Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit *Blue Fluorescence*

Catalog number: 22795
Unit size: 200 Tests

Component	Storage	Amount (Cat No. 22795)
Component A: Caspase 3/7 Substrate (200X Stock Solution)	Freeze (< -15 °C), Minimize light exposure	2 vials (50 µL/vial)
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 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 Caspase 3 activation. Caspase 3 is widely accepted as a reliable indicator for cell apoptosis since the activation of caspase-3 (CPP32/apopain) is important for the initiation of apoptosis. Caspase 3 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). This kit uses Ac-DEVD-AMC as a fluorogenic indicator for caspase-3 activity. Cleavage of AMC peptides by caspase 3 generates strongly fluorescent AMC that is monitored fluorimetrically at 450-480 nm with excitation of 340-370 nm. The kit provides all the essential components with an optimized assay protocol. The assay is robust, and can be readily adapted for high-throughput assays. Using 100 uL of reagents per well in a 96-well format, this kit provides sufficient reagents to perform 200 assays. Using 25 uL of reagents per well in a 384-well format, this kit provides sufficient reagents to perform 800 assays.

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 an equal volume of Caspase 3/7 Substrate working solution.
3. Incubate at room temperature for 1 hour.
4. Monitor fluorescence intensity at Ex/Em = 360/470 nm (Cutoff = 420 nm).

Important Note

Thaw one vial of each kit component at room temperature before starting the experiment.

KEY PARAMETERS

Fluorescence microplate reader

Cutoff	420 nm
Emission	470 nm
Excitation	360 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Top/Bottom read mode

CELL PREPARATION

For guidelines on cell sample preparation, please visit:

https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF WORKING SOLUTION

1. Prepare a Caspase 3/7 Substrate working solution by combining 50 µL of Caspase 3/7 Substrate (Component A) with 10 mL of Assay Buffer (Component B), and mix well.

   Note: Aliquot and store any unused Components A and B at -20°C. Avoid repeated freeze/thaw cycles.

SAMPLE EXPERIMENTAL PROTOCOL

1. To treat cells, add 10 µL per well of 10X test compounds for a 96-well plate or 5 µL per well of 5X test compounds for a 384-well plate into PBS or the desired buffer. For blank wells containing only medium, add an equivalent volume of the compound buffer.
2. Incubate the cell plate at 37°C with 5% CO2 for a desired duration to induce apoptosis (e.g., 4 to 6 hours for Jurkat cells treated with camptothecin). Adjust the time as necessary based on the cell type and treatment conditions.
3. Add 100 µL/well for a 96-well plate or 25 µL/well for a 384-well plate of Caspase 3/7 Substrate working solution.
4. Incubate the plate at room temperature for at least 1 hour, protected from light.

   Note: To confirm the inhibition of the caspase 3/7-like activities, add 1 µL of 1 mM Ac-DEVD-CHO caspase 3/7 inhibitor to selected samples 10 minutes before adding the Caspase 3/7 working solution at room temperature.

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 at Ex/Em = 360/470 nm (Cutoff = 420 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

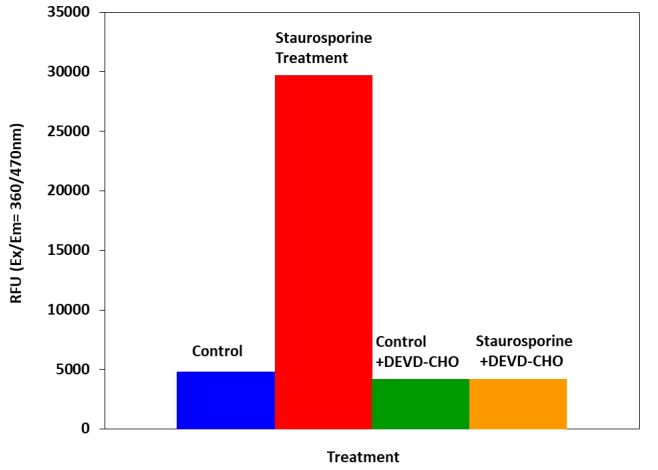

Figure 1. Detection of Caspase 3/7 activity in Jurkat cells with Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit. Jurkat cells were seeded on the same day at 80,000 cells/well/90 µL in a Costar black wall/clear bottom 96-well plate. The cells were treated with or without 1 µM of staurosporine for 4 hours, and with or without 10 µM of the caspase inhibitor AC-DEVD-CHO for 10 minutes. The caspase 3/7 assay solution (100 µL/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 360/470 nm (Cutoff = 420 nm).

DISCLAIMER

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