Amplite™ Fluorimetric Caspase 3/7 Assay Kit *Green Fluorescence*

Catalog number: 13503
Unit size: 500 Tests

Component	Storage	Amount
Component A: Caspase 3/7 Substrate (200X Stock Solution)	Freeze (<-15 °C), Minimize light exposure	1 vial (250 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	50 mL
Component C: DTT	Freeze (<-15 °C), Minimize light exposure	600 µL (1M)
Component D: Ac-DEVD-CHO (Caspase 3/7 Inhibitor)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component E: R110 Standard	Freeze (<-15 °C), Minimize light exposure	50 µL (5 mM)

OVERVIEW

Caspases play important roles in apoptosis and cell signaling. The activation of caspase-3 (CPP32/apopain) is important for the initiation of apoptosis. Caspase 3 is also identified as a drug-screening target. Caspase 3 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). This Amplite™ Caspase-3 Assay Kit uses (Z-DEVD)2R110 as fluorogenic indicator for assaying caspase-3 activity. Cleavage of R110 peptides by caspases generates strongly fluorescent R110 that can be monitored fluorimetrically at 510-530 nm with excitation of 488 nm, the most common excitation light source used in fluorescence instruments. R110-derived caspase substrates are probably the most sensitive indicators widely used for the fluorimetric detection of various caspase activities. This kit can be used to continuously measure the activities of caspase-3 in cell extracts and purified enzyme preparations using a fluorescence microplate reader or fluorometer. It can also be used with flow cytometry for analyzing cell apoptosis and the activities of caspases 3 and 7.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds (100 µL/well/96-well plate or 25 µL/well/384-well plate)
2. Add equal volume of Caspase 3/7 working solution (100 µL/well/96-well plate or 25 µL/well/384-well plate)
3. Incubate at room temperature for 1 hour
4. Monitor fluorescence intensity at Ex/Em = 490/525 nm (Cutoff = 515 nm)

Important Thaw component A, B, C (if desired, Component D and E) at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Caspase 3/7 Inhibitor Ac-DEVD-CHO stock solution (1 mM):
Add 100 µL of DMSO (not provided) directly to the vial of Ac-DEVD-CHO (Component D), and mix well to make 1 mM Caspase 3/7 Inhibitor Ac-DEVD-CHO stock solution. This Caspase 3/7 Inhibitor can be used to confirm the correlation between fluorescence signal intensity and Caspase 3/7-like protease activities.

PREPARATION OF WORKING 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 to make Caspase 3/7 working solution.

Note This Caspase 3/7 working solution is enough for 100 assays using a reaction volume of 100 µL per assay. Protect from light.

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

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

2. Incubate the cell plates in an incubator for desired period of time (4 - 6 hours for Jurkat cells treated with camptothecin) to induce apoptosis.

3. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Caspase 3/7 working solution.

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

   Note If desired, add 1 µL of 1 mM Caspase 3/7 Inhibitor Ac-DEVD-CHO stock solution into selected samples 10 minutes before adding Caspase 3/7 working solution at room temperature to confirm the Caspase 3/7-like activities.

   Note If desired, prepare a R110 standards 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 working 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 3/7 containing reactions.

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

6. Monitor the fluorescence increase at Ex/Em = 490/525 nm (Cutoff = 515 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

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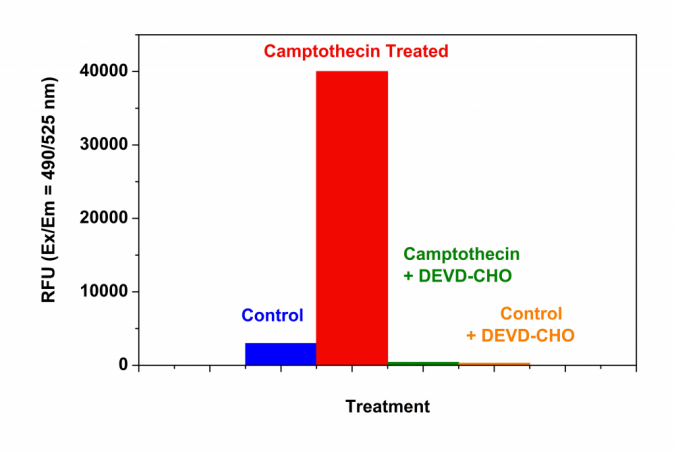

Figure 1. Detection of caspase 3/7 Activity in Jurkat cells. Jurkat cells were seeded on the same day at 80,000 cells/well/90 µL in a black wall/clear bottom 96-well costar plate. The cells were treated with or without 20 µM ofcamptothecin for 5 hours, and/or 5 µM caspase 3/7 inhibitor AC-DEVD-CHO for 10 minutes. The caspase 3/7 assaysolution (100 µL/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity wasmeasured at Ex/Em = 490/525 nm using the NOVOstar instrument (BMG Labtech).