TransDetect® Annexin V-PE/7-AAD Cell Apoptosis Detection Kit

Please read the manual carefully before use.

Cat. No. FA121

Storage: at 2-8℃ in the dark for one year

Description

Annexin V is one of the most sensitive indicators for early apoptosis detection. In normal cells, the phosphatidylserine (PS) is located on the inside of the phospholipid bilayer of the cell membrane. At the early stages of apoptosis, PS is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. The Annexin V, a Ca^{2+} dependent phospholipid-binding protein, can bind specifically to the exposed PS. Therefore, the nature of Annexin V binding to the PS exposed on the outside of cells can be used to detect the early apoptosis of the cells. Annexin V marked with R-Phycoerythrin (R-PE) is used as a probe to detect the occurrence of apoptosis using flow cytometry.

7-AAD is a nucleic acid binding dye, which cannot pass through the normal cells with complete cell membrane and early apoptotic cells, but can stain the nucleus through the cell membrane of apoptotic cells in the middle and late period and necrotic cells, and can be excited by 488 nm with a maximum emission wavelength of 647 nm. By maching Annexin V with 7-AAD, cells in different stages of apoptosis can be distinguished. This kit is easy to operate, highly sensitive and specific, and is suitable for the detection of early apoptosis of the cells.

Kit Contents

Component	FA121-01 (25 rxns)	FA121-02 (50 rxns)
Annexin V-PE	125 µl	250 µl
7-AAD	125 µl	250 µl
1× Annexin V Binding Buffer	12.5 ml	2×12.5 ml

Procedures

Self-prepared

Product Name	Catalogue
PBS (1×)	TransGen, Cat. FG701-01

(1) Induce apoptosis by the desired method. Collect 1~5×10^5 cells by centrifugation at 500×g, 2-8℃ for 5 minutes.

- For suspension cells, directly collect cells by centrifugation.
- For adherent cells, try to treat cells with non-EDTA trypsin. After treatment, terminate the reaction with serum-free medium, and collect cells by centrifugation. After the adherent cells induce apoptosis, if there are some suspension cells, please collect the culture medium into the microcentrifuge tube and centrifuge with the digested cells to ensure the reliability of the experimental results.

(2) Wash cells twice with pre-chilled PBS, and collect cells by centrifugation at 500×g, 2-8℃ for 5 minutes.

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

(4) Add 5 µl Annexin V and 5 µl 7-AAD, and mix gently.

(5) Incubate at room temperature (20℃~25℃) 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, and detect by flow cytometry or fluorescence microscopy within 1 hour.

Sample Analysis

A. Please choose the appropriate voltage and adjust light compensation for flow cytometry analysis, we suggest setting the following control groups.

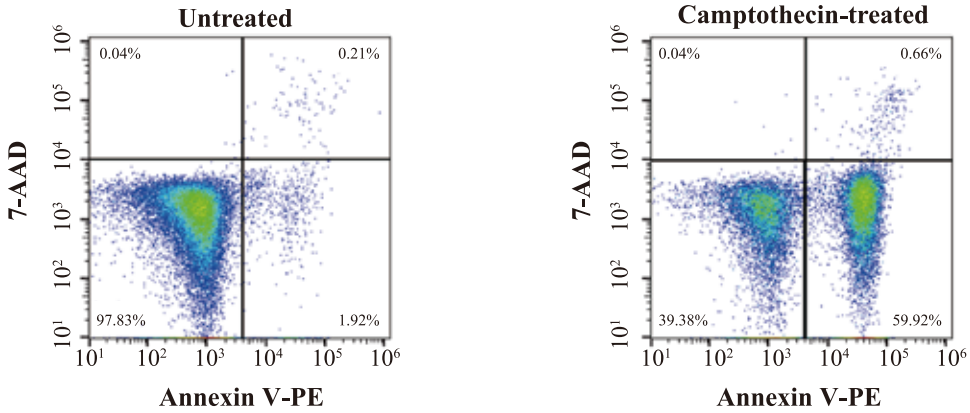
(1) Negative control cells, no dye added.

(2) Apoptotic positive cells, single staining by Annexin V-PE (no 7-AAD).

(3) Apoptotic positive cells, single staining by 7-AAD (no Annexin V-PE).

Example of Analysis by Flow Cytometry

Jurkat T lymphoma cells are treated with 10 μM Camptothecin for four hours. After inducing apoptosis, follow the instruc tion as described above, and detected by flow cytometry, result is shown below.



Notes

- Please gently handle during the whole procedure to avoid the presence of cell debris which can result in a false positive.
- Properly trypsinize cells, either insufficient digestion or excessive digestion can produce cell debris which can result in a 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, Annexin V-PE/7-AAD can directly enter into the permeabilized cells, resulting in errors.
- Cell apoptosis is a constantly changing and dynamic procedure, Annexin V-PE and 7-AAD are photosensitive substances, thus detection should be performed as soon as possible once the 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, the expression level of PS and the extent of its exposure to the cell surface, and the degree of mechanical damage of the cell during the experimental procedure. Therefore, we suggest performing a pre-experiment to optimize the corresponding steps.
- During the experiment, please wear experimental clothes and disposable gloves to ensure safety.

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