AAT Bioquest®

Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Deep Red Fluorescence Optimized for Flow Cytometry*

Catalog number: 22832
Unit size: 100 Tests

Component	Storage	Amount
Component A: Apopxin™ Deep Red (100X stock solution)	Refrigerate (2-8 °C), Minimize light exposure	1 vial (200 µL/vial)
Component B: Assay Buffer (4 °C)	Refrigerate (2-8 °C)	50 mL

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cell viability. 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. Our proprietary Apopxin™ PS sensor used in this kit is small molecule-based PS sensor. It has red fluorescence upon binding to membrane PS. This particular assay kit is optimized to monitor cell apoptosis using a flow cytometer at Cy5 channel (red fluorescence).

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (200 µL/sample)
2. Add Apopxin™ Deep Red assay solution
3. Incubate at room temperature for 30 - 60 minutes
4. Analyze cells using flow cytometer with FL4 channel (Ex/Em = 647/660 nm

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	640 nm laser
Emission:	660/20 nm filter
Instrument specification(s):	APC channel

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

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.

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.

6. **Optional:** add 200 to 300 µL of Assay Buffer (Component B) to increase volume before analyzing the cells with a flow cytometer.

7. Monitor the fluorescence intensity using a flow cytometer with FL4 channel (Ex/Em = 647/660 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

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.

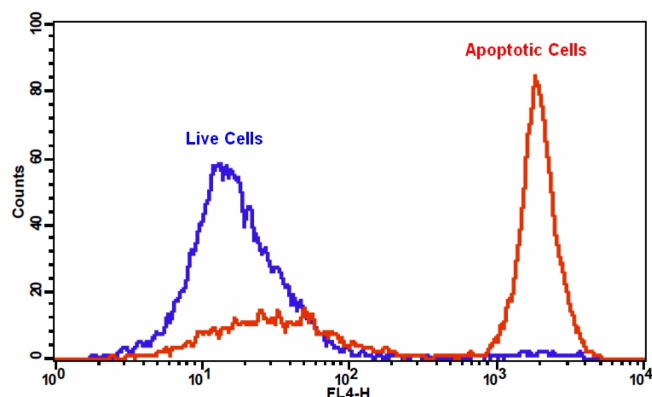

Figure 1. Detection of phosphatidylserine binding activity in Jurkat cells. Jurkat cells were treated without (Blue) or with 20 µM camptothecin (Red) in a 37 °C, 5% CO2 incubator for 4-5 hours, and then loaded with Apopxin™ Deep Red for 30 minutes. The fluorescence intensity of Apopxin™ Deep Red was measured with a FACSCalibur (Becton Dikinson) flow cytometer in FL4 channel.

DISCLAIMER

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