Annexin V-Alexa Fluor 488/PI

Apoptosis Detection Kit

User Manual

Catalog # CAK2003

(Version 1.1A)

Detection phosphatidylserine on the outer leaflet of the cell membrane using flow cytometry.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Annexins are a family of calcium-dependent phospholipid-binding proteins. They are abundant in eukaryotic organisms belonging to a family of ubiquitous cytoplasmic proteins involved in signal transduction. All annexins have been shown to have a putative binding site for protein kinase C (PKC) but only annexin V would possess a potential pseudosubstrate site. Thus annexin V seems to modulate the activity of some PKCs on their substrates.

Annexin V was found to play a major role in matrix vesicle-initiated cartilage calcification as a collage-regulated calcium channel. Annexin V binds to procoagulant phospholipids (Vascular anticoagulant alpha) with high affinity.

Annexin V's preferential binding partner is phosphatidylserine (PS). PS is predominantly located in membrane leaflets, which face the cytosol. However, recent findings show that each cell type has the molecular machinery to expose PS at its cell surface. This machinery is activated during the execution of apoptosis. Once PS is exposed at the cell surface it exhibits procoagulant and proinflammatory activities. Annexin V will bind to the PS-exposing apoptotic cell and can inhibit the procoagulant and proinflammatory activities of the dying cell.

II. PRINCIPLES

Annexin V exhibits anti-phospholipase activity and binds to phosphatidylserine. Alexa Fluor 488 labelling allows simple direct detection by FACS analysis. Counterstaining by propidium iodide allows the discrimination of apoptotic cells.

III. KIT COMPONENTS

Component	20 Assays	50 Assays	100 Assays	Storage
Annexin V-Alexa Fluor 488	100 µl	250 µl	500 µl	4 °C
Binding Buffer (4X)	4 ml	10 ml	20 ml	4 °C
Propidium Iodide (20 µg/ml)	200 µl	500 µl	1000 µl	4 °C
Manual	1	1	1	

Note:

Dilute Binding Buffer (4x): 1:4 in distilled water (1 ml binding buffer and 3 ml distilled water).

IV. MATERIALS REQUIRED BUT NOT PROVIDED

1. 5 ml and 10 ml graduated pipettes
2. 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
3. Beakers, flasks, cylinders necessary for preparation of reagents
4. Glass-distilled or deionized water
5. Bench top centrifuge
6. Flow Cytometer
7. PBS

V. ASSAY PROCEDURE

1. Wash cells in PBS (4 °C) by gentle shaking or pipetting up and down.
2. Resuspend cells in Binding Buffer (1x); cell density should be 1-5 x 10^6/ml.
3. Take 100 µl resuspended cells to the bottom of 5 ml tube; Add 5 µl Annexin V-Alexa Fluor 488 and 10 µl Propidium Iodide (20 µg/ml) to 100 µl cell suspension.
4. Mix and incubate for 15 min at room temperature away from light.
5. Add 400 µl PBS to tube. Perform FACS analysis.

Note:

*It cannot be used trypsin with EDTA when digestive cells.

*Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.

*Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergent before use.

VI. STORAGE/STABILITY

The kit ships on wet ice and storage at 2-8 °C is recommended.

VII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

VIII. NOTES