



## Cell Meter™ Annexin V Binding Apoptosis Assay



### Apoptosis-Induced Changes in Plasma Membrane

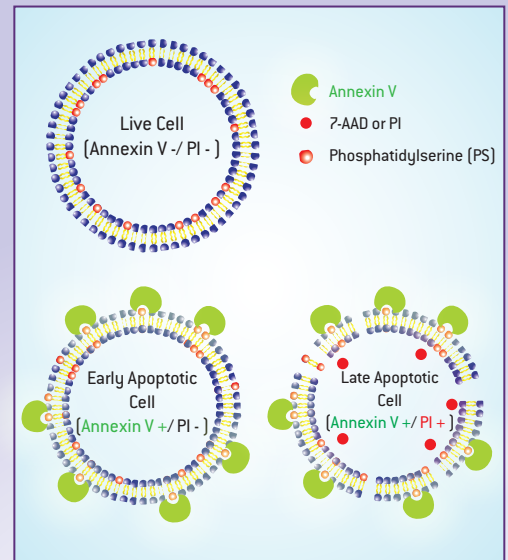
The apoptotic process is characterized by certain morphological features. The features include changes in the plasma membrane (such as loss of membrane symmetry and loss of membrane attachment), a condensation of the cytoplasm and nucleus, protein cleavage, and internucleosomal cleavage of DNA. In the final stages of the process, dying cells become fragmented into "apoptotic bodies" and consequently eliminated by phagocytic cells without significant inflammatory damage to surrounding cells.

### Phosphatidylserine Binding Assays Using Annexin V Conjugates

Changes in the plasma membrane are one of the first characteristics of the apoptotic process detected in living cells. Apoptosis can be detected by the presence of phosphatidylserine (PS), which is normally located on the cytoplasmic face of the plasma membrane. During apoptosis, phosphatidylserine translocates to the outer leaflet of the plasma membrane and can be detected by flow cytometry and cell imaging through binding to fluorochromelabeled Annexin V conjugates when calcium is present. Annexins are a family of calcium-dependent phospholipid-binding proteins. They are abundant in eukaryotic organisms belonging to a family of ubiquitous cytoplasmic proteins involved in signal transduction. Annexin V's preferential binding partner is phosphatidylserine, which is usually kept on the inner-leaflet (the cytosolic side) of cell membranes. In apoptosis, phosphatidylserine is transferred to the outer leaflet of the plasma membrane. The appearance of phosphatidylserine on the cell surface is a universal indicator of the initial/intermediate stages of cell apoptosis and can be detected before morphological changes can be observed.

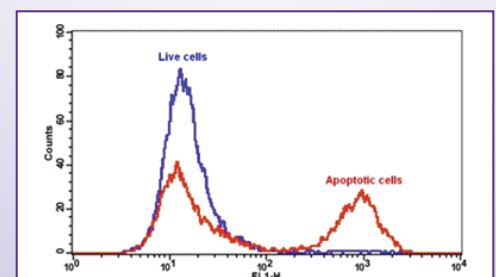
### Cell Meter™ Annexin V Binding Apoptosis Assay

Cell Meter™ Annexin V Binding Apoptosis Assay Kits use our proprietary fluorescent Annexin V-iFluor™ PS sensors that specifically bind PS with good photostability. The kits provide all the essential components with an optimized protocol. Cell Meter™ Phosphatidylserine Apoptosis Assay Kits use Apopxin™ PS sensors. Due to the highly enhanced affinity to phosphatidylserine, the kits are more robust than other commercial Annexin V-based apoptosis kits that are only used with either microscope or flow cytometry platform. The kits can be used with a fluorescence microplate reader besides the microscope and flow cytometry platforms. They have also been used for HTS applications.

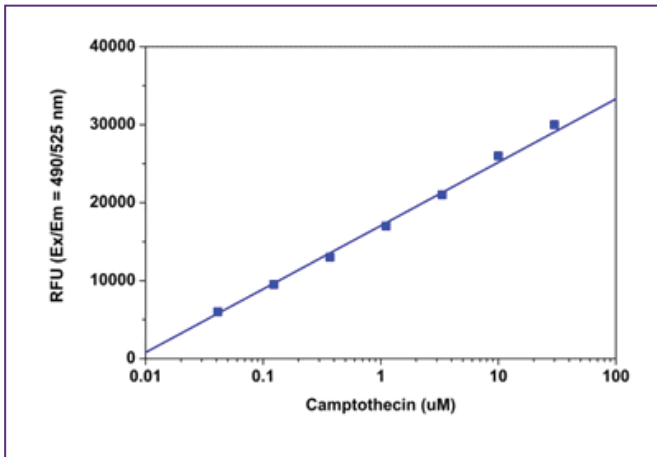


**Figure 1.** Annexin V preferentially binds to negatively charged phospholipids such as phosphatidylserine (PS). In live cells, PS is located on the inner leaflet of the plasma membrane. During early apoptosis, loss of phospholipid asymmetry leading to exposure of PS on the external leaflet of plasma membrane. During late apoptosis, plasma membrane integrity is lost, therefore cell impermeant DNA dyes such as Propidium Iodide (PI) and 7-Aminoactinomycin D (7-AAD) could enter the cell.

