Cell Meter™ FITC-Annexin V Binding Apoptosis Assay Kit

Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22839 (100 assays)	Keep at 4 °C and avoid exposure to light	Flow Cytometer

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 Annexin V- FITC sensor that specifically binds to PS for the early stages of apoptosis, and in pair with uptake of propidium iodide (PI) for late-stage apoptosis. This kit provides all the essential components with an optimized protocol for flow cytometric and fluorescence microscopy applications.

Kit Key Features

Non-Radioactive:	No special requirements for waste treatment.
Convenient:	Include all essential assay components.
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- FITC (100X stock solution)	1 vial (200 µL/vial)
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-FITC assay solution → Incubate at room temperature for 30-60 mintues → Analyze cells with a flow cytometer using FL1 channel (Ex/Em = 490/525 nm)

1. Treat cells with test compounds for a desired period of time (4-6 hours for Jurkat cells treated with camptothecin) to induce apoptosis.
   Note: Annexin V 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).
2. Centrifuge the cells to get 2-5 × 10^5 cells/tube.
3. Resuspend cells in 200 µL of Assay Buffer (Component B).
4. Add 2 µL of Annexin V-FITC (Component A) into the cells.

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Optional: Add 2 µL of 100X Propidium Iodide (Component C) for necrosis cells.

5. Incubate at room temperature for 30 to 60 minutes, protected from light.
6. *Optional:* add 200 to 300 µL of Assay Buffer (Component B) to increase volume before analyzing the cells with a flow cytometer (See Step 7).
7. Monitor the fluorescence intensity of Annexin V-FITC using the FL1 channel (Ex/Em = 490/525 nm), and measure the cell viability with propidium iodide using the FL2 channel.

Data Analysis

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

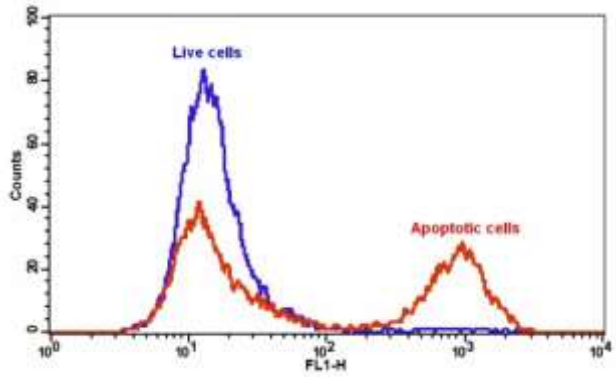

Figure1. The detection of binding activity of Annexin V-FITC to phosphatidylserine in Jurkat cells. Jurkat cells were treated without (Blue) or with 20 µM camptothecin (Red) in a 37 °C, 5% CO_2 incubator for 4-5 hours, and then dye loaded with Annexin V-FITC for 30 minutes. The fluorescence intensity of Annexin V-FITC was measured with a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using the FL1channel.

References

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