Annexin V-PE-Cy5 Apoptosis Detection Kit

(Catalog #: K129-25, -100, -400; Store kit at 4˚C)

I. Introduction:

The Annexin V-PE-Cy5 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 be easily 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 for fixation. Results can be analyzed by flow cytometry or fluorescence microscopy.

II. Kit Contents:

Components	K129-25 25 assays	K129-100 100 assays	K129-400 400 assays	Part Number
Annexin V-PE-Cy5 1X Binding Buffer	125 µl 12.5 ml	500 µl 50 ml	2 ml 2 x 100 ml	K129-XX(X)-1 K129-XX(X)-2

III. Annexin V-PE-Cy5 Assay Protocol:

A. Incubation of cells with Annexin V-PE-Cy5

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-PE-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 Annexin V-PE-Cy5 binding by flow cytometry (Ex = 488 nm; Em = 670 nm).

For analyzing adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-PE-Cy5 (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-PE-Cy5 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 the PE-Cy5 filter.

   Cells which have bound Annexin V-PE-Cy5 will show bright purple staining in the plasma membrane.

IV. Storage and stability:

Store kit at 4˚C. All reagents are stable for one year under proper storage conditions.

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 Fluorometric & Colorimetric Assays & Drug Discovery Kits
- HAT Colorimetric Assay Kit & Reagents
- 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 Activity Assay & Drug Discovery Kits
- 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

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BioVision Research Products
980 Linda Vista Avenue, Mountain View, CA 94043 USA

Tel: 650-428-0236 • Fax: 650-428-0336 • www.biovision.com
Email: tech@biovision.com

GENERAL TROUBLESHOOTIN

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.		

BioVision Research Products
980 Linda Vista Avenue, Mountain View, CA 94043 USA

Tel: 650-428-0236 • Fax: 650-428-0336 • www.biovision.com
Email: tech@biovision.com