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

Catalog number: 22794
Unit size: 100 Tests

Component	Storage	Amount
Component A: Apopxin™ Orange (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

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 orange fluorescent Apopxin™ PS sensor that specifically binds PS with affinity much higher than Annexin V (Kd < 10 nM). The PS sensor used in this kit has orange fluorescence upon binding to membrane PS. The stain has the spectral properties almost identical to those of Cy3® or Alexa Fluor® 555, making it convenient to be used for the common fluorescence instruments equipped with the light sources and filters for Cy3® or Alexa Fluor® 555 (Cy3® or Alexa Fluor® 555 are the trademarks of GE Healthcare and Invitrogen respectively). Due to its highly enhanced affinity to PS, this kit is more robust than the other commercial Annexin V-based apoptosis kits that are only used with either microscope or flow cytometry platform. This kit can be also used with a fluorescence microplate reader besides the microscope and flow cytometry platforms.

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™ Orange working solution
3. Incubate at room temperature for 1 hour
4. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 540/590 nm (Cutoff = 570 nm) or fluorescence microscope with Cy3 filter

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode
Instrument:	Fluorescence microscope
Excitation:	Cy3 filter
Emission:	Cy3 filter
Recommended plate:	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

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

Note 100 µL of Apopxin™ Orange 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™ Orange 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 a fluorescence microplate reader (bottom read mode) at Ex/Em = 540/590 nm (Cutoff = 570 nm) or image cells using fluorescence microscope with Cy3® filter.

EXAMPLE DATA ANALYSIS AND FIGURES

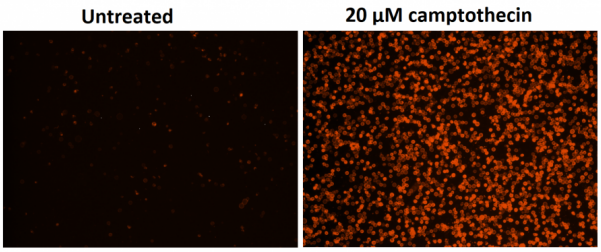

Figure 1. Fluorescence images of Jurkat cells in a Costar black wall/clear bottom 96-well plate stained with the Cell Meter™ Phosphatidylserine Apoptosis Assay Kit. Jurkat cells were treated without (Left) or with 20 µM camptothecin (Right) for 5 hours. The fluorescence intensity was measured using a fluorescence microscope with Cy3® channel.

DISCLAIMER

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