PRODUCT DATA SHEET

Annexin V-FITC/PI Apoptosis Detection Kit

Introduction:

Apoptosis is normal exists in the biological world, not only in physiological process, it also occurs in the pathologic state. Apoptosis is plays an important role in embryonic development and morphogenesis (morphogenesis), maintenence of tissue homeostasis, the body's defense and the stability of the immune response, disease or intoxication induced cell injury, aging, tumor development and progression.

In normal cells, phosphatidylserine (Phosphatidylserine, PS) is located in the inside of the cell membrane, but in the earliest of apoptosis, PS the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, exposed to the extracellular environment. Annexin V is a 35-36 kDa Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS.. Annexin V can be labeled to fluorochromes FITC, to mark the Annexin-V as fluorescent probe, apoptosis can be detect by using flow cytometry and fluorescence microscopy.

Propidium iodide (Propidine Iodide, PI) is a kind of nucleic acid dye, it cannot pass through the intact cell membrane, but in the latest apoptosis.cells and dead cells, PI was able to penetrated the cell membrane and stained the nucleus red. The Annexin-V and PI matching use, can distinguish the earliest or latest stage of apoptosis cells and dead cells.

Using the flow cytometry, fluorescence microscopy or other fluorescence detection device can easily and quickly detection of cell apoptosis by Annexin V-FITC/PI double staining cell apoptosis detection kit,

- Convenient: It can be detect by flow cytometry and fluorescence microscopy;
- Quick: the experiment can be finished in 1 hour;
- Accurate: accurately distinguish living cells, apoptotic cells and necrotic cells, and count the cells in each group.

The kit is suitable for detection of viable cells, not suitable for the tissue samples.

Contents:

Cat No.: BD0073-1(25T)	Cat No.: BD0073-2(50T)	Cat No.: BD0073-3(100T)
Annexin V-FITC,100ul	Annexin V-FITC,250ul	Annexin V-FITC,500ul
Propidium Iodide,200ul	Propidium Iodide,500ul	Propidium Iodide,1ml
10*Binding Buffer,2ml	10*Binding Buffer, 5ml	10*Binding Buffer,10ml

Attention:

1.Annexin V-FITC and propidium iodide should be avoid light storage and use.

2. Propidium iodide is toxic, should be carefully storage and use.

Procedure:

*Attention:

1) Due to the apoptosis is a rapid and dynamic process, so we recommend you shoule analysis just after staining.

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2) You should be put on rubber or disposable gloves while operation process, avoid contact with skin.

3) Cleaning and sterilization treatment should be carefully, the best way is in the 121.5 °C autoclave for 1 hours.

4) If you need fixed cells, please incubated with before fixed cells and use V-FITC liquid wash out unbound Annexin V. Because the fixed cell fragments can be combined with Annexin V, then interference the results.

5) If cell samples containing platelet, such as blood samples, please use the buffer which containing EDTA and 200g centrifugal to washed out the platelet. Because platelet containing PS, which combined Annexin V will interfere the experimental results.

6) Centrifuge the tube If the reagent is installed in rotary cap, make the liquid left to the bottom of pipe, to avoid liquid spilled when opening the cover.

7) It is needs careful operation for cell processing, avoid damage cells.

8) Annexin V-FITC and Propidium iodide are photosensitive material, please protected from prolonged exposure to light.

9) The successfully of the following factors is effect by several factors , such as cell type, cell membrane PS density, PS turn ratio, the method of inducing apoptosis, the reagents, induction time,etc. optimize these factors are improtent to the experimental success.

*Staining

1) 2000RPM, 5 min, collect the suspension cell, disposable the culture medium.

(The digestion time of adherent cells with EDTA free trypsin can not be too long, to prevent causing false positive)

2) With cold PBS washing the cells twice (2000RPM, 5min to collect the cells).

3) Using 10 times water dilution 10* Binding Buffer to 1* Binding Buffer, , 400ul 1* Binding Buffer suspened cells, the concentration was about $1*10^6$ cells/ml.

4) Add 5ul Annexin V-FITC to the cell suspension , mix gently, incubated at 2-8 °C for 15 minutes, protected from prolonged exposure to light.

5) Add 10ul PI, gently mixing, incubated at 2-8 °C for 5 minutes, protected from prolonged exposure to light.

6) Analyze by flow cytometry as soon as possible (within 1 hr).

Flow cytometry analysis

The samples can be analyzed in flow cytometry.

