

Annexin V-Cy3 Apoptosis Detection Kit

(Catalog #: ALX-850-252; 25,100 and 400 tests Store kit at 4°C; Stable for one year)

I. Introduction:

Annexin V Apoptosis Detection Kit is based on the observation that soon after initiating apoptosis, cells translocate the membrane phosphatidyl-serine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can be easily detected by staining with a fluorescent conjugate of Annexin V, a protein that has a high affinity for PS. The one-step staining procedure takes only 10 minutes. Detection can be analyzed by flow cytometry or by fluorescence microscopy.

II. Kit Contents:

Components	ALX-850-252-K101	ALX-850-252-K102	ALX-850-252-K103	Part Number
	25 assays	100 assays	400 assays	
Annexin V-Cy3	125 µl	500 µl	2 ml	ALX-K102-XX(X)-1
1X Binding Buffer	12.5 ml	50 ml	2 x 100 ml	ALX-K102-XX(X)-2

III. Annexin V-Cy3 Assay Protocol:

A. Incubation of cells with Annexin V-Cy3

1. Induce apoptosis by desired method.
2. Collect 1-5 x 10⁵ cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Binding Buffer.
4. Add 5 µl of Annexin V-Cy3.
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-Cy3 binding by flow cytometry (Ex = 543 nm; Em = 570 nm) using the phycoerythrin emission signal detector (usually FL2).

For analyzing adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-Cy3 (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. (Cells must be incubated with Annexin V-Cy3 before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane.)
2. Observe the cells under a fluorescence microscope using a rhodamine filter. Cells that have bound Annexin V-Cy3 will show red staining in the plasma membrane.

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 Set
- Apoptosis siRNA Vectors

Cell Fractionation System

- Mitochondria/Cytosol Fractionation Kit
- Nuclear/Cytosol Fractionation Kit
- Membrane Protein Extraction Kit
- Cytosol/Particulate Rapid Separation Kit
- Mammalian Cell 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
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining 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
- Beta-Secretase Activity Assay Kit

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
- β-Galactosidase Staining Kit & Luciferase Reporter Assay 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

GENERAL TROUBLESHOOTING GUIDE FOR ANNEXIN BASED KITS:

Problems	Cause	Solution
High Background	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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.		