DNA Content (Cell Cycle) Assay kit

(Cat/No.:BC130 Size:100T,50T,20T)

1. Product Description

Cell Cycle is the process by which a cell undergoes continuous mitosis from the end of one mitosis to the end of the next. In this process, the genetic material of the cell replicates and doubles, and is evenly distributed between the two daughter cells at the end of division.Cell Cycle can be divided into interphase and Mitotic phase.Interphase is often divided into dormancy phase (G0) , prophase (G1) , DNA synthesis phase (S) and anaphase (G2) . The whole cycle can be expressed as G1→ S→G2→M.DNA cycle detection can be used to reflect the status of the various stages of the cell cycle, that is, cell proliferation. Because of the ability of intracellular DNA to bind to fluorescent dyes such as propidium iodide, the amount of DNA in a cell varies from time to time, and the intensity of fluorescence detected by Flow cytometry is different.

During apoptosis, the concentration of cytoplasm and Chromatin and karyolysis produce apoptotic bodies, which change the light scattering properties of cells.

At the early stage of apoptosis, the ability of forward angular light scattering was significantly decreased, while the ability of 900 angular light scattering was increased or not changed. At the late stage of apoptosis, the forward angle and 900 angle light scattering signal were decreased. So we can observe the apoptotic cells by measuring the change of light scattering by Flow cytometry.

When the cells were stained with PI, the apoptotic cells showed a low DNA staining cell group before the normal G0/G1 cell group, that is, sub-diploid peak (sub-G1) and apoptosis group before the G1 peak.

The Kit can be used to detect the DNA content (cell cycle) of cultured cells (suspension, adherence cells).

2. Composition

Component	20T	50T	100T	Storage
RNase A Solution	2.0ml	5.0 ml	10.0ml	-20℃
Propidium Iodide（PI）	8.0ml×1 bottle	20.0mL×1 bottle	20.0mL×2 bottles	4℃ and keep avoid light

3. Self-contained instruments and reagents

Low temperature high speed centrifuge, micropipette, 1.5ml centrifuge tube, Flow cytometry, PBS,-20℃ Anhydrous Ethanol

4. Storage

Keep at -20℃,Propidium Iodide（PI）need to be kept at 4℃ and avoid the light.

5. Points to attention

1、Propidium Iodide（PI）need to be kept at 4℃ and avoid the light.

2、For your safety and health, please wear lab coat and disposable gloves.

6. Operating procedure

1、Preparations

a、Place Anhydrous Ethanol in-20 °C refrigerator overnight

b、Dissolve RNase a solution, mix well and place on ice bath for use.

2、Preparation of Samples

a、For suspension cells

The cells were centrifuged (2000 rpm, 5 min) to collect 5×105 cells and carefully sucked out the supernatant, while ensuring that no cells were sucked out as much as possible. PBS was washed once.

Add 0.3ml PBS and resuspend the cells.Go down to step 3 for the experiment.

b、For adherent cells

Digest the adherent cells with trypsin and collect 5×105 cells. Rinse once with PBS, drain and Rinse. Add 0.3ml PBS and resuspend the cells.Go down to step 3 for the experiment.

3、The single cell suspension prepared in step 2 was slowly transferred to 1.2 ml -20 °C anhydrous ethanol, and place in the refrigerator at-20 °C for 1h or overnight.

4、2000 rpm, centrifuged for 5 min, drained the supernatant, add 1.0 ml PBS and re-suspended the cells, left at room temperature for 15 min.

5、2000 rpm, centrifuged for 5 min, drained the supernatant, add 100μl RNase A and re-suspended the cells,and then incubate 30min at 37℃ water bath.

6、Then add 400μl PI and mix well, incubate at 4°C for 30min.

7、Record the Red fluorescence at EX=488nm.