TransDetect® Annexin V-FITC/PI Cell Apoptosis Detection Kit

Cat. No. FA 101

Storage: at 4°C in dark for one year.

Description

The Annexin V-FITC/PI Cell Apoptosis Kit provides a rapid and sensitive method for early apoptosis detection. In normal cells, the membrane phospholipid phosphatidylserine (PS) is located on the cytoplasmic surface of the membrane. In apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. The FITC-conjugated Annexin V, a Ca^{2+} dependent phospholipid-binding protein, can bind specifically to the exposed PS. Propidium iodide (PI) is a nucleic acid binding dye, which binds tightly to the nucleic acids in the cells and stains the cells with red fluorescence. PI is impermeant to live cells and early apoptotic cells, so the combination of Annexin V-FITC and PI staining allows the differentiation among different stage of apoptotic cells and necrosis cells.

Kit Contents

Component	FA101-01 (25 rxns)	FA101-02 (50 rxns)
Annexin V-FITC	125 µl	250 µl
Propidium Iodide (PI)	125 µl	250 µl
1× Annexin V Binding Buffer	12.5 ml	2×12.5 ml

Procedures

(1) Induce apoptosis by desired method. Collect 2×10^5~10^6 cells by centrifugation at 500×g, 4°C for 5 minutes.

- For suspension cells, directly collect cells by centrifugation
- For adherent cells, treat cells with non-EDTA trypsin. After treatment, terminate reaction with serum-containing media, collect cells by centrifugation.

(2) Wash cells twice with pre-chilled PBS, collect cells by centrifugation at 500×g, 4°C for 5 minutes.

(3) Resuspend cells by adding 100 µl of pre-chilled 1×Annexin V Binding Buffer.

(4) Add 5 µl Annexin V-FITC and 5 µl PI, mix gently.

(5) Incubate at room temperature (20°C~25°C) for 15 minutes in the dark.

(6) Add 400 µl of pre-chilled 1×Annexin V Binding Buffer. Gently mix and incubate the sample on ice in the dark, detect by flow cytometry or fluorescence microscopy within 1 hour.

Sample Analysis

A. Analysis by Flow Cytometry

Please choose appropriate voltage and adjust light compensation for flow cytometry analysis, we suggest setting control group as well.

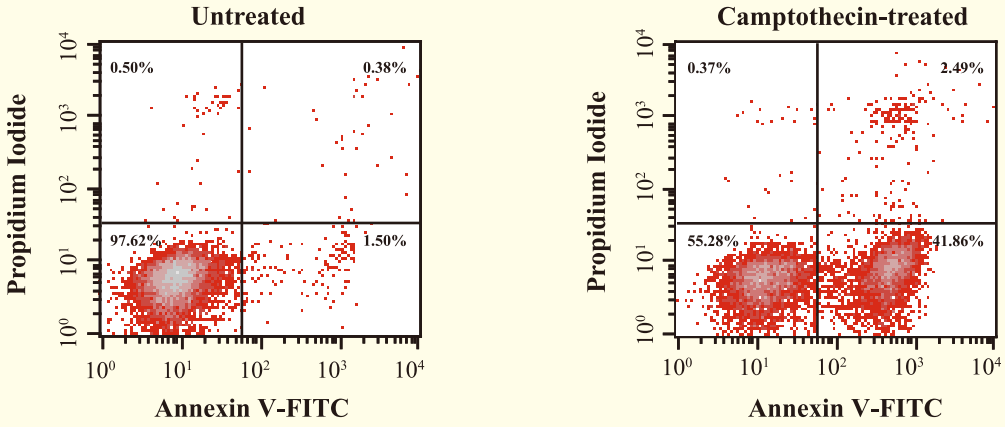
(1) Negative control cells, no dye added.

(2) Apoptotic positive cells, single staining by Annexin V (no PI).

(3) Apoptotic positive cells, single staining by PI (no Annexin V).

Example

Jurkat cells (T-cell leukemia, human) are treated with 10 µM Camptothecin for four hours. After inducing apoptosis, follow the instruction as described above, and detect by flow cytometry, result is shown as below.



B. Detection by Fluorescence Microscopy

Place 10 µl of dual-staining (Annexin V-FITC/PI) cell suspension on a glass slide, cover the cells with a glass coverslip. Observe the cells under a fluorescence microscope using a dual filter, Annexin V-FITC shows green fluorescence, PI shows red fluorescence.

Note: For analyzing adherent cells, grow cells directly on a coverslip and induce apoptosis, then detect with a fluorescence microscopy without fixing the cells. If cell fixation is needed, incubate the cells with Annexin V-FITC before fixation. To analyze loose adherent cells, we suggest to detach the cells into suspension before detection.

Notes

- Please gently handling during the whole procedure to avoid the presence of cell debris which can result in false positive.
- Properly trypsinize cells, either insufficient digestion or excessive digestion can produce cell debris which can result in false positive. After treatment with trypsin, ensure terminating the reaction with serum.
- Washing the cells with pre-chilled PBS cannot be omitted, and residual PBS should be removed as much as possible.
- Cell samples cannot be permeabilized, otherwise AnnexinV-FITC/PI can directly enter into the permeabilized cells, resulting in errors.
- Cell apoptosis is a constantly changing and dynamic procedure, Annexin V-FITC and PI are photosensitive substances, thus detection should be performed as soon as possible once reaction is completed. The whole procedure should be protected from light.
- Please centrifuge the reagents for a short time before use, in order to spin down the liquid from the tube top and wall to the bottom.
- The successful detection of early-stage apoptosis depends on several factors, including cell status and type, the method for inducing apoptosis and dosage, expression level of PS and the extent of its exposure to the cell surface, the degree of mechanical damage of cell during experimental procedure. Therefore, we suggest performing pre-experiment to optimize the corresponding steps.

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