



Annexin-V Biotin Conjugate (Recombinant)

PRODUCT ANALYSIS SHEET

Catalog Number:	PHN1009
Lot Number:	See product label
Quantity:	Biotin conjugated recombinant Annexin-V sufficient for at least 100 tests
Volume:	0.5 mL
Preservation:	0.1% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)
Purification:	Recombinant protein produced in <i>E. coli</i> and purified by HPLC.
Storage:	Store at 2-8°C.
Specificity:	Annexin-V is a 35-36 kDa calcium-dependent phospholipid binding protein with high affinity for phosphatidylserine. The K_d for the binding of Annexin-V to phosphatidylserine has been estimated at 5×10^{-10} M. The Annexin-V binding assay is based on the rapid and selective binding to phosphatidylserine found in the outer cell membrane beginning early in the process of apoptosis (programmed cell death). Viable cells maintain an asymmetric distribution of different phospholipids between the inner and outer leaflets of the plasma membrane. Choline-containing phospholipids such as phosphatidylcholine and sphingomyelin are primarily located on outer leaflet of viable cells and aminophospholipids such as phosphatidylethanolamine and phosphatidylserine are found at the cytoplasmic (inner) face of viable cells. During apoptosis, the plasma membrane changes include a redistribution of phosphatidylserine from the cytoplasmic face to the outer leaflet making it available for Annexin-V binding.
Application:	This product is designed to detect apoptotic cells by flow cytometry.

Apoptosis can be induced by exposure of cells to various agents, including camptothecin, staurosporine, antibody to Fas, and Fas ligand. Following exposure to an apoptosis-inducing agent, cells are washed with PBS and resuspended at $2-3 \times 10^6$ cells/mL in Annexin-V Binding Buffer containing 10 mM HEPES/NaOH, pH 7.4, 140 mM NaCl, 2.5 mM CaCl_2 . Aliquots of cells (100 μL /tube) are incubated with 5 μL of Annexin-V biotin, mixed, and incubated for 15 minutes at room temperature. Cells are washed with 2 mL of Binding Buffer. Cells are resuspended in Binding Buffer to 50 μL then incubated with 50 μL of a Streptavidin conjugate (e.g., FITC or PE) for 15 minutes at room temperature in the dark. At this step, propidium iodide at a concentration of 5 $\mu\text{g}/\text{mL}$, is added to distinguish necrotic cells. Four hundred microliters of Binding Buffer are added to each tube and cells are analyzed by flow cytometry.

This product is for research use only. Not for use in diagnostic procedures.

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PI PHN1009

(Rev 1.1) (DCC-08-1232)

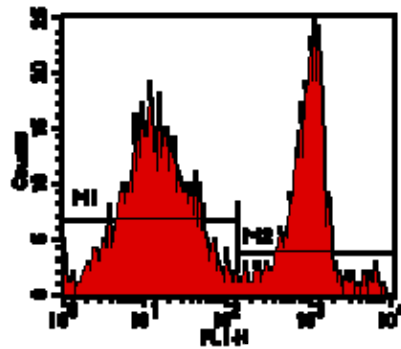
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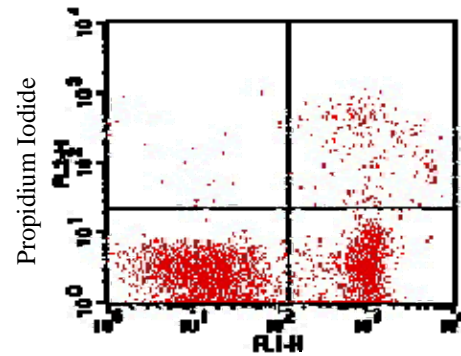
Data Analysis

Annexin-V Biotin binding to cells permits differentiation of apoptotic cells (Annexin-V positive) from non-apoptotic cells (Annexin-V negative) (Figure, left). Annexin-V binding is enhanced under two conditions. The first condition is observed during early apoptosis with the accompanying translocation of phosphatidylserine to the outer leaflet of the cell membrane. The second condition is observed during late apoptosis when cells become necrotic with accompanying membrane permeabilization. This membrane permeabilization allows Annexin-V to enter cells and bind to phosphatidylserine on the cytoplasmic face of the membrane. This membrane permeabilization also permits entry of other materials to the interior of the cell, including the fluorescent DNA binding dye propidium iodide. With the use of an Annexin V-propidium iodide double staining regime, three populations of cells are distinguishable in two-color flow cytometry (Figure, right)

Non-apoptotic cells:	Annexin-V negative and PI negative;
Early apoptotic cells:	Annexin-V positive and PI negative;
Necrotic cells or late apoptotic cells:	Annexin-V positive and PI positive.



Annexin-V Biotin (SAV-FITC)



Annexin-V Biotin (SAV-FITC)

Figure: Jurkat cells were cultured with 2 μ M camptothecin for 5 hours and stained by Annexin-V Biotin and FITC-conjugated streptavidin. Apoptotic and non-apoptotic cells can be differentiated according to annexin-V binding (left). M1 represents non-apoptotic cells and M2 represents apoptotic cells. Staining with propidium iodide differentiates necrotic or late apoptotic cells (right). Propidium iodide stained cells are easily distinguished from early apoptotic population (Annexin-V positive and PI-negative) appearing in the lower right quadrant of the dot plot.

References:

1. Boersma A.W., *et al.* (1996) Quantification of apoptotic cells with fluorescein isothiocyanate-labeled Annexin V in Chinese hamster ovary cell cultures treated with cisplatin. *Cytometry* 24:123-130.
2. Vermes I., *et al.* (1995). A novel assay for apoptosis: flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labeled Annexin V. *J. Immunol. Methods* 184:39-51.
3. Homburg C.H., *et al.* (1995) Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis in vitro. *Blood* 85:532-540.
4. Martin S.J., *et al.* (1995) Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *J. Exp. Med.* 182:1545-1556.

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