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

Catalog number: 13502
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 (1M)	Freeze (<-15 °C), Minimize light exposure	1 vial (600 µL)
Component D: Ac-DEVD-CHO (Caspase 3/7 Inhibitor)	Freeze (<-15 °C), Minimize light exposure	1 vial

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 Ac-DEVD-AMC as fluorogenic indicator for assaying caspase-3 activity. AMC-derived caspase substrates are widely used for fluorimetric detection of various caspase activities. Cleavage of AMC peptides by caspases generates strongly fluorescent AMC that is monitored fluorimetrically at 440-460 nm with excitation of 340-350 nm. 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.

AT A GLANCE

Protocol summary

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

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	350 nm
Emission:	450 nm
Cutoff:	420 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.

(Optional) Caspase 3/7 Inhibitor Ac-DEVD-CHO stock solution (1 mM):
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.

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.

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. Keep 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 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.

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.

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 the 1 mM stock solution of the Caspase 3/7 Inhibitor Ac-DEVD-CHO to selected samples 10 minutes before adding the assay solution at room temperature to confirm the caspase 3/7-like protease activities.

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

6. Monitor the fluorescence increase at Ex/Em = 350/450 nm.

EXAMPLE DATA ANALYSIS AND FIGURES

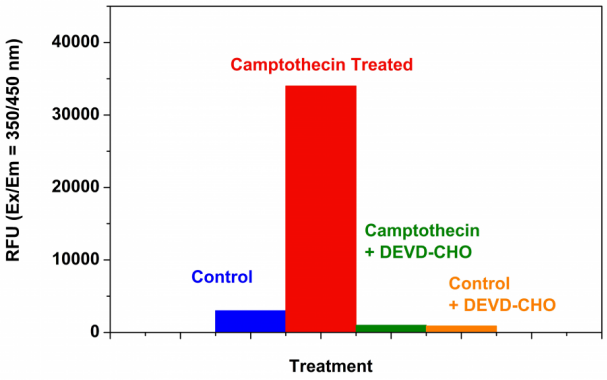

Figure 1. Detection of Caspase 3/7 activity in Jurkat cells with Amplite™ Fluorimetric Caspase 3/7 Assay Kit. Jurkat cells were seeded on the same day at 80,000 cells/well/90 µL in a Costar black wall/clear bottom 96-well plate. The cells were treated with or without 20 µM of camptothecin for 5 hours, and with or

without 5 μM of the caspase 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 = 350/450 nm.

DISCLAIMER