Product Information

Annexin V-Cy3 Apoptosis Kit Plus

I. Kit Contents:

Component	K2058-25 25 assays	K2058-100 100 assays	K2058-400 400 assays	Part Number
Annexin V-Cy3	125 µl	500 µl	2 ml	K2058-C-1
SYTOX Green Dye	25 µl	100 µl	400 µl	K2058-C-2
Binding Buffer	12.5 ml	50 ml	2 x 100 ml	K2058-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-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. Following the staining the cell population with annexin V-Cy3 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 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).

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℃. All reagents are stable for one year under proper storage conditions.

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 • Use of extremely confluent cells • Contaminated cells	• Refer to data sheet and use the suggested cell number • Use calibrated pipettes accurately • Refer to data sheets 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) • 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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