Cell cycle and apoptosis detection kit

Packing specification

Product number: IN0001

Specifications: 50T

Storage conditions

Store at -20°, valid for two years

product manual:

Cell Cycle and Apoptosis Analysis Kit (Cell Cycle and Apoptosis Analysis Kit) is a method that uses classic Propidium Iodide staining to analyze cell cycle and apoptosis.

Propidium Iodide (PI) is a fluorescent dye for double-stranded DNA. The combination of propidium iodide and double-stranded DNA can produce fluorescence, and the fluorescence intensity is proportional to the content of double-stranded DNA. After the DNA in the cell is stained with propidium iodide, the cell can be used to determine the DNA content of the cell with a flow cytometer. Then, according to the distribution of the DNA content, cell cycle and apoptosis analysis can be performed.

After propidium iodide staining, assuming that the fluorescence intensity of cells in G0/G1 phase is 1, then the theoretical value of fluorescence intensity of cells in G2/M phase containing double genomic DNA is 2, and the fluorescence of cells in S phase that is undergoing DNA replication The intensity is between 1-2. The apoptotic cells are lost during the staining process due to the condensation of the nucleus and DNA fragmentation. Therefore, the apoptotic cells exhibit weak staining after staining with propidium iodide, that is, the fluorescence intensity is less than 1. The so-called sub-G1 peak, that is, apoptotic cell peak, appears on the fluorescence image of flow cytometry.

When a cell undergoes apoptosis, apoptotic bodies are produced due to the condensation of cytoplasm and chromatin and nuclear fragmentation, which changes the light scattering properties of the cell. In the early stage of cell apoptosis, the ability of cells to scatter forward angle light is significantly reduced, and the ability to scatter side light is increased or unchanged. In the late stage of apoptosis, the signal of forward and side light scattering decreases. Therefore, the changes in light scattering of cells can be measured by flow cytometry to observe cell apoptosis.

This kit is usually applied to the cell cycle and apoptosis detection of cultured adherent or suspended cells. If it is used to detect the cell cycle and apoptosis of the tissue, the tissue must be digested into a single cell state before it can be detected.

This kit is enough to detect 50 samples, and the number of cells in each sample can be 100,000 to 1 million.

Kit components:

Product number	product name	50T	100T	save
CE00-1	Staining buffer	25ml	50ml	-20° C protected from light for 2 ye
CE00-2	Propidium iodide staining solut (20X)	1.25ml	2.5ml	-20° C protected from light for 2 ye
CE00-3	RNase A(50X)	0.5ml	1ml	-20° C protected from light for 2 ye

Precautions:

1) This kit requires flow cytometry for detection. You need to bring your own PBS and 70% ethanol.

2) Cell handling needs to be gentle, try to avoid artificial damage to cells.

3) In order to prevent different batches of cells from being in different cycles during the experiment resulting in poor repeatability, the cells can be synchronized before the experiment. The experimental cells should be in the logarithmic growth phase. Adherent cells are generally collected at 50-80% confluence.

4) The 400-mesh screen filter is used to filter out the stuck cell clusters, leaving single cells, otherwise artificial polyploidy interference will occur. If there is no condition to filter, please flick the cells to disperse before staining, and then stain.

5) Fluorescent dyes all have quenching problems. Please try to avoid light during storage and use to slow down fluorescence quenching.

6) Propidium iodide is irritating to the human body. When handling propidium iodide, take care to protect your eyes and avoid inhalation.

7) For your safety and health, please wear lab coats and disposable gloves for operation.

Instructions:

1. Preparation of cell samples: The number of cells is controlled at 1×105~1×106.

a) Adherent cells: Carefully aspirate the cell culture medium, digest the cells with trypsin to prepare a single cell suspension. Centrifuge at 1000 g for 5 min to pellet the cells, discard the supernatant, rinse the cells once with 1 mL of pre-cooled PBS, and collect the cells by centrifugation.

b) Suspended cells: Centrifuge at 1000 g for 5 min to pellet the cells and carefully aspirate the supernatant. Add 1 mL of pre-cooled PBS, resuspend the cells, and centrifuge again to collect the cells.

c) Tissue cells: After cutting the tissue block into small pieces as small as possible with scissors, digest it with 0.25% trypsin for 0.5-1 h, and filter through a 200-400 mesh screen to obtain a single cell suspension. Centrifuge at 1000 g for 5 min to pellet the cells. Add about 1 mL of pre-cooled PBS, resuspend the cells, and centrifuge again to pellet the cells. If the tissue is difficult to digest, add proper amount of collagenase.

2. Cell fixation:

The cell pellet was mixed gently with 1 mL of pre-cooled 70% ethanol, and fixed at 4°C for more than 2 hours or overnight. After centrifugation at 1000 g for 5 minutes to pellet the cells, carefully aspirate the supernatant. About 50 microliters of 70% ethanol can remain to avoid aspirating the cells.

Add 1 mL of pre-cooled PBS to resuspend. Then centrifuge again at 1000g for 5 min to pellet the cells. Carefully aspirate the supernatant, about 50 microliters of PBS can remain to avoid aspiration of cells. Gently flick the bottom of the centrifuge tube to properly disperse the cells to avoid cell clumping.

3. Preparation of propidium iodide staining solution:

For 1 sample, add 25uL propidium iodide stock solution and 10uL RNase A solution to 0.5 mL staining buffer, mix

well and set aside. For other numbers of samples, refer to the following table, and prepare an appropriate amount of propidium iodide staining solution according to the number of samples to be tested:

name	1 sample	6 sample	12sample
Staining buffer	0.5mL	3mL	6mL
Propidium iodide staining solution (20X)	25uL	150uL	300uL
RNase A(50X)	10uL	60uL	120uL
Final volume	0.535mL	3.21mL	6.42mL

Note: The prepared propidium iodide staining solution can be stored at 4°C for a short time and should be used on the same day.

4. Dyeing:

Add 0.5 ml of propidium iodide staining solution to each tube of cell samples, mix gently to resuspend the cell pellet, and incubate at 37°C for 30 minutes in the dark, then flow cytometry can be performed. Flow cytometry is best completed within 5 hours.

5. Streaming detection and analysis:

A flow cytometer was used to detect the red fluorescence at the excitation wavelength of 488nm, while detecting the light scattering. Use appropriate analysis software for cell DNA content analysis and light scattering analysis.