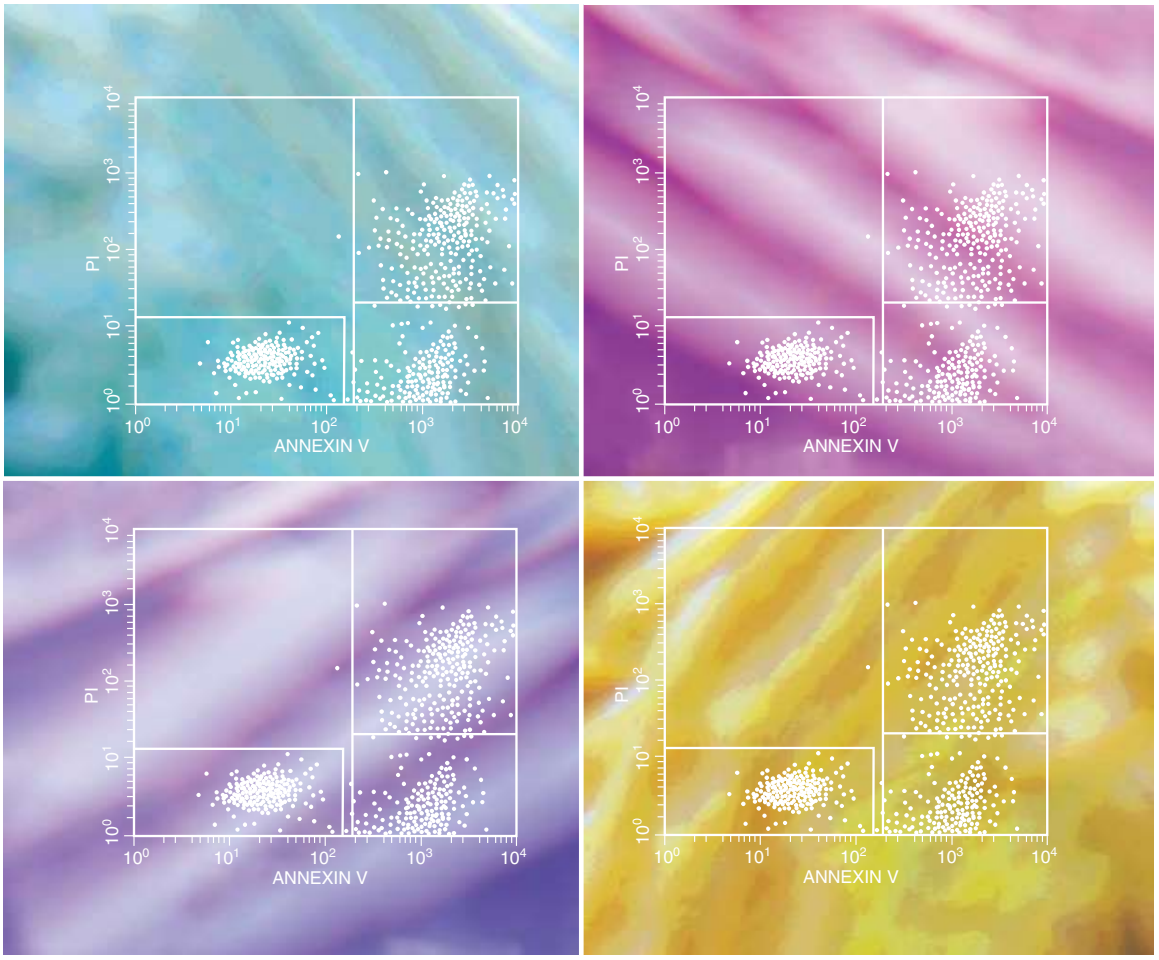
ANNEXIN V
KITS and REAGENTS



TREVIGEN®

ANNEXIN V PRODUCTS

amsbio

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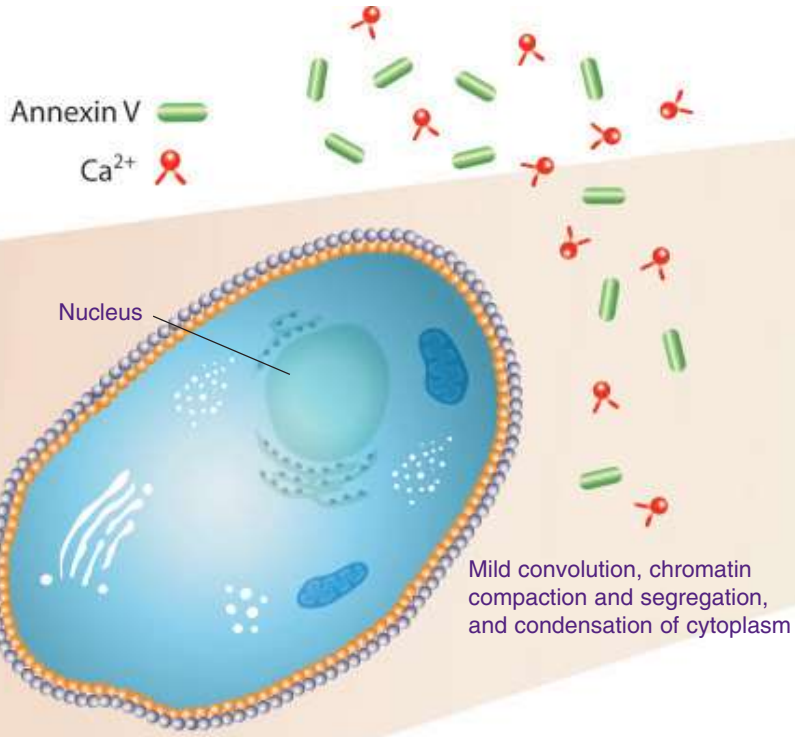
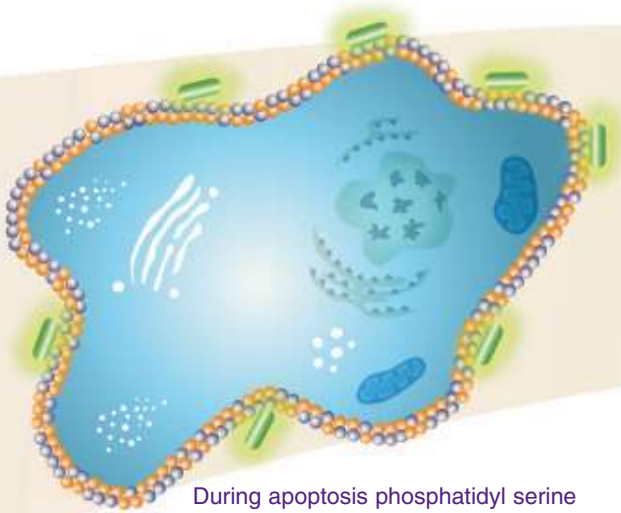
Apoptosis is a programmed cell death process characterized by morphological and biochemical features occurring at different stages. Once triggered, apoptosis proceeds with different kinetics depending on cell types and culminates with cell disruption and formation of apoptotic bodies. Cell surface changes of the dying cells results in the recognition and the uptake of these cells by phagocytes. Phospholipids are asymmmetrically distributed between inner and outer leaflets of the plasma membrane with phosphatidylcholine and sphingomyelin exposed on the external leaflet of the lipid bilayer, and phosphatidylserine predominantly observed on the inner surface facing the cytosol. Cells undergoing apoptosis break up the phospholipid asymmetry of their plasma membrane and expose phosphatidylserine, which is translocated to the outer layer of the membrane. This

