Amplite™ Fluorimetric Caspase 3/7 Assay Kit

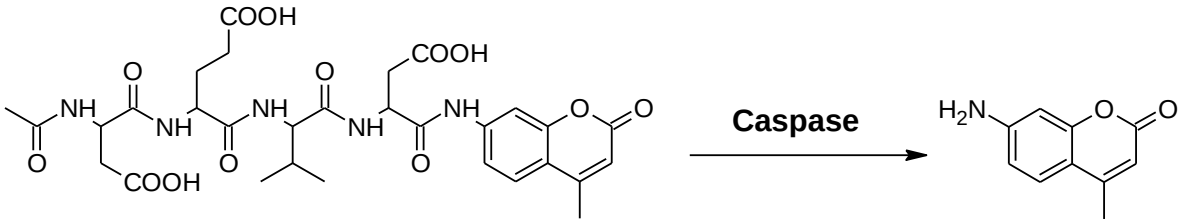
Blue Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13502 (500 assays)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

Introduction

Caspases play important roles in apoptosis and cell signaling. The activation of Caspase 3/7 (CPP32/apopain) is important for the initiation of apoptosis. Caspase 3/7 is also identified as a drug-screening target. Caspase inhibitors have anti-cancer and other pharmalogical potentials. It has been proven that Caspase 3/7 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD).

Our Amplite™ Fluorimetric Caspase 3/7 Assay Kit uses Ac-DEVD-AMC as a fluorogenic indicator for Caspase 3/7 activity. AMC peptides are almost non-fluorescent. Cleavage of AMC peptides by Caspase 3/7 generates strongly fluorescent AMC that is fluorimetrically monitored at 440-460 nm with excitation of 340-350 nm. It can be used to continuously measure the activities of Caspase 3/7 in cell extracts and purified enzyme preparations with a fluorescence microplate reader or fluorometer.



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

Kit Components

Components	Amount
Component A: Caspase 3/7 Substrate (200X stock solution)	1 vial (250 µL)
Component B: Assay Buffer	50 mL
Component C: DTT (1M)	1 vial (600 µL)
Component D: Ac-DEVD-CHO (Caspase 3/7 Inhibitor)	1 vial

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

Materials Required (but not provided)

- 96 or 384-well microplates: Tissue culture microplate with black wall and clear bottom is recommended.
- A fluorescence microplate reader: Capable of detecting fluorescence intensity at Ex/Em = 350±10/450±10 nm.

Assay Protocol for One 96-Well Plate

Brief Summary

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

1. Prepare Cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 20,000 to 80,000 cells/well/90 µL for a 96-well plate or 5,000 to 20,000 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 80,000 to 200,000 cells/well/90µL for a 96-well poly-D lysine plate or 20,000 to 50,000 cells/well/20µL for a 384-well poly-D lysine plate. Centrifuge the plates 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 stock solutions:

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

2.2 If desired, prepare a 1 mM stock solution of the Caspase 3/7 Inhibitor Ac-DEVD-CHO: Add 100 µL of DMSO (not provided) directly to the vial of Caspase 3/7 Inhibitor Ac-DEVD-CHO (Component D). This inhibitor can be used to confirm the correlation between fluorescence signal intensity and Caspase 3/7-like protease activities.
Note: The un-used inhibitor stock solution should be aliquoted and stored desiccated at -20 °C.

3. Prepare Caspase 3/7 assay solution:

Add 50 µL of 200X Caspase 3/7 Substrate stock solution (Component A), and 100 µL of 1M DTT solution (Component C) into 10 mL of Assay buffer (Component B), and mix well.
Note: 50 µL of the 200X Caspase 3/7 Substrate stock solution is enough for 100 assays using a reaction volume of 100 µL per assay. The unused 200X Caspase 3/7 Substrate stock solution should be aliquoted and stored desiccated at -20 °C. Keep from light.

4. Run Caspase 3/7 assay:

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

4.2 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.

4.3 Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Caspase 3/7 assay solution (from Step 3).

4.4 Incubate the plate at room temperature for at least 1 hour, protected from light.

Note 1: If desired, add 1μL of the 1 mM stock solution of the Caspase 3/7 Inhibitor Ac-DEVD-CHO (from Step 2.2) to selected samples 10 minutes before adding the assay solution at room temperature to confirm the caspase 3/7-like activities.

