Annexin V-FITC Apoptosis Detection Kit

User Manual

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I. Introduction

A. Product description

The ACTGene Annexin V-FITC Apoptosis Detection Kit is designed to employ FITC conjugated Annexin V with Propidium Iodide (PI) to allow the researchers to identify early apoptotic cells (Annexin V-FITC positive, PI negative). This kit contains FITC conjugated Annexin V, allowing for convenient quantitative assays before morphological changes associated with apoptosis has occurred and before membrane integrity has been lost. The secondary dye, propidium iodide (PI), can be used to distinguish apoptotic cells with intact membranes from lysed necrotic cells. The one-step staining procedure takes only 10 minutes. Detection can be analyzed by flow cytometry or by fluorescence microscopy.

These reagents must be used on live cells and are not applicable to previously sectioned, scraped, fixed or permeabilized cell samples. However, treatments that impact the cell membrane can be performed after the protocol.

B. Background

Apoptosis is a normal physiologic process, which occurs during embryonic development and in maintenance of tissue homeostasis. It's also a cell death process characterized by certain morphological and biochemical features, including loss of plasma membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. In early apoptosis, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner face of the plasma membrane to the cell surface, and exposed to the external cellular environment. Annexin V is a 35-36 kDa Ca$^{2+}$ dependent phospholipid-binding protein that has a high affinity to PS, and easily binds to cells with exposed PS$^{1}$. Annexin V may be conjugated with fluorochromes such as FITC, to serve as a sensitive probe for flow cytometric analysis of cells that are undergoing apoptosis$^{2-5}$. 


Propidium Iodide (PI) is a standard flow cytometric viability probe and is used to distinguish viable from nonviable cells. Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells become increasingly permeable to PI. PI can readily move across the cell membrane and bind to DNA. Cells that stain positive for Annexin V-FITC and negative for PI are undergoing apoptosis. Cells that stain positive for both Annexin V-FITC and PI are either in the end stage of apoptosis, are undergoing necrosis, or are already dead. Cells that stain negative for both Annexin V-FITC and PI are alive and not undergoing measurable apoptosis.

The stained cells can be analyzed with two-color flow cytometry, or a fluorescence microscope equipped with FITC and rhodamine filter sets.

C. Reagents Provided With The Kit

†Annexin-V-FITC: 110 µl
Propidium iodide: 120 µl
Annexin V Binding buffer: 60 ml

†One mol Annexin V conjugates 1- 2 mol FITC
*The product is stable until expiry date (see lot-specific label imprint) at 2-8°C

D. Materials Required But Not Supplied

- Cells undergo apoptosis.
- Phosphate buffered saline (PBS)

For cytometry analysis
- Two-color flow cytometer

For fluorescence microscope analysis
- Glass microscope slides and coverslips
- Fluorescence microscope equipped with FITC and rhodamine filter sets
II. Annexin V-FITC Apoptosis Detection Kit Protocol

A. Staining Solution Preparation

For 10 assays, prepare Staining Solution by adding 20 µl Annexin V-FITC labeling reagent (green cap bottle) and 20 µl propidium iodide (red cap bottle) into 1 ml Annexin V Binding buffer.

B. Staining And Analysis

Proceed to B.I or B.II or B.III below depending on method of analysis.

B.I  Quantification by Flow Cytometry

1. Trypsinize cells if using adherent cells. Collect 1x 10^6 cells by centrifugation.
2. Wash cells with PBS and centrifuge at 1000 xg for 5 min to get cell pellete.
3. Discard supernatant and resuspend the cell pellet in 100 µl of Staining Solution.
5. According to the cell density, add 0.4-0.8 ml Annexin V Binding buffer.
6. Analyze cells by flow cytometry with Ex.= 488 nm and Em.= 530 nm for Annexin V-FITC detection (FL1 channel). Using a filter > 600 nm for PI detection (FL2 channel). Electronic compensation of the instrument is required to exclude overlapping of the two emission spectra.
7. Events falling in the FITC (+)/ PI (-) region of the lower right quadrant are counted as apoptotic cells.
B.II  Detection By Fluorescence Microscopy Of Suspended cells

1. Trypsinize cells if using adherent cells. Collect 1x 10^6 cells by centrifugation.
2. Wash cells with PBS and centrifuge at 1000 xg for 5 min to get cell pellete.
3. Discard supernatant and resuspend the cell pellet in 100 µl of Staining Solution.
5. Transfer 25- 50 µl of cell suspension on a glass microscope slide, and cover with a glass coverslip.
6. Observe the cells under a fluorescence microscope using a dual filter set for FITC and rhodamine, or separate filters. Cells that have bound Annexin V-FITC will show green staining on the plasma membrane. Cells that have lost membrane integrity will show red PI staining throughout the nuclei and a halo of green staining (FITC) on the plasma membrane.

B.III  Detection By Fluorescence Microscopy Of Adherent cells

1. Grow cells directly on a coverslip, and induce apoptosis.
2. Remove medium and cover the coverslip with Staining Solution. *(Note: the volume of Staining Solution should be enough to cover the surface of whole coverslip).*
4. Invert the coverslip on a glass microscope slide and visualize cells.
5. Observe the cells under a fluorescence microscope using a dual filter set for FITC and rhodamine, or separate filters. Cells that have bound Annexin V-FITC will show green staining on the plasma membrane. Cells that have lost membrane integrity will show red PI staining throughout the nuclei and a halo of green staining (FITC) on the plasma membrane.
III. Troubleshooting

1. Apoptosis is an ongoing process so that cells stained with Annexin V should not be kept for prolonged times before measurement. Cells which still maintain membrane integrity for longer incubation times may become positive for PI since this dye will enter intact cells although very slowly. Analyze cells as soon as possible after 10-15 min incubation.

2. Even in the absence of induced apoptosis, most cell populations will contain low percentages of apoptotic and necrotic cells (Annexin V-FITC positive, PI negative or Annexin V-FITC and PI positive). Thus, it is important to note that the basal level of apoptosis and necrosis varies. The percentage of cells that have been induced to undergo apoptosis is then determined by subtracting the percentage of apoptotic cells in the untreated population from percentage of apoptotic cells in the treated population.

3. Since cell death is the eventual outcome of cells undergoing apoptosis, cells in the late stages of apoptosis will have a damaged membrane and stain positive for PI as well as for Annexin V-FITC. Thus the assay does not distinguish between cells that have already undergone an apoptotic cell death and those that have died as a result of necrotic pathway, because in either case the dead cells will stain with both Annexin V-FITC and PI.
IV. References


