

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>

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.