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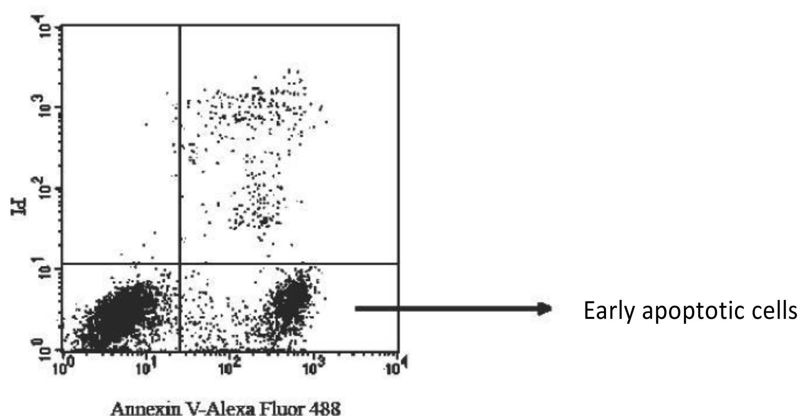
AnnexinV-AlexaFluor 488/PI Apoptosis detection Kit

Cat: CA1040
Size: 20T/50T/100T
Storage: Store at 2-8°C protected from light, do not freeze.

Product content:	CA1040-20	CA1040-50	CA1040-100
4 x (Binding Buffer 4x)	2ml	5ml	10ml
Propidium Iodide	0.1ml	0.25ml	0.5ml
rh Annexin V/Alexa Fluor 488	0.1ml	0.25ml	0.5ml

Product introduction:

Early changes in apoptosis occur on the cell membrane surface. One of these cell membrane surface changes is the transfer of phosphatidylserine (PS) from the inside of the cell membrane to the outside of the cell membrane, exposing PS on the outer surface of the cell membrane. PS is a negatively charged phospholipid, which normally mainly exists on the inner surface of the cell membrane. When the cell undergoes apoptosis, the asymmetry of the phospholipid distribution on the cell membrane is destroyed and PS is exposed to the outside of the cell membrane. Annexin V has the characteristics of being easy to bind to phospholipids such as PS, and has a high affinity for PS. Therefore, the protein can act as a sensitive probe to detect PS exposed on the cell membrane surface. The transfer of PS to the outside of the cell membrane is not unique to apoptosis, but can also occur in cell necrosis. The difference between the two methods of cell death is that the cell membrane is intact in the initial stage of apoptosis, while the integrity of the cell membrane is destroyed in the early stage of cell necrosis. Therefore, the double staining method of AnnexinV and 7-AAD can be used to detect early cell apoptosis by flow cytometry.



Flow cytometric analysis of Jurkat cells using Annexin V-Alexa Fluor 488/PI double staining after UV-induced apoptosis

Method of operation: (for reference only)

1. Preparation of cell samples：

a) For adherent cells: carefully collect the cell culture medium into a centrifuge tube for later use. Digest the cells with trypsin without EDTA. When the cells can be gently pipetted down with a pipette or pipette tip, add the previously collected cell culture solution, pipette down all the adherent cells, and gently blow off the cells. Collect again into the centrifuge tube. Centrifuge at about 1000 rpm for 5 minutes to pellet the cells. For specific cells, if the cells cannot be completely centrifuged to the bottom of the centrifuge tube, you can appropriately extend the centrifuge time or slightly increase the centrifugal force. Carefully aspirate the supernatant. About 50μl of culture fluid can remain to avoid aspirating the cells. Add about 1ml of 4°C pre-cooled PBS, resuspend the cells, centrifuge again to pellet the cells, carefully aspirate the supernatant;;

b) For suspension cells: Centrifuge at about 1000 rpm for 5 minutes to pellet the cells. For specific cells, if the cells cannot be completely centrifuged to the bottom of the centrifuge tube, you can appropriately extend the centrifuge time or slightly increase the centrifugal force. Carefully aspirate the supernatant. About 50μl of culture fluid can remain to avoid aspirating the cells. Add about 1ml of 4°C pre-cooled PBS, resuspend the cells, centrifuge again to pellet the cells, carefully aspirate the supernatant;

2. Dilute the binding buffer 1:3 with deionized water (2ml 10x binding buffer + 18ml deionized water) ;
3. Resuspend the cells with 1x binding buffer and adjust the concentration to 1-5×10^6/ml;
4. Take 100μl of cell suspension into a 5ml flow tube, add 5μl Annexin V/Alexa Fluor 488 to mix well, and incubate for 5 minutes at room temperature in the dark;
5. Add 5μl of 20ug/ml PI and 400μl of PBS to perform flow detection immediately.

Experimental design:

1) Untransfected cells

Blank tube: Negative control cells, without Annexin V/Alexa Fluor 488, PI, used to adjust voltage.

Single staining tube: positive control cells, only with Annexin V/Alexa Fluor 488 or only PI for adjustment and compensation.

Detection tube: treated cells, add Annexin V/Alexa Fluor 488, PI. After adjusting the voltage compensation with the blank tube and the single dye tube, the required flow data can be obtained.

Precautions:

1. Annexin V is compatible with phosphatidylserine (PS), and PS has no difference between different species. In normal cells, PS is only distributed on the inner side of the lipid bilayer of the cell membrane. In the early stage of apoptosis, PS turns from the inner side of the lipid membrane to the outer side.
2. Digest with low-concentration trypsin, gently blow the adherent cells 2 to 3 times, centrifuge at 4°C 1000 rpm for 5 minutes, if handled properly, the damage caused by trypsin can be controlled within 5%. If there is a control group, the experimental results Will not cause significant impact.
3. Add PI first. Not only is it difficult to judge whether the staining is uniform and sufficient for each group, but PI itself is also toxic to cells and will have a greater impact on the experimental results than pancreatin. This is not recommended.

4. Annexin V is a Ca-dependent protein, so EDTA cannot be added to prevent EDTA from chelating Ca ions and thus affecting Annexin V, thereby affecting the results.
5. When using flow cytometry to detect apoptosis, PI is greatly affected by time. Because PI is labeled, it will increase cytotoxicity. As time goes on, it will increase the staining of PI, especially when detecting early apoptosis. In addition to increasing the gap between the cell populations on the flow cytometer, the error will increase significantly. Generally, PI is added and the machine is immediately on the machine, and then the test is completed within an hour. Both methods are ok, but the error caused by following our operating steps will be smaller.

Related Products:

CA1050	AnnexinV-Alexa Fluor647/PI	Apoptosis detection Kit
CA1030	Annexin V-PE/ 7AAD Kit	Apoptosis detection Kit

Note: For more documents using this product, please refer to the www.solarbio.com.