

Cell Meter™ Annexin V Binding Apoptosis Assay Kit

**Red Fluorescence Optimized for Flow Cytometry **

Ordering Information	Storage Conditions	Instrument Platform
Product Number: 22826 (100 assays)	Keep at 4 °C and avoid exposure to light	Flow cytometry 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 cell apoptosis through measuring the translocation of phosphatidylserine (PS).

This kit uses our proprietary orange fluorescent Annexin V-iFluor™ 594 PS sensor that specifically binds PS with orange fluorescence. The red fluorescence sensor has Ex/Em = 590/620 nm with high photostability. The spectral property is compatible with Texas Red® filter set (Texas Red® is the trademarks of Invitrogen). This 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: Annexin V-iFluor™ 594 (100X stock solution)	1 vial (200 µL/vial)
Component B: Assay Buffer	50 mL

Assay Protocol

Brief Summary

**Prepare cells with test compounds (200 µL/sample) → Add Annexin V-iFluor™ 594 assay solution
→ Incubate at room temperature for 30-60 minutes → Analyze cells using a flow cytometer
or fluorescence microscope with Ex/Em = 590/620 nm filter set**

1. Prepare and incubate cells with Annexin V-iFluor™ 594:

- 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-iFluor™ 594 (Component A) into the 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 = 590/620 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- iFluor™ 594 binding by using a flow cytometer at Ex/Em = 590/620 nm.

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-iFluor™ 594 (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-iFluor™ 594 and visualized under a microscope.

- 3.2 Analyze the apoptotic cells with Annexin V-iFluor™ 594 under a fluorescence microscope using TRITC or Texas Red channel. The orange staining on the plasma membrane indicates the Annexin V-iFluor™ 594 binding to PS on cell surface.

Data Analysis

In live non-apoptotic cells, Annexin V-iFluor™ 594 detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells, Annexin V-iFluor™ 594 binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulted in increased staining intensity.

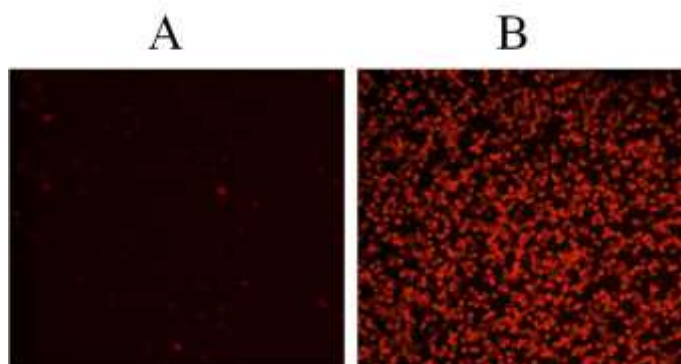


Figure1. Images of Jurkat cells in a Costar black wall/clear bottom 96-well plate stained with the Cell Meter™ Annexin-V Binding Apoptosis Assay Kit *Red Fluorescence* A: Untreated control cells B: Cells treated with 1 μM staurosporine for 5 hours.

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 if you have any questions.