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Annexin V-mCherry Apoptosis Detection Kit

1 Contents

Components	HY-K1076-20T	HY-K1076-50T
Annexin V-mCherry	100 μL	250 μL
Binding Buffer	12 mL	30 mL

2 Introduction

MCE Annexin V-mCherry 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 Vis 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. After staining cells with Annexin V-mCherry, live cells show little or no fluorescence, apoptosis cells and necrosis cells show red fluorescence.

3 General Protocol

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. Incubate the cells at room temperature for 10-20 minutes in the dark.

5. Detection

5.1 Detect the fluorescence by flow cytometer directly.

Note: The maximum excitation wavelength and emission wavelength of Annexin-mCherry were 587 nm and 610 nm respectively.

5.2 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.

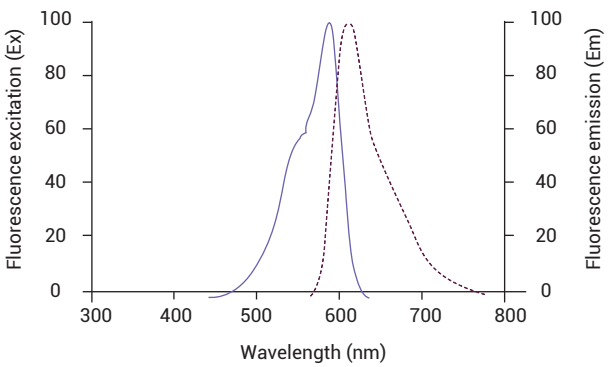

Figure 1. Excitation/Emission spectrum of Annexin V-mCherry.
(Solid line: excitation spectrum; Dotted line: emission spectrum)

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 V-mCherry is sensitive to light, please operate away from light.
3. Annexin V-mCherry is 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.