

Annexin V-FITC Apoptosis Detection Kit Plus

(Catalog #: K201-25, -100, -400; 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:

Components	K201-25	K201-100	K201-400
	25 assays	100 assays	400 assays
Annexin V-FITC	125 µl	500 µl	2 ml
SYTOX Green Dye	25 µl	100 µl	400 µl
Binding Buffer	12.5 ml	50 ml	2 x 100 ml

III. Annexin V-FITC Plus Assay Protocol:

1. Induce apoptosis by desired method. Concurrently incubate a control culture without induction.
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-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.

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- 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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- 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

Adipocyte & Lipid Transfer

- Recombinant Adiponectin, Survivin, & Leptin
- CETP & PLTP Activity Assay & Drug Discovery Kits
- 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

Growth Factors and Cytokines

- Adiponectin/Resistin/Leptin and their Antibodies
- Recombinant Protein A and Protein G
- Recombinant Cytokines and Growth Factors
- Recombinant Complement C5a

Monoclonal and Polyclonal Antibodies