Cell Meter™ Phosphatidylserine Apoptosis Assay Kit

Blue Fluorescence Excited at 405 nm

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22835 (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 blue fluorescent Apopxin™ Violet 450 PS sensor that specifically binds PS with blue fluorescence. The stain has the spectral properties similar to those of Pacific Blue® at Ex/Em= ~405/450 nm (Pacific Blue® is the trademark of Invitrogen). The blue fluorescence stain is well excited with the violet laser at 405 nm, and emits intense blue fluorescence at ~450 nm. The kit is optimized to be used with a flow cytometer equipped with a violet laser. It is particularly suitable for multicolor flow cytomytric analysis of cells. The kit provides all the essential components with an optimized protocol for flow cytometer and fluorescence microscope applications.

Kit Key Features

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

Kit Components

Components	Amount
Component A: Apopxin™ Violet 450 (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 Apopxin™ Violet 450 assay solution → Incubate at room temperature for 15-30 mintues → Analyze with a flow cytometer or a fluorescence microscope at Ex/Em = 405/450 nm

1. Prepare and incubate cells with Apopxin™ Violet 450:

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 1-5×10^5 cells/tube.

1.3 Resuspend cells in 200 µL of Assay Buffer (Component B).

1.4 Add 2 µL of Apopxin™ Violet 450 (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 = 405/450 nm by using a flow cytometer or a fluorescence microscope (See Step 2 or 3 below).

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

2. Analyze by using a flow cytometer:

Quantify Apopxin™ Violet 450 binding by using a flow cytometer at Ex/Em = 405/450 nm. Measure the cell viability by using the FL2 channel when propidium iodide is added into the cells.
Note: Apopxin™ 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 Casiola-Rosen et al. and van Engelend 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 Apopxin™ Violet 450 (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 Apopxin™ Violet 450 and visualized under a microscope.

3.2 Analyze the apoptotic cells with Apopxin™ Violet 450 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 blue staining on the plasma membrane indicates the Apopxin™ Violet 450 binding to PS on cell surface.

Data Analysis

In live non-apoptotic cells, Apopxin™ Violet 450 detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells Apopxin™ Violet 450 binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulted in increased staining intensity.

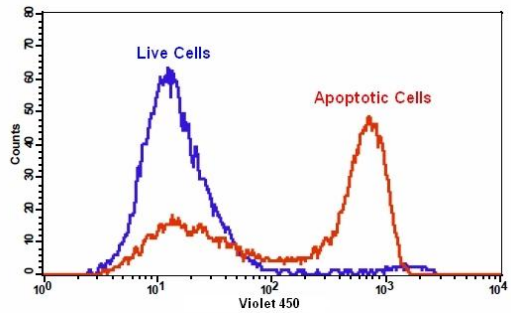

Figure 1. The detection of binding activity of Apopxin™ Violet 450 and phosphatidylserine in Jurkat cells. Jurkat cells were treated without (Blue) or with 1 μM staurosporine (Red) in a 37 °C, 5% CO_2 incubator for 5 hours, and then dye loaded with Apopxin™ Violet 450 for 30 minutes. The fluorescence intensity of Apopxin™ Violet 450 was measured with a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using violet laser at Ex/Em = 405/450 nm.

References

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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.
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