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

Catalog number: 22791
Unit size: 100 Tests

Component	Storage	Amount
Component A: Apopxin™ Green (100X Stock Solution)	Refrigerate (2-8 °C), Minimize light exposure	1 vial (100 µL/vial)
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 kit uses a fluorescent sensor that specifically binds 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 green 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™ Green working solution
3. Incubate at room temperature for 1 hour
4. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 490/525 nm (Cutoff = 515 nm) or fluorescence microscope with FITC filter

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Instrument specification(s):	Bottom read mode
Recommended plate:	Black wall/clear bottom

Instrument:	Fluorescence microscope
Excitation:	FITC filter
Emission:	FITC filter
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

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

Note 100 µL of Apopxin™ Green 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 desired test compounds by adding 10 µL/well (96-well plate) of 10X test compound solution into cell preparation buffer, for a total volume of 100 µL/well. For a 384-well plate, use 5 µL/well of 5X test compound solution into cell preparation buffer, for a total volume of 25 µL/well. 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 camptothecin) to induce apoptosis.

3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Apopxin™ Green 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 = 490/525 nm (Cutoff = 515 nm) or image cells using fluorescence microscope with FITC filter.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Camptothecin samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

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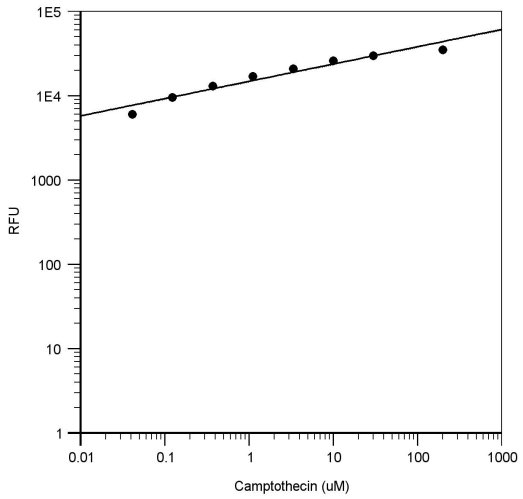

Figure 1. Detection of phosphatidylserine binding activity in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with different doses of camptothecin for 5 hours as indicated. The Apopxin™ Green assay solution (100 µL/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 490/525 nm with NOVOstar instrument (from BMG Labtech) using bottom read mode.

DISCLAIMER