

# Annexin V-EGFP/PI Apoptosis Detection Kit

## 1 Components

Components	HY-K1074-20T	HY-K1074-50T
Annexin V-EGFP	100 µL	250 µL
Binding Buffer	10 mL	26 mL
PI Stain	220 µL	550 µL

## 2 Introduction

MCE Annexin V-EGFP/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<sup>2+</sup>-dependent phospholipid-binding protein that has a high affinity for PS. Annexin V labeled with EGFP 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-EGFP 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-EGFP and PI

1. Collect  $1-5 \times 10^6$  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-EGFP.
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-EGFP binding by flow cytometer (Ex = 488 nm; Em = 507 nm) using EGFP 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-EGFP or PI; b: cells with only Annexin-EGFP; 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.

## 6 Storage

-20°C, 1 year

Protect from light

Avoid repetitive freeze-thaw cycles

## 7 Precautions

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