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Annexin V-FITC/PI Apoptosis Detection Kit

1 Components

Components	HY-K1073-20T	HY-K1073-50T
Annexin V-FITC	100 μL	250 μL
Binding Buffer	12 mL	30 mL
PI Stain	220 μL	550 μL

2 Introduction

MCE Annexin V-FITC/PI Apoptosis Detection Kit provides a rapid and convenient method to detect cell apoptosis and necrosis. In normal live cells, Phosphatidylserine (PS) is located on the cytoplasmic surface of the cell membrane. Upon initiation of apoptosis, PS translocates from the inner to the outer leaflet of the membrane. Annexin V is a 35-36 kDa Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for PS. Annexin V labeled with FITC can identify apoptotic cells by binding to PS exposed on the outer leaflet. Propidium Iodide (PI) is a cell-membrane impermeable dye to live cells and early apoptosis cells, but stains late apoptosis cells and necrosis cells with red fluorescence. After staining cells with Annexin V-FITC and PI, live cells show little or no fluorescence (Annexin V-/PI-), early apoptosis cells show green fluorescence (Annexin V+/PI-), late apoptosis cells and necrosis cells show red and green fluorescence (Annexin V+/PI+).

3 General Protocol

Incubation of cells with Annexin V-FITC and PI

1. Collect 1-5 × 10^5 cells.

For suspension cells: Centrifuge at 1000 g for 5 minutes and then discard the supernatant. Add 1 mL of pre-cooled PBS to resuspend the cells, centrifuge at 1000 g for 5 minutes and then discard the supernatant.

For adherent cells: Collect the cell culture medium. Wash cells with PBS and add trypsin to dissociate cells. Add the medium and gently suspend the cells to make a single-cell suspension. Centrifuge at 1000 g for 5 minutes and then discard the supernatant. Add 1 mL of pre-cooled PBS to resuspend the cells, centrifuge at 1000 g for 5 minutes and then discard the supernatant.

Note: It is recommended to use trypsin containing no EDTA.

2. Resuspend the cells in 195 μL of Binding Buffer.
3. Add 5 μL of Annexin V-FITC.
4. Add 10 μL of PI Stain.
5. Incubate the cells at room temperature for 10-20 minutes in the dark.

Detection by flow cytometer

Analyze Annexin-FITC binding by flow cytometer (Ex = 488 nm; Em = 525 nm) using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2 or FL3).

Note: It is recommended to perform three controls: a: cells with no Annexin-FITC or PI; b: cells with only Annexin-FITC; c: cells with only PI.

Detection by fluorescence microscope

Detect the fluorescence by fluorescence microscope: Centrifuge at 1000 g for 5 minutes, discard the supernatant, resuspend the cells with 50-100 μL of Binding Buffer and then detect the fluorescence by fluorescence microscope.

4 Storage

-20°C, 1 year

Protect from light

Avoid repetitive freeze-thaw cycles

5 Precautions

1. Detect the fluorescence as soon as possible to avoid fluorescence quenching.
2. Annexin-FITC and PI are sensitive to light, please operate away from light.
3. Annexin-FITC and PI are both harmful, 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.