

SIZE: BV-1001-13 200 assays (0.2 ml)
BV-1001-5 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, Ca²⁺-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 x 10⁵ cells by centrifugation.
3. Resuspend cells in 500 µl of 1X Annexin V Binding Buffer (Code.#:BV-1035-3).
4. Add 1 µl of Annexin V-FITC and 1 µl Propidium Iodide (PI, Code.#: JM-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 µM 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.