Amplite™ Colorimetric Caspase 3/7 Assay Kit
Yellow Color

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 13507 (200 tests)	Keep at -20ºC and protect from light	Absorbance 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™ Colorimetric Caspase 3/7 Assay Kit uses $(Z\text{-DEVD})_2$R110 as the chromogenic indicator for assaying caspase 3/7 activity. R110 peptides are colorless. Cleavage of R110 peptides by caspases generates R110, a yellow color dye that can be monitored at 490-520 nm. The increase in the absorbance of caspase-induced R110 hydrolysis is proportional to the activities of caspases. This kit can be used to continuously measure the activities of caspase 3/7 in cell extracts and purified enzyme preparations with an absorbance microplate reader with much higher sensitivity than the similar commercial kits from other vendors that use the DEVD-pNA peptide.

Kit Components

Components	**Amount**
Component A: Caspase 3/7 Substrate (200X Stock Solution)	2 vials (50 µL/vial)
Component B: Assay Buffer	20 mL

Assay Protocol for One 96-Well Plate

Brief Summary

Prepare cells with test compounds (100 µL/96-well plate or 25 µL/384-well plate) →
Add equal volume of caspase 3/7 assay solution → Incubate at room temperature for 1-2 hour →
Monitor absorbance at 490 nm

1. Prepare Cells:

1.1 For non-adherent cells: Centrifuge the cells from the culture medium and then suspend the cell pellet in culture medium at 1,000,000 to 5,000,000 cells/mL for a 96-well poly-D lysine plate (100 µL/well). 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 Caspase 3/7 assay solution:

Add 50 µL of 200X caspase 3/7 Substrate stock solution (Component A) into 10 mL 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. The unused 200X caspase 3/7 Substrate stock solution should be aliquoted, stored desiccated at -20°C, and protected from light.

3. Run Caspase 3/7 assay:

4.1 Treat cells by adding 10 µL of 10X test compounds (96-well plate) into PBS or desired buffer. For blank wells (medium without the cells), add the corresponding amount of compound buffer.

4.2 Incubate the cell plates in an incubator for a desired period of time to induce apoptosis.
Note: We treated Jurkat cells with staurosporine (SS) for 4 hours at 37ºC to induce cell apoptosis. See Figure 1 for details.

4.3 Add 100 µL/well of caspase 3/7 assay solution (from step 2).

4.4 Incubate the plate at room temperature for at least 1 hour, kept from light.

4.5 Centrifuge cell plates at 800 rpm for 2 minutes with brake off.

4.6 Monitor the absorbance increase with an absorbance plate reader at OD =490 nm.

Data Analysis

The absorbance in blank wells with the growth medium only is subtracted from the values for those wells with cells. The absorbance of the blank wells may vary depending on the sources of the microtiter plates or the growth media.

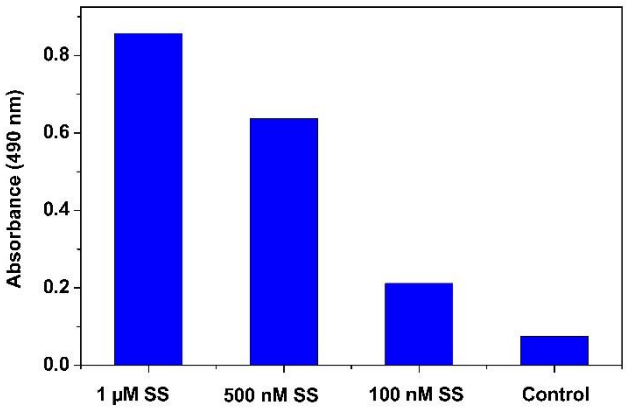

Figure 1. Detection of caspase 3/7 Activity in Jurkat cells. The cells were treated with staurosporine (SS) at the concentration of 0-1 µM for 4 hours at 37ºC. After treatment, cells were incubated with caspase 3/7 assay solution for 2 hours. The absorbance was measured at 490 nm using a SpectraMax reader (Molecular Devices).

Reference

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