Cell Meter™ Caspase 3 Activity Apoptosis Assay Kit
Green Fluorescence

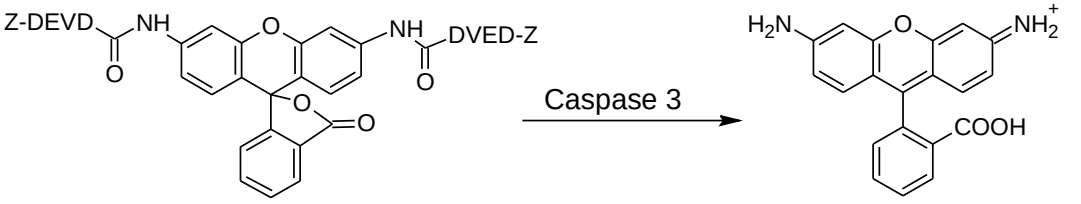
Ordering Information:
Product Number: #22796 (200 assays)

Instrument Platform:
Fluorescence microplate readers

Storage Conditions:
Keep in freezer and protect from light.

Introduction

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 Z-DEVD-Rh 110-DVED-Z as a fluorogenic indicator for caspase 3 activity. Cleavage of Rh 110 peptides by caspase 3 generates strongly fluorescent Rh 110 that is monitored fluorimetrically at 520-530 nm with excitation of 480-500 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 in a wide variety of fluorescence platforms such as microplate assays, immunocytochemistry and flow cytometry. The kit can be quantification of activated caspase in apoptotic cells, or screening for caspase inhibitors.



Non-Fluorescent Substrate — Strongly fluorescent product

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 3 activity.
Enhanced value: Less expensive than the sum of individual components.

Kit Components

Component	Amount
Component A: Caspase 3 substrate (100X stock solution)	2 vials (100 µL/vial)
Component B: Assay buffer	20 mL
Component C: Ac-DEVD-CHO (Caspase 3 inhibitor)	50 µL (1 mM)
Component D: Rh 110 (Fluorescence reference standard)	25 µl (5 mM)

Materials Required (but not provided)

- 96 or 384-well microplate: Tissue culture microplate with black wall and clear bottom is recommended.
- Fluorescence microplate reader: Capable of detecting emission at 520 ± 30 nm with excitation at 494 ± 30 nm.

Assay Protocol (for 1 plate)

Brief Summary

Prepare cells with test compounds (100 µL /96-well-plate or 25 µL 384-well-plate)
→ Add equal volume of caspase 3 assay solution → Incubate at room temperature for 1 hr
→ Read Fluorescence at Ex 494/Em 525

1. Preparation of Cells

1.1 For adherent cells, plating cells overnight in growth medium at 20,000 cells/well/90 µL for 96-well or 5,000 cells/well/20 µL for 384-well plates.

1.2 For non-adherent cells, centrifuging the cells from the culture medium and then suspension of the cell pellet in culture medium at 80,000 cells/well/90 µL for 96-well or 20,000 cells/well/20 µL for 384-well poly-D lysine plates. Centrifuge the plates at 800 rpm for 2 minutes with break 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. Preparation of Caspase 3 assay loading solution

2.1 Thaw Component A and B (if desired, Component C and D) to room temperature before use.

2.2 Add 100 µL caspase 3 substrate (Component A) into 10 mL assay buffer (Component B), mix well.

3. Assay Procedures

3.1 Treat cells with test compounds by adding 10 µL (for 96-well plates) or 5 µL (for 384-plates) compounds in PBS or your desired buffer. For blank wells (medium without the cells), add the corresponding amount of compound buffer.

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

3.3 Add 100 µL (96-well plate) or 25 µL (384-well plate) per well of Caspase 3 assay loading solution (from step 2.2).

3.4 Incubate the assay solution loading plate at room temperature for at least 1 hr, avoiding light if possible.
Note 1*: If desired, add 1μL of the 1 mM Ac-DEVD-CHO inhibitor (Component C) to selected samples 10 minutes before adding the assay solution at room temperature for confirming the caspase 3-like activities.*
Note 2*: If desired, prepare an Rh110 reference standard curve by diluting 5 mM Rh 110 stock (Component D) into growth medium to yield Rh110 solution ranging from 0-50 μM. Add 100 μL of each reference standard into the wells containing 100 μL of Caspase 3 assay loading solution at any time prior to measuring the fluorescence. This standard curve could be used to determine the moles of product produced in the caspase 3 containing reactions.*

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

3.6 Monitor the fluorescence at 525 nm (excitation: 496 nm).

4. 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 can be varied depending upon the sources of the growth media or the microtiter plates.

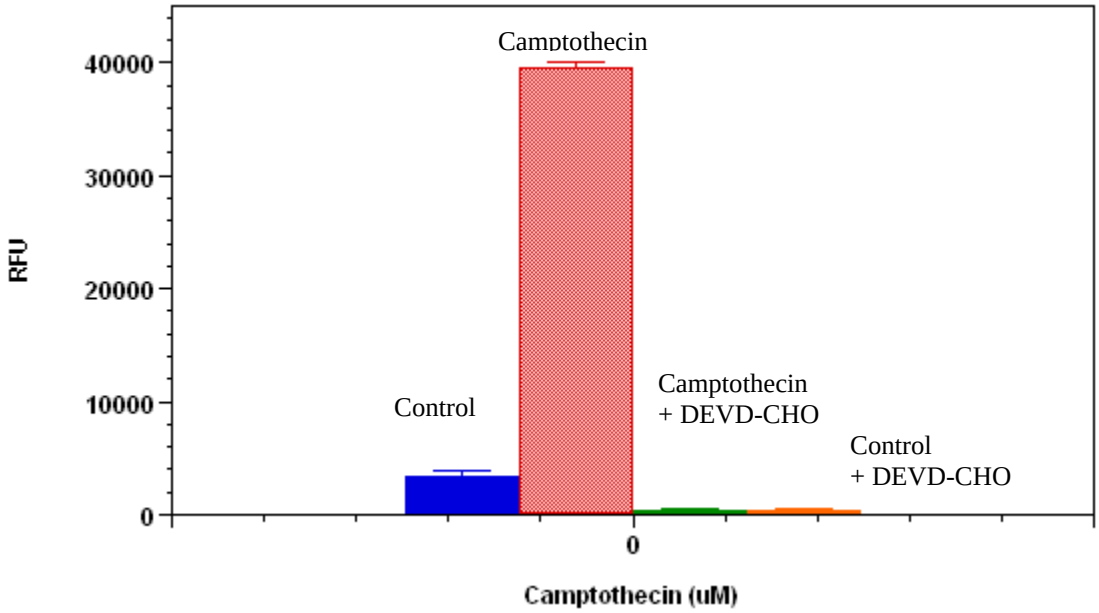


Figure 1. Detection of Caspase 3 Activity in Jurket cells. Jurkat cells were seeded on the same day at 80,000 cells per 90 μL per well in a 96-well black wall/clear bottom Costar plate. The cells were treated with or without 20 μM camptothecin for 5 hr, and/or 5 μM of the caspase inhibitor AC-DEVD-CHO (Component C 1 μL) for 10 min. The caspase 3 assay solution (100 μL /well) was added and incubated at room temperature for 1 hr. The fluorescence intensity was measured at Ex485/Em520 using NOVOstar instrument (from BMG LabTech).

References:

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