Product Information

Annexin V-FITC Apoptosis Kit Plus

I. Kit Contents:

Component	K2057-25 25 assays	K2057-100 100 assays	K2057-400 400 assays	Part Number
Annexin V-FITC	125 µl	500 µl	2 ml	K2057-C-1
SYTOX Green Dye	25 µl	100 µl	400 µl	K2057-C-2
Binding Buffer	12.5 ml	50 ml	2 x 100 ml	K2057-C-3

II. Introduction:

Soon after the apoptosis is activated, most cell types transfer the membrane phospholipid phosphatidylserine (PS) from the plasma membrane inner face to the cell surface. Detection of the cell-surface PS can be easily done by staining with a fluorescent conjugate of protein Annexin V which has a robust natural affinity for PS. The one-step staining process needs just 10 minute. This assay can be directly carried out on live cells without 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. Following the staining the cell population with annexin V-FITC and SYROX Green dye in the given binding buffer, apoptotic cells exhibit green fluorescence, dead cells exhibits a higher level of green florescence and lives cells exhibits little or no fluorescence.

Those cell populations can be differentiated with flow cytomery at excitation of 488 nm and emission of 530 nm.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.

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.

General Troubleshooting Guide:

Problems	Cause	Solution
High Background	• Cell density is higher than recommended • Increased volumes of components added • Incubation of cell samples for extended periods	• Refer to data sheet and use the suggested cell number • Use calibrated pipettes accurately • Refer to data sheets and incubate for exact times

	• Use of extremely confluent cells • Contaminated cells	• 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) • Cell lysate contains interfering substances • 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 • Use the cell lysis buffer in the kit or refer data sheet for instructions • Determine the time-point for initiation of apoptosis after induction (time-course experiment) • Refer to data sheet for appropriate cell number • Refer to data sheet 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 data sheet & 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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Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com