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

4.6 Monitor the fluorescence increase 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 varies depending upon the sources of the growth media or the microtiter plates.

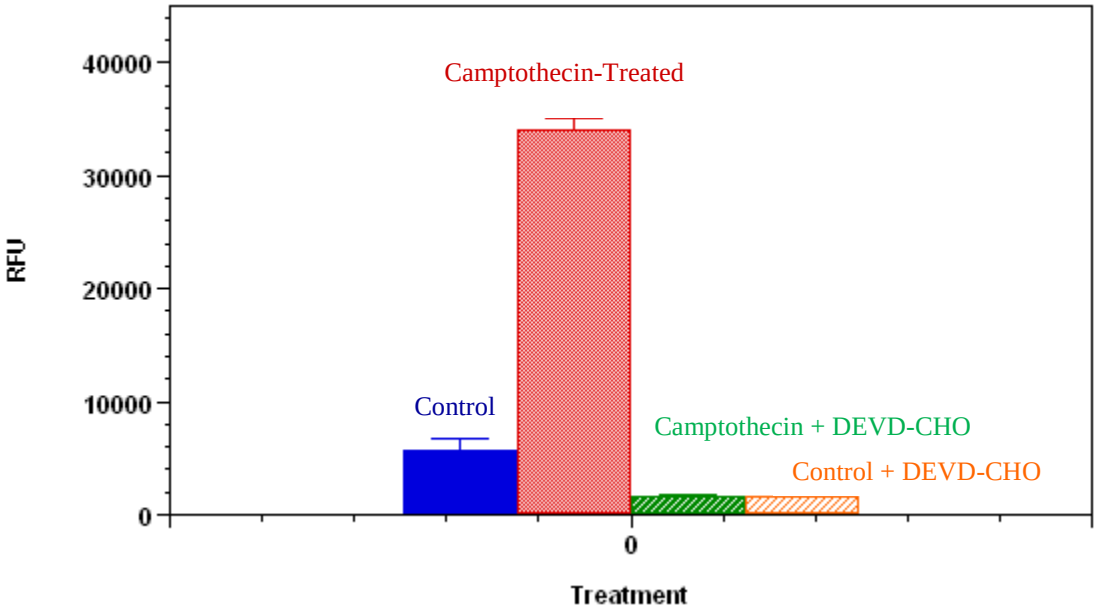


Figure 1. Detection of Caspase 3/7 Activity in Jurkat cells. Jurkat cells were seeded on the same day at 80,000 cells/well/90 μL in a black wall/clear bottom 96-well Costar plate. The cells were treated with or without 20 μM camptothecin for 5 hours. The caspase 3/7 assay solution (100 μL/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 350/450 nm using NOVOStar instrument (from BMG Labtech).

References

1. N. A. Thornberry and Y. Lazebnik, *Science* 281, 1312-1316 (1998).
2. J. C. Reed, *J.Clin.Oncol.* 17, 2941-2953 (1999).
3. Y. A. Lazebnik, S. H. Kaufmann, S. Desnoyers, G. G. Poirier, W. C. Earnshaw, *Nature* 371, 346-347 (1994).
4. P. Villa, S. H. Kaufmann, W. C. Earnshaw, *Trends Biochem.Sci.* 22, 388-393 (1997).
5. Y. Liu et al., *Anal.Biochem.* 267, 331-335 (1999).
6. M. Sakaue, Y. Motoyama, K. Yamamoto, T. Shiba, T. Teshima, K.Chiba. Biochem Biophys Res Commun, 350, 878 (2006)
7. T. Kume, R. Taguchi, H. Katsuki, M. Akao, H. Sugimoto, S. Kaneko, A. Akaike. Eur J Pharmacol, 542, 69 (2006) (2006)
8. M. Fennell, H. Chan , A Wood. J Biomol Screen, 11, 296 (2006)
9. X. Wu, J. Simone, D. Hewgill, R. Siegel, PE. Lipsky, L. He. Cytometry A, 69, 477 (2006)