Product Information

Annexin V-APC

Catalog Number: A6080S, A6080L

Product Size: 0.1 mL, 1 mL

Storage

Store at 4℃ and protect from light. Do not freeze. Expiration date marked on the outer packing.

Spectral Characteristics

Annexin V-APC: Abs/Em: 650/660 nm

Description

Fluorescent conjugates of Annexin V can be used to label apoptotic cells. In normal viable cells, PS is located on the inner leaflet of the cytoplasmic membrane. However, in apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, where it is available for binding to fluorescently labeled Annexin V, which can be detected by fluorescence microscopy or flow cytometry.

Annexin V-APC can be used in combination with 7-AAD/PI to detect apoptosis. 7-AAD/PI is excluded by live cells and early apoptotic cells, but stains necrotic and late apoptotic cells with compromised membrane integrity, so as to distinguish living cells, early dead cells and late dead cells.

Protocol

1. Apoptosis was induced according to the experimental requirements. The test sample should contain the untreated samples. The cells were taken as negative control. In addition, set a group of samples for single staining to adjust the compensation.

2. Collecting cells.Suspension cell: 1000rpm, centrifuge for 5 minutes.Adherent cells: Trypsin without EDTA is used to digest the Centrifugation 5 min precipitation cells. In order to prevent the occurrence of false positive apoptosis, trypsin digestion time should not be too long . Cells are Collected by centrifugation at 1000 rpm for 5 min. For specific cells, if the cells can not be completely centrifuged to the bottom of the tube, centrifugal time or centrifugal force can be extended appropriately. In addition, the supernatant should be carefully removed, and about 50 μL of cell culture medium is reserved.

Note: the cells were digested with trypsin and then cultured under the optimum conditions. In order to avoid false positives, the cells were recovered for about 30 min and then stained again.

3. Add 1 ml of 4℃ precooled 1 × PBS, suspension cells, centrifugation and precipitation again. Then carefully remove the supernatant.

4. Resuspend the cells with 1× binding buffer and adjust the cell concentration to 1~5×10^6cells/mL.

5. Take 100 μL of the cell suspension into a 1.5 mL Ep tube, add 5 μL Annexin V-APC and 5 μL PI, mix and incubate at room temperature for 10-15 min in the dark (Prepare two tubes of apoptotic cells, and add only one dye to each tube: Annexin V-APC Or PI, used for compensation) .

6. Add 10 μL of 20 μg/mL 7-AAD or PI, and add 400 μL of PBS, Immediately for flow cytometry detection. Annexin V-APC is used Excitation with 633 nm exciter, maximum emission wavelength of 660 nm, it is recommended to use FL4 or RL1.

UElandy Inc.
Tel:0512-88965152
Web:www. uelandy.com

Notes

1. Fluorescent dyes have quenching problems. Please keep away from light during storage and use to slow down fluorescence quenching.

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