



# Annexin V-Biotin Apoptosis Detection Kit

*Manufactured by BioVision.*

**ALX-850-251-KI01: ~25 tests**  
**ALX-850-251-KI02: ~100 tests**  
**ALX-850-251-KI03: ~400 tests**

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

## I. Introduction:

The **Annexin V-Biotin 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 easily bind to Biotin-conjugated annexin V, a protein that has a strong natural affinity for PS. Annexin V-Biotin can be detected in conjunction with conventional dye-staining using any streptavidine- or avidin-dye reagents, such as (strept)avidine-fluorescein, -peroxidase, -alkaline phosphatase (AP), and - $\beta$ -gal, etc.

## II. Kit Contents:

Components	850-251-KI01	850-251-KI02	850-251-KI01
	25 tests	100 tests	400 tests
Annexin V-Biotin	125 $\mu$ l	500 $\mu$ l	2 ml
1x Binding Buffer	12.5 $\mu$ l	50 ml	2 x 100 ml
Propidium Iodide (PI)	125 $\mu$ l	500 $\mu$ l	2 ml

## III. Annexin V-Biotin Assay Protocol

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

1. Induce apoptosis by desired method.
  2. Collect  $1-5 \times 10^5$  cells by centrifugation.
  3. Resuspend cells in 200  $\mu$ l of 1x Binding Buffer.
  4. Add 5  $\mu$ l of Annexin V-Biotin and 5  $\mu$ l of Propidium Iodide (PI, optional)
  5. Incubate at room temperature for 5 minutes in the dark.
  6. Wash the cells once in 200  $\mu$ l of 1x Binding Buffer. Centrifuge to remove the buffer.
  7. Fix cells with 2% formaldehyde in PBS for 15 minutes and wash cells once with PBS.  
Resuspend cells in 100  $\mu$ l of PBS + 1 mg/ml BSA.
- Note:** Cells must be incubated with Annexin V-Biotin 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.
8. Add 5  $\mu$ g/ml of avidin-fluorescein (not provided) and incubate for 15 minutes.
  9. Collect cells by centrifugation and resuspend in PBS.  
Proceed to B or C below depending on method of analysis

### B. Quantification by Flow Cytometry

Analyze samples by Flow Cytometry (Ex = 488 nm; Em = 530 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-Biotin (A.3-5).

### **C. Detection by Fluorescence Microscopy**

1. Place the cell suspension from Step A.9 on a glass slide. Cover the cells with a glass coverslip.

For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.9), invert coverslip on glass slide and visualize cells.

2. Observe the cells under a fluorescence microscope using a dual filter set for FITC & rhodamine.

Cells that have bound Annexin V-Biotin and stained with (strept)avidine-FITC will show green staining in the plasma membrane.

Cells which 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).

### **IV. Storage and Stability:**

- Store kit at +4°C.
- All reagents are stable for at least 1 year under proper storage conditions.