Cell Meter™ Phosphatidylserine Apoptosis Assay Kit

Deep Red Fluorescence Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22832 (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 red 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 red fluorescence upon binding to membrane PS. The stain has the spectral properties almost identical to those of Cy5® or Alexa Fluor® 647 (Cy5™ and Alexa Fluor® are the trademarks of GE Healthcare and Invitrogen respectively), making it convenient to be used with the common fluorescence instruments equipped with the light sources and filters for Cy5® or Alexa Fluor® 647. Due to its highly enhanced affinity to PS, this kit is more robust than the other commercial Annexin V based apoptosis kits and can be also used with a flow cytometry platform.

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.

Components	**Amount**
Component A: Apopxin™ Deep Red (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 Apopxin™ Deep Red assay solution → Incubate at room temperature for 30-60 mintues → Analyze cells with a flow cytometer using FL4 channel (Ex/Em = 647/660 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).
4. Add 2 µL of Apopxin™ Deep Red (Component A) into the cells.
5. Incubate at room temperature for 30 to 60 minutes, protected from light.

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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™ Deep Red using the FL4 channel (Ex/Em = 647/660 nm).

Data Analysis

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

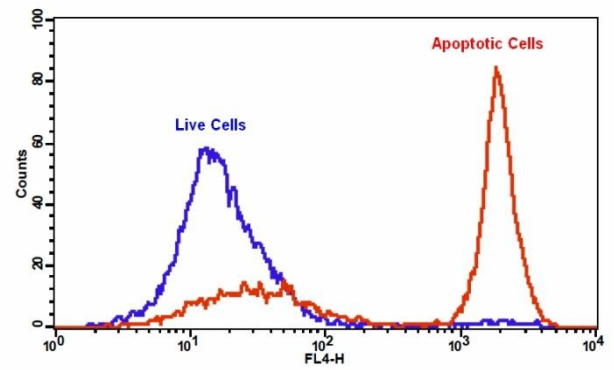

Figure1. Detection of phosphatidylserine binding activity in Jurkat cells. Jurkat cells were treated without (Blue) or with 20 µM camptothecin (red) in a 37°C, 5% CO_2 incubator for 4-5 hours, and then loaded with Apopxin™ Deep Red for 30 minutes. The fluorescence intensity of Apopxin™ Deep Red was measured with a FACSCalibur (Becton Dikinson, San Jose, CA) flow cytometer in FL4 channel.

References

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