Flow cytometry excitation wavelength using Ex.= 488nm dual wavelength excitation, Em.= 530 nm emission detection of FITC fluorescence and >575 nm emission of PI. The green fluorescence of Annexin V-FITC through the FITC channel (FL1) detection; the red fluorescence of PI through the PI channel(FL2 or FL3) detection, recommend use FL3. Cells can be divided into three subgroups: the low fluorescence

intensity of living cells, strong green fluorescence of apoptosis cells, green and red fluorescent double staining of necrotic cells (including very late apoptotic cells).

It is requirement the instrument remove the emitting superposition between two dyes by Using flow cytometry to correct fluorescence compensation of Annexin V-FITC/PI double staining. Because the fluorescent voltage compensation settings directly associated with PMT, so there are different compensation between different instruments. Suggested first analysis the Annexin V, PI single stained cells to adjust compensation removal the fluorescence spectral overlap at the beginning of the experiment. Setting cross door according to the untreated cell control and the Annexin V, PI respectively after cell staining of single dye control .

1) Accession unstained cells, circle population door with the target cell in the linear FS-SS points.

2) Establish LogFL1-LogFL2 (preferably FL3) double parameter point diagram and analysis of the above light scattering diagram gating cells; ensure >98% cells in the lower left center of the X, Y axis and Log 1 quadrant region boundary.

3) Detect the Annexin V-FITC single stained cells and check the FL1-FL2 (or FL3) plot, guarantee in the upper left and upper right quadrant there is no particles. If the particles appear in the upper quadrant indicate fluorescence leakage; the fluorescence of FL1 was detected by FL2 (or FL3) PMT. In order to correct this phenomenon, increase of FL1 leakage into the fluorescence compensation of FL2 (or FL3) (which may be 1-5%). If the regulation is not effectively remove the positive signal of FL2, at this time should reduce the FL2 (or FL3) PMT voltage.

4) Detect the PI single stained cells and check the FL1-FL2 (or FL3) plot, guarantee in the upper right and lower right quadrant without particles. If the particles appear in the right quadrants indicate fluorescence leakage; the fluorescence of PI was detected by FL1 PMT. In order to correct this phenomenon, increased FL2 (or FL3) leakage into the fluorescence compensation of FL1 (which may be 15-25%). If the regulation of positive signal does not remove FL1 effectively, reduce the voltage at the FL1 PMT.

Note: If the adjustment of compensation process changes the PMT voltage in the above, suggestion repeat step3 and step4, to ensure there was no excessive fluorescence compensation. Excessive compensation phenomenon can be observed from the positive cells were very close to the standard. An appropriate compensation should be the fluorescence intensity in the lower left quadrant of Log 1 as the boundary of negative cell is consistent with the fluorescence intensity of single positive cells.

5) Set the location of cross gate, FL1 and FL2 (or FL3) following delimitation:

5.1) Set FL1 rod position: the group of cells in the left lower quadrant is Annexin V staining negative cells (the cells would rise to 2 log coordinates in the FL1 axis direction). The FL1 scale is vertically set in close proximity to the Annexin V negative group right 0.1-0.2Log local.

5.2) Set of FL2 (or FL3) rod position: double positive cells of certain data to distinguish between PI+ and PI-.Two populations cells may identify in this condition, one is in the lower right plot (ANN+/PI-), another is the upper right side (ANN+/PI+). The horizontal line can be placed in the middle of the two group of cells. If no PI+ cells in the analysis of the cell population, reference double negative cells group differentiating PI+ cells is the best, the horizontal line should be above the 0.1-0.3 Log unit in the double

negative cells.

Note: Cells in addition to negative group door can be identified as Annexin V or Annexin V and PI positive cells.

Fluorescence microscopy

1) Suspened cells after dyeing, add a drop of the cell suspension in glass slide, covered with cover glass cell, fluorescence microscopy.

2) Adherent cells can be stained as suspension cells, add a drop of the cell suspension in glass slide, covered with cover glass cell, fluorescence microscopy.

Adherent cells can be cultured on glass coverslips (according to the glass size, in 24-well or 12-well cell culture plates), and induce cell apoptosis. Staining the cells in cell culture plate. Use PBS rinse twice, then add 400 μL Binding Buffer in the well. Add 5 μ L Annexin V-FITC and 10 μ L Propidium Iodide, mixed, protected from prolonged exposure to light, incubation at room temperature for 10 min

3) Put the coverslips invert to the slide, observed under the fluorescence microscope . Detect the green fluorescence of V-FITC and Annexin PI red fluorescent signal.

Storage & Shelf life:

All components should be storage at 2-8 ℃ and keep in dark place, quality guarantee period is one year.

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