Hoechst 33342/PI Double Staining Kit

Introduction

Hoechst 33342/PI Double Staining Kit is a kit that can rapidly detect cell apoptosis and necrosis. This kit provides two dyes, Hoechst 33342 and propidium iodide (PI). Hoechst 33342 can penetrate cell membranes, staining normal cells with a weak blue color. Chromatin condensation is a hallmark of apoptosis. Hoechst 33342 can stain condensed chromatin of apoptotic cells more brightly than normal cells. PI cannot penetrate the cell membrane and cannot stain normal cells or apoptotic cells red; for necrotic cells (whose membrane loses integrity), PI can stain them red. When using these two dyes for double staining, normal cells are weak blue + weak red fluorescence, apoptotic cells are strong blue + weak red fluorescence, and necrotic cells are strong blue + strong red fluorescence.

Components and Storage

Components	K2237-100 T	K2237-500 T
Hoechst 33342 staining solution	250 μL	1.25 mL
PI staining solution	250 μL	1.25 mL
Staining buffer	100 mL	5 x 100 mL

Store the kit at -20°C, stable for 1 year. Hoechst 33342 staining solution and PI staining solution should be stored away from light.

Protocol

1. **Sample preparation:** Collect 1 x 10^5-1 x 10^6 cells per sample and centrifuge at 500 g for 2 min. Discard the supernatant and resuspend cells with 1 mL of staining buffer to a density of 0.1-1 x 10^6 cells/mL.

***Note:** Adherent cells do not need digestion and harvest if detected in situ. For 6-well plates, discard the culture medium and wash twice with PBS, add 1 mL of staining buffer per well and follow the steps below for staining and observation.

2. **Staining:** Add 2.5 μL of Hoechst 33342 staining solution and 2.5 μL of PI staining solution to each 1 mL of cell suspension, mix gently and incubate in an ice bath protected from light for 20-30 min。

*Note: To obtain ideal results, mix every 5 minutes during incubation.

3. **Assay:** After incubation, wash cells with PBS two times. Resuspend the cells with an appropriate amount of PBS. Then detect the fluorescence signal by fluorescence microscopy or flow cytometry. Hoechst 33342 (Ex/Em: 352/461 nm, when binding to DNA); PI (Ex/Em: 535/617 nm, when binding to DNA). If observed using fluorescence microscopy, DAPI and Cy3 filters can be used respectively.

*Note: Detection should be performed as soon as possible after staining.

4. **Results analysis:**

   Normal cells: weak blue + weak red fluorescence;

   Apoptotic cells: strong blue + weak red fluorescence;

   Necrotic cells: strong blue + strong red fluorescence.

Note

1. Detection should be performed as soon as possible after staining.
2. Hoechst 33342 and PI are harmful to the human body, please pay attention to protection when using.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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