Amplite™ Colorimetric Caspase 3/7 Assay Kit *Yellow Color*

Catalog number: 13507
Unit size: 200 Tests

Component	Storage	Amount
Component A: Caspase 3/7 Substrate (200X Stock Solution)	Freeze (< -15 °C), Minimize light exposure	2 vials (50 µL/vial)
Component B: Assay Buffer	Freeze (< -15 °C)	1 bottle (20 mL)

OVERVIEW

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-DEVD)2R110 as the chromogenic indicator for assaying caspase 3/7 activity. R110 peptide sustrates 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 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 other commercial kits that use DEVD-pNA peptide.

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds (100 µL/well/96-well plate or 25 µL/well/384-well plate)
2. Add equal volume of Caspase 3/7 working solution (100 µL/well/96-well plate or 25 µL/well/384-well plate)
3. Incubate at room temperature for 1 - 2 hours
4. Monitor absorbance at 490 nm

KEY PARAMETERS

Absorbance microplate reader

Absorbance	490 nm
Recommended plate	Clear bottom

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF WORKING SOLUTION

Add 50 µL of 200X Caspase 3/7 Substrate stock solution (Component A) into 10 mL Assay Buffer (Component B), and mix well to make Caspase 3/7 working solution. **Note:** This Caspase 3/7 working solution is enough for 100 assays using a reaction volume of 100 µL per assay. Before opening the vial, do brief centrifuge to accumulate the stock solution to the bottom of the tube.

SAMPLE EXPERIMENTAL PROTOCOL

1. Treat cells with 10 µL of 10X test compounds (for a 96-well plate) or 5 µL of 5X test compound (for a 384-well plate) in PBS or desired buffer. For blank wells (medium without the cells), add the corresponding amount of compound buffer.

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 37°C to induce cell apoptosis. See Figure 1 for details.

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.

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

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

EXAMPLE DATA ANALYSIS AND FIGURES

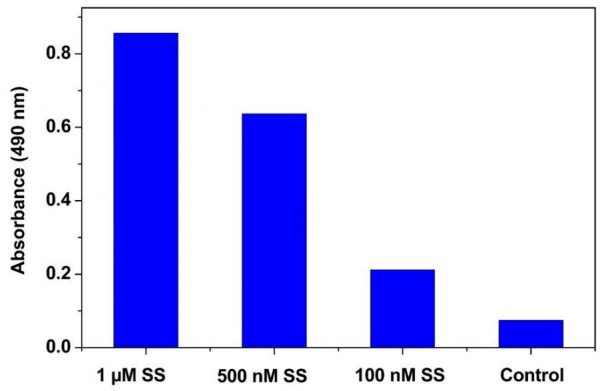

Figure 1.
Detection of caspase 3/7 Activity in Jurkat cells. The cells were treated with staurosporine (SS) at the concentration of 0-1 µM for 4 hours at 37ºC. After treatment, cells were incubated with caspase 3/7 assay solution for 2 hours. The absorbance was measured at 490 nm using a SpectraMax reader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

DISCLAIMER

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