Annexin V-FITC Apoptosis Kit Plus (BN00473-74)

(Catalog BN00473 -25, -100, -400 ; Store kit at 4˚C)

I. Introduction:

The Annexin V-FITC Apoptosis Detection Kit Plus 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 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-FITC Apoptosis Detection Kit Plus includes annexin V-FITC, 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-FITC and SYTOX Green dye in the provided binding buffer, apoptotic cells show green fluorescence, dead cells show a higher level of green fluorescence and live cells show little or no fluorescence. These populations can easily be distinguished using a flow cytometry with the 488 nm line of an argon-ion laser for excitation. Both annexin V-FITC and SYTOX Green dye emit green fluorescence that can be detected in the FL1 channel, freeing the other channels for the addition of other probes in multi-color labeling experiments.

II. Kit Contents:

Component	BN00473 25 assays	BN00473 100 assays	BN00473 400 assays
Annexin V-FITC	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-FITC 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-FITC 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 (Ex = 488 nm; Em = 530 nm).

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

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

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

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
	• Cell lysate contains interfering substances	• Use the cell lysis buffer in the kit or refer datasheet for instructions
	• 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.		