Product Information

Annexin V-EGFP Apoptosis Kit

I. Kit Contents:

Component	K2006-25 25 assays	K2006-100 100 assays	K2006-400 400 assays	Part Number
Annexin V-EGFP	125 µl	500 µl	2 ml	K2006-C-1
1X Binding Buffer	12.5 ml	50 ml	2 x 100 ml	K2006-C-2
Propidium Iodide	125 µl	500 µl	2 ml	B7758

II. Introduction:

Annexin V is a cellular protein, which plays important roles in the inhibition of the activity of phospholipase A1 and blood coagulation by competing for phosphatidylserine (PS) binding sites with prothrombin. Annexin V has a high affinity to PS and is used as a probe to detect cells that have expressed PS on the cell surface. After initiating apoptosis, cells translocate phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface soon, which can be easily detected by Annexin V. Enhanced green fluorescent protein (EGFP) is a fluorescent reagent that is more brighter and more photo-stable.

Annexin V-EGFP Apoptosis Detection Kit uses an EGFP fusion of annexin V that can easily detect PS on the cell surface after initiating apoptosis. The one-step staining procedure needs only 10 minutes. The result can be analyzed by fluorescence microscopy or by flow cytometry with a FITC filter. The Annexin V-EGFP Apoptosis Detection Kit can differentiate apoptosis vs necrosis when performing both Annexin V-EGFP and PI staining. In addition, this kit can be directly performed on live cells.

III. Annexin V-EGFP Assay Protocol:

A. Incubation of cells with Annexin V-EGFP

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-EGFP and 5 µl of propidium iodide (PI 50µg/ml, optional).
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-EGFP binding by flow cytometry (Ex = 488 nm; Em = 530 nm) using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2).

For adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-EGFP (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.

Note:

Cells must be incubated with Annexin V-EGFP before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane.

General Troubleshooting Guide For Annexin Based Kits:

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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