TREVIGEN® ANNEXIN V –



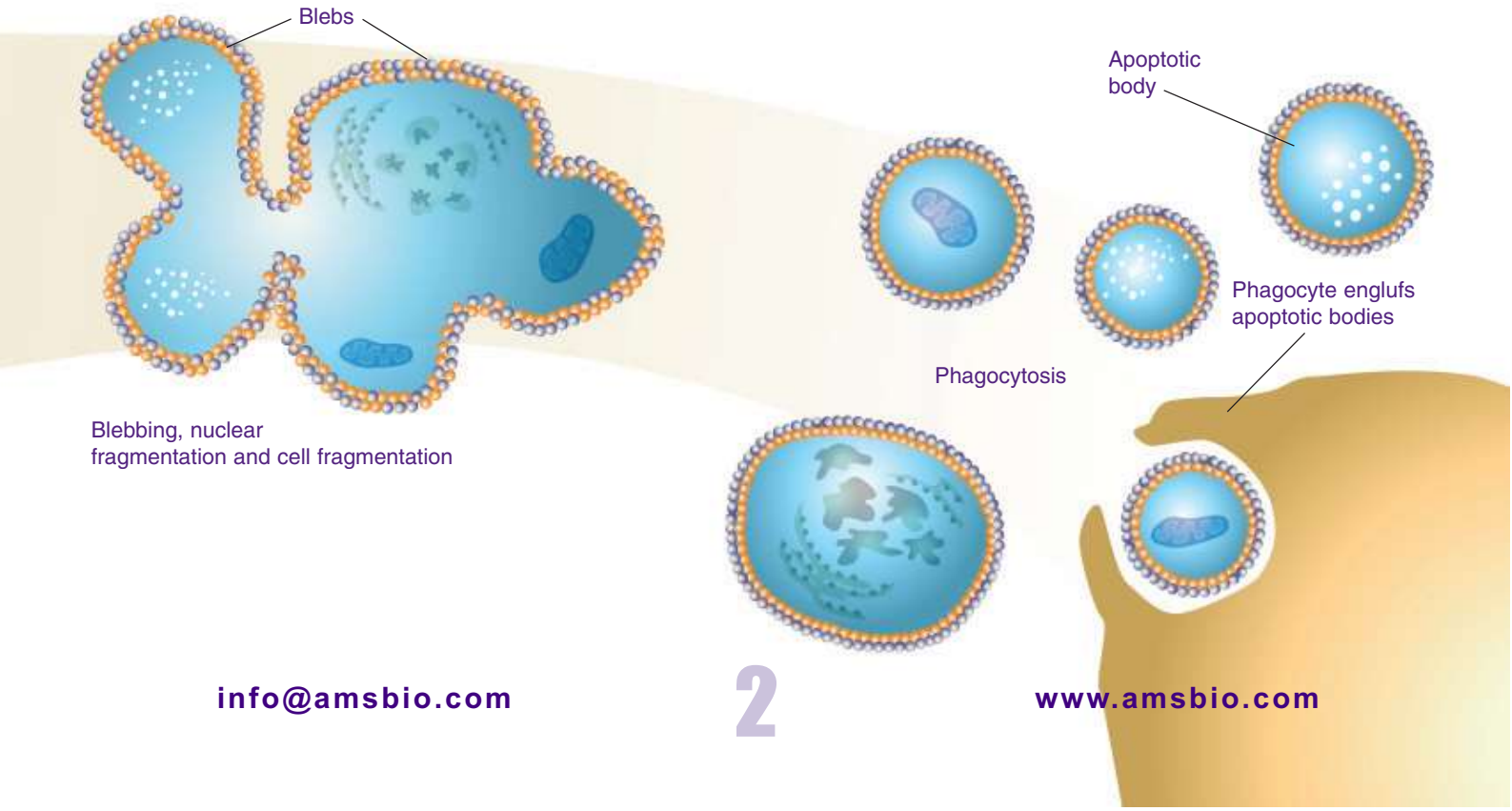
Mild convolution, chromatin compaction and segregation, and condensation of cytoplasm



During apoptosis phosphatidyl serine flips to the outer leaflet of the membrane and is subsequently detected by FITC or Biotin labeled Annexin

occurs in the early phases of apoptotic cell death during which the cell membrane remains intact. The flipping of phosphatidyl serine from the inner leaflet to the outer leaflet of the cell membrane represents a hallmark (early and widespread) in detecting dying cells. The anticoagulant properties of Annexin V have proven to be a useful tool in detecting apoptotic cells. It preferentially binds to negatively charged phospholipids like PS in the presence of Ca^{2+} and shows minimal binding to phosphatidylcholine and sphingomyeline. Changes in PS asymmetry, which is analyzed by measuring Annexin V binding to the cell membrane, are detected before morphological changes associated with apoptosis occur and before membrane integrity has been lost.

