

Cell Cycle and Apoptosis Analysis Kit

P1373478

Storage -20°C. Protect from light. Do not freeze

Introduction

The Cell Cycle and Apoptosis Kit (Cell Cycle and Apoptosis Kit) is a test kit that uses the classic propidium iodide (PI) staining method to analyze the cell cycle and apoptosis. Propidium iodide is a fluorescent dye for double-stranded DNA. When propidium iodide binds to double-stranded DNA, it can produce fluorescence, and the fluorescence intensity is proportional to the content of double-stranded DNA. After the DNA in the cells is stained with propidium iodide, the DNA content of the cells can be measured using a flow cytometer, and then based on the distribution of DNA content, the cell cycle and apoptosis can be analyzed. After staining with propidium iodide, if the fluorescence intensity of G0/G1 phase cells is 1, then the theoretical fluorescence intensity of G2/M phase cells containing double genomic DNA is 2, and the fluorescence intensity of S phase cells undergoing DNA replication is between 1 and 2. Apoptotic cells, due to the condensation of the cell nucleus and the fragmentation of DNA (DNA fragmentation), cause some genomic DNA fragments to be lost during staining, so the iodine staining of apoptotic cells presents a significantly weak color, that is, the fluorescence intensity is less than 1, and a so-called sub-G1 peak appears on the fluorescence graph of flow cytometry, which is the peak of apoptotic cells. When cells undergo apoptosis, due to the concentration of cytoplasm and chromatin, apoptotic bodies are produced, causing changes in the light scattering properties of the cells. In the early stage of cell apoptosis, the ability of the cell to forward-angle light scattering significantly decreases, while the ability of lateral light scattering increases or remains unchanged. In the late stage of cell apoptosis, the signals of forward and lateral light scattering both decrease, so the changes in cell light scattering can be measured using a flow cytometer to observe the apoptosis situation.

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

Product components

P1373478	Component	20 T	50 T	100 T	Storage	Quantity Per Test
P1373478A	Dyeing buffer solution	10 mL	25 mL	50 mL	-20°C.	500 µL per 1×10^6 cells.
P1373478B	Propidium Iodide staining solution (20X)	100 µL	250 µL	500 µL	-20°C. Store in the dark.	5 µL per 1×10^6 cells.
P1373478C	RNase A (50X)	200 µL	500 µL	1 mL	-20°C.	10 µL per 1×10^6 cells.

Note: The recommended number of cells to stain per test is 1×10^6 cells.

Instructions for use

1. Collect cells and wash twice with ice-cold PBS; resuspend in ice-cold PBS at a density of $0.1\text{--}1.0 \times 10^7$ cells/mL.
2. In the cell suspension, anhydrous ethanol was added dropwise while shaking until the final ethanol concentration reached 70%.
3. Mix gently and fix overnight at 4 °C.
4. Wash cells twice with ice-cold PBS, then resuspend in dyeing buffer solution of 2×10^6 cells/mL.
5. Add 5 µL of Propidium iodide and 10 µL RNase A to 500 µL of the cell suspension.
6. Resuspend cells gently and thoroughly, then incubate at room temperature for 30 min in the dark.
7. Analyze samples by flow cytometry.

Note: Please bring your own PBS and absolute ethanol.

Precautions

1. Fluorescent dyes all have the problem of quenching. Please try to avoid light during storage and use to slow down fluorescence quenching;
2. Propidium iodide is irritating to the human body. Please take appropriate protective measures;
3. Propidium iodide is a known mutagen, so this solution needs to be treated with activated carbon before being discarded;
4. When fixing cells with 70% ethanol, make sure it is thorough; otherwise, uneven staining will result in unclear or inaccurate results. It is recommended to fix the cells overnight at 4°C with 70% ethanol;



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5. After cell fixation, ensure that the cells are in a single-cell suspension; cell adhesion may affect our results;
 6. For your safety and health, please wear laboratory coats and disposable gloves during operation;
 7. This product is only for scientific research and cannot be used for clinical diagnosis or treatment, nor for food or medicine. It must not be stored in ordinary residences.

