Cell Cycle and Apoptosis Analysis Kit

Introduction:

Cell Cycle and Apoptosis Analysis Kit is use the principle that intracellular DNA can be Combined with fluorescent dye. The binding of fluorescent dye is different ,becanse of in different period, the content of DNA is different. Fluorescence intensity of flow cytometry is not the same to detect the cell cycle.

The cytoplasm and chromatin condensation, nuclear fragmentation, produce apoptotic bodies, the light scattering properties of cells changed while cell apoptosis. In the early stage of apoptosis, the ability of the cells to forward angle light scattering is significantly reduced, the angle of 90 °capacity increase or no change in light scattering. In the cell apoptosis of late, forward angle and 90 °angle light scattering signals are reduced. Thus the changes in light scattering cells of the cell apoptosis was observed by flow cytometry. The cells were stained by PI, due to the total DNA decreased of apoptosis cells, the emergence of low DNA stained cells would detected before normal G0/G1 cells, G1 peak appears in front of hypodiploid peak (sub-G1) group is the apoptosis cells.

Contents:

Cat No.: BD0062-1(20T)	Cat No.: BD0062-2(50T)	Cat No.: BD0062-3(100T)
Rnase A,400ul	Rnase A,1ml	Rnase A,2ml
Propidium Iodide,8ml	Propidium Iodide,20ml	Propidium Iodide,40ml

Procedure:

1. Collect the cells, the numbers should be in $10*10^5$.

2. Use cold PBS washing the cells twice.

3. 75% ethanol frozen the samples at -20 ℃ for 1 hours.

4. Use cold PBS washing the cells once.

5. Use 200-500ul cold PBS suspension the cells.

6. Add the Rnase A 20ul solution for 30 minutes at 37 ℃ water.

7. 400 mesh filter.

8. Add 400ul PI dye solution, mix gently 4 ℃avoid from light incubation of 30 minutes -1 hours.

9. Detect with flow cytometry , The maximum excitation wavelength is 488nm.

Attention：

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

2) Fixed the sample in the oscillator, oscillation cells gently, and slowly add 75% ethanol. Added directly may leads to cell aggregation, it is difficult to resuspend into single cells. Or frist suspension the cells with cold PBS into single cells. Then slowly adding anhydrous alcohol, finally concentration of 70-75% ethanol. It must be free of precipitation after ethanol fixed cells.

3) In order to prevent poor reproducibility because cells in the different cycles of the different batches,the

cells can be synchronized before the experiment, reduce the difference. The cells can not be completely covered in the logarithmic growth phase, in general 50 ~ 80% was good.

4) When analysis it is need single cell, 400 mesh filter is used to stick the cell mass filter, or will appear otherwise the polyploid anthropogenic interference, but the experienced operators can also gate. If there is no filter conditions, please resuspend into single cells before staining cells

5) Methods of tissue processing: cut the tissue to pieces use scissors, digestion of 30min - 1H use 0.25% trypsin, 200 - 400 mesh filter cells, to obtain single cell suspension. We can join the collagenase as the organization difficult to digest.

Storage & Shelf life:

Store at -20℃ and keep in dark place, quality guarantee period is one year.

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