

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	K129-100	K129-400
	25 assays	100 assays	400 assays
Annexin V-PE-Cy5	125 µl	500 µl	2 ml
1X Binding Buffer	12.5 ml	50 ml	2 x 100 ml

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 10⁵ 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:

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- 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

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- Senescence Detection Kit
- High Throughput Apoptosis/Cell Viability Assay Kits
- LDH-Cytotoxicity Assay Kit
- Bioluminescence Cytotoxicity Assay Kit
- Live/Dead Cell Staining Kit

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- HAT Colorimetric Assay Kit & Reagents
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- Glutathione & Nitric Oxide Fluorometric & Colorimetric Assay Kits

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- Akt & JNK Activity Assay Kits
- Beta-Secretase Activity Assay Kit

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- CETP Activity Assay & Drug Discovery Kits
- PLTP Activity Assay & Drug Discovery Kits
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- 5 Minutes DNA Ligation Kit
- 20 Minutes Gel Staining/Destaining Kit
- β-Galactosidase Staining Kit & Luciferase Reporter Assay Kit

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