

Annexin V-FITC Apoptosis Detection Kit

(Catalog #: K101-25, -100, -400; Store at 4°C; Lot# 41101 Stable for one year)

I. Introduction:

Annexin V Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, cells translocate the membrane phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can be easily detected by staining with a fluorescent conjugate of Annexin V, a protein that has a high affinity for PS. The one-step staining procedure takes only 10 minutes. Detection can be analyzed by flow cytometry or by fluorescence microscopy. The kit can differentiate apoptosis vs necrosis when performing both Annexin V-FITC and PI staining.

II. Kit Contents:

Components	K101-25	K101-100	K101-400
	25 assays	100 assays	400 assays
Annexin V-FITC	125 µl	500 µl	2 ml
1X Binding Buffer	12.5 ml	50 ml	2 x 100 ml
Propidium Iodide (PI)	125 µl	500 µl	2 ml

III. Annexin V-FITC Assay Protocol:

A. Incubation of cells with Annexin V-FITC

1. Induce apoptosis by desired method.
2. Collect $1-5 \times 10^5$ cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Binding Buffer.
4. Add 5 µl of Annexin V-FITC and 5 µl of propidium iodide (PI 50 µg/ml, optional.)
5. Incubate at room temperature for 5 min in the dark.
Proceed to B or C below depending on method of analysis.

B. Quantification by Flow Cytometry

Analyze Annexin V-FITC binding by flow cytometry (Ex = 488 nm; Em = 530 nm) using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2).

For adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-FITC (A.3-5).

C. Detection by Fluorescence Microscopy

1. Place the cell suspension from Step A.5 on a glass slide. Cover the cells with a glass coverslip.

For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.5), invert coverslip on a glass slide and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization. (Cells must be incubated with Annexin V-FITC before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane.)

2. Observe the cells under a fluorescence microscope using a dual filter set for FITC & rhodamine.

Cells that have bound Annexin V-FITC will show green staining in the plasma membrane. Cells that have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (FITC) on the cell surface (plasma membrane).

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