

## Amplite™ Fluorimetric Caspase 3/7 Assay Kit \*Red Fluorescence\*

Catalog number: 13504  
Unit size: 100 tests

Component	Storage	Amount
Component A: Z-DEVD-ProRed™	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	10 mL
Component C: DTT	Freeze (<-15 °C), Minimize light exposure	200 µL (1M)
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 Z-DEVD-ProRed™ as the fluorogenic indicator for assaying caspase-3 activity. Cleavage of R110 peptides by caspases generates strongly red fluorescent ProRed™ that can be monitored fluorimetrically at ~620 nm with excitation of ~530 nm. Z-DEVD-ProRed™ is recognized as the most sensitive red fluorogenic caspase 3/7 substrate. 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
2. Add equal volume of caspase 3/7 working solution
3. Incubate at room temperature for 1 hour
4. Monitor fluorescence intensity at Ex/Em = 535/620 nm

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

### KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	535 nm
Emission:	620 nm
Cutoff:	610 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. **Z-DEVD-ProRed™ stock solution (200X):**  
Add 65 µL of DMSO (not provided) into the vial of Component A.
2. **(Optional) Caspase 3/7 Inhibitor Ac-DEVD-CHO stock solution (1 mM):**  
Add 100 µL of DMSO 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.

### PREPARATION OF WORKING SOLUTION

Add 50 µL of 200X Z-DEVD-ProRed™ stock solution 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 Z-DEVD-ProRed™ stock solution is enough for 100 assays using a reaction volume of 100 µL per assay.

### 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) into PBS or desired buffer. For blank wells (medium without the cells), add the same amount of compound buffer.
  2. Incubate the cell plates in an incubator for a desired period of time (3 - 5 hours for Jurkat cells treated with staurosporine) 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, kept from light.
- Note** If desired, add 1 µL of the 1 mM stock solution of the caspase 3/7 Inhibitor Ac-DEVD-CHO into selected samples 10 minutes before adding the caspase 3/7 assay working solution at room temperature to confirm the caspase 3/7-like activities.
5. Monitor the fluorescence intensity at Ex/Em = 535/620 nm (cut off at 610 nm) with either top or bottom read mode.

**Note** Sometimes, bottom read gives better signal to background ratio, centrifuge cell plate (especially for the nonadherent cells) at 800 rpm for 2 minutes (brake off) if using bottom read mode.

### EXAMPLE DATA ANALYSIS AND FIGURES

Example data analysis and images of this product can be found on the web at: <https://www.aatbio.com/products/amplite-fluorimetric-caspase-3-7-assay-kit-red-fluorescence>

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