



## **Annexin V-PE Apoptosis Detection Kit**

*Manufactured by BioVision.*

**ALX-850-256-KI01: ~25 tests**  
**ALX-850-256-KI02: ~100 tests**

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

## I. Introduction:

The **Annexin V-PE Apoptosis Detection Kit** 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 be easily 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 for fixation. The Annexin V-PE Apoptosis Detection Kit contains the bright orange-red PE fluorescent probe that can be easily detected by flow cytometry or fluorescence microscopy.

## II. Kit Contents:

Components	850-256-KI01	850-256-KI02
	25 tests	100 tests
Annexin V-PE 1x Binding Buffer	125 µl 12.5 ml	500 µl 50 ml

## III. Annexin V-PE Assay Protocol:

### A. Incubation of cells with Annexin V-PE

1. Induce apoptosis by desired method.
  2. Collect  $1-5 \times 10^5$  cells by centrifugation.
  3. Resuspend cells in 500 µl of 1x Binding Buffer.
  4. Add 5 µl of Annexin V-PE.
  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 Annexin V-PE binding by flow cytometry (Ex = 488 nm; Em = 578 nm) using the phycoerythrin emission signal detector (usually FL2).

For analyzing adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-PE (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 (A.5), invert coverslip on 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-PE before fixation since 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 rhodamine filter.  
Cells which have bound Annexin V-PE will show orange-red staining in the plasma membrane.

**IV. Storage and stability:**

Store kit at 4°C.