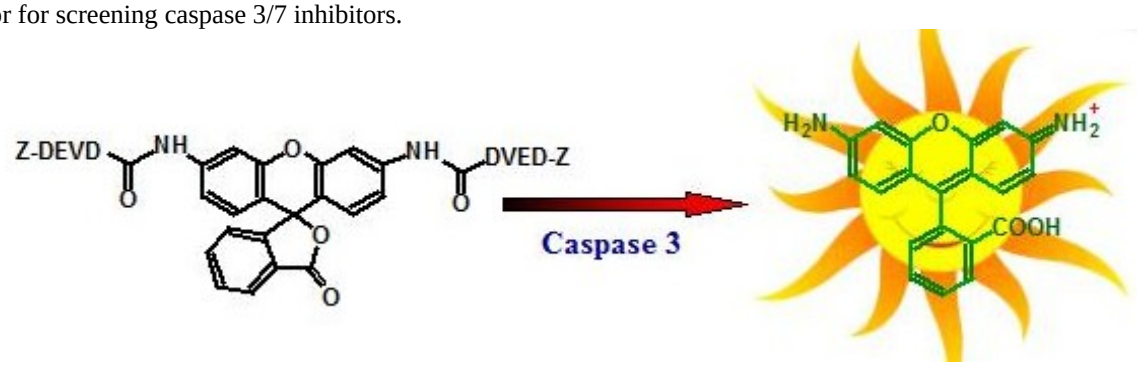
Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit

Green Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: #22796 (2 plates) #22796B (50 plates)	Keep in freezer and avoid light	Fluorescence microplate readers

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 3/7 activation. Caspase 3/7 is widely accepted as a reliable indicator for cell apoptosis since the activation of caspase 3/7 (CPP32/apopain) is important for the initiation of apoptosis. Caspase 3/7 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). This kit uses Z-DEVD-Rh110-DVED-Z as a fluorogenic indicator for caspase 3/7 activity. Cleavage of Rh110 peptides by caspase 3/7 generates strongly fluorescent Rh110 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 used for the quantification of activated caspase 3/7 activities in apoptotic cells, or for screening caspase 3/7 inhibitors.



Non-Fluorescent Rh110 Substrate — Highly Fluorescent Rh110

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:	Optical conditions for the detection of caspase 3/7 activity.
Enhanced value:	Less expensive than the sum of individual components.

ABD Bioquest, Inc., 923 Thompson Place, Sunnyvale, CA 94085. Tel: 408-733-1055; Fax: 408-733-1304
Ordering: sales@abdbioquest.com; 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support: support@abdbioquest.com; 408-733-1055

Kit Components

Component	#22796 200 assays (96-well) 800 assays (384-well)	#22796B 5,000 assays (96-well) 20,000 assays (384-well)
Component A: Caspase 3/7 Substrate (200X stock solution)	2 vials (50 µL/vial)	5 vials (0.5 mL/vial)
Component B: Assay Buffer	20 mL	5 bottles (100 mL/bottle)

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 3/7 assay solution (100 µL/96-well plate or 25 µL/384-well plate) → Incubate at room temperature for 1 hr → Read Fluorescence at Ex/Em = 490/525 nm

1. Preparation of cells:

1.1 For adherent cells, plating cells overnight in growth medium at 20,000 cells/well/90µL for 96-well or 5,000cells/well/20µL 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/90µL for 96-well or 20,000 cells/well/20µL 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 3/7 assay loading solution:

2.1 Thaw Component A and B to room temperature before use.

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

3. Assay procedures:

3.1 Treat cells with test compounds by adding 10 µL (for 96-well plates) 10X or 5 µL (for 384-plates) 5X compounds in PBS or 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 3/7 assay loading solution (from step 2.2).

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

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 Ex/Em = 490/525 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 can be varied depending upon the sources of the growth media or the microtiter plates.

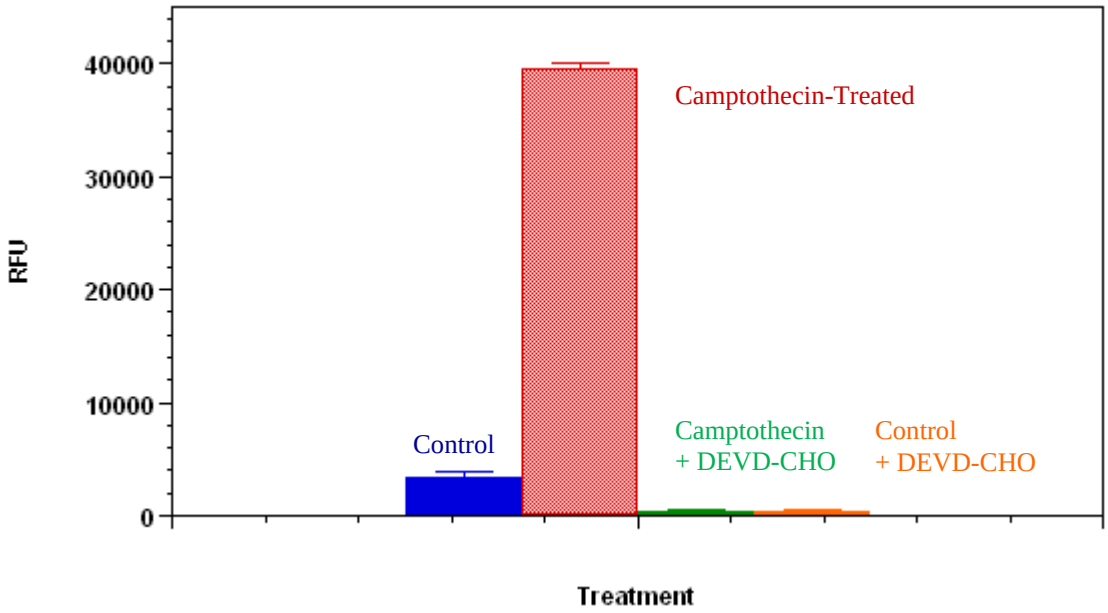


Figure 1. Detection of Caspase 3/7 Activity in Jurket cells. Jurkat cells were seeded on the same day at 80,000 cells per 90 µL per well in a 96-well black wall/clear bottom Costar plate. The cells were treated with or without 20 µM camptothecin for 5 h, and/or 5 µM of the caspase 3/7 inhibitor AC-DEVD-CHO for 10 min. The caspase 3/7 assay solution (100 µL/well) was added and incubated at room temperature for 1 hr. The fluorescence intensity was measured at Ex/Em = 490/525 using NOVOStar instrument (from BMG Labtech).

References:

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