Annexin V-Cy5 A

(Catalog #: K103-25, -100, -400; Store kit at 4°C)

I. Introduction:

Annexin V Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, cells translocate the membrane phosphatidyl-serine (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.

II. Kit Contents:

Components	K103-25 25 assays	K103-100 100 assays	K103-400 400 assays	Part Number
Annexin V-Cy5 1X Binding Buffer	125 µl 12.5 ml	500 µl 50 ml	2 ml 2 x 100 ml	K103-XX(X)-1 K103-XX(X)-2

III. Assay Protocol:

A. Incubation of Cells with Annexin V-Cy5:

1. Induce apoptosis by desired methods.
2. Collect 1-5 x 10^5 cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Annexin V Binding Buffer.
4. Add 5 µl of Annexin V-Cy5.
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 cells by flow cytometry (Ex = 649 nm; Em = 670 nm) using a Helium-Neon Laser.

For adherent cells, trypsinize and gently wash cells with serum-containing medium before incubation with Annexin V-Cy5 (A.3-5).

C. Detection by Fluorescence Microscopy:

1. Place the cell suspension from Step A.5 on a glass slide, and cover 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 with 1X Annexin V binding Buffer and fixed in 2% formaldehyde before visualization. (Cells must be incubated with Annexin V-Cy5 before fixation because 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 Cy5 filter, or FITC/Cy3/Cy5 triple band filter (Chroma Technology) if performing triple labeling using these dyes, or detect cells using a CCD camera.

   Cells that have bound Annexin V-Cy5 will show bright red-blue staining on the plasma membrane.

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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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