

# Annexin V-Cy5

(Catalog #: K103-25, -100, -400; Store kit at 4°C)

## 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	K103-25	K103-100	K103-400	Part Number
	25 assays	100 assays	400 assays	
Annexin V-Cy5	125 µl	500 µl	2 ml	K103-XX(X)-1
1X Binding Buffer	12.5 ml	50 ml	2 x 100 ml	K103-XX(X)-2

## III. Assay Protocol:

### A. Incubation of Cells with Annexin V-Cy5:

1. Induce apoptosis by desired methods.
2. Collect 1-5 x 10<sup>5</sup> cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Annexin V Binding Buffer.
4. Add 5 µl of Annexin V-Cy5.
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 cells by flow cytometry (Ex = 649 nm; Em = 670 nm) using a Helium-Neon Laser.

For adherent cells, trypsinize and gently wash cells with serum-containing medium before incubation with Annexin V-Cy5 (A.3-5).

### C. Detection by Fluorescence Microscopy:

1. Place the cell suspension from Step A.5 on a glass slide, and cover with a glass coverslip.  
For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.5), invert coverslip on a glass slide and visualize cells. The cells can also be washed with 1X Annexin V binding Buffer and fixed in 2% formaldehyde before visualization. (Cells must be incubated with Annexin V-Cy5 before fixation because 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 Cy5 filter, or FITC/Cy3/Cy5 triple band filter (Chroma Technology) if performing triple labeling using these dyes, or detect cells using a CCD camera.  
Cells that have bound Annexin V-Cy5 will show bright red-blue staining on the plasma membrane.

## 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:**

<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>• 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>• 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.		