Annexin V-PE Apoptosis Detection Kit

(Catalog #: K128-25, -100, -400; Store kit at 4˚C)

I. Introduction:

The Annexin V-PE 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 a fluorescent conjugate 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, without the need for fixation. The Annexin V-PE Apoptosis Detection Kit contains the bright orange-red PE fluorescent probe that can be easily detected by flow cytometry or fluorescence microscopy.

II. Kit Contents:

Components	K128-25 25 assays	K128-100 100 assays	K128-400 400 assays	Part Number
Annexin V-PE 1X Binding Buffer	125 µl 12.5 ml	500 µl 50 ml	2 ml 2 x 100 ml	K128-XX(X)-1 K128-XX(X)-2

III. Annexin V-PE Assay Protocol:

A. Incubation of cells with Annexin V-PE

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-PE.
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-PE binding by flow cytometry (Ex = 488 nm; Em = 578 nm) using the phycoerythrin emission signal detector (usually FL2).

For analyzing adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-PE (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.

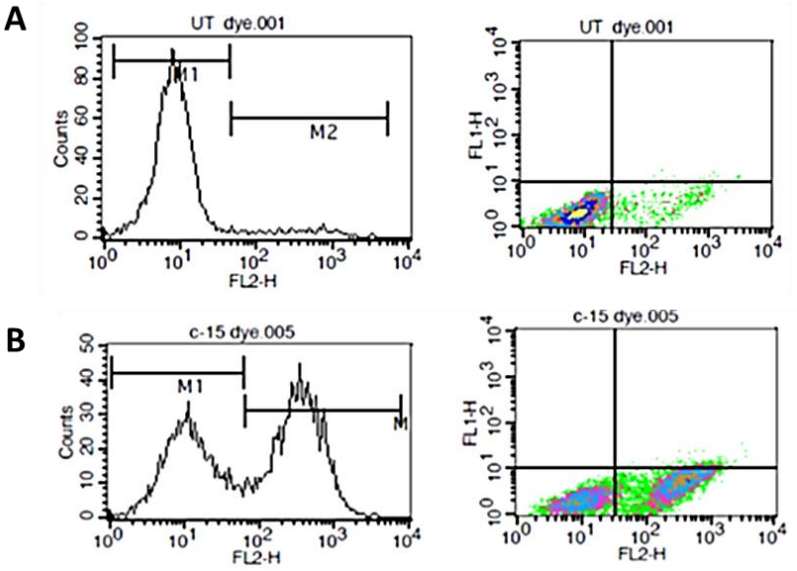
   For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.5), invert coverslip on glass slide and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization. (Cells must be incubated with Annexin V-PE before fixation since any cell 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 rhodamine filter.

   Cells which have bound Annexin V-PE will show orange-red staining in the plasma membrane.

IV. Storage and stability:

Store kit at 4˚C. All reagents are stable for one year under proper storage conditions.



Analysis of Apoptosis with the Annexin V-PE Apoptosis Detection Kit: Apoptosis was induced in Jurkat cells by 4-6 hrs incubation with 2 µM Camptothecin. The resultant apoptosis was quantified in un-induced cells **(A)** and the induced cells **(B)** using this detection kit.

RELATED PRODUCTS:

- Annexin V-PE Apoptosis Kit Plus **(K203-25, -100, -400)**
- Annexin V-PE-Cy5 Apoptosis Detection Kit **(K129-25, -100, -400)**
- Annexin V-FITC Apoptosis Kit Plus **(K201-25, -100, -400)**
- Annexin V-FITC Apoptosis Kit **(K101-25, -100, -400)**
- Annexin V-EGFP Apoptosis Kit **(K104-25, -100, -400)**
- Annexin V-Cy5 Apoptosis Kit **(K103-25, -100, -400)**
- Annexin V-Cy3 Apoptosis Kit **(K102-25, -100, -400)**
- Annexin V-Cy3 Apoptosis Kit Plus **(K202-25, -100, -400)**
- Annexin V-Biotin Apoptosis Kit **(K109-25, -100, -400)**

FOR RESEARCH USE ONLY! Not to be used on humans.

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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.

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