Therefore, by staining cells with a combination of fluorescently labelled Annexin V and PI/7-AAD, it is possible to detect **non-apoptotic live cells (annexin V negative/PI negative)**, **early apoptotic cells (annexin V positive, PI negative)** and **late apoptotic or necrotic cells (PI positive)** by flow cytometry.



**Figure 2.** The detection of binding activity of Annexin V-iFluor™ 488 [Cat# 20071] to phosphatidylserine in Jurkat cells. Jurkat cells were treated without (blue) or with 20 μM camptothecin (red) in a 37 °C, 5% CO<sub>2</sub> incubator for 4-5 hours, and then dye loaded with Annexin V-iFluor™ 488 for 30 minutes. The fluorescence intensity of Annexin V-iFluor™ 488 was measured with a FACSCalibur flow cytometer using the FL1 channel.



**Figure 3.** Detection of phosphatidylserine binding activity in Jurkat cells with Cell Meter™ Phosphatidylserine Apoptosis Assay Kit (Cat# 22791). Jurkat cells were seeded on the same day at 200,000 cells/90  $\mu$ L/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with different doses of camptothecin for 5 hours as indicated. The Apopxin™ Green assay solution (100  $\mu$ L/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/ Em = 490/525 nm with NOVostar instrument using bottom read mode. Our Cell Meter™ Phosphatidylserine Apoptosis Assay Kits are the only commercial products available for monitoring phosphatidylserine binding in live cells using a microplate reader.

## Annexin V Binding Based Cell Apoptosis Reagents and Assay Kits

Cat. #	Product Name	Size
20030	Annexin V, FITC Labeled	100 tests
20031	Annexin V, TRITC Labeled	100 tests
20070	Annexin V-iFluor™ 350 Conjugate	100 tests
20071	Annexin V-iFluor™ 488 Conjugate	100 tests
20072	Annexin V-iFluor™ 555 Conjugate	100 tests
20073	Annexin V-iFluor™ 594 Conjugate	100 tests
20074	Annexin V-iFluor™ 647 Conjugate	100 tests
20075	Annexin V-iFluor™ 680 Conjugate	100 tests
20077	Annexin V-iFluor™ 700 Conjugate	100 tests
20076	Annexin V-iFluor™ 750 Conjugate	100 tests
20080	Annexin V-mFluor™ Violet 450 Conjugate	100 tests
20081	Annexin V-mFluor™ Violet 510 Conjugate	100 tests
20082	Annexin V-mFluor™ Violet 540 Conjugate	100 tests
22828	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Blue Fluorescence Excited at 405 nm*	100 tests
22827	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Deep Red Fluorescence Optimized for Flow Cytometry*	100 tests
22829	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Green Fluorescence Excited at 405 nm*	100 tests
22824	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests
22830	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Orange Fluorescence Excited at 405 nm*	100 tests
22825	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Orange Fluorescence Optimized for Flow Cytometry*	100 tests
22826	Cell Meter™ Annexin V Binding Apoptosis Assay Kit *Red Fluorescence Optimized for Flow Cytometry*	100 tests
22850	Cell Meter™ Live Cell Caspase 3/7 and Phosphatidylserine Detection Kit *Triple Fluorescence Colors*	100 tests
22835	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Blue Fluorescence Excited at 405 nm*	100 tests
22790	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Blue Fluorescence Optimized for Microplate Readers*	100 tests
22832	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Deep Red Fluorescence Optimized for Flow Cytometry*	100 tests
22793	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Deep Red Fluorescence Optimized for Microplate Readers*	100 tests
22836	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Green Fluorescence Excited at 405 nm*	100 tests
22831	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Green Fluorescence Optimized for Flow Cytometry*	100 tests
22791	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Green Fluorescence Optimized for Microplate Readers*	100 tests
22794	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Orange Fluorescence Optimized for Microplate Readers*	100 tests
22792	Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Red Fluorescence Optimized for Microplate Readers*	100 tests