



50 tests

ApoDETECT™

Annexin V-FITC Kit

Catalog No.: 33-1200

Lot No. See product label

ApoDETECT™ ANNEXIN V-FITC KIT

BACKGROUND

In normal cells phosphatidylserine (PS) is located on the inner leaflet of the plasma membrane. During the early stages of apoptosis, PS is translocated to the outer layer and is exposed on the external surface of the cell. This early event in apoptosis can be detected by using a sensitive method in which to detect PS exposure. Translocation of PS to the external surface of the plasma membrane is not a unique property of apoptotic cells, as this phenomenon also occurs during cell necrosis. However, during apoptosis, the cell membrane remains intact; whereas, during necrosis, the cell becomes leaky and loses its integrity. Therefore, it is necessary to assess membrane integrity together with PS translocation.

Annexin V is a Ca²⁺-dependent phospholipid binding protein. This protein can bind to a variety of phospholipids, but it has the highest affinity for phosphatidylserine (PS). Based on its affinity for PS, Annexin V can be utilized as a sensitive probe for cell surface exposure of PS. To use the Annexin V protein as a probe for apoptotic cells, the protein has been labeled with fluorescein isothiocyanate (FITC). In this form, the protein can be used directly for quantification of apoptotic cells. The measurement of Annexin V binding when performed simultaneously with a dye exclusion test (such as propidium iodide) can be used to effectively discriminate between apoptotic and necrotic cells.

REAGENTS PROVIDED:

1. ANNEXIN V-FITC

QUANTITY: 0.5 ml. Sufficient for 50 tests.

SOURCE: E. coli

MOLECULAR WEIGHT: 35.8 kDa

FORM:

Liquid. Solution contains 50 mM TRIS, 100 mM NaCl, 1% BSA, 0.01% Sodium Azide, pH 7.4.

PURITY:

>98% pure as demonstrated by SDS-gel electrophoresis and reverse phase HPLC.

2. BINDING BUFFER

QUANTITY: 16 ml (2X Concentrate). Dilute in deionized water.

FORM: Liquid. After dilution-10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂.

3. PROPIDIUM IODIDE

QUANTITY: 20 µg/ml (0.5 ml).

(cont'd)

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APPLICATIONS:

Annexin V can be used to detect apoptotic cells by flow cytometry or immunofluorescent cytology.

Recommended Procedure:

- Spin cells down, 1 minute @ 3,000 rpm, decant supernatant.
- Wash cells in ice cold PBS, pH 7.4. Gently resuspend pellet.
- Spin cells down, 1 minute @ 3,000 rpm, decant supernatant, remove excess PBS with pipettor.
- Resuspend cells in 1X binding buffer (10 mM Hepes/NaOH, pH, 7.4, 140 mM NaCl, 2.5 mM CaCl₂, filtered through 0.2 μm pore filter); adjust cell density to 2-5 x 10⁵ cells/ml.
- Add 10 μl of Annexin V-FITC to 190 μl of cell suspension. Mix gently.
- Incubate 10 min at room temperature.
- Wash cells 1X with binding buffer. Spin cells down, 1 minute @ 3,000 rpm, decant supernatant, and resuspend in 190 μl binding buffer.
- Add 10 μl of 20 μg/ml propidium iodide stock solution.
- Analyze cells by flow cytometry or fluorescent microscopy.

STORAGE:

Store at 2-8°C. After opening Annexin V-FITC, aliquot contents and freeze at -20°C.

For research use only. CAUTION: Not intended for human or animal therapeutic or diagnostic use.

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