Annexin V-Biotin Apoptosis Detection Kit

(Catalog #: K109-25, -100, -400; Store kit at 4°C; Stable for one year)

I. Introduction:

The Annexin V-Biotin Apoptosis Detection Kit 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 bind to Biotin-conjugated Annexin V, a protein that has a strong natural affinity for PS. Annexin V-Biotin can be detected in conjunction with conventional dye-staining using any streptavidine- or avidin-dye reagents, such as (strept)avidine-fluorescein, -peroxidase, -alkaline phosphatase (AP), and -β -gal, etc.

II. Kit Contents:

Components	K109-25 25 assays	K109-100 100 assays	K109-400 400 assays	Part Number
Annexin V-Biotin	125 µl	500 µl	2 ml	K109-XX(X)-1
1X Binding Buffer	12.5 ml	50 ml	2 x 100 ml	K109-XX(X)-2
Propidium Iodide (PI)	125 µl	500 µl	2 ml	K109-XX(X)-3

III. Annexin V-Biotin Assay Protocol:

A. Incubation of cells with Annexin V-Biotin

1. Induce apoptosis by desired method.
2. Collect 1-5 x 10^5 cells by centrifugation.
3. Resuspend cells in 200 µl of 1X Binding Buffer.
4. Add 5 µl of Annexin V-Biotin and 5 µl of propidium iodide (PI, optional)
5. Incubate at room temperature for 5 min in the dark.
6. Wash the cells once in 200 µl of 1X Binding Buffer. Centrifuge to remove the buffer.
7. Fix cells with 2% formaldehyde in PBS for 15 min and wash cells once with PBS. Resuspend cells in 100 µl of PBS + 1 mg/ml BSA.

   Note: Cells must be incubated with Annexin V-Biotin before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane.
8. Add 5 µg/ml of avidin-fluorescein (not provided) and incubate for 15 min.
9. Collecting cells by centrifugation and resuspend in PBS.

   Proceed to B or C below depending on method of analysis.

B. Quantification by Flow Cytometry

Analyze samples 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-Biotin (A.3-5).

C. Detection by Fluorescence Microscopy

1. Place the cell suspension from Step A.9 on a glass slide. Cover the cells with a glass coverslip. For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.9), invert coverslip on glass slide and visualize cells.
2. Observe the cells under a fluorescence microscope using a dual filter set for FITC & rhodamine.

   Cells that have bound Annexin V-Biotin and stained with (strept)avidine-FITC will show green staining in the plasma membrane. Cells which have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (FITC) on the cell surface (plasma membrane).

IV. Related Products:

Apoptosis Detection Kits & Reagents
- Annexin V Kits & Bulk Reagents
- Caspase Assay Kits & Reagents
- Mitochondrial Apoptosis Kits & Reagents
- Nuclear Apoptosis Kits & Reagents
- Apoptosis Inducers and Apoptosis siRNA Vectors

Cell Fractionation System
- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- FractionPREP Fractionation System

Cell Proliferation & Senescence
- Quick Cell Proliferation Assay Kit
- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit

Cell Damage & Repair
- HDAC & HAT Fluorometric & Colorimetric Assays & Drug Discovery Kits
- DNA Damage Quantification Kit
- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

Signal Transduction
- cAMP & cGMP Assay Kits
- Akt & JNK Activity Assay Kits

Adipocyte & Lipid Transfer
- Recombinant Adiponectin, Survivin, & Leptin
- CETP & PLTP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

Molecular Biology & Reporter Assays
- siRNA Vectors
- Cloning Insert Quick Screening Kit
- Mitochondrial & Genomic DNA Isolation Kits
- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit

Growth Factors and Cytokines
- Adiponectin/Resistin/Leptin and their Antibodies
- Recombinant Protein A and Protein G
- Recombinant Complement C5a
- Recombinant Cytokines and Growth Factors

Monoclonal and Polyclonal Antibodies

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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
	• 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.		

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