

## Annexin V-FITC Apoptosis Detection Kit Plus

(Catalog #: ALX-850-255; 25 and 100 assays Store kit at 4°C)

### I. Introduction:

The Annexin V-FITC Apoptosis Detection Kit Plus 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 easily be 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 of fixation. The Annexin V-FITC Apoptosis Detection Kit Plus includes annexin V-FITC, SYTOX green dye, and binding buffer. The SYTOX green dye is impermeant to live cells and apoptotic cells, but stains necrotic cells with intense green fluorescence by binding to cellular nucleic acids. After staining a cell population with annexin V-FITC and SYTOX Green dye in the provided binding buffer, apoptotic cells show green fluorescence, dead cells show a higher level of green fluorescence and live cells show little or no fluorescence. These populations can easily be distinguished using a flow cytometry with the 488 nm line of an argon-ion laser for excitation. Both annexin V-FITC and SYTOX Green dye emit green fluorescence that can be detected in the FL1 channel, freeing the other channels for the addition of other probes in multi-color labeling experiments.

### II. Kit Contents:

| Component       | ALX-850-255- | ALX-850-255- | Part Number      |
|-----------------|--------------|--------------|------------------|
|                 | K101         | K102         |                  |
|                 | 25 assays    | 100 assays   |                  |
| Annexin V-FITC  | 125 µl       | 500 µl       | ALX-K201-xx(x)-1 |
| SYTOX Green Dye | 25 µl        | 100 µl       | ALX-K201-xx(x)-2 |
| Binding Buffer  | 12.5 ml      | 50 ml        | ALX-K201-xx(x)-3 |

### II. Annexin V-EGFP Assay Protocol:

1. Induce apoptosis by desired method. Concurrently incubate a control culture without induction.
  2. Collect  $1-5 \times 10^5$  cells by centrifugation.
  3. Resuspend cells in 500 µl of 1X Binding Buffer.
  4. Add 5 µl of Annexin V-FITC and 1 µl of SYTOX Green dye
- Note:** Thaw the SYTOX Green dye in room temperature before use.
5. Incubate at room temperature for 5-10 min in the dark.
  6. Analyze the stained cells by flow cytometry (Ex = 488 nm; Em = 530 nm).

The cell population should separate into three groups: live cells with only a low level of fluorescence, apoptotic cells with moderate green fluorescence and necrotic cells with high-intensity green fluorescence.

For adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-FITC and SYTOX dye.

### 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 & HAT Fluorometric & Colorimetric Assays & Drug Discovery
- 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 & PLTP Activity Assay & Drug Discovery Kits
- Total Cholesterol Quantification Kit

#### Molecular Biology & Reporter Assays

- siRNA Expression 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

#### Growth Factors and Cytokines

#### Monoclonal and Polyclonal Antibodies

**GENERAL TROUBLESHOOTING GUIDE FOR ANNEXIN BASED KITS:**

| <b>Problems</b>   | <b>Cause</b>  | <b>Solution</b>   |
|---|---|---|
| <b>High Background</b>  | <ul style="list-style-type: none"> <li>• Cell density is higher than recommended</li> <li>• Increased volumes of components added</li> <li>• Incubation of cell samples for extended periods</li> <li>• Use of extremely confluent cells</li> <li>• Contaminated cells</li> </ul>   | <ul style="list-style-type: none"> <li>• Refer to datasheet and use the suggested cell number</li> <li>• Use calibrated pipettes accurately</li> <li>• Refer to datasheets and incubate for exact times</li> <li>• Perform assay when cells are at 80-95% confluency</li> <li>• Check for bacteria/ yeast/ mycoplasma contamination</li> </ul>  |
| <b>Lower signal levels</b>  | <ul style="list-style-type: none"> <li>• Washing cells with PBS before/after fixation (adherent cells)</li> <li>• Cell lysate contains interfering substances</li> <li>• Cells did not initiate apoptosis</li> <li>• Very few cells used for analysis</li> <li>• Incorrect setting of the equipment used to read samples</li> <li>• Use of expired kit or improperly stored reagents</li> </ul> | <ul style="list-style-type: none"> <li>• Always use binding buffer for washing cells</li> <li>• Use the cell lysis buffer in the kit or refer datasheet for instructions</li> <li>• Determine the time-point for initiation of apoptosis after induction (time-course experiment)</li> <li>• Refer to data sheet for appropriate cell number</li> <li>• Refer to datasheet and use the recommended filter setting</li> <li>• Always check the expiry date and store the components appropriately</li> </ul> |
| <b>Erratic results</b>  | <ul style="list-style-type: none"> <li>• Uneven number of cells seeded in the wells</li> <li>• Adherent cells dislodged at the time of experiment</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> <li>• Increased or random staining observed in adherent cells</li> </ul>   | <ul style="list-style-type: none"> <li>• Seed only healthy cells (correct passage number)</li> <li>• Perform experiment gently and in duplicates or triplicates for each treatment</li> <li>• Refer to datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> <li>• Always stain cells with Annexin before fixation (makes cell membrane leaky)</li> </ul>  |
| <p><b>Note#</b> The most probable cause is listed under each section. Causes may overlap with other sections.</p> |   |   |