

Store at
4°C

Annexin V-PE Early Apoptosis Detection Kit



#40295

1 Kit
(100 assays)Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/supportOrders: 877-616-2355 (U.S.)
orders@cellsignal.comEntrez-Gene ID #308
UniProt ID #P08758

New 10/19

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity
Annexin V-PE Conjugate	17629	200 µl
10X Annexin V Binding Buffer	11732	2 x 1.5 mL
7-AAD Cell Staining Solution	47501	1 x 1 mL

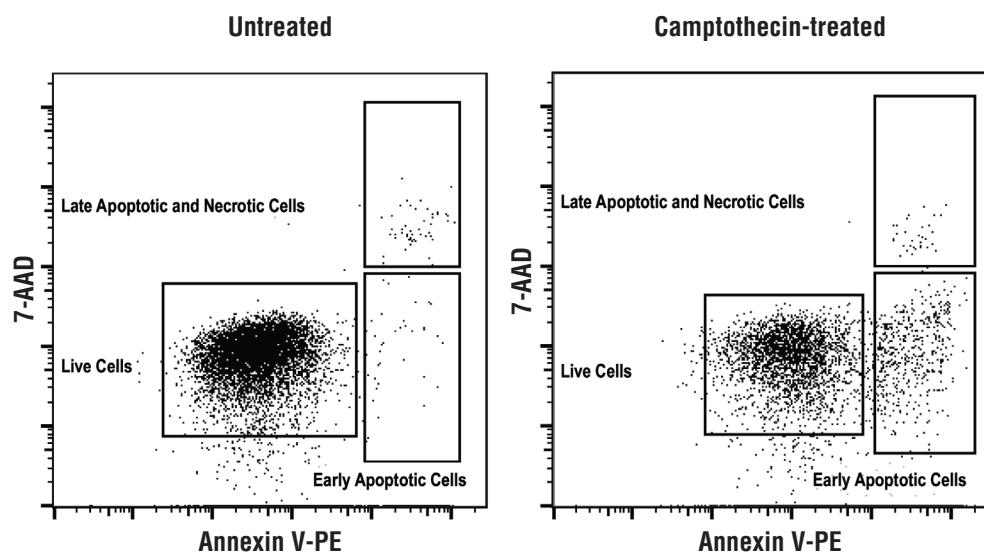
Description: The Annexin V-PE Early Apoptosis Detection Kit enables researchers to identify early apoptotic cells within a cell population. Annexin V-PE conjugated protein binds to cell surfaces expressing phosphatidylserine, an early apoptosis marker. Cells stained with 7-Aminoactinomycin D (7-AAD), a non-cell-permeable DNA dye, indicate necrotic cells. Cells stained with both 7-AAD and annexin V-PE demonstrate later stage apoptosis and early necrosis. This kit provides enough reagent to perform 100 assays, based on a 250 µl assay volume.

Background: Annexin V belongs to the annexin family of proteins consisting of over 160 members. All annexin family members share the same characteristic of Ca²⁺-dependent binding to negatively charged phospholipid surfaces (1). During early apoptosis, phosphatidylserine (PS) is translocated from the cytosolic side of the plasma membrane to the cellular surface. This translocation exposes PS to the extracellular environment with the plasma membrane left intact (2). Annexin V protein demonstrates high affinity, specificity, and sensitivity for PS and can be used as a marker of early apoptosis. In order to rule out "leaky" necrotic cells, annexin V must be used in tandem with reagents that determine the integrity of the cell membrane, such as propidium iodide (2).

Storage: The components within the Annexin V-PE Early Apoptosis Detection Kit are stable for 1 year at 4°C. *Protect from light.*

Background References:

- (1) Boersma, H.H. et al. (2005) *J Nucl Med* 46, 2035-50.
- (2) Vermes, I. et al. (1995) *J Immunol Methods* 184, 39-51.



Flow cytometric analysis of Jurkat cells, untreated (left) or treated with camptothecin (10 µM, 4 hr; right), using Annexin V-PE Early Apoptosis Detection Kit.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

Flow Cytometry, Annexin V-PE Conjugate, 7aad Protocol

A Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS) (#9808):** To prepare 1L 1X PBS: add 50 ml 20X PBS to 950 ml dH₂O, mix.
2. **10X Annexin-V Binding Buffer:** To prepare 1L 1X Annexin-V Binding Buffer: add 1ml 10X Binding Buffer to 9ml dH₂O, mix.

B Staining

1. Collect cells of interest by centrifugation.
2. Wash Cells with ice-cold PBS.
3. Resuspend cells at 10⁵ or 10⁶ cells/ml with 1x Annexin V Binding Buffer. Aliquot 96µl of cell suspension in an assay tube.
4. Add 2µl Annexin V-PE Conjugate and 10µl 7aad staining solution to each 96µl cell suspension. Allow cells to incubate 10 minutes on ice in the dark.
5. Dilute the cell suspension to a final volume of 250µl/assay with ice-cold 1X Annexin V Binding Buffer. Analyze immediately.