Cell Meter™ Phosphatidylserine Apoptosis Assay Kit

Green Fluorescence Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22831 (100 assays)	Keep at 4 °C and avoid exposure to light	Flow Cytometer

Introduction

Our Cell Meter™ assay kits are a set of tools for monitoring cellular functions. There are a variety of parameters that can be used for monitoring cell viability. This particular kit is designed to monitor cell apoptosis through measuring the translocation of phosphatidylserine (PS). 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

This kit uses our proprietary green fluorescent Apopxin™ PS sensor that specifically binds PS with affinity much higher than Annexin V (K_d < 10 nM). The PS sensor used in this kit has green fluorescence upon binding to membrane PS. The stain has the spectral properties almost identical to those of FITC with much higher photostability, making it convenient to be used with the common fluorescence instruments equipped with the light sources and filters for FITC, the most common fluorophore. This kit is optimized for flow cytometry applications while Kit 22791 is optimized to be used with a fluorescence microplate reader or a fluorescence microscope. This kit is a convenient alternative to Annexin V-FITC conjugate. The 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: Apopxin™ Green (100X stock solution)	1 vial (200 µL/vial)
Component B: Assay Buffer	50 mL
Component C: 100X Propidium Iodide	1 vial (200 µL)

Assay Protocol

Brief Summary

Prepare cells with test compounds (200 µL/sample) → Add Apopxin™ Green 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: 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).
2. Centrifuge the cells to get 1-5 × 10^5 cells/tube.
3. Resuspend cells in 200 µL of Assay Buffer (Component B).

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4. Add 2 µL of Apopxin™ Green (Component A) into the cells.
   Optional: Add 2 µL of 100X Propidium Iodide (Component C) into the cells for necrosis cells.
5. Incubate at room temperature for 10 to 20 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 Apopxin™ Green 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, Apopxin™ Green detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells, Apopxin™ Green binds to phosphatidylserine, which is located on the outer leaflet of the cell membrane, resulted in increased staining intensity.

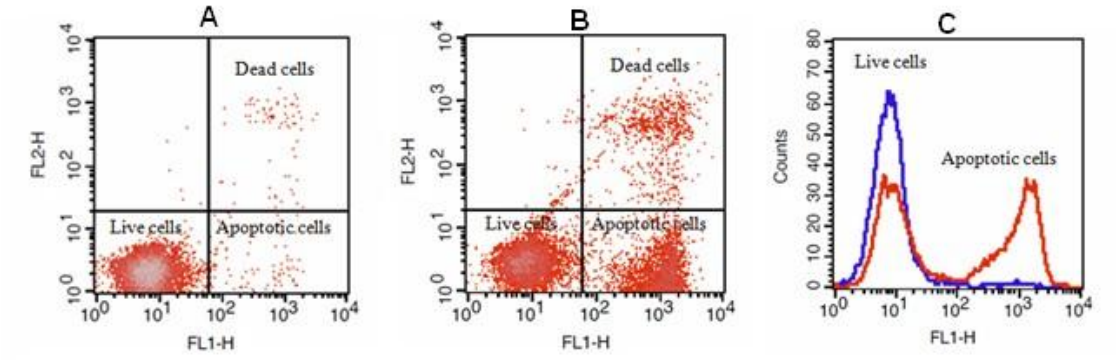

Figure 1. The detection of binding activity of Apopxin™ Green and phosphatidylserine in Jurkat cells. Jurkat cells were treated without (A, and C-Blue line) or with 20 µM camptothecin (B and C-Red line) in a 37 ^{o}C, 5% CO_2 incubator for 4-5 hours, and then dye loaded with Apopxin™ Green and Propidium Iodide for 30 minutes. The fluorescence intensity of Apopxin™ Green was measured with a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer using the FL1 channel and the fluorescence intensity of Propidium Iodide was measured using the FL2 channel.

References

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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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