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Annexin V-iFluor 488/PI Apoptosis Detection Kit

1 Components

Component	HY-K1080-25T	HY-K1080-50T	HY-K1080-100T
Annexin V-iFluor 488	125 μL	250 μL	500 μL
10× Binding Buffer	2 mL	2 × 2 mL	3 × 2 mL
PI Solution	125 μL	250 μL	500 μL

2 Introduction

MCE Annexin V-iFluor 488/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 iFluor 488 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-iFluor 488 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-iFluor 488 and PI

1. Collect 1-5 × 10^5 cells

For suspension cells: Centrifuge at 2000 rpm for 5 minutes and then discard the supernatant. Add 1 mL of pre-cooled PBS to resuspend the cells, centrifuge at 2000 rpm for 5 minutes for two times 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 2000 rpm for 5 minutes and then discard the supernatant. Add 1 mL of pre-cooled PBS to resuspend the cells, centrifuge at 2000 rpm for 5 minutes for two times and then discard the supernatant.

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

2. Resuspend the cells in 500 μL of 1× Binding Buffer.

Note: Dilute 0.1 mL of 10× Binding Buffer in 0.9 mL ddH_2O to prepare 1× Binding Buffer.

3. Add 5 μL of Annexin V-iFluor 488.

4. Add 5 μL of PI Solution.

5. Incubate the cells at room temperature for 15-20 minutes in the dark.

Detection by flow cytometer

Analyze Annexin V-iFluor 488 binding by flow cytometer (Ex = 488 nm; Em = 514 nm) using iFluor 488 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 V-iFluor 488 or PI; b: cells with only Annexin V-iFluor 488; c: cells with only PI.

Detection by flow cytometer

Detect the fluorescence by fluorescence microscope: Centrifuge at 2000 rpm for 5 minutes, discard the supernatant, resuspend the cells with 50-100 μL of 1× Binding Buffer and then detect the fluorescence by fluorescence microscope.

4 Storage

4°C, 2 years.
Protect from light.

5 Precautions

1. Detect the fluorescence as soon as possible to avoid fluorescence quenching.
2. Annexin V-iFluor 488 and PI are sensitive to light, please operate away from light.
3. Annexin V-iFluor 488 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.