For monitoring apoptosis via flow cytometry

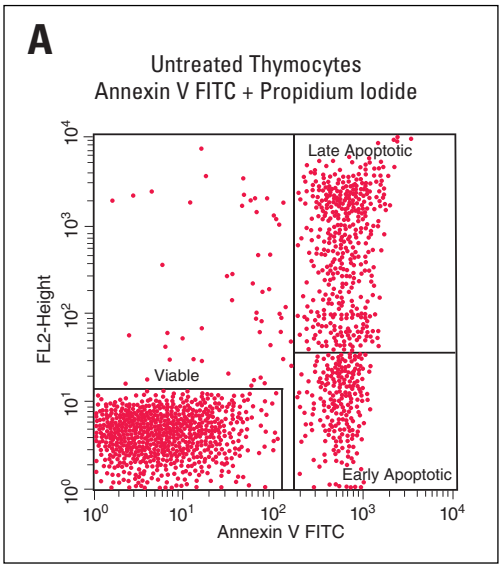


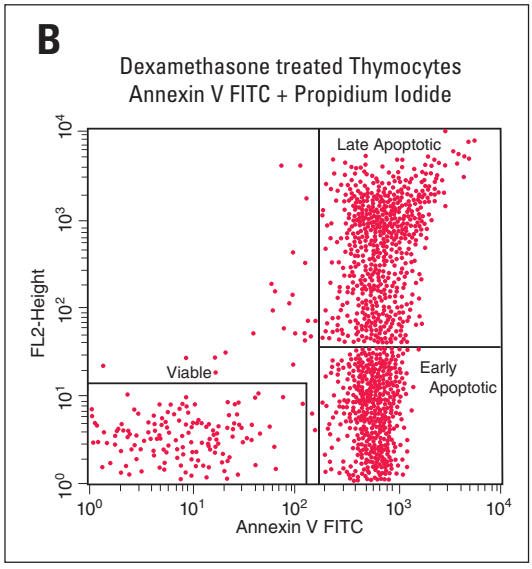
PRODUCTS

Kit Contents

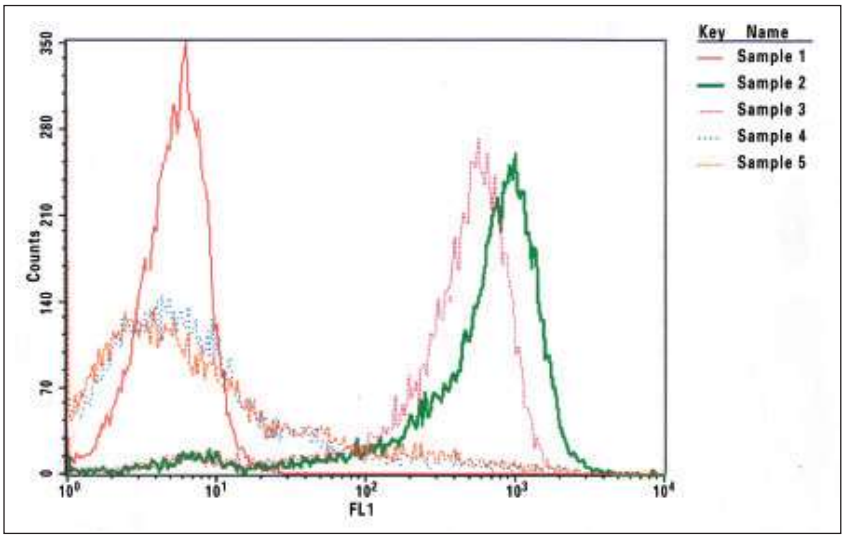
- Annexin V FITC or Annexin V Biotin
- Binding Buffer
- Propidium Iodide

