

Annexin V detection protocol for apoptosis

Procedure for early detection of apoptosis using annexin V.

The appearance of phosphatidylserine (PS) residues (normally hidden within the plasma membrane) on the surface of the cell is an early event in apoptosis, and can also be used to detect and measure apoptosis. During apoptosis, 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 can be used as a probe for detecting apoptosis.

Example protocol – Protocol for annexin V-FITC apoptosis detection kit (ab14085)

A. Incubation of cells with annexin V-FITC

1. Induce apoptosis by desired method.
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 5 µL of propidium iodide (PI, optional).
5. Incubate at room temperature for 5 min in the dark.
6. Proceed to B or C below depending on method of analysis.

B. Quantification by flow cytometry

Analyze annexin V-FITC binding by flow cytometry (Ex = 488 nm; Em = 350 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-FITC (Steps 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 (Step A.5), invert coverslip on a glass slide and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization. (Cells must be incubated with annexin V-FITC before fixation since any cell membrane disruption can cause non-specific 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. Cells that have bound annexin V-FITC will show green staining in the plasma membrane. Cells that have lost membrane integrity will show red staining (PI) throughout the nucleus and a halo of green staining (FITC) on the cell surface (plasma membrane).

The above is an example protocol for PS exposure detection using annexin V, based on the protocol provided in [Annexin V-FITC Apoptosis Detection Kit \(ab14085\)](#). Please be aware that, when using a kit, you should always use the protocol provided as it has been specifically designed for the product.