



Annexin V-Cy5 Apoptosis Detection Kit

Manufactured by BioVision.

ALX-850-254-KI01: ~25 tests
ALX-850-254-KI02: ~100 tests

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For laboratory use only. Not for human or diagnostic use.

I. Introduction:

The **Annexin V-Cy5 Apoptosis Detection Kit** is based on the observation that soon after initiating apoptosis, cells translocate the membrane phosphatidyl-serine (PS) from the inner face of the plasma membrane to the cell surface. Once on the cell surface, PS can be easily detected by staining with a fluorescent conjugate of Annexin V, a protein that has a high affinity for PS. The one-step staining procedure takes only 10 minutes. Detection can be analyzed by flow cytometry or by fluorescence microscopy.

II. Kit Contains:

Components:	ALX-850-254-KI01	ALX-850-254-KI02
		25 tests
Annexin V-Cy5	125 μ l	500 μ l
1x Binding Buffer	12.5 ml	50 ml

III. Assay Procedure:

A. Incubation of Cells with Annexin V-Cy5:

1. Induce apoptosis by desired methods.
2. Collect $1-5 \times 10^5$ cells by centrifugation.
3. Resuspend cells in 500 μ l of 1x Annexin V Binding Buffer.
4. Add 5 μ l of Annexin V-Cy5.
5. Incubate at room temperature for 5 minutes 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 = 649 nm; Em = 670 nm) using a Helium-Neon Laser.

For adherent cells, trypsinize and gently wash cells with serum-containing medium before incubation with Annexin V-Cy5 (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 1 x Annexin V Binding Buffer and fixed in 2% formaldehyde before visualization. (Cells must be incubated with Annexin V-Cy5 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 Cy5 filter, or FITC/Cy3/Cy5 triple band filter (Chroma Technology) if performing triple labeling using these dyes, or detect cells using a CCD camera.
Cells that have bound Annexin V-Cy5 will show bright red-blue staining on the plasma membrane

IV. Storage:

- Store kit at +4°C