

DESCRIPTION

Annexin V belongs to the annexin family of intracellular proteins that bind to phosphatidylserine (PS) in a calcium dependent manner. PS is located on the inner layer of the phospholipid bilayer of the plasma membrane in viable cells. Cells undergoing early apoptosis will transfer PS to the external layer while maintaining an intact plasma membrane. Fluorescently labeled Annexin V may be used to bind the PS specifically and identify cells engaged in the early stages of apoptosis. During late apoptosis or necrosis, plasma membrane integrity is lost, which allows for the access of live/dead cell differential dyes such as 7-ADD, Propidium Iodide or DRAQ7. The staining of cells with Annexin V will effectively resolve viable apoptotic cell populations by flow cytometry.

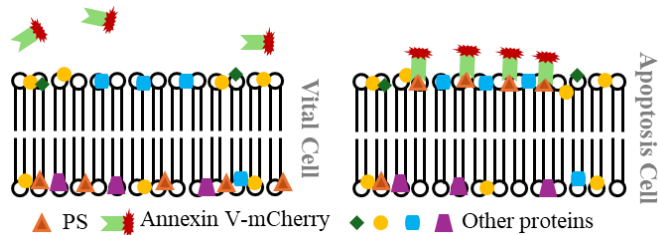


Fig 1: The principle of apoptosis cells stained by Annexin V

APPLICATIONS

- 1) Cell Proliferation.
- 2) Apoptosis and Cytotoxicity in cells.

THE CONTENTS

Annexin V-mCherry: 500 µL for 50 tests.
Apoptosis binding buffer: 25 mL

STORAGE CONDITION

Store the Reagent at 4°C protected from light. Shelf life: 6 months.

Materials Not Included

Flow Buffer: 1% BSA in PBS

PROCEDURES

1. Cells are induced or treated according to experiment design before starting the staining procedure.
2. Wash cells twice with cold PBS, and then resuspend cells in 100ul Apoptosis Binding Buffer at a concentration of 0.25-1.0 x 10⁷ cells/mL.
3. Add 10 µL of Annexin V-mCherry. Gently vortex the cells and incubate for 30-60 min at room temperature (25°C) in the dark.
4. Wash cells twice with cold PBS, resuspend cells in 300 µL of flow buffer to each tube. Analyze by flow cytometry with proper machine settings.

EXCITATION LASERS

5. mCherry: excitation peak at 587 nm and emission peak at 610 nm.

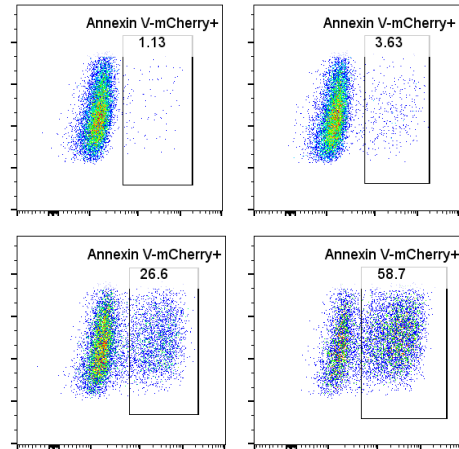


Fig. 2: Jurkat cells (T cell leukemia, human) are induced by 1.25ug/ml puromycin at 2 hours (upper right), 4 hours (lower left) and 6 hours (lower right) and the control (upper left) which is not treated with puromycin. Then all of them are stained by Annexin V-mCherry and analyzed by flow cytometry. The percentages of Aennix V-mCherry positive cells among live Jurkat cells are shown on the tops of each sample picture. Apoptotic cells are increased after puromycin inductions, and the percentiles of apoptotic cells are higher with the treatment time extensions.

RELATIVE PRODUCTS

- Resazurin Cell Viability Kit (TBS2001)
- CCK-8 Cell Viability Assay (TBS2022)
- ATP Colorimetric/Fluorometric Assay Kit (TBS2010)
- ADP/ATP Ratio Assay Kit (Bioluminescent (TBS2015)
- ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
- Caspase-3 Colorimetric Assay kit (TBS2030)
- Caspase-3 Fluorometric Assay kit (TBS2035)

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