ANNEXIN V-FITC/PI Apoptosis Detection Kit

Note: This product has a change in reagent concentration, please read the instructions carefully before use.

Cat: CA1020
Size: 20T/50T /100T
Storage: 2-8°C, avoid light, valid for 1 year.

Product Contents:	CA1020-20	CA1020-50	CA1020-100	Storage
Annexin V-FITC	100μL	250μL	500μL	2-8°C, avoid light
Propidium iodide(PI)	100μL	250μL	500μL	2-8°C, avoid light
Binding Buffer (10×)	6mL	15mL	30mL	2-8°C, long storage at -20°C

Product Description:

Early apoptotic changes occur on the surface of the cell membrane, and 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.

Protocols:

1. Preparation of cell sample:

a) For adherent cells: carefully collect the cell culture solution into a centrifuge tube for later use. Digest the cells with pancreatic enzymes without EDTA. When the cells can be gently blown down with a 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 of PBS pre-cooled at 4°C, suspend the cells, centrifuge the precipitated cells again, and carefully remove the supernatant.

b) 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, leaving about 50μL of culture solution to avoid cell absorption. Add about 1mL of PBS pre-cooled at 4 ° C, suspend the cells, centrifuge the precipitated cells again, and carefully remove the supernatant.

2. Dilute the Binding Buffer with deionized water 1:9 (2mL 10x Binding Buffer+18mL deionized water);

3. The cells were re-suspended with 1x binding buffer and the concentration was adjusted to 1-5×10^6/mL;

4. 100μL cell suspension was placed in a 5mL flow tube, mixed with 5μL Annexin V/FITC, and incubated at room temperature for 5min away from light;

5. Add 5μL Propium iodide solution (PI) and add 400μL PBS for immediate flow or fluorescence microscopy detection.

Experimental design:

Blank tube: negative control cells, Annexin V/FITC without Annexin V/FITC, propyl iodide solution (PI). For regulating voltage.

Single dye tube: Positive control cells, Annexin V/FITC only added. For regulatory compensation.

Detection tube: Treated cells, plus Annexin V/FITC, Propyl iodide solution (PI). After voltage compensation was adjusted with blank tube and single dye tube, the required flow data was obtained.

Analysis of experimental results:

A. Flow cytometry analysis:

The maximum excitation wavelength of FITC was 488nm, and the maximum emission wavelength was 525nm. The green fluorescence of FITC was detected in FL1 channel. The maximum excitation wavelength of the PI-DNA complex is 535nm, the maximum emission wavelength is 615nm, and the red fluorescence of PI is detected in the FL2 or FL3 channel. flowjo, CellQuest and other software were used for analysis, and the double dispersion plot was drawn, with FITC as the abscissa and PI as the ordinate. In a typical experiment, the cells could be divided into three subgroups, living cells only had low intensity background fluorescence, early apoptotic cells only had strong green fluorescence, and late apoptotic cells had double staining of green and red fluorescence.

B. Fluorescence microscope analysis:

1) Place a drop of cell suspension double-stained with Annexin V-FITC/PI on a slide and cover the cells with a cover slide.

Note: For adherent cells, cells can be cultured directly on a cover slide and apoptosis induced.

2) Observe with a two-color filter under a fluorescence microscope. Annexin V-FITC fluorescent signal is green and PI fluorescent signal is red.

Questions:

1. Can the Annexin V/ PI apoptosis detection kit detect apoptosis in animals other than humans?

Agreed. Because Annexin V is a phosphatidylserine (PS) affinity, and PS does not differ between species. In normal cells, PS is only distributed in the inner lipid bilayer of the cell membrane, but during early apoptosis, PS flips from the inner lipid membrane to the outer lipid membrane.

2. Do adherent cells undergo apoptosis and damage the cell membrane with pancreatic enzymes?

Digestion of pancreatic enzyme with low concentration, gently blow the adherent cells for 2 to 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 when there is a control group.

3. Can adherent cells be first stained with PI and then digested? In this way, can we reduce the error of PI infected by cell membrane damage caused by digestive fluid?

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

4. Why can only EDTA-free pancreatic enzyme be used to digest adherent cells? What is the effect of EDTA-containing pancreatic enzyme on the results?

Since Annexin V is a CA-dependent protein, Annexin V cannot be added to prevent Annexin V from chelating Ca ions and thus affecting the Annexin V results.

5. Annexin V and PI in some manufacturers' instructions are annexin V and PI. Why do you add Annexin V before PI?

When using flow cytometry to detect apoptosis, PI is greatly affected by time, because the labeling of PI will increase cytotoxicity, and the staining of PI will increase 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 cell population gap on flow cytometry. Generally, PI is added immediately after the machine, and then the detection is completed within one hour. Either way is fine, but the error caused by following our steps will be smaller.

Related literature:

[1] Chenguang Wang, Yukun Guan, Mengze lv, et al. Manganese Increases the Sensitivity of the cGAS-STING Pathway for Double-Stranded DNA and Is Required for the Host Defense against DNA Viruses. Immunity. April 2018. (IF 21.522)
[2] Wei Ling, Guoguang Li, Ya Li, et al. Materials and Techniques for Implantable Nutrient Sensing Using Flexible Sensors Integrated with Metal-Organic Frameworks. Advanced Materials. 2018. (IF 25.809)
[3] Hao Huang, Lizhen He, Wenhua Zhou, et al. Stable black phosphorus/Bi2O3 heterostructures for synergistic cancer radiotherapy. Biomaterials. July 2018. (IF 10.273)
[4] Chang Yu, Binbin Ding, Xinyang Zhang, et al. Targeted iron nanoparticles with platinum-(IV) prodrugs and anti-EZH2 siRNA show great synergy in combating drug resistance in vitro and in vivo. Biomaterials. February 2018. (IF 10.273)
[5] Lina Zhang, Hui Tian, XiuLi Zhou, et al. Upregulation of microRNA-351 exerts protective effects during sepsis by ameliorating skeletal muscle wasting through the Tead-4–mediated blockade of the Hippo signaling pathway. Faseb Journal. November 2018. (IF 5.391)

Note: Please refer to Solarbio website for more literature on the use of this product.