

## Annexin-V-Biotin

Cat. No. 11 828 690 001 250 tests

<b>Type</b>	Indirect fluorescence staining for flow cytometric, fluorescence or light microscopic analysis
<b>Useful for</b>	Detection of apoptotic cells with membrane alterations (phosphatidylserine translocation); differentiation of apoptotic from necrotic cells
<b>Samples</b>	Cell lines (adherent and suspensions), freshly isolated cells
<b>Method</b>	Simultaneous staining of cell surface phosphatidylserine (with Annexin-V-Biotin) and necrotic cells (with propidium iodide), followed by detection of biotin (with streptavidin/avidin conjugate)
<b>Time</b>	Approx. 75 min (after induction of apoptosis)

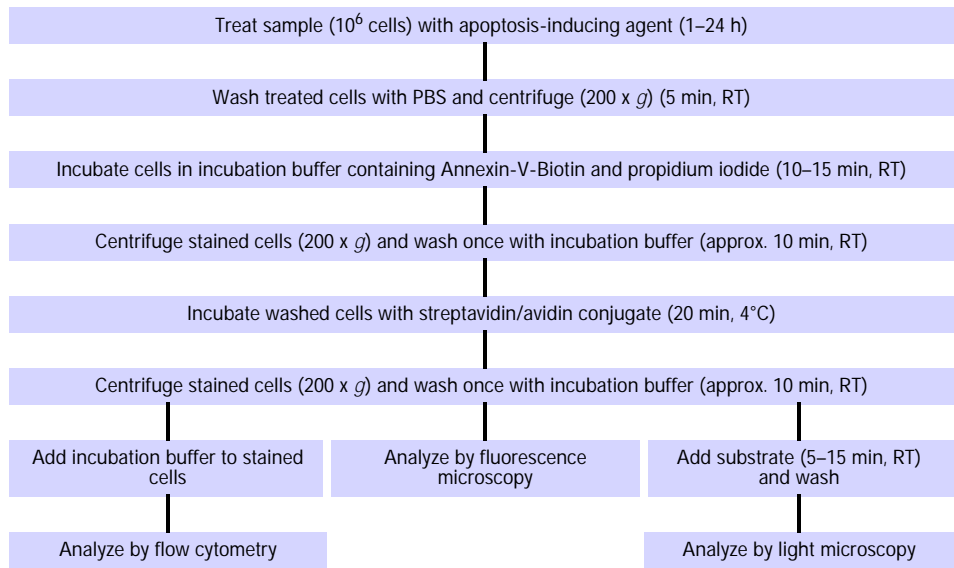
**Significance of reagent:** Annexin-V is a phospholipid-binding protein with a high affinity for phosphatidylserine (PS). During apoptosis, PS translocates to the outer surface of apoptotic cells. Detection of cell-surface PS with annexin-V thus serves as a marker for apoptotic cells. Labeling of cells with the Biotin-conjugate of Annexin-V allows fixation after Annexin-V binding for further analysis of additional cellular parameters in combination with detection of apoptosis (van Engeland, M., Ramaekers FCS, Schutte, B & Reutelingsperger, CPM (1996): A Novel Assay to Measure Loss of Plasma Membrane Asymmetry During Apoptosis of Adherent Cells in Culture. *Cytometry* 24: 131–139). For distinguishing apoptosis using Annexin-V, see Table 7, page 45.

**Test principle:** Annexin-V-Biotin serves as a probe for apoptotic cells. It will not bind normal, intact cells. However, since necrotic cells are leaky enough to give Annexin-V-Biotin access to inner membrane PS, apoptotic cells have to be differentiated from necrotic cells. Thus, the assay involves simultaneous staining with both Annexin-V-Biotin, Avidin-Fluorescein and propidium iodide. Exclusion of propidium iodide, coupled with binding of Annexin-V-Biotin, indicates an apoptotic cell. Annexin-V-Biotin is visualized with a streptavidin/avidin conjugate. Analysis may be by flow cytometry, by fluorescence microscopy, or by light microscopy. The procedure (Flow Chart 10) involves:

- 1 Washing suspended cells, then pelleting the cells.
- 2 Resuspending cells in a staining solution containing Annexin-V-Biotin and propidium iodide.  
*Note:* Cells may also be labeled with other membrane stains, such as a fluorescein-, phycoerythrin- or TRITC-labeled monoclonal antibody simultaneously.
- 3 Washing labeled cells.
- 4 Incubating cells with a streptavidin (SA)/avidin conjugate (Table 8).
- 5 Analyzing samples in a flow cytometer, under a fluorescence microscope, or under a light microscope (depending on the SA/avidin conjugate).

**Specificity:** Annexin-V-Biotin binds apoptotic cells and leaky necrotic cells





▲ Flow Chart 10: Assay procedure, Annexin-V-Biotin.

**Can be used to assay:**

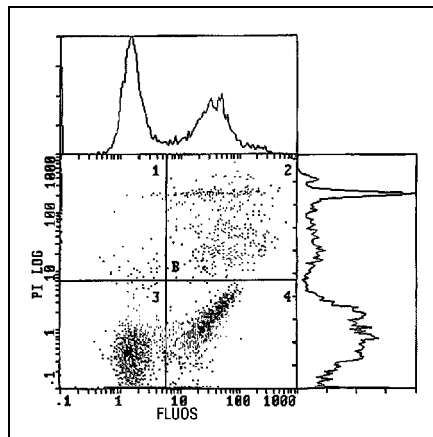
- Cell lines (adherent/suspensions)
- Freshly isolated cells

**Reagent contents:**

- Annexin-V-Biotin solution, 50 x concentrated.

**Typical results:** see Figure 36.

**Other applications:** For more examples of how the Annexin-V-Biotin can be used in the lab, see Appendix, pages 141–142.



◀ **Figure 36: Flow cytometric analysis of apoptotic U937 cells stained with Annexin-V-Biotin, Avidin-FLUOS and propidium iodide.** U937 cells (a leukemic cell line) were cultivated for 4 h with 4 µg/ml camptothecin. Cells were stained with Annexin-V-Biotin and propidium iodide (PI), then incubated with Avidin-fluorescein and analyzed. Single parameter histograms are shown at the top (Annexin-V-Biotin/Avidin-FLUOS) and on the right side (PI) of the diagram. Two parameter histograms are shown in quadrants 1–4. PI, propidium iodide; FLUOS, fluorescein.

**Result:** Flow cytometric analysis clearly differentiates normal cells (quadrant 3) with low FLUOS and low PI staining, apoptotic cells (quadrant 4) with high FLUOS and low PI staining, and necrotic cells (quadrant 2) with high FLUOS and high PI staining.

Product	Application	Cat. No.	Pack Size
Avidin-Fluorescein	fluorescence microscopy, flow cytometry	11 975 595 001	1 mg
Avidin-Rhodamine	fluorescence microscopy, flow cytometry	11 975 609 001	1 mg
SA-Peroxidase	light microscopy	11 089 153 001	500 U (1 ml)
SA-Alkaline Phosphatase	light microscopy	11 089 161 001	1000 U (1 ml)
SA-β-Galactosidase	light microscopy	11 112 481 001	500 U

▲ Table 8: Streptavidin (SA) /Avidin conjugates available for the indirect assay of apoptotic cells with Annexin-V-Biotin.

**Note:** Additional substrates can be found in Table 6.