

Annexin V-FITC Reagent

CATALOG #: 1001-200 200 assays (0.2 ml)
1001-1000 1000 assays (1.0 ml)

STORAGE CONDITIONS: Store at 4°C. Do not freeze. Stable for 1 year.

FORMULATION: 0.15 mg/ml in PBS containing 1% BSA, 0.03% Proclin.

DESCRIPTION:

Reagent for detecting 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 is used as a probe for detecting apoptosis. The Annexin V-FITC conjugate can be used for detection of apoptosis by fluorescence microscopy or by flow cytometry.

ASSAY PROTOCOL:

A. Incubation of cells with Annexin V-FITC:

1. Induce apoptosis by desired methods.
2. Collect 1×10^5 cells by centrifugation.
3. Resuspend cells in 500 μ l of 1X Annexin V Binding Buffer (Cat.#: 1035-100).
4. Add 1 μ l of Annexin V-FITC and 1 μ l Propidium Iodide (PI, Cat.#: 1056-1).
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. = 488 nm; Em. = 530 nm) using FL1 channel for detecting Annexin V-FITC staining and FL2 channel for detecting PI staining.

For adherent cells, trypsinize and gently wash cells with serum-containing medium before incubation with Annexin V-FITC (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-FITC 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 dual filter set for FITC and rhodamine, or separate filters.

Cells that have bound Annexin V-FITC will show green staining on the plasma membrane. Cells that have lost membrane integrity will show red PI staining throughout the nuclei and a halo of green staining (FITC) on the plasma membrane.

TESTING RESULTS:

Jurkat cells (treated with 2 μ M camptothecin for 6 hours) were collected for annexin V assay according to the kit instructions. Results show 40-60% apoptotic cells as analyzed by flow cytometry.

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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