Amplite™ Fluorimetric Caspase 3/7 Assay Kit

Green Fluorescence

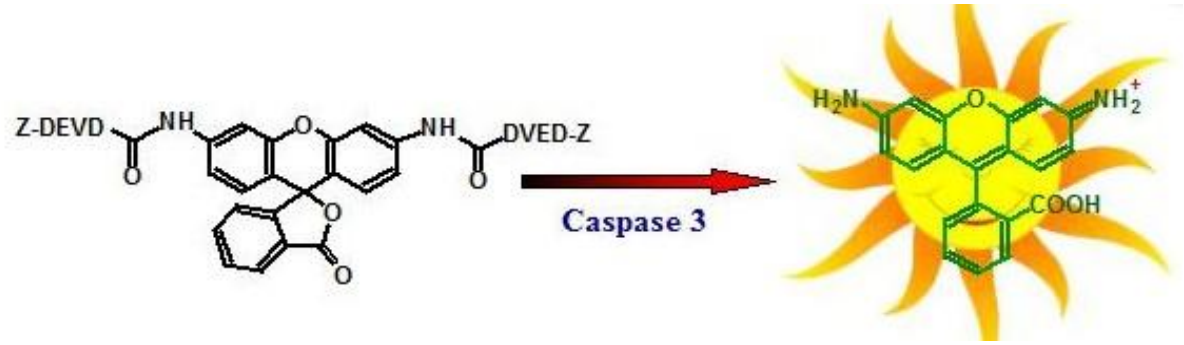
Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13503 (500 assays)	Keep in freezer Avoid exposure to light	Fluorescence microplate readers

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 $(Z\text{-}DEVD)_2$ R110 as fluorogenic indicator for assaying caspase 3/7 activity. R110-derived caspase substrates are probably the most sensitive indicators used for the fluorimetric detection of various caspase activities. R110 peptides are colorless and non-fluorescent. Cleavage of R110 peptides by caspases generates strongly fluorescent R110 that can be monitored fluorimetrically at 510-530 nm with excitation at 488 nm, the most common excitation light source. The increase in fluorescence of caspase-induced R110 hydrolysis is proportional to the activities of caspases.

This kit 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. Many labs have used this kit for screening aspase 3/7 inhibitors.



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:	Include all essential assay components.
Optimized Performance:	Optimal conditions for the detection of casepase 3 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	600 µL (1M)
Component D: Ac-DEVD-CHO (Caspase 3/7 Inhibitor)	1 vial
Component E: R110 Standard	50 µL (5 mM)

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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 monitoring fluorescence intensity at Ex/Em = 490±10/520±10 nm

Assay Protocol for One 96-Well 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 → Incubate at room temperature for 1 hour → Monitor fluorescence intensity at Ex/Em = 490/525 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 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 stock solutions:

2.1 Thaw Component A, B, C (if desired, Component D and E) 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 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 unused 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 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, stored desiccated at -20 °C, and protected 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) into PBS or desired buffer. For blank wells (medium without the cells), add the corresponding amount of compound buffer.

4.2 Incubate the cell plates in an 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, kept 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) into selected samples 10 minutes before adding the assay solution at room temperature to confirm the Caspase 3/7 -like activities.
Note 2: If desired, prepare an R110 standard curve by diluting 5 mM R110 Standard (Component E) into growth medium to yield serially diluted R110 standards ranging from 0-50 µM. Add 100 µL of the serially diluted R110 standards into the wells containing 100 µL of Caspase 3/7 assay solution at any time prior to

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measuring the fluorescence. This standard curve could be used to determine the moles of product produced in the caspase 3/7 containing reactions.

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

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

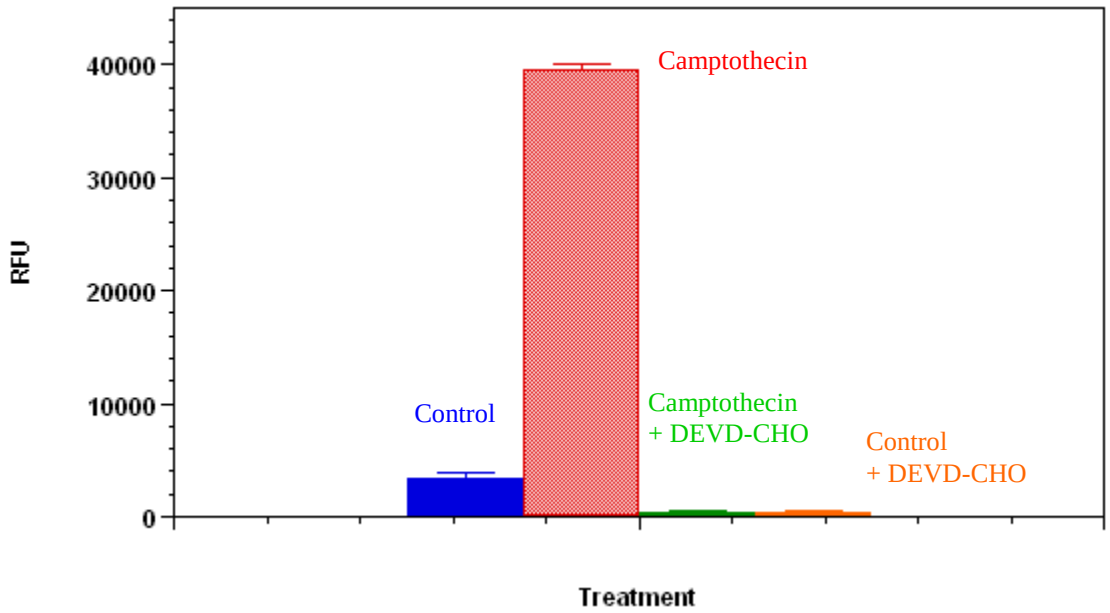

Figure 1. Detection of caspase 3/7 Activity in Jurkat cells. Jurkat cells were seeded on the same day at 80,000 cells /well/90 µL in a black wall/clear bottom 96-well costar plate. The cells were treated with or without 20 µM of camptothecin for 5 hours, and/or 5 µM caspase 3/7 inhibitor AC-DEVD-CHO for 10 minutes. 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 = 490/525 nm using the NOVOstar instrument (BMG Labtech).

References

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