Cell Meter™ Annexin V Binding Apoptosis Assay Kit

**Orange Fluorescence Optimized for Flow Cytometry **

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22825 (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™ 555 PS sensor that specifically binds PS with orange fluorescence. The spectral property is almost identical to those of Cy3® or Alexa Fluor® 555, making it convenient to be used for common fluorescence instruments equipped with the light sources and filters for Cy3® or Alexa Fluor® 555(Cy3® or Alexa Fluor® 555 are the trademarks of GE Healthcare and Invitrogen respectively). This kit provides all the essential components with an optimized protocol for flow cytometric 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™ 555 (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™ 555 assay solution → Incubate at room temperature for 30-60 mintues → Analyze cells using a flow cytometer or Fluorescence microscope with Ex/Em = 555/ 590 nm filter set

1. Prepare and incubate cells with Annexin V-iFluor™ 555:

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 Annexin V-iFluor™ 555 (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 = 555/590 nm by using a flow cytometer or a fluorescence microscope (See Step 2 or 3 below).

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2. Analyze by using a flow cytometer:

Quantify Annexin V- iFluor™ 555 binding by using a flow cytometer at Ex/Em = 555/590 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 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 Annexin V-iFluor™ 555 (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™ 555 and visualized under a microscope.

3.2 Analyze the apoptotic cells with Annexin V-iFluor™ 555 under a fluorescence microscope using the Violet channel. Measure the cell viability by using the Cy5 channel when Nuclear Red™ DCS1 (AAT catalog# 17552) is added into the cells. The orange staining on the plasma membrane indicates the Annexin V-iFluor™ 555 binding to PS on cell surface.

Data Analysis

In live non-apoptotic cells, Annexin V-iFluor™ 555 detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells, Annexin V-iFluor™ 555 binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulted in increased staining intensity.

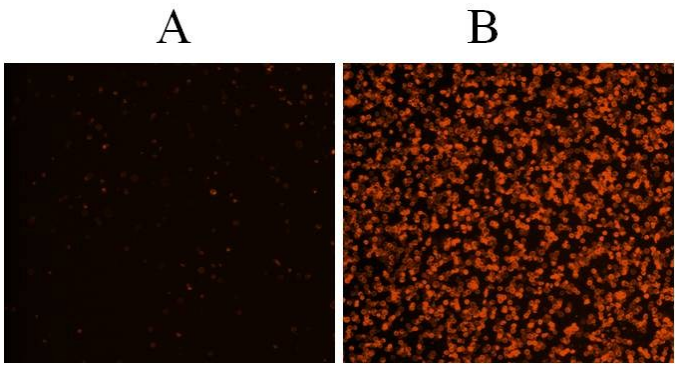

Figure1. Images of Jurkat cells in a Costar black wall/clear bottom 96-well plate stained with the Cell Meter™ Annexin-V Binding Apoptosis Assay Kit *Orange Fluorescence* A: Untreated control cells B: Cells treated with 1 μM staurosporine for 5 hours.

References

1. van Engeland M, Ramaekers FCS, Schutte B, Reutelingsperger CPM: A novel assay to measure loss of plasma membrane asymmetry during apoptosis of adherant 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.