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

Catalog number: 22793
Unit size: 100 Tests

Component	Storage	Amount
Component A: Apopxin™ Deep Red (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. Our proprietary Apopxin™ PS sensor used in this kit is small molecule-based PS sensor. It has red 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™ Deep Red working solution
3. Incubate at room temperature for 1 hour
4. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 650/680 nm (Cutoff = 665 nm) or fluorescence microscope with Cy5 filter

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	650 nm
Emission:	680 nm
Cutoff:	665 nm
Instrument specification(s):	Bottom read mode
Recommended plate:	Black wall/clear bottom
Instrument:	Fluorescence microscope
Excitation:	Cy5 filter
Emission:	Cy5 filter
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

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

Note 100 µL of Apopxin™ Deep Red 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 camptothecin) to induce apoptosis.
3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Apopxin™ Deep Red 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 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 = 650/680 nm (Cutoff = 665 nm) or image cells using a fluorescence microscope with Cy5® filter.

EXAMPLE DATA ANALYSIS AND FIGURES

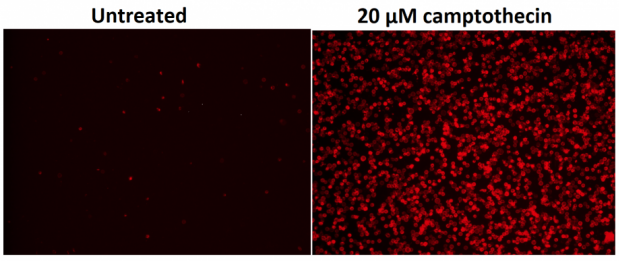

Figure 1. Fluorescence Images of Jurkat cells in a Costar black wall/clear bottom 96-well plate stained with the Cell Meter™ Phosphatidylserine Apoptosis Assay Kit. Jurkat cells were treated without (Left) or with 20 µM camptothecin (Right) for 5 hours. The fluorescence intensity was measured using a fluorescence microscope with Cy5® channel.

DISCLAIMER

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