Annexin V-Cy3

(Catalog #: K102-25, -100, -400; Store kit at 4˚C; 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 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	K102-25 25 assays	K102-100 100 assays	K102-400 400 assays	Part Number
Annexin V-Cy3 1X Binding Buffer	125 µl 12.5 ml	500 µl 50 ml	2 ml 2 x 100 ml	K102-XX(X)-1 K102-XX(X)-2

III. Annexin V-Cy3 Assay Protocol:

A. Incubation of cells with Annexin V-Cy3

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-Cy3.
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-Cy3 binding by flow cytometry (Ex = 543 nm; Em = 570 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-Cy3 (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-Cy3 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 that have bound Annexin V-Cy3 will show red staining in the plasma membrane.

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