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Annexin V-FITC/PI Apoptosis Detection Kit

This product is for R&D use only, not for drug, household, or other uses.

Cat Number: CA1020

Size: 20T/50T /100T

Storage: 2-8°C for 1 year. Avoid lighting .

Kit component

	CA1020-20	CA1020-50	CA1020-100	Storage
Annexin V-FITC	100μl	250μl	500μl	4°C, avoid lighting
Propidium iodide(PI)	100μl	250μl	500μl	4°C, avoid lighting
Binding Buffer(10×)	6ml	15ml	30ml	4°C, -20°C for longer time

Product Description:

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.

Procedure:

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℃ pre-cooled PBS, resuspend the cells, centrifuge again to pellet the cells, carefully aspirate the supernatant;

2. Dilute the binding buffer 1:9 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/PE to mix well, and incubate for 5 minutes at room temperature in the dark;

5. Add 5μl PI and 400μl of PBS to perform flow-type or fluorescence-based microscopy detection detection immediately.

Experimental design:

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

Single staining tube: positive control cells, only with Annexin V/FITC for adjustment and compensation.

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

Interpretation

A. Flow cytometry analysis

FITC maximum excitation wavelength is 488nm, maximum emission wavelength is525nm, FITC's green fluorescence can be detected in FL1. PI-DNA maximum excitation wavelength is 535nm, maximum emission wavelength is 615nm, PI's red fluorescence can be detected in FL2 or FLI3. Analysis by flowjo,CellQuest, draw a double dispersion plot with FITC abscissa and PI ordinate. Typical of the real In the test, the cells can be divided into three subsets, the living cells only have a very low intensity of background fluorescence, and the early apoptotic cells only have a strong green screen Light, late apoptotic cells have double staining with green and red fluorescence.

B. Fluorescent microscopy for the analysis

1)A single drop of the cell suspension double-stained with Annexin V/FITC / PI was placed onto the slides, and the cells were covered with the coverslips.

Note: For adherent cells, cells can be directly cultured with cover slips and induce apoptosis.

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

FAQ

1. Whether the Annexin V/PI apoptosis detection kit can detect cell apoptosis in animals other than humans.

Yes, because 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. Adherent cells do apoptosis and trypsin digestion will damage the cell membrane?

Digest with low-concentration trypsin, gently pipette the adherent cells 2-3 times, centrifuge at 4°C 1000rpm for 5 minutes, if handled properly, the damage caused by trypsin can be controlled within 5%. If there is a control group, the experiment The result will not have a noticeable impact.

3. Why can we only digest cells with trypsin without EDTA, and what effect does the use of trypsin with EDTA have on the results?

Because Annexin V is a calcium-dependent protein, EDTA cannot be added to prevent EDTA from chelating calcium ions, thereby affecting Annexin V and affecting the results.

4. Can adherent cells be stained with PI first and then digested? Can this reduce the error of PI infection due to cell membrane damage caused by digestive juice?

Adding PI first is not only 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, so this is not recommended.

5. Some manufacturers add Annexin V and PI together? Why did you add Annexin V first and then PI?

When using flow cytometry to detect apoptosis, PI is greatly affected by time, because it will increase cytotoxicity after PI is labeled, and it will increase the staining of PI as time increases. Especially when detecting early apoptosis, if the time is prolonged, It will cause the gap in the cell grouping on the flow cytometer to increase, and the error will increase significantly. Generally, the 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 our operation steps will be smaller.

Related Documents:

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. (IF21.522)

[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. (IF10.273)

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