Annexin V-Alexa Fluor 488/PI Kit Apoptosis Detection Kit

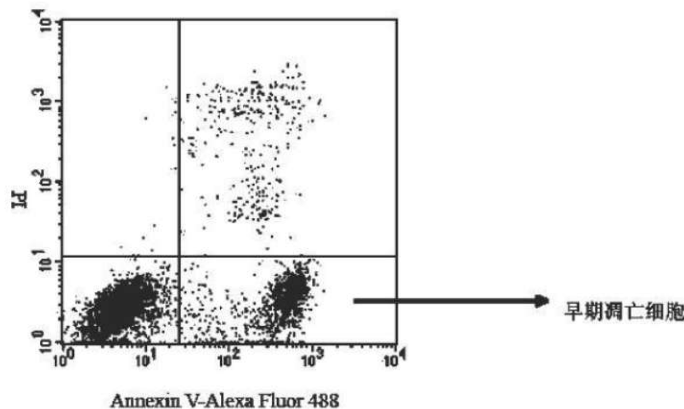
Cat: CA1040
Size: 20T/50T/100T
Storage: 2-8°C, avoid light

Product components:

Components	CA1040-20	CA1040-50	CA1040-100
10× Binding Buffer	2mL	5mL	10mL
Propidium Iodide	0.1mL	0.25mL	0.5mL
rh Annexin V/Alexa Fluor 488	0.1mL	0.25mL	0.5mL

Product description:

Early apoptotic changes occur on the surface of the cell membrane. One of these changes on the surface of the cell membrane is the transfer of phosphatidylserine (PS) from inside the cell membrane to outside the cell membrane, exposing PS to the outer surface of the cell membrane. PS is a negatively charged phospholipid, which normally exists mainly in the inner surface of the cell membrane. During cell apoptosis, the asymmetry of this phospholipid distribution on the cell membrane is destroyed, and PS is exposed to the outer surface of the cell membrane. Annexin V has Annexin V properties for easy binding to phospholipids such as PS, with high affinity for PS. Thus, the protein acts as a sensitive probe to detect PS exposed on the surface of the cell membrane. The transfer of PS to the outside of the cell membrane is not unique to apoptosis and can also occur during cell necrosis. The difference between the two modes of cell death is that the membrane is intact during the initial stage of apoptosis, while the integrity of the membrane is destroyed during the early stages of cell necrosis. Therefore, the Annexin V and PI double staining method can be used to detect early apoptosis by flow cytometry.



Apoptosis of Jurkat cells was induced by UV with Annexin V-Alexa Fluor 488/PI double staining flow analysis

Protocols: (only for reference) :

1. Preparation of cell sample:

① For adherent cells: carefully collect the cell culture fluid into a centrifuge tube for use. Digest the

cells with pancreatic enzyme without EDTA, and when the cells can be gently blown down by pipette or gun, add the cell culture solution collected earlier, blow down all the adherent cells, and gently blow away the cells. The cells are collected again into the centrifuge tube. Centrifuge at about 1000rpm for 5min and precipitate the cells. For specific cells, if the cells cannot be completely centrifuged to the bottom of the centrifuge tube, the centrifuge time can be extended appropriately or the centrifugal force can be slightly increased. Carefully remove the supernatant, leaving about 50μL of culture solution to avoid cell absorption. Add about 1mL PBS pre-cooled at 4℃, re-suspend the cells, centrifuge the precipitated cells again, and carefully remove the supernatant;

② For suspended cells: centrifuge at about 1000rpm for 5min and precipitate cells. For specific cells, if the cells cannot be completely centrifuged to the bottom of the centrifuge tube, the centrifuge time can be extended appropriately or the centrifugal force can be slightly increased. Carefully remove the supernatant, and about 50μL of culture solution can be left to avoid cell absorption. Add about 1mL PBS pre-cooled at 4℃, suspend the cells, centrifuge the precipitated cells again, and carefully remove the supernatant;

2. Dilute the binding buffer with deionized water at 1:9 (2mL 10x binding buffer +18mL deionized water);

3. Re-suspend the cells with 1× binding buffer and adjust the concentration to 1-5×10^6/mL;

4.100μL cell suspension was placed in a 5mL flow tube, mixed with 5μL Annexin V/Alexa Fluor 488, and incubated at room temperature for 5min away from light;

5. Add 5μL of 20μg/mL Propium iodide solution (PI) and add 400μL PBS for flow detection immediately.

Experimental design:

1. Blank tube: negative control cells without Annexin V/Alexa Fluor 488, propyl iodide solution (PI). For regulating voltage;

2. Single dye tube: positive control cells, Annexin V/Alexa Fluor 488 only added. For regulating compensation;

3. Detection tube: for treated cells, add Annexin V/Alexa Fluor 488, Propyl iodide solution (PI). After voltage compensation was adjusted with blank tube and single dye tube, the required flow data was obtained.

Note:

1. Annexin V is binding with phosphatidylserine (PS), which does not differ between species. In normal cells, PS is only distributed in the medial lipid bilayer of the cell membrane, but in the early stage of apoptosis, PS turns from the medial lipid membrane to the lateral.

2. Digestion of pancreatic enzyme at low concentration, gently blow the adherent cells 2-3 times, centrifuge at 4℃ 1000rpm for 5min, if properly treated, the damage caused by pancreatic enzyme can be controlled within 5%, and the experimental results will not be significantly affected in the case of a control group.

3. Adding PI first is not only difficult to judge whether the staining is uniform and sufficient in each group, but also PI itself is toxic to cells, which will have a greater impact on the experimental results than pancreatic enzyme, so it 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, which in turn affects the results.

5. When using flow cytometry to detect apoptosis, PI is greatly affected by time, because the labeling of PI will increase cytotoxicity, which will lead to increased PI staining with time, especially in the detection of early apoptosis, if the time is extended, the error will be significantly increased in addition to the widening of the difference in cell group on flow cytometry. Generally, PI is added immediately after the machine, and then the detection is completed within one hour. Both methods are OK, but the error caused by following our steps will be smaller.

Related products:

CA1050 AnnexinV-Alexa Fluor647/PI apoptosis detection Kit

CA1030 Annexin V-PE /7AAD Apoptosis detection Kit