CYDN19-CHANGYU Annexin V-FITC Apoptosis Detection Kit

Catalog number	Package
CYDN1901	50 reactions
CYDN1902	25 reactions

Storage and shipping: 2-8 °C. Valid for one year.

Description

Apoptosis is a basic characteristic of cells and plays an important role in embryonic development, tissue repair and maintenance of the internal environment. Annexin V is a Ca^{2+}-dependent phospholipid-binding protein with a molecular weight of 35-36 kD that binds with high affinity and specificity to phosphatidylserine (PS) translocated to the outer leaflet of the plasma membrane during apoptosis. Using FITC-labelled Annexin V as a fluorescent probe, apoptosis can be detected by flow cytometry or fluorescence microscopy.

Propidium iodide (PI) is a nucleic acid dye that cannot pass through an intact plasma membrane, but in mid-to-late apoptotic cells and dead cells PI can enter the cells and stain the nuclei red. Using Annexin V together with PI allows early apoptotic cells to be distinguished from late apoptotic cells and dead cells.

Kit components

Component	50 reactions (CYDN1901)	25 reactions (CYDN1902)
Annexin V-FITC (20 μg/ml)	500 μl	250 μl
Binding Buffer	40 ml	20 ml
Propidium Iodide (PI, 50 μg/ml)	250 μl	125 μl

Notes

Note: To obtain satisfactory experimental results, read these notes carefully before using the kit.

Note: Briefly centrifuge the reagents (especially reagents in small vials) before opening to collect liquid from the inner wall of the cap at the bottom of the tube, so that no liquid is lost when opening.

Note: Propidium iodide (PI) can be absorbed through the skin and irritates the eyes.

Note: This kit is for research use only and is not suitable for clinical diagnosis.

Note: The PBS provided with this kit is supplied as a gift and is not a true component of the kit; wash cells by standard methods.

Important notes for the procedure

Note: Handle the cells as gently as possible throughout the procedure; do not pipette vigorously, and perform the operations at 4 °C as far as possible.

Note: Detect the samples as soon as possible after the reaction, because apoptosis is a dynamic process and the fluorescence intensity begins to decay 1 hour after the reaction.

Note: Annexin V-FITC and propidium iodide are light-sensitive; protect them from light during the procedure.

Note: Successful apoptosis detection is affected by factors such as the cell type, the density of PS on the cell membrane, the proportion of PS flipped during apoptosis, the method used to induce apoptosis, the reagents used and the induction time. Optimizing these factors is essential for a successful experiment.

Note: In the last cell-washing step, remove the supernatant as completely as possible so that residual PBS does not affect the experimental result.

Note: Prolonged PI staining may cause the detected apoptosis rate to be too high; it is recommended to stain with Annexin V-FITC first and add PI 5 minutes before acquisition.

❖ Procedure

1. For adherent cells, detach the cells with trypsin first.
2. After counting, take 5×10^5-1×10^6 cells/ml and centrifuge at 1,000 rpm at 4 °C for 10 minutes; discard the supernatant.
3. Add 1 ml of cold PBS and agitate gently to resuspend the cells.
4. Centrifuge at 1,000 rpm at 4 °C for 10 minutes and discard the supernatant.
5. Repeat steps 3 and 4 twice.
6. Resuspend the cells in 200 μl of Binding Buffer.
7. Add 10 μl of Annexin V-FITC, mix gently and incubate at room temperature for 15 minutes in the dark, or at 4 °C for 30 minutes.
8. Add 300 μl of Binding Buffer (total reaction volume 500 μl) and 5 μl of PI, and acquire on the instrument within 1 hour (it is recommended to filter the cell suspension through a 200-mesh sieve before adding PI).
9. For detection by fluorescence microscopy, centrifuge the cell mixture, smear the cell pellet and observe.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.