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Annexin V-mCherry/SYTOX Green Apoptosis Detection Kit

1 Contents

Components	HY-K1077-20T	HY-K1077-50T
Annexin V-mCherry	100 μL	250 μL
Binding Buffer	12 mL	30 mL
SYTOX Green Stain	20 μL	50 μL

2 Introduction

MCE Annexin V-mCherry/SYTOX Green 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 is translocated 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 mCherry can identify apoptotic cells by binding to PS exposed on the outer leaflet. SYTOX Green is an excellent green-fluorescent nuclear and chromosome counterstain that only works on dead cells. After staining cells with Annexin V-mCherry and SYTOX Green, live cells show little or no fluorescence, apoptosis cells show red fluorescence, necrosis cells show red and green fluorescence.

3 General Protocol

Incubation of cells with Annexin V-mCherry and SYTOX Green

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-mCherry.
4. Add 1 μL of SYTOX Green.
5. Incubate the cells at room temperature for 10-20 minutes in the dark.

Detection by flow cytometer

The maximum excitation wavelength and emission wavelength of mCherry were 587 nm and 610 nm respectively, the maximum excitation wavelength and emission wavelength of SYTOX Green-DNA were 504 nm and 523 nm respectively.

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

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.

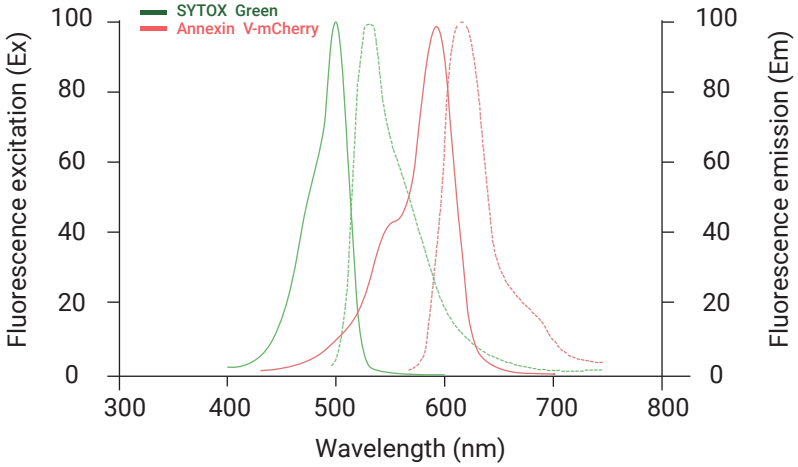

Figure 1. Excitation/Emission spectrum of Annexin V-mCherry and SYTOX Green.
(Solid line: excitation spectrum; Dotted line: emission spectrum)

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-mCherry and SYTOX Green are sensitive to light, please operate away from light.
3. Annexin-mCherry and SYTOX Green 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.