

# Cell Meter™ Annexin V Binding Apoptosis Assay Kit

## \*Orange Fluorescence Excited at 405 nm\*

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 22830 (100 assays)	Keep at 4 °C and avoid exposure to light	Flow cytometer Fluorescence microscope

### Introduction

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 (PS), which is usually kept on the inner-leaflet (the cytosolic side) of cell membranes. In apoptosis, PS 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. Our Cell Meter™ assay kits are a set of tools for monitoring cellular functions, which are designed to monitor cell apoptosis through measuring the translocation of phosphatidylserine.

This kit uses our proprietary orange fluorescent Annexin-V Violet 550 PS sensor that specifically binds PS with orange fluorescence. The stain has the spectral properties similar to those of Pacific Orange® at Ex/Em= ~405/550 nm (Pacific Orange® is the trademark of Invitrogen). The orange fluorescence stain is well excited with the violet laser at 405 nm, and emits intense orange fluorescence at ~550 nm. The kit is optimized to be used with a flow cytometer equipped with a violet laser. It is particularly suitable for multicolor flow cytometric analysis of cells. The kit provides all the essential components with an optimized protocol for flow cytometer and fluorescence microscope applications.

### Kit Key Features

<b>Non-Radioactive:</b>	No special requirements for waste treatment.
<b>Convenient:</b>	All essential assay components are included.
<b>Optimized Performance:</b>	Provide optimal conditions for detecting the translocation of phosphatidylserine.
<b>Enhanced Value:</b>	Less expensive than the sum of individual components.

### Kit Components

Components	Amount
Component A: Annexin V-mFluor Violet™ 550 (100X stock solution)	1 vial (200 µL)
Component B: Assay Buffer	50 mL
Component C: 100X Propidium Iodide	1 vial (100 µL)

### Assay Protocol

#### Brief Summary

**Prepare cells with test compounds (200 µL/sample) → Add Annexin V-mFluor Violet™ 550 assay solution → Incubate at room temperature for 15-30 minutes → Analyze with a flow cytometer or a fluorescence microscope at Ex/Em = 405/ 550 nm**

#### 1. Prepare and incubate cells with Annexin V-mFluor Violet™ 550:

- 1.1 Treat cells with test compounds for a desired period of time (4-6 hours for Jurkat cells treated with staurosporine) to induce apoptosis.
- 1.2 Centrifuge the cells to get  $1-5 \times 10^5$  cells/tube.
- 1.3 Resuspend cells in 200 µL of Assay Buffer (Component B).
- 1.4 Add 2 µL of Annexin V-mFluor Violet™ 550 (Component A) into the cells.  
*Optional: Add 2 µL of 100X Propidium Iodide (Component C) into the cells for necrosis cells.*
- 1.5 Incubate at room temperature for 30 to 60 minutes, protected from light.
- 1.6 Add 300 µL of Assay Buffer (Component B) to increase volume before analyzing the cells with a flow cytometer or fluorescence microscope (see Step 1.7 below).
- 1.7 Monitor the fluorescence intensity at Ex/Em = 405/550 nm by using a flow cytometer or a fluorescence microscope (See Step 2 or 3 below).

## 2. Analyze by using a flow cytometer:

Quantify Annexin V-mFluor Violet™ 550 binding by using a flow cytometer at Ex/Em = 405/550 nm. Measure the cell viability by using the FL2 channel when propidium iodide is added into the cells.

*Note: Annexin V binding flow cytometric analysis on adherent cells is not routinely tested since specific membrane damage may occur during cell detachment or harvesting. However, methods for utilizing Annexin V for flow cytometry on adherent cell types have been previously reported by Casciola-Rosen et al. and van Engeland et al (see Refs 1 and 2).*

## 3. Analyze by using a fluorescence microscope:

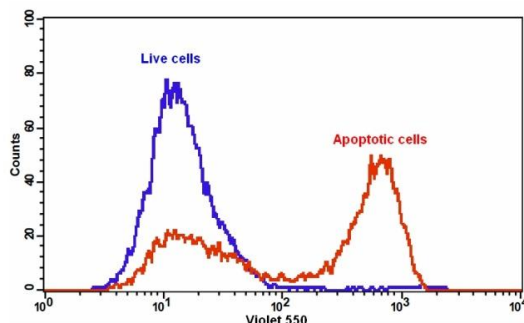
- 3.1 Pipette the cell suspension from Step 1.5, rinse 1-2 times with assay buffer, and then resuspend the cells with assay buffer. Add the cells on a glass slide that is covered with a glass cover slip.

*Note: For adherent cells, it is recommended to grow the cells directly on a cover slip. After incubation with Annexin V-mFluor Violet™ 550 (Step 1.5), rinse 1-2 times with assay buffer, and add assay buffer back to the cover slip. Invert cover slip on a glass slide and visualize the cells. The cells can also be fixed in 2% formaldehyde after the incubation with Annexin V-mFluor Violet™ 550 and visualized under a microscope.*

- 3.2 Analyze the apoptotic cells with Annexin V-mFluor Violet™ 550 under a fluorescence microscope using the Violet channel. Measure the cell viability by using the TRITC channel when propidium iodide is added into the cells. The orange staining on the plasma membrane indicates the Annexin V-mFluor Violet™ 550 binding to PS on cell surface.

## Data Analysis

In live non-apoptotic cells, Annexin V-mFluor Violet™ 550 detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells Annexin V-mFluor Violet™ 550 binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulted in increased staining intensity.



**Figure 1.** The detection of binding activity of Annexin V-mFluor Violet™ 550 and phosphatidylserine in Jurkat cells. Jurkat cells were treated without (Blue) or with 1  $\mu$ M staurosporine (Red) in a 37 °C, 5% CO<sub>2</sub> incubator for 5 hours, and then dye loaded with Annexin V-mFluor Violet™ 550 for 30 minutes. The fluorescence intensity of Annexin V-mFluor Violet™ 550 was measured with a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using violet laser at Ex/Em = 405/550 nm.

## References

1. van Engeland M, Ramaekers FCS, Schutte B, Reutelingsperger CPM: A novel assay to measure loss of plasma membrane asymmetry during apoptosis of adherent cells in culture. *Cytometry* 24:131–139, 1996.
2. L Casciola-Rosen, A Rosen, M Petri, and M Schlissel. Surface blebs on apoptotic cells are sites of enhanced procoagulant activity: implications for coagulation events and antigenic spread in systemic lupus erythematosus. *Proc Natl Acad Sci U S A*. 1996 February 20; 93(4): 1624–1629.
3. Hanshaw RG, Lakshmi C, Lambert TN, Johnson JR, Smith BD. (2005) Fluorescent detection of apoptotic cells by using zinc coordination complexes with a selective affinity for membrane surfaces enriched with phosphatidylserine. *Chembiochem*, 6, 2214.
4. Koulov AV, Stucker KA, Lakshmi C, Robinson JP, Smith BD. (2003) Detection of apoptotic cells using a synthetic fluorescent sensor for membrane surfaces that contain phosphatidylserine. *Cell Death Differ*, 10, 1357.

**Warning: This kit is only sold to end users. Neither resale nor transfer to a third party is allowed without written permission from AAT Bioquest. Chemical analysis of the kit components is strictly prohibited. Please call us at 408-733-1055 or e-mail us at [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.**