Annexin V-FITC Apoptosis Detection Kit (50 assay)

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Cat. No. AVK050

Description:

Strong's Annexin V-FITC Apoptosis Detection kit allows for fluorescent detection of Annexin V bound to apoptotic cells and quantitative determination by flow cytometry. The process needs only a 15-minute incubation procedure.

Annexin V has a high affinity in a Ca^{2+}-dependent manner to negatively charged phospholipid phosphatidylserine, which is found on the outer cell membrane early during apoptosis. The Annexin V-FITC kit employs FITC conjugated Annexin V in concert with propidium iodide (PI). As the cell membrane becomes increasingly permeable during the later stage of apoptosis, propidium iodide can readily move across the cell membrane and bind to DNA. This combination allows the differentiation among 3 populations of cells in two-color flow cytometry:

- Normal cells: Annexin V negative and PI negative
- Early apoptotic cells: Annexin V positive and PI negative
- Necrotic cells or late apoptotic cells: Annexin V positive and PI positive

Alternatively, the cell can be examined with a fluorescence microscope equipped with FITC and rhodamine filter sets.

Source:

Recombinant human Annexin V is produced in *E. coli*. Annexin V-FITC contains 1-2 moles FITC per mole Annexin V.

Comments:

- Detects apoptosis earlier in the process than DNA-based assays such as TUNEL.
- Rapid labeling of cells. Cell staining takes only 10 minutes.
- No cell fixation or processing required, reducing the detection time and allowing the cells to be used for further study.
- Propidium iodide secondary dye is included with the kit to differentiate apoptotic cells from viable and necrotic cells.

Required products: Cells to undergo apoptosis
Apoptosis inducers, e.g., staurosporine; phosphate buffered saline (PBS)

Kit contents: 3 solutions, ready-to-use

Bottle	Content	Volume	Cap Color
1	**Annexin V-FITC**	110 µl	Green
2	**Propidium Iodide**	120 µl	Red
3	**Annexin V Binding Buffer**	60 ml	Natural

Kit Stability:

The product is stable until expiry date (see lot-specific label imprint) at 2-8°C.

Application:

The assay procedure involves：

Step1: Washing the cells in PBS.
Step2: Incubation of cells with Annexin V-FITC in a Binding buffer containing PI.
Step3: Analysis of the samples under a fluorescence microscope or on a flow cytometry.

Preparation of the staining solution:

For 10 assays, predilute 20 µl Annexin V-FITC (green cap bottle) in 1000 µl Binding buffer (natural cap bottle) and add 20 µl propidium iodide (red cap bottle).

Staining procedures:

A. Flow cytometry or fluorescence microscopy

- Wash the 10^6 cells with PBS and centrifuge cells at 200 × g for 5 min.
- Resuspend the cell pellet in 100 µl of staining solution and incubate for 10-15 min at 15 to 25°C.
- Analyze as analysis step a or b.

B. Adherent cells

- Before staining grow cells on chamber slides, induce apoptosis.
- Remove chambers and silicon borders.
- Remove medium and cover slides with staining solution (100 µl / chamber)
- Put cover slips on slides and incubate for 10-15 min at 15 to 25°C.
- Analyze as analysis step a or b.

Analysis:

a. Fluorescence microscopy

For evaluation by fluorescence microscopy use an excitation wavelength in the range of 450-500 nm (e.g. 488 nm) and detection in the range of 515-565 nm (green).

b. Flow cytometry

According to the cell density, add 0.4-0.8 ml binding buffer (bottle 3) and analyze on a flow cytometer using 488 nm excitation and a 515 nm band pass filter for FITC detection and a filter >600 nm for PI detection. Electronic compensation of the instrument is required to exclude overlapping of the two emission spectra.

Storage Temp: Store at 2-8ºC.

References:

1. Pigault, C., et al., Formation of two-dimensional arrays of Annexin V on phosphatidylserine-containing liposomes. J. Mol. Biol., 236, 199-208 (1994).
2. Dachary-Prigent, J., et al., Calcium involvement in aminophospholipid exposure and microparticle formation during platelet activation: a study using Ca^{2+}-ATPase inhibitors. Biochemistry, 34, 11625-11634 (1995).
3. Kuypers, F.A., et al., Detection of altered membrane phospholipid asymmetry in subpopulations of human red blood cells using fluorescently labeled Annexin V. Blood, 87, 1179-1187 (1996).
4. Koopman, G., et al., Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood, 84, 1415-1420 (1994).