Store at 4°C

#6592

Annexin V-FITC Early Apoptosis Detection Kit

1 Kit
(100 assays)

Orders ■ 877-616-CELL (2355)
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For Research Use Only. Not For Use In Diagnostic Procedures.

Entrez Gene ID # 308
UniProt ID # P08758

Products Included	Product #	Quantity
Annexin V-FITC Conjugate	4894	100 µl
10X Annexin V Binding Buffer	11732	2 x 1.5 ml
Propidium Iodide (PI) Solution	11733	1 x 1.3 ml

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

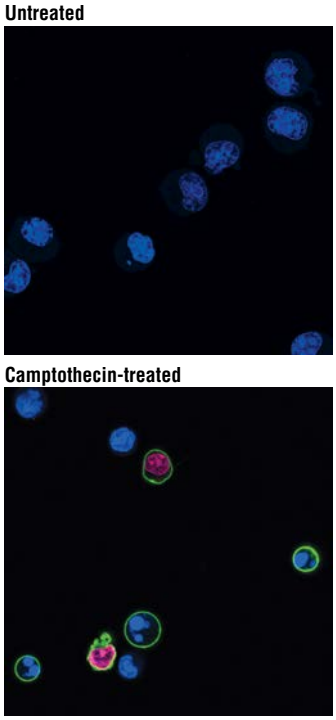
Background References:

(1) Boersma, H.H. et al. (2005) *J Nucl Med* 46, 2035-50.

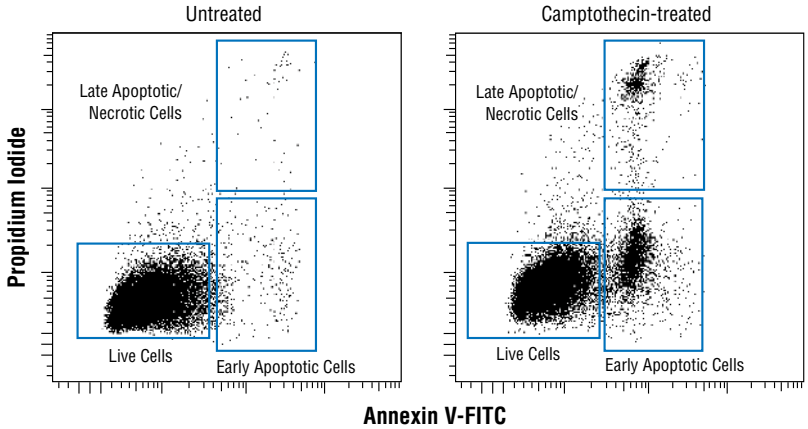
(2) Vermes, I. et al. (1995) *J Immunol Methods* 184, 39-51.

Description: The Annexin V-FITC Early Apoptosis Detection Kit enables researchers to identify early apoptotic cells within a cell population. Annexin V-FITC conjugated protein binds to cell surfaces expressing phosphatidylserine, an early apotosis marker. Cells stained with propidium iodide (PI), a non-cell-permeable DNA dye, indicate necrotic cells. Cells stained with both PI and annexin V-FITC 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 charactersistic of Ca^{2+}-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).



Confocal immunofluorescent analysis of live Jurkat cells, untreated (upper) or camptothecin-treated (lower), using Annexin V-FITC Conjugate (green). Red = Propidium Iodide (fluorescent DNA dye). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



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

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The Annexin V-FITC conjugate is a product manufactured for Cell Signaling Technology by TAU Technologies BV.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
Species Cross-Reactivity Key: **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.

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Flow Cytometry, Annexin V-FITC Conjugate, Propridium Iodide 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 50ml 20X PBS to 950ml dH_20, mix.
2. 10X Annexin-V Binding Buffer: To prepare 1L 1X Annexin-V Binding Buffer: add 1ml 10X Binding Buffer to 9ml dH_20, mix.

B Test Procedure

1. Collect cells of interest by centrifugation.
2. Wash cells with ice cold PBS or culture medium.
3. Resuspend cells at 10^5 or 10^6 cells/ml with 1x Annexin V Binding Buffer. Aliquot 96µl of cell suspension in an assay tube.
4. Add 1 µl Annexin V-FITC Conjugate and 12.5 µl Propidium Iodide (PI) Solution to each 96µl cell suspension. Allow cells to incubate 10 min on ice in the dark.
5. Dilute the cell suspension to a final volume of 250 µl/ assay with ice-cold, 1X Annexin V Binding Buffer. Analyze immediately.

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