Annexin V-FITC Apoptosis Detection Kit (with PI)

Catalog # A211

Version 5.1

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1. Introduction

In healthy cells, phosphatidylserine (PS) only distributes in the inner layer of the cell membrane lipid bilayer. At the early stage of apoptosis, PS is turned from the inside of the lipid membrane to the outside. Annexin V, a Ca^{2+} dependent phospholipid binding protein with a molecular weight of 35-36 kD, has a high affinity to PS and can be used to detect to detect the externalization of PS. Annexin V is recognized as one of the sensitive indicators of early apoptosis. Apoptosis can be detected by fluorescence microscopy or flow cytometry using Annexin V probe conjugated to FITC (a green fluorescent).

This kit also includes propidium iodide (PI), a dye for nucleic acid that cannot penetrate the intact cell membrane of healthy or early apoptotic cells. PI can penetrate the membrane of late apoptotic and necrotic cells, and stain these cells with red fluorescence. Using both Annexin V and PI, this kit can used to distinguish cells at different apoptotic periods, i.e. live cells (Annexin V^-/PI^-), early apoptotic cells (Annexin V^+/PI^-), and late apoptotic/dead cells (Annexin V^+/PI^+).

2. Contents of Kit

Component	A211-01 (50 rxn)	A211-02 (100 rxn)
Annexin V-FITC	250 µl	500 µl
PI Staining Solution	250 µl	500 µl
1× Binding Buffer	25 ml	2 × 25 ml

3. Storage

Store all components at 4-8℃ and protect from light.
The Annexin V-FITC can be stored at -20℃ for longer use.

4. Protocol

4.1. Staining

(1). Centrifuge cells at 300 × g for 5 min at 4℃. For adherent cells, digest cells with EDTA-free trypsin before centrifugation. To avoid false positive, do not over-digest the cells.

(2). Wash cells twice with cold phosphate-buffered saline (PBS). Centrifuge at 300 × g for 5 min at 4℃. Collect 1-5 × 10^5 cells.

(3). Resuspend cells in 100 µl of 1× Binding Buffer.

(4). Add 5 µl of Annexin V-FITC and 5 µl of PI Staining Solution to each 100 µl of cell suspension and mix gently.

(5). Incubate the cells at room temperature for 10 min and protect from light.

(6). After incubation, add 400 µl of 1× Binding Buffer. Mix gently and keep the samples on ice. Analyze the stained cells by flow cytometry or fluorescence microscopy within 1 hour.

4.2. Sample Analysis

A. Flow Cytometry

Set the excitation wavelength at 488 nm. Detect the FITC-green fluorescence in channel FL1. Detect the PI-red fluorescence in channel FL2 or FL3 (with FL3 preferred). Analyze and plot two-color dot plots with FL1 as the X-axis and FL3 as Y-axis in CellQuest. Collects 1000 events for each sample. Typically, the population should separate into three groups: live cells will show only a very low background fluorescence (Annexin V^-/PI^-), early apoptotic cells show only strong green fluorescent (Annexin V^+/PI^-), and late apoptotic cells show both green fluorescent and red fluorescent (Annexin V^+/PI^+).

B. Fluorescence Microscope

Add a drop of cell suspension stained by both Annexin V-FITC and PI on a slide. Cover cells with a coverslip. Observe cells under a fluorescence microscope using appropriate filters for Annexin V-FITC (green) and PI (red).

Note: Adherent cells can be cultured and induced apoptosis directly on slides.

5. Reference Results

Jurkat T lymphocytes were treated with 0, 0.5, 1.5, 4.5, 13.5 ng/ml TNF-related apoptosis-inducing ligand (TRAIL) for 3 hours, respectively. The results are shown in **Fig. 1**.

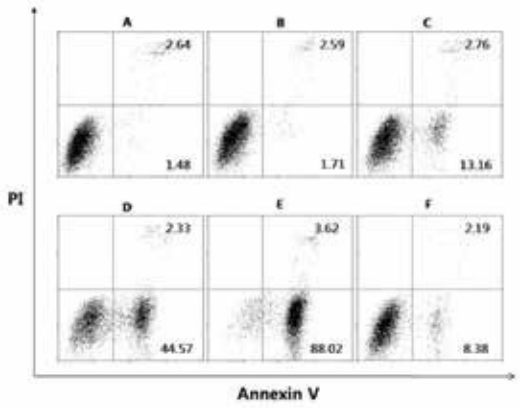

Fig. 1. Detection of TRAIL-induced apoptosis of Jurkat cells by flow cytometry

(A) 0 ng/ml. (B) 0.5 ng/ml. (C) 1.5 ng/ml. (D) 4.5 ng/ml. (E) 13.5 ng/ml. (F) Jurkat cells were induced apoptosis for 3 hours by 13.5 ng/ml TRAIL and then treated with 10 µg unlabeled Annexin V for 10 min, followed by Annexin V-FITC/PI staining. Unlabeled Annexin V, competing with Annexin V-FITC, was used to confirm the specificity of staining.

6. Notes

(1). Apoptosis is a rapid process. It is recommended to analyze the cells within 1 hour after staining.

(2). For adherent cells, a proper digestion and harvest of cells is crucial for final results. Suspended cells that result from apoptosis should also be collected. These cells should be combined together with adherent cells for staining. During digestion, carefully handle the adherent cells to avoid cell damage. An appropriate digesting time is necessary for both the penetration of PI and the binding of Annexin V-FITC with PS. Under-digested cells are hard to detach from culture surface, while over-digestion leads to damage of cell membrane. **DO NOT** use solutions with EDTA for digestion, for EDTA affects the binding between Annexin V and PS.

(3). For fixed cells, please use Annexin V-FITC rather than Annexin V-EGFP for apopotosis detection. Before fixation, incubate cells with Annexin V-FITC and remove the unbound Annexin V-FITC with Binding Buffer.

(4). For blood samples, please remove platelets before staining by a centrifugation at 200 × g in the buffer containing EDTA. Platelets contain PS which binds to Annexin V and affects the result.

(5). Briefly centrifuge the kit before use. Protect Annexin V-FITC and PI from light.

7. Trouble Shooting

(1). Annexin V-FITC staining failed or low positive rate

First, confirm whether proper apoptosis-inducer is used by setting a positive control.

Use solutions without EDTA for digestion and digest the adherent cells properly (refer to **6. Notes**).

A low density of PS on cell membrane may also lead to poor staining results. It is recommended to replace cell lines or use TUNEL to detect apoptosis.

(2). False positive

False positive means a high Annexin V+/PI+ double positive rate found in the control group without induction of apoptosis. A poor viability of cells may cause this result. It is recommended to calculate cell viability by Trypan blue staining. Three times of cell passage are needed for newly resuscitated cells. Improper cell operation such as repeated violent blow may also cause this situation. An excessive induction time (longer than 48 hours) may also lead to false positives.