Description	Size	Catalog No.
TACS™ Annexin V FITC	100 Samples	4830-01-K
TACS™ Annexin V FITC	250 Samples	4830-250-K
TACS™ Annexin V Biotin	100 Samples	4835-01-K
TACS™ Annexin V Biotin	250 Samples	4835-250-K





Analysis of dexamethasone-treated thymocytes using Annexin V FITC and propidium iodide. Cells were treated with 100 nM dexamethasone for 15.5 hours. The results obtained typically show a distinct population of cells that have bound Annexin V (lower right quadrant of a dot or density plot). These cells are early apoptotic. Annexin V positive cells that also take up propidium iodide are either late apoptotic or necrotic (upper right quadrant of dot plot). There may also be a population of cells that are negative for both Annexin V and propidium iodide (lower left quadrant of dot plot). These are normal viable cells. This dot plot of untreated (Panel A) and treated (Panel B) thymocytes shows viable, early apoptotic (Annexin V FITC positive) and late apoptotic or necrotic cells. Analysis courtesy of Dr. C.M. Knudson, Howard Hughes Medical Institute, St. Louis, MO.



Flow cytometry analysis of WEHI 7.1 cells labeled with Annexin V Biotin and detected by streptavidin FITC. WEHI 7.1 cells treated with 25 µM etoposide for two hours with an overnight recovery produce a peak approximately log of 103 in the fluorescence channel 1 (FL1) (samples 2 and 3). Healthy WEHI 7.1 cells produce a peak less than log 101 in the FL1 channel which is similar to unlabeled cells (samples 4, 5, and 1 respectively). Analysis was tested using two different populations of cells (samples 1, 2, 4 and 3, 5 respectively).

CITATIONS

Recent Citations using Trevigen Annexin Products –
Product descriptions and catalog numbers on page 3

Cationic gradient reversal and cytoskeletal independent volume regulatory pathways define an early stage of apoptosis

Annexin V FITC

Carl D. Bortner, Maria I. Sifre, and John A. Cidlowski
J. Biol. Chem., Jan 2008; 10.1074/jbc.M707809200.

MAPK and heat shock protein 27 activation are associated with respiratory syncytial virus induction of human bronchial epithelial monolayer disruption

Annexin V FITC

Divyendu Singh, Kelly L. McCann, and Farhad Imani
Am J Physiol Lung Cell Mol Physiol., Aug 2007; 293: L436 - L445.

Effects on neurite outgrowth and cell survival of a secreted fibroblast growth factor binding protein upregulated during spinal cord injury

Annexin V FITC

Elena Tassi, Sharon Walter, Achim Aigner, Rafael H. Cabal-Manzano, Ranjan Ray, Paul J. Reier, and Anton Wellstein
Am J Physiol Regulatory Integrative Comp Physiol., Aug 2007; 293: R775 - R783.

Selective Regulation of Bone Cell Apoptosis by Translational Isoforms of the Glucocorticoid Receptor

Annexin V FITC

Nick Z. Lu, Jennifer B. Collins, Sherry F. Grissom, and John A. Cidlowski
Mol. Cell. Biol., Oct 2007; 27: 7143 - 7160.

vFLIP from KSHV inhibits anoikis of primary endothelial cells

Annexin V Biotin

Sofia Efklidou, Ranbir Bailey, Nigel Field, Mahdad Noursadeghi, and Mary K. Collins
J. Cell Sci., Feb 2008; 121: 450 – 457.

