AAT Bioquest®

PRODUCT INFORMATION SHEET

Cell Meter™ Phosphatidylserine Apoptosis Assay Kit *Blue Fluorescence Optimized for Microplate Readers*

Catalog number: 22790
Unit size: 100 Tests

Component	Storage	Amount
Component A: Apopxin™ Violet 450 (100X stock solution)	Refrigerate (2-8 °C), Minimize light exposure	1 vial (100 µL)
Component B: Assay Buffer (4 °C)	Refrigerate (2-8 °C)	10 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. This particular kit is designed to monitor cell apoptosis through measuring the translocation of phosphatidylserine (PS). This kit uses our proprietary fluorescent small molecule-based Apopxin™ PS sensor that specifically binds PS with affinity much higher than Annexin V (Kd < 10 nM). It has blue fluorescence upon binding to membrane PS. It can be used in the formats of microplate, microscope and flow cytometer while most of other commercial apoptosis assay kits are only used with either microscope or flow cytometry platform.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (100 µL/well/96-well plate or 25 µL/well/384-well plate)
2. Add equal volume of Apopxin™ Violet 450 working solution
3. Incubate at room temperature for 1 hour
4. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 405/450 nm (Cutoff = 420 nm) or fluorescence microscope with Violet filter

Important Warm Assay Buffer (Component B) at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	405 nm
Emission:	450 nm
Cutoff:	420 nm
Instrument specification(s):	Bottom read mode
Recommended plate:	Black wall/clear bottom
Instrument:	Fluorescence microscope
Instrument specification(s):	FITC channel
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

Add 10 µL of 100X Apopxin™ Violet 450 (Component A) into 1 mL of Assay Buffer (Component B) and mix well to make Apopxin™ Violet 450 working solution.

Note 100 µL of Apopxin™ Violet 450 working solution is enough for one well. Prepare fresh before use.

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 by adding 10 µL/well (96-well plate) or 2.5 µL/well (384-well plate) of 10X test compound stock solution into PBS or the desired buffer. For blank wells (medium without the cells), add the same amount of compound buffer.

2. Incubate the cell plate in a 5% CO_2, 37°C incubator for a desired period of time (4 - 6 hours for Jurkat cells treated with staurosporine) to induce apoptosis.

   Note Some compounds such as camptothecin might give false positive response due to the broad emission spectrum from 380 to 490 nm when excited at 405 nm.

3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Apopxin™ Violet 450 working solution into each well.

4. Incubate the cell plate at room temperature for at least 1 hour, protected from light.

5. Centrifuge cell plate (especially for the non-adherent cells) at 800 rpm for 2 minutes (brake off).

6. Monitor the fluorescence intensity with a fluorescence microplate reader (bottom read mode) at Ex/Em = 405/450 nm (Cutoff = 420 nm) or image cells using fluorescence microscope with Violet filter.

EXAMPLE DATA ANALYSIS AND FIGURES

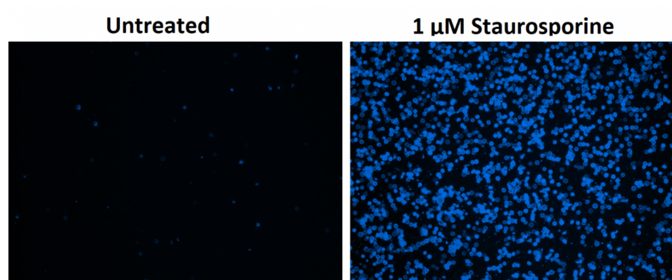

Figure 1. Fluorescence image of HeLa cells stained with Apopxin™ Violet 450 conjugate. Jurkat cells were treated without (Left) or with 1 µM staurosporine (Right) at 37 ºC for 4 hours. The fluorescence intensity was measured using a microscope with a violet filter set (Excitation=405 nm).

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