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Apoptosis and Necrosis Assay Kit

1 Components

Components	HY-K1070-100T
Cell Stain Buffer	100 mL
Hoechst 33342 Stain	0.5 mL
PI Stain	0.5 mL

2 Introduction

MCE Apoptosis and Necrosis Assay Kit provides a rapid and convenient method to detect cell apoptosis and necrosis. This kit contains two ready-to-use dyes bound to DNA. Hoechst 33342, a type of blue fluorescence dye (excitation/emission maxima ≈ 352/461 nm when bound to DNA), stains the condensed chromatin in apoptotic cells more brightly than the chromatin in normal cells. Propidium Iodide (PI) is a cell-membrane impermeable dye with characteristic excitation maximum at 535 nm and emission maximum at 617 nm which intercalates with nucleic acids. The simultaneous use of these two dyes makes it possible to distinguish normal, apoptotic, and dead cell populations by flow cytometer or fluorescence microscope. Normal cells show weak blue fluorescence + weak red fluorescence, apoptotic cells show strong blue fluorescence + weak red fluorescence, necrotic cells show strong blue fluorescence + strong red fluorescence.

This kit takes only 20-30 minutes to complete one-step staining and there is no need for dilution or preparation of other solutions when detected by flow cytometer. Each kit contains enough reagents for 100 samples.

3 General Protocol

1. Collect cells

For suspension cells: Add 10^5-10^6 cells in a 1.5 mL centrifuge tube. Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Add 1 mL of pre-cooled PBS to resuspend the cells, centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant.

For adherent cells: Discard the cell culture medium, and add trypsin to dissociate cells to make a single-cell suspension. Centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant. Add 1 mL of pre-cooled PBS to resuspend the cells, centrifuge at 1000 g at 4°C for 3-5 minutes and then discard the supernatant.

2. Double Staining

2.1 Resuspend the cell pellet with 0.8-1 mL of Cell Stain Buffer.

2.2 Add 5 μL of Hoechst 33342 Stain.

2.3 Add 5 μL of PI Stain.

2.4 Mix thoroughly and incubate the cells on ice or at 4°C for 20-30 minutes.

3. Detection

3.1 Detect the fluorescence by flow cytometer directly.

3.2 Detect the fluorescence by fluorescence microscope: centrifuge at 1000 g at 4°C for 3-5 minutes, discard the supernatant, wash with PBS and then detect the fluorescence by fluorescence microscope.

Note: When combined with DNA, the maximum excitation wavelength and emission wavelength of Hoechst 33342 were 352 nm and 461 nm respectively, the maximum excitation wavelength and emission wavelength of PI were 535 nm and 617 nm respectively.

4 Storage

4°C, 6 months; -20°C, 1 year

Protect from light

5 Precautions

1. Detect the fluorescence as soon as possible to avoid fluorescence quenching.
2. Hoechst 33342 and PI are sensitive to light, please operate away from light.
3. Hoechst 33342 and PI are both harmful to human, take care when handling.
4. This product is for R&D use only, not for drug, household, or other uses.
5. For your safety and health, please wear a lab coat and disposable gloves to operate.