WHAT CUSTOMERS SAY

Donna Stefanick
Biologist
Dr. Samuel Wilson's Lab
Laboratory of Structural Biology
National Institute of
Environmental Health Sciences

"We have routinely used the Trevigen Annexin V FITC kit for around seven years in order to study the time course and mechanism of cell death in a number cell lines treated with a DNA methylating agent (MMS) combined with a PARP inhibitor. Use of Annexin V staining, in conjunction with propidium iodide (both provided with the kit), has allowed us to quickly and easily distinguish apoptotic from necrotic cells. The protocol included in the kit is easy to follow; however for adherent human fibroblasts used here, we have found it necessary to harvest the cells by brief trypsinization using 0.05% tiypsin-EDTA, then combine these cells with those already released into the medium. We highly recommend this kit for any lab that is interested in studying apoptosis by flow cytometry."

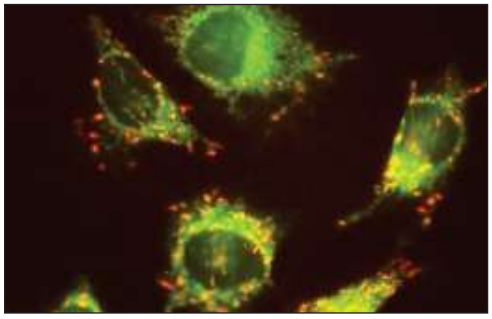
Mike Fisher,
Laboratory Research Specialist
Pharmacology Department
University of North Carolina

"I have been using the Trevigen Annexin V FITC apoptosis detection kit and found the product to be excellent in terms of quality, sensitivity and reproducibility. In addition this kit was fast, precise and simple to perform. Trevigen provides a responsive customer support service and excellent technical competence. I highly recommend this product."

RELATED PRODUCTS

Researchers who purchased Annexin V products also purchased these...

Product	Description	Size	Catalog No.
FlowTACS™	For the detection of DNA fragmentation by flow cytometry	60 Samples	4817-60-K
DePsipher™	For the detection of mitochondrial membrane potential by flow cytometry and fluorescent microscopy	100 Tests	6300-100-K
MitoShift™	For the assessment of mitochondrial membrane potential by flow cytometry and fluorescent microscopy	100 Tests	6305-100-K



Identification of apoptotic cells using DePsipher™. INT407 human cells were treated with 25 µM etoposide for 8 hours, and treated with the DePsipher™ reagent in Reaction Buffer for 30 minutes prior to visualization. Healthy cells (containing red aggregates) can be differentiated from apoptotic cells (containing mostly green monomers).

FAQS

1. Can Annexin V FITC be used with cells expressing green fluorescent protein (GFP)?

TACS™ Annexin V FITC kit cannot be used with cells expressing GFP since the fluorescence molecules have similar excitation and emission spectra. However, the TACS™ Annexin V Biotin Kit (cat # 4835-01-K and cat # 4835-250-K) offers flexibility in your flurophore preference. Trevigen supplies Streptavidin Conjugates compatible with the Annexin V Biotin kit, flow cytometric-based assays, and for in situ labeling. Conjugates supplied by Trevigen include Strep-AMCA, Strep-DTAF, and Strep-HRP.

2. What is Propidium Iodide (cat # 4830-01-02) (PI)?

Propidium Iodide (cat # 4830-01-02) is used as a fluorescent marker of late apoptosis or necrosis. In healthy cells, PI cannot cross the plasma membrane, but once the membrane becomes compromised, PI enters the cell and intercalates into the DNA.

3. At what stage during the apoptosis process should I expect Annexin V to generate a detectable signal?

Signals are often detectable at 1 hr post apoptosis stimulus. Annexin V detects one of the earliest events in the apoptosis process.

4. Since Annexin V binds to phosphatidylserine, is this binding exclusively for apoptotic cells?

No. Annexin V will also label cells undergoing necrosis. Trevigen provides propidium iodide (PI) in order to label necrotic cells.

5. Can non-specific binding be a problem with Annexin V?

Yes. A solution of Annexin V and tripotassium EDTA (final concentration 0.1 mg/ml) can be used for determining non-specific Annexin V binding. Citrated saline + 10 mM $CaCl_2$ + 0.5% human serum albumin can be used to block non-specific Annexin V binding.