Amplite™ Fluorimetric Caspase 3/7 Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13504 (100 assays)	Keep in freezer and protect from 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-DEVD-ProRed™ as the fluorogenic indicator for caspase-3 activity. Cleavage of ProRed™ DEVD blocking peptide residue by Caspase 3/7 generates strongly red fluorescent ProRed™ that is monitored fluorimetrically at ~620 nm with excitation of ~535 nm. The increase in fluorescence of caspase-induced ProRed™ hydrolysis is proportional to the activities of caspases. The kit provides all the essential components with an optimized assay protocol. It can be used to measure the activities of caspase 3/7 in cell extracts and purified enzyme, as well as to screen the caspase 3/7 inhibitors. Using 100 µl of reagents per well in a 96-well format, this kit provides sufficient reagents to perform 100 assays. Using 25 ul of reagents per well in a 384-well format, this kit provides sufficient reagents to perform 400 assays.

Kit Components

Components	Amount
Component A: Z-DEVD-ProRed™	1 vial
Component B: Assay Buffer	10 mL
Component C: DTT	200 µL (1M)
Component D: Ac-DEVD-CHO (Caspase 3/7 Inhibitor)	1 vial

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells with test compounds (100 µL/well/96-well plate or 25 µL/well/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 = 535/620 nm

1. Prepare Cells:

1.1 For adherent cells: Plate cells overnight in growth medium at 20,000 cells/well/90µL for a 96-well or 5,000cells/well/20µL for a 384-well plate black wall/clear bottom 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 or 20,000 to 50,000 cells/well/20µL for a 384-well black wall/clear bottom 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) at room temperature before use.

2.2 Make 200X Z-DEVD-ProRed™ stock solution by adding 65 µL DMSO (not provided) into the vial of Component A.

2.3 If desired, prepare a 1 mM stock solution of the caspase 3/7 Inhibitor Ac-DEVD-CHO: 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.
Note: The unused inhibitor stock solution should be aliquoted and stored desiccated at -20 °C.

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Technical Support: support@aatbio.com; Tel: 408-733-1055

3. Prepare Caspase 3/7 assay solution:

Add 50 µL of 200X Z-DEVD-ProRed™ stock solution (from Step 2.2), and 100 µL of 1M DTT solution (Component C) into10 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. The unused 200X Z-DEVD-ProRed™ stock solution (from Step 2.2) 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 same amount of compound buffer.
4.2 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.
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 caspase 3/7*assay solution at room temperature to confirm the caspase 3/7 -like activities.*
4.5 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 non-adherent cells) at 800 rpm for 2 minutes (brake off) if using bottom read mode.

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 may vary depending on the sources of the growth media or the microtiter plates.

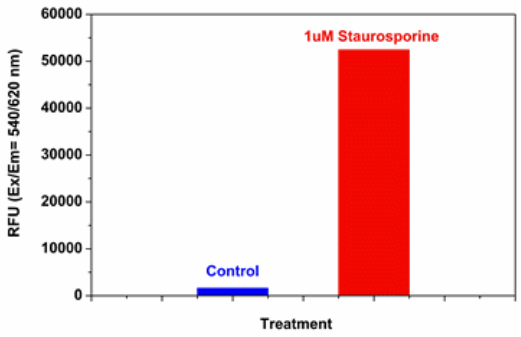

Figure 1. Detection of Caspase 3/7 Activities in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with staurosporine at the final concentration of 1 µM for 5 hours while the untreated cells were used as control. 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 = 540/620 nm with FlexStation fluorescence microplate reader (Molecular Devices).

References

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3. Y. A. Lazebnik, S. H. Kaufmann, S. Desnoyers, G. G. Poirier, W. C. Earnshaw, *Nature* 371, 346-347 (1994).
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