PRODUCT INFORMATION & MANUAL

Annexin V Apoptosis Detection Kit

NBP2-54834

For research use only.

Not for diagnostic or therapeutic procedures.

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Annexin V-EGFP Apoptosis Detection Kit

(Catalog #: NBP2-54834 Store kit at 4˚C; Stable for one year)

I. Introduction:

The Annexin V-EGFP Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, most cell types translocate the membrane phospholipid 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 an enhanced green fluorescent protein (EGFP) fusion of annexin V, a protein that has a strong natural affinity for PS. The one-step staining procedure takes only 10 minutes. In addition, the assay can be directly performed on live cells. Detection can be analyzed by flow cytometry or by fluorescence microscopy with a FITC filter. EGFP is brighter and more photo-stable than other fluorescent reagents. The kit can differentiate apoptosis vs necrosis when performing both annexin V-EGFP and PI staining.

II. Kit Contents:

Components	NBP2-54834	NBP2-54834	NBP2-54834
	25 assays	100 assays	400 assays
Annexin V-EGFP	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-EGFP Assay Protocol:

A. Incubation of cells with Annexin V-EGFP

1. Induce apoptosis by desired method.
2. Collect 1-5 x 10^5 cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Binding Buffer.
4. Add 5 µl of Annexin V-EGFP and 5 µl of propidium iodide (PI 50µg/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-EGFP 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-EGFP (A.3-5).

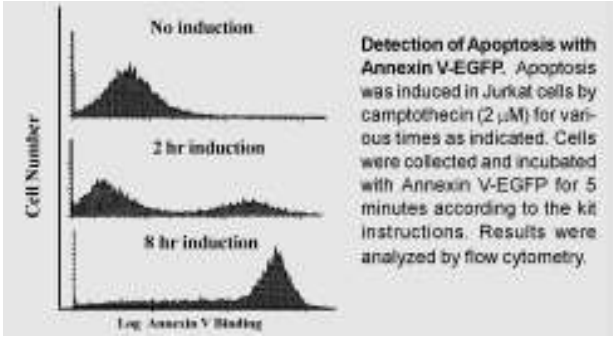
C. Detection by Fluorescence Microscopy

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 glass slide and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization.

Note: Cells must be incubated with Annexin V-EGFP 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.



Detection of Apoptosis with Annexin V-EGFP. Apoptosis was induced in Jurkat cells by camptothecin (2 µM) for various times as indicated. Cells were collected and incubated with Annexin V-EGFP for 5 minutes according to the kit instructions. Results were analyzed by flow cytometry.

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

GENERAL TROUBLESHOOTING GUIDE FOR ANNEXIN BASED KITS:

Problems	Cause	Solution
High Background	• Cell density is higher than recommended	• Refer to datasheet and use the suggested cell number
	• Increased volumes of components added	• Use calibrated pipettes accurately
	• Incubation of cell samples for extended periods	• Refer to datasheets and incubate for exact times
	• Use of extremely confluent cells	• Perform assay when cells are at 80-95% confluency
	• Contaminated cells	• Check for bacteria/ yeast/ mycoplasma contamination
Lower signal levels	• Washing cells with PBS before/after fixation (adherent cells)	• Always use binding buffer for washing cells
	• Cells did not initiate apoptosis	• Determine the time-point for initiation of apoptosis after induction (time-course experiment)
	• Very few cells used for analysis	• Refer to data sheet for appropriate cell number
	• Incorrect setting of the equipment used to read samples	• Refer to datasheet and use the recommended filter setting
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
Erratic results	• Uneven number of cells seeded in the wells	• Seed only healthy cells (correct passage number)
	• Adherent cells dislodged at the time of experiment	• Perform experiment gently and in duplicates or triplicates for each treatment
	• Incorrect incubation times or temperatures	• Refer to datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
	• Increased or random staining observed in adherent cells	• Always stain cells with Annexin before fixation (makes cell membrane leaky)
Note# The most probable cause is listed under each section. Causes may overlap with other sections.		