Annexin V-Cy3 Apoptosis Kit Plus (BN00476)

(Catalog BN00476 -25, -100, -400; Store kit at 4˚C)

I. Introduction:

The assay is based on the observation that soon after initiating apoptosis, cells 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 easily be 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 of fixation. The Annexin V-Cy3 Apoptosis Detection Kit Plus includes annexin V-Cy3, SYTOX green dye, and binding buffer. The SYTOX green dye is impermeant to live cells and apoptotic cells, but stains necrotic cells with intense green fluorescence by binding to cellular nucleic acids. After staining a cell population with annexin V-Cy3 and SYTOX Green dye in the provided binding buffer, apoptotic cells show red fluorescence, dead cells show green fluorescence and live cells show little or no fluorescence. These populations can easily be distinguished by Fluorescence microscopy using FITC and rhodamine filters or by flow cytometry using the FL1 channel (Ex. 488 nm/Em. 530 nm) for SYTOX Green dye and FL2 channel for Annexin V-Cy3 (Ex. 543 nm/Em. 570 nm).

II. Kit Contents:

Component	BN00476 25 assays	BN00476 100 assays	BN00476 400 assays
Annexin V-Cy3	125 µl	500 µl	2 ml
SYTOX Green Dye	25 µl	100 µl	400 µl
Binding Buffer	12.5 ml	50 ml	2 x 100 ml

III. Annexin V-Cy3 Plus Assay Protocol:

1. Induce apoptosis by desired method. Concurrently incubate a control culture without induction.
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 and 1 µl of SYTOX Green dye.
   Note: Thaw the SYTOX Green dye in room temperature before use.
5. Incubate at room temperature for 5-10 min in the dark.
6. Analyze the stained cells by flow cytometry using FL1 channel for SYTOX Green dye (Ex = 488 nm; Em = 530 nm) and FL2 channel for Annexin V-Cy3 (Ex = 543 nm; Em = 570 nm).

   The cell population should separate into three groups: live cells with only a low level of fluorescence, apoptotic cells with red fluorescence and necrotic cells with green fluorescence.

   The flow cytometric results can also be confirmed by viewing the cells under a fluorescence microscope using FITC filter for SYTOX and rhodamine filter for Annexin V-Cy3.

   For adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-Cy3 and SYTOX dye.

IV. Storage and Stability:

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

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

Problems	Cause	Solu
High Background	• Cell density is higher than recommended	• Refe
	• Increased volumes of components added	• Use
	• Incubation of cell samples for extended period	• Refe
	• Use of extremely confluent cells	• Perfo
	• Contaminated cells	• Chec
Lower signal levels	• Washing cells with PBS before/after fixation (adherent cells)	• Alwa
	• Cell lysate contains interfering substances	• Use
	• Cells did not initiate apoptosis	• Dete experi
	• Very few cells used for analysis	• Refe
	• Incorrect setting of the equipment used to read samples	• Refe
	• Use of expired kit or improperly stored reagents	• Alwa
Erratic results	• Uneven number of cells seeded in the wells	• Seed
	• Adherent cells dislodged at the time of experiment	• Perfo
	• Incorrect incubation times or temperatures	• Refe
	• Incorrect volumes used	• Use
	• Increased or random staining observed in adherent cells	• Alwa
Note: The most probable cause is listed under each section. Causes may overlap with other sections.		

GENERAL TROUBLESHOOTING GUIDE FOR ANNEXIN BASED KITS: