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)	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

Instrument:	Absorbance microplate reader
Absorbance:	490 nm
Recommended plate:	Clear bottom

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.

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 with 10 µL of 10X test compounds (for a 96-well plate) or 5 µL of 5X test compound (for a 384-well plate) into 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

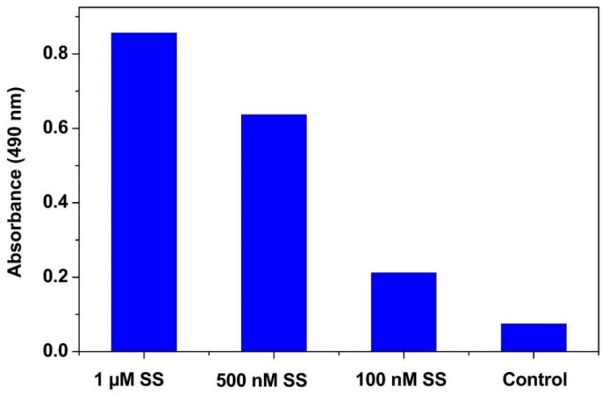

Figure 1.

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