Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit

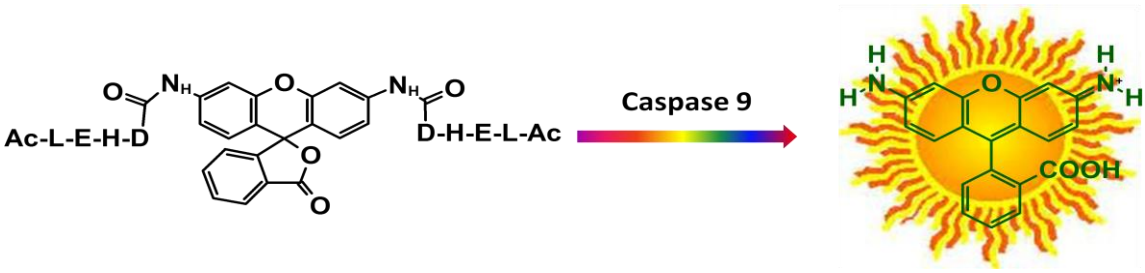
Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22799 (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. This particular kit is designed to monitor cell apoptosis by measuring caspase 9 activity. Caspase 9 is a member of the CED-3 subfamily. Activated Caspase-9 cleaves downstream caspases such as caspase-3, -6 and -7, initiating the caspase cascade. It is essential for apoptosis during normal development of the central nervous system.

Caspase 9 is proven to have selectivity for the peptide sequence Leu-Glu-His-Asp (LEHD). This kit uses $(Ac\text{-}LEHD)_2$-R110 as a fluorogenic indicator for caspase 9 activity. Cleavage of R110 peptides by caspase 9 generates strongly fluorescent rhodamine 110 (R110) which is monitored at the emission between 520 nm and 530 nm with the excitation between 480 nm and 500 nm. The kit provides all the essential components. The assay is robust and can be readily adapted for high throughput screening. It can be used to either quantify the activated caspase 9 activities in apoptotic cells or screen the caspase 9 inhibitors. Quite a few labs have used this kit for high throughput screenings.



Non-Fluorescent R110 Substrate Highly Fluorescent R110

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 are included.
Optimized Performance:	Optimal conditions for the detection of caspase 9 activity.
Enhanced Value:	Less expensive than the sum of individual components.

Kit Components

Components	**Amount**
Component A: Caspase 9 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 9 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 the fluorescence at Ex/Em = 490/525 nm

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 200,000 cells/well/90µL for a 96-well poly-D lysine plate or 50,000 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 9 assay loading solution:

2.1 Thaw all the kit components to room temperature before use.

2.2 Make caspase 9 assay loading solution: Add 50 µL of Caspase 9 Substrate (Component A) into 10 mL of Assay Buffer (Component B), and mix them well.
Note: Aliquot and store the unused Caspase 9 Substrate (Component A) and Assay Buffer (Component B) at -20 ^{o}C. Avoid repeated freeze/thaw cycles.

3. Run caspase 9 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 to induce apoptosis.

3.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of caspase 9 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 1 µL of the 1 mM Ac-LEHD-CHO caspase 9 inhibitor to selected samples 10 minutes before adding the assay loading solution at room temperature to confirm the inhibition of the caspase 9-like activity.

3.5 Centrifuge the 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 = 490/525 nm.

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Data Analysis

The fluorescence in blank wells with the growth medium is subtracted from the values for 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.

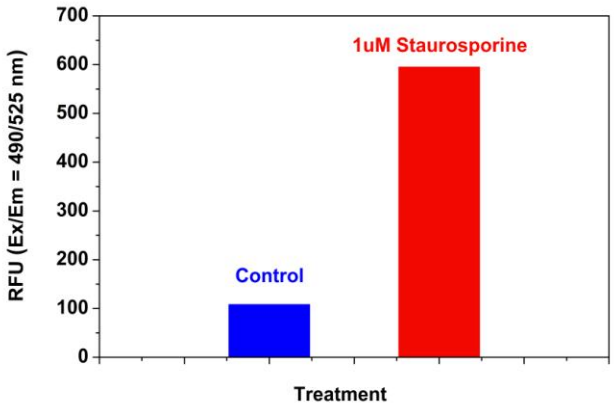

Figure1: Detection of Caspase 9 Activities in Jurkat cells. Jurkat cells were seeded at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with 1µM staurosporine for 5 hours while the untreated cells were used as control. The caspase 9 assay loading solution (100 µL/well) was added and incubated at room temperature for 1hour. The fluorescence intensity was measured at Ex/Em = 490/525 nm with a FlexStation™ microplate reader (Molecular Devices).

References

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