For research use only

Annexin V-Cy3 Reagent

CATALOG #: GWB-AXR006 400 assays

STORAGE CONDITIONS: Store at 4°C. Do not freeze.

SHELF LIFE: 1 year under proper storage conditions

DESCRIPTION:
The bright red color fluorescent reagent for detecting of the early stages of apoptosis. During apoptosis, phosphatidylserine (PS) is translocated from the cytoplasmic face of the plasma membrane to the cell surface. Annexin V has a strong, Ca^{2+}-dependent affinity for PS and therefore serves as a probe for detecting apoptosis. The Annexin V-Cy3 conjugate contains the bright red fluorescent probe that can be used for detection of apoptosis by fluorescence microscopy with a rhodamine filter or by flow cytometry. Cy3 yields red fluorescence with a λ_{max} emission of 570 nm.

ASSAY PROTOCOL:

A. Incubation of cells with Annexin V-Cy3:

1. Induce apoptosis by desired methods.
2. Collect 1 x 10^5 cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Annexin V Binding Buffer
4. Add 1 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 cells by flow cytometry (Ex = 543 nm; Em = 570 nm) using FL2 channel. For adherent cells, trypsinize and gently wash cells with serum-containing medium 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, 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-Cy3 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 a rhodamine filter.

   Cells that have bound Annexin V-Cy3 will show bright red staining on the plasma membrane.

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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

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- 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

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

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- PLTP Activity Assay & Drug Discovery Kits
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