Hoechst 33342/PI Apoptosis Detection Kit

H1373483

Storage -20℃. Store in the dark. Avoid repeated freezing and thawing.

Introduction

The Hoechst 33342/PI Double Staining Apoptosis Detection Kit provides a classic, rapid, and straightforward method for distinguishing between apoptotic and necrotic cells. This kit utilizes a dual-staining approach with Hoechst 33342 and propidium iodide (PI). The nuclear dye Hoechst 33342 permeates cell membranes and emits blue fluorescence upon binding to double-stranded DNA. In normal cells, limited dye entry results in weak blue fluorescence. In apoptotic cells, however, increased membrane permeability allows significantly more Hoechst 33342 to enter, leading to higher fluorescence intensity. Additionally, chromatin condensation during apoptosis enhances the efficiency and concentration of DNA binding by the dye. Impaired function of the P-glycoprotein pump on the membrane of apoptotic cells further prevents effective efflux of Hoechst 33342, resulting in its intracellular accumulation. These characteristics collectively contribute to a markedly stronger fluorescence in apoptotic cells compared to normal cells. In contrast, propidium iodide (PI), another nuclear dye, cannot penetrate the intact membranes of normal or apoptotic cells, making viable cells impermeable to PI staining. Necrotic cells, which lose membrane integrity at an early stage, are readily stained by PI.

When dual-stained with these dyes, normal cells exhibit weak blue fluorescence primarily from Hoechst 33342. Apoptotic cells display intense blue fluorescence due to enhanced Hoechst 33342 staining, while necrotic cells show strong red fluorescence from PI.

Component

H1373483	Component	100T	500T	Storage	Quantity Per Test
H1373483A	Hoechst 33342 Staining Solution	1 mL	5 mL	-20℃. Store in the dark.	10 μL per 0.5-1.0x10^6
H1373483B	Propidium iodide Staining Solution (PI)	1 mL	5 mL	-20℃. Store in the dark.	10 μL per 0.5-1.0x10^6

Note: The recommended number of cells to stain per test 0.5-1.0x10^6 cells.

Usage method

1. Cell Preparation

Adherent cells: Grow cells in 6-well plates to the logarithmic growth phase. Wash twice with PBS and add 1 mL of PBS.

Suspension cells: Wash cells twice with pre-cooled PBS and resuspend in PBS at a density of 0.5–1.0×10^6 cells/mL.

2. Staining

Adherent cells: Add 10 μL of Hoechst 33342 and 10 μL of PI directly to each well of the 6-well plate. Incubate at 4 °C in the dark for 20–30 minutes.

Suspension cells: Take 1 mL of the cell suspension, add 10 μL of Hoechst 33342 and 10 μL of PI, and incubate at 4 °C in the dark for 20–30 minutes.

Note: After staining, proceed with fluorescence detection as soon as possible. The number of cells for each detection should not exceed 1×10^6.

3. Fluorescence Microscopy Detection and Analysis

Adherent cells: Remove the staining solution, wash twice with PBS, add an appropriate amount of PBS, and observe under a fluorescence microscope.

Suspension cells: Wash twice with PBS, resuspend the cells in PBS, transfer to a culture dish, and observe for red and blue fluorescence.

Matters needing attention

1. After the staining process is completed, the detection should be carried out as soon as possible.
2. Hoechst 33342 and PI are harmful to the human body. Please take protective measures when using them.
3. For your safety and health, please wear a laboratory coat and put on disposable gloves when operating.
4. This product is exclusively for scientific research purposes and must not be used for clinical diagnosis or treatment.