Cell Cycle and Apoptosis Analysis Kit

Cat #：orb1566777 (manuals)

Size：50T

Product composition

Reagents	Specifications
Propidium Iodide (PI) Staining Solution (20×)	1.25 ml
RNase A (50×)	0.5 ml
Staining Buffer	25 ml
Manual	1 copy

Product introduction

The Cell Cycle and Apoptosis Analysis Kit is a classical propidium iodide (PI) staining method, which is used to detect cell cycle and apoptosis by flow cytometry analysis.

The cell cycle is the whole process from the completion of one division to the end of the next division, in which the cytogenetic material is replicated and doubled and distributed equally to both daughter cells at the end of division. The cell cycle is divided into two stages: Interval (G0), DNA synthesis phase (G1), DNA synthesis phase (S) and DNA synthesis phase (G2). According to the binding characteristics of intracellular DNA and fluorescent dyes (such as propidium iodide), the DNA content of the cells at different stages is different and the fluorescence intensity detected is also different, which reflects the status of each stage of the cell cycle, that is, the status of cell proliferation.

Propidium Iodide is a fluorescent dye that can be chimeric and bound to base pairs of double-stranded DNAs and RNAs, with no base specificity. When PI binds to double-stranded DNA, fluorescence is produced, and the fluorescence intensity is proportional to the content of double-stranded DNA. DNA in cells stained with PI can be assayed by flow cytometry for cell cycle and apoptosis. Assuming that the fluorescence intensity of the G0/G1-phase cells is one, the theoretical value of the fluorescence intensity of the G2/M-phase cells containing two copies of genomic DNAs is two, and the fluorescence intensity of the S-phase cells undergoing replication of DNAs is between 1-2.

Due to the concentration of cell nuclei and the DNA fragmentation of apoptotic cells, some genomic DNA fragments were lost during staining, so apoptotic cells showed obvious weak staining after propidium iodide staining, that is, the fluorescence intensity was less than one, and the so-called sub-G1 peak, that is apoptotic cell peak, appeared on the fluorescence map of flow detection. PI staining also differentiates between apoptotic and necrotic cell peak patterns. When apoptosis occurs, apoptotic cells shrink, chromatin concentrate, nuclear fragmentation occur, and apoptotic bodies are produced, so that the forward light scattering (FSC) of cells is lower than normal. At the early stage of apoptosis, the ability of cells to forward angular light scattering (FSC) was significantly reduced, and the ability to lateral angular light scattering (SSC) was increased or unchanged. In the late phase of apoptosis, both FSC and SSC signals decreased. Cells tend to be swollen when necrotic, so FSC is higher than normal and SSC is higher than normal.

This kit is suitable for the detection of cell cycle and apoptosis of cultured adherent or suspended cells, and can also be used to distinguish between apoptosis and cell necrosis. When used to detect cell cycle and apoptosis in tissues, tissues must be digested into a single-cell state. The kit typically ranges from 10^5 to 10^6 cells per sample, which is sufficient for 50 samples.

Operating Steps

Cell Collection:

1. a. For adherent cells: Collect the cell culture medium into the centrifuge tube for use. Cells were routinely digested with trypsin, the digestion was terminated with the previously collected cell culture medium, and cells were gently blown away and collected into centrifuge tubes. (Note: Blow gently after digestion to avoid cell breakage.)

b. For suspended cells: Carefully pipette all media containing cells into the centrifuge tube.

2. Centrifuge about 1000g of the above collected cells for 3-5min to pellet the cells. (If the cell pellet is not sufficient, you can appropriately extend the centrifugation time or increase the centrifugal force slightly) Carefully aspirate and discard the supernatant, leaving about 50μl to avoid sucking away the cells.

3. Add 1 ml of pre-chilled PBS, resuspend the cells, and transfer to a 1.5 ml centrifuge tube. Centrifuge at about 1000g again for 3-5min, pellet the cells, carefully aspirate the supernatant, and leave about 50μl to avoid aspirating the cells. Gently flick the bottom of the centrifuge tube to properly disperse the cells and avoid clumping of cells.

Cell fixation:

1. Add 1ml of PBS to resuspend well to be single cells, gently vortex and slowly add 3ml of pre-chilled absolute ethanol dropwise to a final concentration of 75%, fix at 4°C for more than 4h, or let stand at 4°C overnight (18-24h) for better results. (Fixed-cell cells can be stored at -20°C for one month)

2. Centrifuge about 1000g of the fixed cell suspension for 3-5min to pellet the cells. (If the cell pellet is not sufficient, you can appropriately extend the centrifugation time or increase the centrifugal force slightly) Carefully aspirate and discard the supernatant, leaving about 50μl to avoid sucking away the cells.

3. Add 1ml of pre-chilled PBS, resuspend the cells, centrifuge the cells again at about 1000g for 3-5min, carefully aspirate the supernatant, and leave about 50μl to avoid aspirating the cells. Gently flick the bottom of the centrifuge tube to properly disperse the cells and avoid clumping of cells.

Preparation of dyeing working solution:

According to the ratio of Staining Buffer: Propidium Iodide (PI) Staining Solution (20X): RNase A (50X) = 100:5:2, please refer to the following table to select the appropriate working solution volume according to the number of samples:

	1 sample	n samples
Staining Buffer	500 μl	500×n μl
Propidium Iodide (PI) Staining Solution (20X)	25 μl	25×n μl
RNase A(50X)	10 μl	10×n μl
Total Volume	535 μl	535×n μl

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

Staining:

Add 500μl of staining solution to each tube of cell sample, gently and thoroughly resuspend the cell pellet, and take a 37 °C dark bath 30min。 After the staining is completed, it should be stored at 4°C in the dark, and the flow cytometry detection should be completed within 24 hours.

Flow cytometry detection and analysis:

The flow cytometry was used to detect the red fluorescence at the excitation wavelength of 488 nm, and the light scattering was detected at the same time. Cell DNA content analysis and light scattering analysis were performed using appropriate analysis software.

Save the condition

Store at -20°C for one year. Propidium iodide (PI) staining solution (20X) should be stored in the dark. The kit can be stored at 4°C and is valid for one month.

Precautions

1. Please use a flow cytometer for detection, and adjust the injection speed to low speed, if the peak shape is wide, it may be because the injection speed is too fast.

2. Follow the cell fixation method provided in the instructions to obtain as many cells as possible and avoid DNA degradation.

3. Minimize the generation of debris in the cell sample, please be careful not to over-digest, not over-pipette, and not too high centrifugation speed.

4. You need to bring your own PBS and absolute ethanol.

5. There is a quenching problem in fluorescent dyes, so please pay attention to the light during the storage and use of propidium iodide PI staining solution to slow down the fluorescence quenching. It is recommended to complete the test on the same day as possible after staining.

6. Propidium iodide is irritating to the human body, please be careful when handling it, and pay attention to appropriate protection to avoid direct contact with the human body or inhalation.

7. For your safety and health, please wear a lab coat and disposable gloves when operating.