Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit

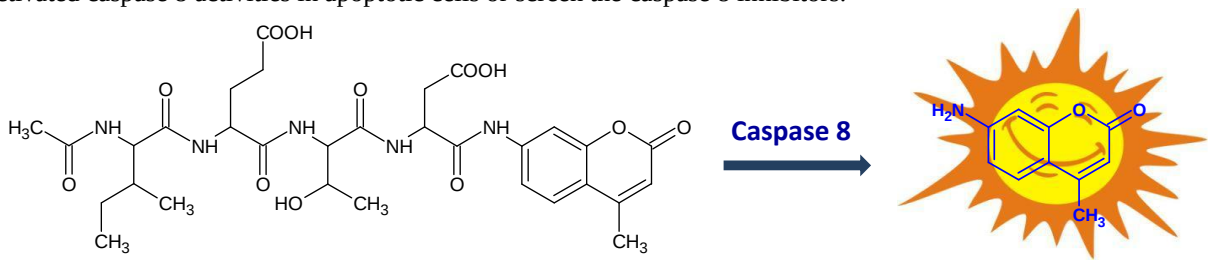
Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22812 (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. There are a variety of parameters that can be used to monitor cell apoptosis. This particular kit is designed to monitor cell apoptosis by measuring caspase 8 activity. Caspase 8 is a caspase protein, encoded by the CASP8 gene. Caspase 8 also plays an important role in neurodegenerative diseases, such as Huntington disease.

Caspase 8 is proven to have substrate selectivity for the peptide sequence Ile-Glu-Thr-Asp (IETD). This kit uses Ac-IETD-AMC as a fluorogenic indicator for caspase 8 activity. Cleavage of AMC peptides by caspase 8 generates strongly fluorescent AMC which is monitored at the emission between 450 nm and 460 nm with the excitation between 350 nm and 370 nm. The kit provides all the essential components with an optimized assay protocol. The assay can be readily adapted for high throughput screenings. It can be used to either quantify the activated caspase 8 activities in apoptotic cells or screen the caspase 8 inhibitors.



Non-Fluorescent AMC Substrate — 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:	All essential assay components included.
Optimized Performance:	Optimal conditions for the detection of caspase 8 activity.
Enhanced Value:	Less expensive than the sum of individual components.

Kit Components

Components	**Amount**
Component A: Caspase 8 Substrate (200X stock solution)	2 vials (50 µL/vial)
Component B: Assay Buffer	20 mL

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

Assay Protocol for One 96-well 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 8 assay solution (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Incubate at room temperature for 30 min to 1 hour → Monitor fluorescence increase at Ex/Em = 370/450 nm

1. Prepare cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 20,000 cells/well/90 µL for a 96-well plate or 5,000 cells/well/20 µL 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 200,000 cells/well/90 µL for a 96-well poly-D lysine plate or 50,000 cells/well/20 µL 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 8 assay loading solution:

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

2.2 Prepare caspase 8 assay loading solution: Add 50 µL of Caspase 8 Substrate (Component A) into 10 mL of Assay Buffer (Component B), and mix them well.

Note 1: Caspasae 8 assay loading solution is not stable, use promptly.
Note 2: Aliquot and store unused Caspase 8 Substrate (Component A) and Assay Buffer (Component B) at -20 ^{o}C. Avoid repeated freeze/thaw cycles.

3. Run caspase 8 assay:

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 5% CO_2, 37 °C incubator for a desired period of time (4-6 hours for Jurkat cells treated with staurosporine) to induce apoptosis.

3.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of caspase 8 assay loading solution (from Step 2.2).

3.4 Incubate the caspase 8 assay loading solution plate at room temperature for 30 min to 1 hour, protected from light.
Note: If desired, add 1 µL of the 1 mM Ac-IETD-CHO caspase 8 inhibitor to selected samples 10 minutes before adding the assay loading solution at room temperature to confirm the inhibition of the caspase 8-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 increase at Ex/Em = 370/450 nm.

Data Analysis

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

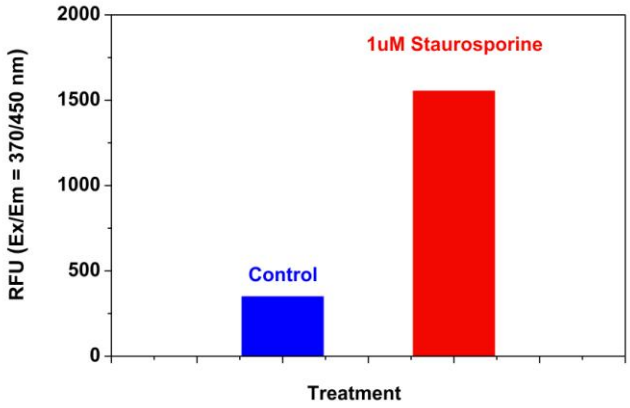


Figure1: Detection of Caspase 8 Activities in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with 1 µM staurosporine for 5 hours while the untreated cells were used as control. The caspase 8 assay loading solution (100 µL/well) was added and incubated at room temperature for 30 minutes. The fluorescence intensity was measured at Ex/Em = 370/450 nm with a FlexStation™ microplate reader (Molecular Devices).

References

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