

## Annexin V-EGFP Reagent

**CATALOG #:** 1004-200 200 assays  
1004-1000 1000 assays

**LOT #:** \_\_\_\_\_

**STORAGE COITIONS:** Store at 4°C (short term), -20°C (long term).

**SHELF LIFE:** 1 year under proper storage conditions

### DESCRIPTION:

The bright and photo-stable 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 serves as a probe for detecting apoptosis. The annexin V-EGFP fusion is brighter and photo-stable, ideal for detecting apoptosis by fluorescence microscopy with a FITC filter or by flow cytometry.

### ASSAY PROTOCOL:

#### A. Incubation of cells with Annexin V-EGFP

1. Induce apoptosis by desired method.
2. Collect 1-5 x 10<sup>5</sup> cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Binding Buffer (Cat.# 1035-100).
4. Add 1 µl of Annexin V-EGFP and 1 µl of 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 Annexin V-EGFP binding by flow cytometry (Ex = 488 nm; Em = 530 nm) using FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2).

For adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-EGFP (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.

Note: Cells must be incubated with Annexin V-EGFP 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 a dual filter set for FITC & rhodamine. Cells which have bound Annexin V-EGFP will show green staining in the plasma membrane. Cells which have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (EGFP) on the cell surface (plasma membrane).

### 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
- $\beta$ -Galactosidase Staining Kit & Luciferase Reporter Assay Kit

#### Growth Factors and Cytokines

#### Monoclonal and Polyclonal Antibodies