Propidium Iodide Staining Solution (50 μg/ml)

Cat #: A-CSH392

Size: 10mL

Storage: -20°C, avoid light (6 months)

Product Description

Propidium Iodide (PI) staining is used for the analysis of cell cycle and cell apoptosis. Propidium Iodide (PI) is a fluorescent dye that can intercalate into the base pairs of double-stranded DNA and RNA, without base specificity. After binding to double-stranded DNA, Propidium Iodide generates fluorescence, and the fluorescence intensity is proportional to the amount of double-stranded DNA. By staining the cellular DNA with Propidium Iodide, the DNA content of the cells can be measured using flow cytometry. Based on the distribution of DNA content, analysis of cell cycle and cell apoptosis can be performed. After Propidium Iodide staining, assuming the fluorescence intensity of G0/G1 phase cells is 1, the theoretical fluorescence intensity of G2/M phase cells with twice the genomic DNA content is 2, and the fluorescence intensity of S phase cells undergoing DNA replication is between 1 and 2. Due to nuclear condensation and DNA fragmentation, apoptotic cells lose some genomic DNA fragments during the staining process. Therefore, apoptotic cells exhibit a significantly weaker staining, with fluorescence intensity less than 1, and appear as a distinct sub-G1 peak on the fluorescence histogram obtained from flow cytometry, known as the apoptotic peak.

During cell apoptosis, flow cytometry can reveal characteristics of a sub-diploid nuclear pattern. Based on the characteristics of light scattering, PI staining can distinguish between cell apoptosis and cell necrosis. During cell apoptosis, there is cell shrinkage, chromatin condensation, nuclear fragmentation, and the formation of apoptotic bodies, leading to a decrease in forward light scattering compared to normal cells. In the early stages of cell apoptosis, cells exhibit a significant decrease in forward angle light scattering, an increase or no change in side scatter. In the late stages of cell apoptosis, both forward and side light scattering signals decrease. In contrast, cell necrosis is characterized by cell swelling, resulting in higher forward light scattering and higher side light scattering than normal cells.

Propidium Iodide Staining Solution (50 μg/ml) is primarily composed of PI, membrane permeabilizers, and other

components. It is commonly used for cell cycle and cell apoptosis analysis in adherent or suspension cells in culture. It is also useful for distinguishing between cell apoptosis and cell necrosis. The working concentration of PI staining solution is typically between 20-50μg/ml. It does not contain RNase and is recommended for RNA staining. The range of cell detection is generally between $0.1\sim1\times10^6$.

Usage

Preparation of Cell Samples:

Adherent Cells:

1. Carefully collect the cell culture medium into a sterile centrifuge tube and set it aside.
2. Digest the cells with trypsin until they can be gently dislodged by pipetting or tapping the side of the flask. Add the previously collected cell culture medium to dislodge all the adherent cells and gently disperse the cells.
3. Collect the cell suspension obtained in the previous step into a centrifuge tube.
4. Centrifuge at 4 °C, 1000g for 3-5 minutes to pellet the cells at the bottom of the tube. Carefully aspirate and discard the supernatant, leaving approximately 50 μl of the culture medium to avoid aspirating the cells.
5. Add approximately 1ml of pre-chilled PBS, resuspend the cells, and transfer them to a sterile 1.5ml centrifuge tube. Centrifuge at 4 °C, 1000g for 3-5 minutes to pellet the cells at the bottom of the tube.
6. Carefully aspirate and discard the supernatant, leaving approximately 50 μl of PBS to avoid aspirating the cells. Gently tap the bottom of the centrifuge tube to disperse the cells evenly and prevent cell clumping.

Suspension Cells:

1. Centrifuge at 4 °C, 1000g for 3-5 minutes to pellet the cells at the bottom of the tube.
2. Carefully aspirate and discard the supernatant, leaving approximately 50 μl of the culture medium to avoid aspirating the cells.
3. Add approximately 1ml of pre-chilled PBS, resuspend the cells, and transfer them to a sterile 1.5ml centrifuge tube. Centrifuge at 4 °C, 1000g for 3-5 minutes to pellet the cells at the bottom of the tube.
4. Carefully aspirate and discard the supernatant, leaving approximately 50 μl of PBS to avoid aspirating the cells. Gently tap the bottom of the centrifuge tube to disperse the cells evenly and prevent cell clumping.

Cell Fixation:

Add 1ml of ice-cold 70% ethanol, gently mix by pipetting, and fix at 4 °C for 2 hours or longer. Fixing at 4 °C for 12-24 hours may yield better results.

Cell Washing:

1. Centrifuge at 4 °C, 1000g for 3-5 minutes to pellet the cells at the bottom of the tube.
2. Carefully aspirate and discard the supernatant, leaving approximately 50 µl of solution to avoid aspirating the cells.
3. Add approximately 1ml of pre-chilled PBS, resuspend the cells, and transfer them to a sterile 1.5ml centrifuge tube.
4. Centrifuge at 4 °C, 1000g for 3-5 minutes to pellet the cells at the bottom of the tube.
5. Carefully aspirate and discard the supernatant, leaving approximately 50 µl of PBS to avoid aspirating the cells.
6. Gently tap the bottom of the centrifuge tube to disperse the cells evenly and prevent cell clumping.

PI Staining:

Add 500 µl of the prepared PI staining working solution to each cell sample, gently resuspend the cell pellet, and incubate in a light-protected water bath at 37 °C for 30 minutes. Staining should be performed or analyzed by flow cytometry within 24 hours.

Detection and Analysis:

Use a flow cytometer to detect red fluorescence at an excitation wavelength of 488nm and simultaneously measure light scattering. Employ appropriate analysis software for cell DNA or RNA content analysis and light scattering analysis.

Staining Results

The peak type of subdiploid cell population appeared on the left side of the peak of apoptotic cell G1, and the forward light scattering was lower than normal, and the lateral light scattering was higher than normal.

Notes

1. Fluorescent dyes are prone to fading, so it is recommended to perform the detection as soon as possible after staining.

2. To minimize fluorescence fading, the use of anti-fade mounting medium is advised.

3. During the centrifugation process to pellet the cells, for certain cell types, if the cell pellet is not sufficient, you may increase the centrifugation force or extend the centrifugation time accordingly.

4. If conducting cell cycle and apoptosis analysis for tissues, it is necessary to digest the tissue and prepare a single-cell suspension before the detection can be performed.

5. During cell apoptosis, one of the indicators is a decrease in DNA stainability. However, this is not an absolute rule, as a decrease in DNA content or a decrease in the binding capacity between DNA and the dye can also lead to reduced DNA stainability. Therefore, special attention should be paid during the analysis.

6. PI has a certain irritant effect on the human body, so please take appropriate precautions.

7. For your safety and health, please wear laboratory attire and disposable gloves while performing the procedure.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.