

## Annexin V Biotin-conjugated

**Biotin** - conjugated recombinant Annexin V

Cat-No: **31490012X2**

2 ml for 400 tests

**please note: store at 4°C**

Biotin-conjugated recombinant chicken Annexin V (AxV) for the detection of phosphatidylserine exposed in the membrane of apoptotic cells. There is a 85 % homology of recombinant chicken Annexin V to the human Annexin V and a 100 % identity in the phosphatidylserine binding sites. Annexin V-Biotin binding to PS is  $\text{Ca}^{2+}$  dependent.

**Introduction:** Apoptosis and necrosis are the two main forms of cell death. Apoptosis is mostly a physiological process and plays an essential role in the development and homeostasis of all multi-cellular organisms. Apoptosis can be induced by several stimuli like UV- and gamma-irradiation or DNA damaging substances. Apoptotic cells change the structure of their membrane, which leads to the exposure of phosphatidylserine (PS) on the membrane surface. Annexins are ubiquitous homologous proteins that bind phospholipids in the presence of calcium. Since the redistribution of phosphatidylserine from the internal to the external membrane surface represents an early indicator of apoptosis, Annexin V and its conjugates can be used for the detection of apoptosis because they interact strongly and specifically with exposed phosphatidylserine. Detection of apoptotic cells with Annexin V can be achieved earlier than analysis of apoptosis by DNA-based assays.

**Buffer/Additives/Preservative:** Each vial contains fluorescein conjugated annexin with 0.1 % BSA in PBS.  
Preservative: 0.09 % w/v sodium azide.

**Application:** An early event in apoptosis is the flipping of phosphatidylserine of the plasma membrane from the inside surface to the outside surface. Annexin V binds specifically to phosphatidylserine and Biotin-conjugated Annexin V can be used as a fluorescent probe to label apoptotic cells. Binding of Annexin V to the exposed charged head groups of PS is a  $\text{Ca}^{2+}$  dependent process. Propidium Iodide is used in conjunction with Annexin V-Biotin. The cell membrane integrity excludes Propidium Iodide in viable and apoptotic cells, whereas necrotic cells are permeable to Propidium Iodide. Thus dual parameter FACS analysis allows for the discrimination between viable, apoptotic and necrotic cells.

**Staining procedure for flow cytometry and fluorescence microscopy:**

Wash cells (up to  $10^6$ ) in 500  $\mu\text{l}$  binding buffer (PBS with  $\text{Ca}^{2+}$  = add 0.33 g/l to PBS)  
Spin at 250 xg for 5 minutes and discard supernatant,  
Resuspend the cell pellet in 70  $\mu\text{l}$  binding buffer,  
Add 5  $\mu\text{l}$  of AnnexinV-Biotin, incubate 15 minutes at room temperature in the dark.

**References:** Savill J, Fadok V, Henson P, Haslett C: Phagocyte recognition of cells undergoing apoptosis. Immunol Today 14:131, 1993; Reutelingsperger CP, van Heerde WL: Annexin V, the regulator of phosphatidylserine-catalyzed inflammation and coagulation during apoptosis. Cell Mol Life Sci 53: 527, 1997; DeFrancesco L: Dead Again: Adventures in Apoptosis. The Scientist 13:17, 1999

**Warning:** Sodium azide is harmful if swallowed (R22). Keep out of reach of children (S2). Keep away from food, drink and animal feedingstuff (S13). Wear suitable protective clothing (S36). If swallowed, seek medical advice immediately and show this container or label (S46). Contact with acids liberates very toxic gas (R32). Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop.

This material is offered for research only. Not for use in human. For in vitro use only. ImmunoTools will not be held responsible for patent infringement or other violations that may occur with the use of our products.

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