Annexin V-Alexa Fluor 647/PI
Apoptosis Detection Kit

Packing specification

Product number: FL0020、FL0050、FL0100

Specifications: 20T, 50T, 100T

Storage conditions

Stored at 4ºC, valid for one year

Precautions:

1. This kit is for research use only.
2. Micro-reagents need to be centrifuged for a few seconds to collect the reagents to the bottom of the tube before opening the cap for use.
3. Propidium Iodide (PI) is toxic. Wear gloves when handling it. Avoid contact with skin, eyes and mucous membranes.
4. Annexin V-Alexa Fluor 647 contains highly toxic sodium azide (NaN3). Wear gloves when handling and avoid contact with skin, eyes and mucous membranes.
5. This kit is used to detect live cells, the number of cells should not be less than 1x106 during flow cytometry.
6. It is advisable to detect as soon as possible after staining, too long may lead to an increase in the number of apoptosis or necrotic cells.
7. The Annixin V method for detecting apoptotic cells is suitable for the detection of cells growing in suspension, such as lymphocytes. For the adherent cells, the cell membrane will be damaged during the digestion process such as trypsin, which will cause high false positives, and the use of cell scrapers will cause the cells to adhere and form clumps, which will affect the detection. The digested cells are stored in PBS containing 2% BSA to prevent further damage. Although at present, some units including foreign countries also use this method to detect adherent growth of cells. I do not recommend using this method to detect. Because of its poor repeatability and the need to be very careful when operating.
8. The remaining trypsin digesting adherent cells will digest and degrade Annexin V-Alexa Fluor 647, which will eventually lead to staining failure.
9. After cells are fixed, fluorescence may be quenched. Please do not fix the sample.

Reagents:

Reagents	20 assays	50 assays	100 assays	Storage
Annexin V-Alexa Fluor 647 product code:FL001	100 µl	250 µl	500 µl	4°C in the dark
Propidium Iodide, PI product code:FL002	200 µl	500 µl	1000 µl	4°C in the dark
Binding Buffer (4×) product code:FL003	4 ml	10 ml	20 ml	4°C

Instructions:

1. Preparation of cell samples:

a) Suspension cells:

1) Collect the cells in a centrifuge tube and centrifuge at 1000-2000 rpm for 5 minutes, and carefully remove the supernatant.
2) Gently resuspend the cells with 1ml of 4°C pre-cooled PBS and count, centrifuge at 1000-2000rpm for 5min, carefully aspirate the supernatant.

3) Add 1ml of 4℃ pre-cooled PBS to resuspend the cells, centrifuge at 1000-2000rpm for 5min, carefully aspirate the supernatant.

b) Adherent cells:

1) Aspirate the cell culture medium into a centrifuge tube, wash the adherent cells once with PBS, and add an appropriate amount of trypsin cell digestion solution without EDTA to digest the cells.

2) Incubate at room temperature until the adherent cells can be blown down by gentle pipetting, aspirate the trypsin cell digestion solution. To avoid excessive digestion of pancreatin.

3) Add the cell culture solution collected in the above steps, mix it well, transfer to a centrifuge tube, centrifuge at 1000-2000rpm for 5 minutes, carefully aspirate the supernatant.

Note: On the one hand, the added cell culture fluid can collect suspended cells that have undergone apoptosis or necrosis. On the other hand, the serum in the cell culture fluid can effectively inhibit or neutralize the residual pancreatin; the residual pancreatin will be digested and degraded The subsequent addition of Annexin V-Alexa Fluor 647 caused staining failure.

4) Gently resuspend the cells with 1ml of 4°C pre-cooled PBS and count, centrifuge at 1000-2000rpm for 5min, carefully aspirate the supernatant.

5) Add 1ml of 4℃ pre-cooled PBS to resuspend the cells, centrifuge at 1000-2000rpm for 5min, carefully aspirate the supernatant.

2. Dilute the Binding Buffer 1:4 with deionized water (4ml Binding Buffer+12ml deionized water);

3. Resuspend the cells with 250l binding buffer and adjust the concentration to 1×106/ml;

4. Take 100l of cell suspension in a 5ml flow tube, add 5l Annexin V-Alexa Fluor 647, and mix gently;

5. Incubate at room temperature (20-25oC) in the dark for 10 minutes;

6. Add 10l propidium iodide solution 5min before getting on the machine and mix gently;

7. Before getting on the machine, add 400l PBS to the reaction tube to resuspend the cells, store in the dark, and then perform FACS detection. Annexin V-Alexa Fluor 647 and PI are red fluorescence.

For scientific research use only.