# Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Blue Fluorescence*

Catalog number: 22813
Unit size: 200 Tests

| Component | Storage | Amount |
|---|---|---|
| Component A: Caspase 9 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

Our Cell Meter™ assay kits are a set of tools for monitoring cellular functions. There are a variety of parameters that can be used. This particular kit is designed to monitor cell apoptosis by measuring caspase 9 activity. Caspase 9 is a member of the CED-3 subfamily. Activated Caspase-9 cleaves downstream caspases such as caspase-3, -6 and -7, initiating the caspase cascade. It is essential for apoptosis during normal development of the central nervous system. Caspase 9 is proven to have selectivity for the peptide sequence Leu-Glu-His-Asp (LEHD). This kit uses Ac-LEHD-AMC as a fluorogenic indicator for caspase 9 activity. Cleavage of AMC by caspase 9 generates strongly fluorescent AMC. The kit provides all the essential components. The assay is robust and can be readily adapted for high throughput screening. It can be used to either quantify the activated caspase 9 activities in apoptotic cells or screen the caspase 9 inhibitors. Quite a few labs have used this kit for high throughput screenings.

## 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 9 Substarte working solution (100 µL/well/96-well plate or 25 µL/well/384-well plate)
3. Incubate at room temperature for 30 - 60 minutes
4. Monitor the fluorescence at Ex/Em = 375/435 nm (Cutoff = 420 nm)

**Important** Thaw all the kit components at room temperature before starting the experiment.

## KEY PARAMETERS

| | |
|---|---|
| Instrument: | Fluorescence microplate reader |
| Excitation: | 375 nm |
| Emission: | 435 nm |
| Cutoff: | 420 nm |
| Recommended plate: | Black wall/clear bottom |
| Instrument specification(s): | Top/Bottom read mode |

## PREPARATION OF WORKING SOLUTION

Add 50 µL of Caspase 9 Substrate (Component A) into 10 mL of Assay Buffer (Component B) and mix well to make Caspase 9 Substrate working solution. Protect from light.

***Note*** Aliquot and store the unused Caspase 9 Substrate (Component A) and Assay Buffer (Component B) at -20 °C. Avoid repeated freeze/thaw cycles.

## 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/well of 10X test compounds (96-well plate) or 5 µL/well of 5X test compounds (384-well plate) into PBS or the desired buffer. For blank wells (medium without the cells), add the same amount of compound buffer.
2. Incubate the cell plate in a 5% $CO_2$, 37°C incubator for a desired period of time (4-6 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 9 Substrate working solution.
4. Incubate the Caspase 9 Substrate working solution plate at room temperature for at least 1 hour, protected from light.

   ***Note*** If desired, add 1 µL of the 1 mM Ac-LEHD-CHO caspase 9 inhibitor to selected samples 10 minutes before adding Caspase 9 Substrate working solution at room temperature to confirm the inhibition of the caspase 9-like activity.
5. Centrifuge the cell plate (especially for the non-adherent cells) at 800 rpm for 2 minutes (brake off).
6. Monitor the fluorescence intensity with a fluorescence microplate reader at Ex/Em = 375/435 nm (Cutoff = 420 nm).

## EXAMPLE DATA ANALYSIS AND FIGURES

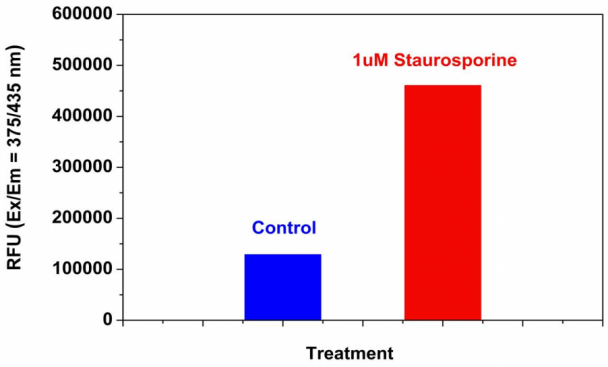


**Figure 1.** Detection of Caspase 9 Activities in Jurkat cells using Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Blue Fluorescence*. Jurkat cells were seeded at 300,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with or without 1µM of staurosporine for 4 hours. The caspase 9 Substrate working solution (100 µL/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 375/435 nm (Cutoff = 420 nm) with a FlexStation™ microplate reader (Molecular Devices).

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