Product Information

Catalog number: REK0001

Size: 20T/50T/100T

Storage : Please store at 4℃ for 6 months, Avoid freeze / thaw cycles.

Product components:

Components	25 Tests	50 Tests	100 Tests
AnnexinV-FITC	125μl	250μl	500μl
10×Binding Buffer	1.5ml	2.5ml	6.5ml
Propidium Iodide	125μl	250μl	500μl

Product Description

In normal cells, phosphatidylserine is only distributed on the inner side of the lipid bilayer of the cell membrane. In the earliest stage of cell apoptosis, membrane phosphatidylserine (PS) flips from the inner side of the lipid membrane to the outer side. This change precedes apoptotic phenomena such as cell shrinkage, chromatin condensation, DNA fragmentation, and increased cell membrane permeability. Annexin V is a phospholipid binding protein that has a high affinity for phosphatidylserine. Therefore, it can bind to the cell membrane of early apoptotic cells through the phosphatidylserine exposed on the outer side of the cell. Therefore, Annexin V is used as one of the sensitive indicators for detecting early cell apoptosis. Propidium iodide (PI) is a nucleic acid dye that cannot penetrate the intact cell membrane. However, due to the increased cell membrane permeability of cells in the middle and late stages of apoptosis and dead cells, PI can penetrate the cell membrane and stain the cell nucleus red. Therefore, by matching Annexin V with PI, cells in different stages of apoptosis can be distinguished.

Operation steps

Adherent cells need to be digested with 0.25% trypsin. Note that over-digestion may damage cells. Adding 2% BSA during digestion can prevent over-digestion. If digesting with trypsin containing EDTA, be sure to completely remove the EDTA: wash with 1×PBS or 1×binding buffer before labeling to remove EDTA, so as to prevent residual EDTA from chelating with Ca^{2+} and affecting the binding of Annexin V.

1. Dilute 10×Binding Buffer to 1×Binding Buffer with deionized water;
2. Cell collection:

   Suspended cell collection: Centrifuge for 5 minutes;

Adherent cells: Digest with EDTA-free trypsin and centrifuge at 2000rpm for 5-10 minutes at room temperature to collect cells;

(Note: Trypsin digestion time should not be too long, otherwise it will affect the binding of phosphatidylserine on the cell membrane with Annexin V-FITC)

3. Cell washing: Resuspend the cells once with pre-cooled 1×PBS (4℃), centrifuge at 2000rpm for 5-10 minutes, discard the supernatant and wash the cells;
4. Add 300μL of 1×Binding Buffer to suspend the cells;
5. Annexin V-FITC labeling: Add 5μL of Annexin V-FITC and mix well, protect from light, and incubate at room temperature for 15 minutes;
6. PI labeling: Add 5 minutes before the machine 5μL of PI staining;
7. Before loading, add 200μL of 1×Binding Buffer. Note: The recommended number of cells for each reaction system is 1×10^4 to 1×10^6

Instructions

1. Centrifuge at low speed before use to prevent liquid from accumulating on the tube cap and tube wall;
2. This reagent is for research use only.
3. PI (propidium iodide) is toxic, can be absorbed through the skin, and is irritating to the eyes. Wear gloves when using it;
4. Annexin V-FITC and PI are photosensitive substances. Please avoid light when operating. When handling and labeling, try to do it in a dark room. During the incubation stage, wrap the container with aluminum foil or place it in a drawer. After cell labeling, observe with a microscope in a dark room;
5. The entire operation process should be as gentle as possible, do not blow the cells hard, and try to operate at 4℃ to avoid affecting the cell state.
6. In the last step of cell washing, please try to discard the supernatant to avoid PBS residue, which may affect the experimental results.
7. To prevent fluorescence decay, it is advisable to perform flow detection within 1 hour.
8. If PI staining is performed for too long, the apoptosis rate may be too high. It is recommended to perform Annexin V-FITC staining first, and then add PI staining at least 5 minutes before the instrument is put on.

Notes

This product is for research use only.