

# Annexin V-Cy3 Apoptosis Detection Kit Plus

(Catalog #: ALX-850-257, 25 tests, 100 tests; Store kit at 4°C)

## I. Introduction:

The assay is based on the observation that soon after initiating apoptosis, cells 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 be detected by staining with a fluorescent conjugate of Annexin V, a protein that has a strong natural affinity for PS. The one-step staining procedure takes only 10 minutes. In addition, the assay can be directly performed on live cells, without the need of 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. After staining a cell population with annexin V-Cy3 and SYTOX Green dye in the provided binding buffer, apoptotic cells show red fluorescence, dead cells show green fluorescence and live cells show little or no fluorescence. These populations can easily be distinguished by Fluorescence 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).

## II. Kit Contents:

Component	ALX-850-257-25	ALX-850-257-100	Part Number
	25 assays	100 assays	
Annexin V-Cy3	125 µl	500 µl	ALX-K202-xx-1
SYTOX Green Dye	25 µl	100 µl	ALX-K202-xx-2
Binding Buffer	12.5 ml	50 ml	ALX-K202-xx-3

## 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<sup>5</sup> 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°C. All reagents are stable for one year under proper storage conditions.

## V. 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

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 and 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
- Luciferase and Beta-Galactosidase Assay Kits

Growth Factors and Cytokines

Monoclonal and Polyclonal Antibodies

**GENERAL TROUBLESHOOTING GUIDE FOR ANNEXIN BASED KITS:**

<b>Problems</b>	<b>Cause</b>	<b>Solution</b>
<b>High Background</b>	<ul style="list-style-type: none"> <li>• Cell density is higher than recommended</li> <li>• Increased volumes of components added</li> <li>• Incubation of cell samples for extended periods</li> <li>• Use of extremely confluent cells</li> <li>• Contaminated cells</li> </ul>	<ul style="list-style-type: none"> <li>• Refer to datasheet and use the suggested cell number</li> <li>• Use calibrated pipettes accurately</li> <li>• Refer to datasheets and incubate for exact times</li> <li>• Perform assay when cells are at 80-95% confluency</li> <li>• Check for bacteria/ yeast/ mycoplasma contamination</li> </ul>
<b>Lower signal levels</b>	<ul style="list-style-type: none"> <li>• Washing cells with PBS before/after fixation (adherent cells)</li> <li>• Cell lysate contains interfering substances</li> <li>• Cells did not initiate apoptosis</li> <li>• Very few cells used for analysis</li> <li>• Incorrect setting of the equipment used to read samples</li> <li>• Use of expired kit or improperly stored reagents</li> </ul>	<ul style="list-style-type: none"> <li>• Always use binding buffer for washing cells</li> <li>• Use the cell lysis buffer in the kit or refer datasheet for instructions</li> <li>• Determine the time-point for initiation of apoptosis after induction (time-course experiment)</li> <li>• Refer to data sheet for appropriate cell number</li> <li>• Refer to datasheet and use the recommended filter setting</li> <li>• Always check the expiry date and store the components appropriately</li> </ul>
<b>Erratic results</b>	<ul style="list-style-type: none"> <li>• Uneven number of cells seeded in the wells</li> <li>• Adherent cells dislodged at the time of experiment</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> <li>• Increased or random staining observed in adherent cells</li> </ul>	<ul style="list-style-type: none"> <li>• Seed only healthy cells (correct passage number)</li> <li>• Perform experiment gently and in duplicates or triplicates for each treatment</li> <li>• Refer to datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> <li>• Always stain cells with Annexin before fixation (makes cell membrane leaky)</li> </ul>
<b>Note#</b> The most probable cause is listed under each section. Causes may overlap with other sections.		