Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit

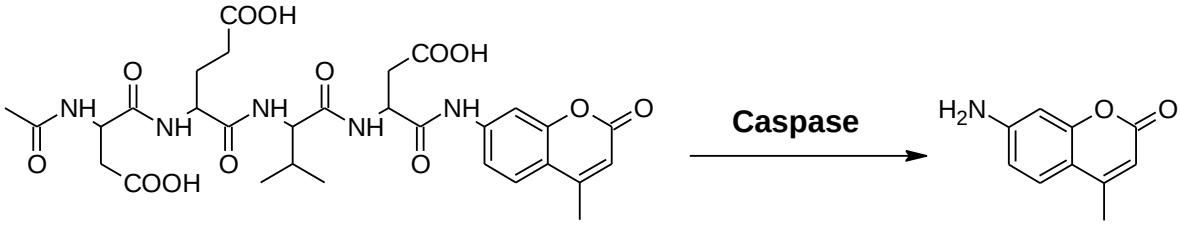
Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Numbers: 22795 (2 plates),	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

Our Cell Meter™ assay kits are a set of tools for monitoring cellular functions. A variety of parameters can be used to monitor cell functions. This particular kit is designed to monitor cell apoptosis by measuring Caspase 3/7 activation. Caspase 3/7 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 3/7 is proven to have 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 3/7 generates strongly fluorescent AMC that is monitored at the emission between 440 nm and 460 nm with the excitation at 350 nm. It is robust and can be readily adapted for high throughput screenings. The kit can be used to either quantify the activated caspase 3/7 activities in apoptotic cells or screen the caspase 3/7 inhibitors.



Non-Fluorescent Ac-DEVD-AMC Highly Fluorescent AMC

Kit Key Features

Non-Radioactive:	No special requirements for waste treatment.
Continuous:	Easily adapted to automation with minimal hands on time.
Convenient:	Include all essential assay components.
Optimized Performance:	Optimal conditions for the detection of caspase 3/7 activities.
Enhanced Value:	Less expensive than the sum of individual components.

Kit Components

Components	Amount
Component A: Caspase 3/7 Substrate (200X Stock Solution)	2 vials (50 µL/vial)
Component B: Assay Buffer	20 mL

Assay Protocol (for one plate)

Brief Summary

Prepare cells with test compounds (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Add equal volume of caspase 3/7 assay solution (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Incubate at room temperature for 1 hour → Monitor fluorescence intensity at Ex/Em = 350/450 nm

Ordering: sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@aatbio.com; Tel: 408-733-1055

1. Prepare cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 20,000 cells/well/90μL for a 96-well plate or 5,000cells/well/20μL for a 384-well plate.

1.2 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellet in culture medium at 80,000 cells/well/90μL for a 96-well poly-D lysine plate or 20,000 cells/well/20μL for a 384-well poly-D lysine plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.
Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

2. Prepare caspase 3/7 assay loading solution:

2.1 Thaw both of the kit components at room temperature before use.

2.2 Make caspase 3/7 assay loading solution by adding 50 μL of Caspase 3/7 Substrate (Component A) into 10 mL of Assay Buffer (Component B), and mix well.
Note: Aliquot and store the unused Components A and B at -20 ^{o}C. Avoid repeated freeze/thaw cycles.

3. Assay procedures:

3.1 Treat cells by adding 10 μL/well of 10X test compounds (96-well plate) or 5 μL/well of 5X test compounds (384-well plate) into PBS or the desired buffer. For blank wells (medium without the cells), add the same amount of compound buffer.

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

3.3 Add 100 μL/well (96-well plate) or 25 μL/well (384-well plate) of caspase 3/7 assay loading solution (from Step 2.2).

3.4 Incubate the assay loading solution plate at room temperature for at least 1 hour, protected from light.
***Note**: If desired, add 1μL of the 1 mM Ac-DEVD-CHO caspase 3/7 inhibitor into selected samples 10 minutes before adding the assay loading solution at room temperature to confirm the inhibition of the caspase 3/7-like activities.*

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

3.6 Monitor the fluorescence intensity at Ex/Em = 350/450 nm.

Data Analysis

The fluorescence in blank wells with the growth medium is subtracted from the values for those wells with the cells. The background fluorescence of the blank wells may vary depending on the sources of the growth media or the microtiter plates.

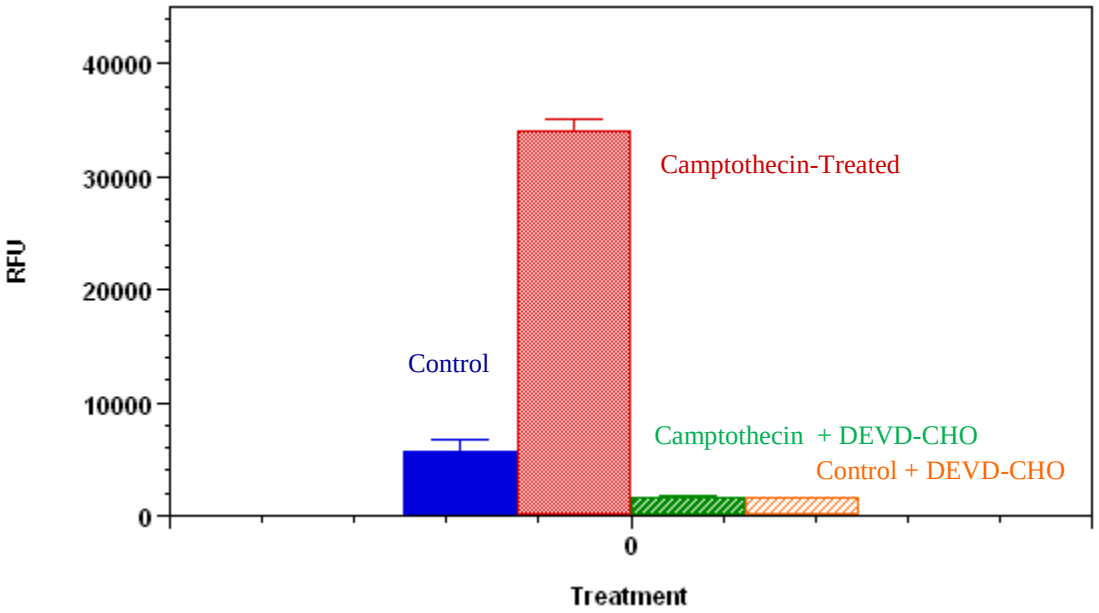


Figure 1. Detection of Caspase 3/7 Activities in Jurkat cells. Jurkat cells were seeded on the same day at 80,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with 20 µM camptothecin for 5 hours while the untreated cells were used as control. The treated cells and controls were incubated with or without the caspase 3/7 inhibitor AC-DEVD-CHO (5 µM) 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 = 350/450 nm with NOVOStar instrument (from BMG Labtech).

References

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