their cell membrane integrity in the late stages of apoptosis, showing red fluorescence. For necrotic cells, since the integrity of the cell membrane has been lost, Annexin V-FITC can enter the cytoplasm and bind to phosphatidylserine located on the inner side of the cell membrane, thus causing the necrotic cells to display green fluorescence. Therefore, by matching Annexin V with PI, cells in different stages of apoptosis can be distinguished.

2. Kit composition:

Reagent	10T	20T	50T	Storage conditions
Annexin V-FITC	50μl	100μl	250μl	Store at 4℃ protect from light
Binding solution	7.5 ml	15 ml	40 ml	4℃
Propidium iodide （PI）	50μl	100μl	250μl	Store at 4℃ protect from light

3. Prepare your own instruments and reagents other than test kits

Flow cytometer or fluorescence microscope, low-speed centrifuge, pipette, 1.5ml centrifuge tube, slide, cover slip, PBS, EDTA-free trypsin digestion solution

4. Storage conditions

Store at 4°C. Annexin V-FITC and propidium iodide staining solutions must be protected from light and are valid for one year. For long-term storage, the Annexin V-FITC and propidium iodide staining solutions can be appropriately packed and stored at -20°C. The Annexin V-FITC combined solution can be directly stored at -20°C.

5. Things to note：

① If there is bacterial or fungal contamination, it will seriously affect the detection effect.

②Testing should be done as soon as possible after staining. Too long time may lead to an increase in the number of apoptotic or necrotic cells.

③ Fluorescent substances are prone to quenching. When conducting fluorescence observation, the observation time should be shortened as much as possible, and at the same time, try to avoid light during operation and storage.

④You need to bring your own PBS.

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

⑥ This kit is suitable for detecting living cells. When detected by flow cytometry, the number of cells should not be less than 1×105. It is not suitable for detecting tissue samples.

⑦ Due to the different types of cells being detected, the types of apoptosis inducers, and the detection instruments used, the fluorescence compensation of flow cytometry detection is also different.

Therefore, it is recommended that cells without apoptosis induction treatment be used as a control for each detection to adjust fluorescence compensation.

⑧ Detect with flow cytometer, excitation wavelength Ex=488 nm; emission wavelength Em=530 nm. The green fluorescence of Annexin V-FITC is detected through the FITC channel (FL1); the red fluorescence of PI is detected through the PI channel (FL2 or FL3). It is recommended to use FL3. Fluorescence compensation adjustment: Use normal cells without apoptosis induction treatment as a control to perform fluorescence compensation adjustment to remove spectral overlap and set the position of the cross gate.

6. Operating procedures

1. For suspension cells:

A. After stimulating cell apoptosis, centrifuge at 1000g (about 1000~2000rpm) for 5 minutes, discard the supernatant, collect the cells, gently resuspend the cells in PBS and count. (Note: PBS resuspension cannot be omitted. The PBS resuspension process also plays a role in washing the cells, which can ensure the subsequent binding of Annexin V-FITC.)

B. Take 1~5×105 resuspended cells, centrifuge at 1000g for 5 minutes, discard the supernatant, add 500μl binding solution and gently resuspend the cells.

C. Add 5μl Annexin V-FITC and mix gently; then add 5μl propidium iodide and mix gently.

D. Incubate at room temperature (20-25°C) away from light for 10 minutes. Aluminum foil can be used to protect from light.

E. Then perform flow cytometry detection. Annexin V-FITC has green fluorescence and PI has red fluorescence. If it is used for detection under a fluorescence microscope, put a drop of the above-stained cell suspension on a glass slide and cover the cells with a coverslip for detection.

2. For adherent cells:

A. Aspirate the cell culture medium into a suitable centrifuge tube, wash the adherent cells once

with PBS, and digest the cells with trypsin cell digestion solution (preferably not containing EDTA). (Note: Trypsin digestion time should not be too long, otherwise it will easily cause false positives)

B. After the cells are digested, add the cell culture medium collected in step 2A, mix slightly, transfer to a centrifuge tube, centrifuge at 1000g for 5 minutes, discard the supernatant, collect the cells, gently resuspend the cells in PBS and count. .

C. Take 1~5×105 resuspended cells, centrifuge at 1000g for 5 minutes, discard the supernatant, add 500μl binding solution and gently resuspend the cells.

D. Add 5μl Annexin V-FITC and mix gently; then add 5μl propidium iodide and mix gently.

E. Incubate at room temperature (20-25°C) away from light for 10 minutes. Aluminum foil can be used to protect from light.

F. Immediately perform flow cytometry detection. Annexin V-FITC has green fluorescence and PI has red fluorescence. If it is used for detection under a fluorescence microscope, put a drop of the above-stained cell suspension on a glass slide and cover the cells with a coverslip for detection.

3. In situ fluorescence microscopy detection of adherent cells:

[Note]: The advantage of this method is that it can observe cell apoptosis in situ, but the disadvantage is that some apoptosis cannot be detected because it does not adhere to the wall.

A. (Optional) If conditions permit, culture the cells in a 24-well plate, 48-well plate or 96-well plate; or grow the cells on a coverslip, use an appropriate apoptosis inducer to induce apoptosis, and establish Negative control group.

B. Aspirate the cell culture medium, add PBS and wash twice; or wash the coverslip twice with PBS.

C. Add 5 μl of Annexin V-FITC and 5 μl of propidium iodide to 500 μl of binding solution, and mix gently.

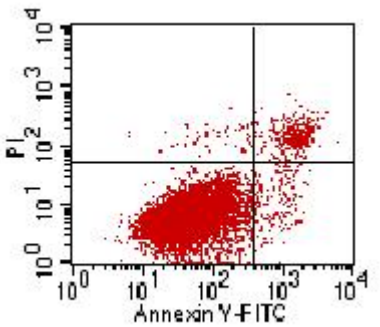
D. Add the above solution dropwise into the well of the culture plate or onto the surface of the coverslip to evenly cover the surface of the coverslip with cells.

E. Incubate at room temperature (20~25℃) away from light for 10 minutes

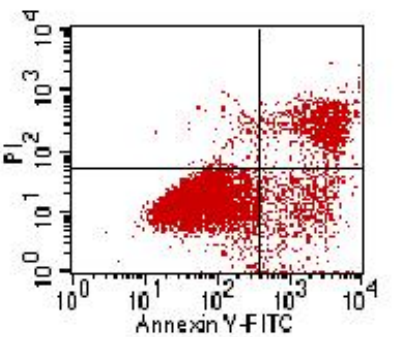
F. Then observe under a fluorescence microscope. Annexin V-FITC has green fluorescence and PI has red fluorescence.

7. Application examples:

Use apoptosis inducers to induce apoptosis in K562 cells. After culturing for 4 to 6 hours in a 37°C, 5% CO2 incubator, perform the detection according to the instructions in the instructions. The results of the flow cytometry detection are shown in the figure below:



Negative control group



Inducer treatment group