Hoechst 33258/PI Apoptosis Detection Kit

H1492197

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

Introduction:

The Hoechst33258/PI Apoptosis Assay Kit is a detection kit that uses a dual fluorescence staining method with Hoechst 33258 and Propidium Iodide (PI) to analyze cell cycle and cell necrosis. While PI staining alone can observe the sub-G1 peak of apoptotic cells on the DNA histogram, it can only represent apoptosis in the G0/G1 phase and cannot observe apoptosis in the S and G2 phases. Moreover, after fixation, it is impossible to distinguish between live and dead cells. Hoechst 33258 can penetrate the cell membrane and bind to DNA in both normal and apoptotic cells, showing blue fluorescence under ultraviolet light. After staining, the fluorescence of apoptotic cells is significantly enhanced compared to normal cells. PI cannot penetrate the cell membrane and cannot stain normal or apoptotic cells with intact cell membranes. However, for necrotic cells, where the integrity of the cell membrane is lost, PI can penetrate the cell membrane and color the necrotic cells to produce red fluorescence. When these two dyes are used for double staining, normal cells appear weak blue, apoptotic cells appear bright blue, and necrotic cells appear bright blue and red.

Component

H1492197	Component	100T	Storage	Quantity Per Test
H1492197A	Hoechst 33258 Staining Solution	1 mL	-20℃, Store in the dark.	10 μL per 0.5-1.0x10^6
H1492197B	Propidium iodide Staining Solution (PI)	0.5 mL	-20℃, Store in the dark.	5 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 33258 and 5 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 33258 and 5 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 33258 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.