Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Red Fluorescence*

Catalog number: 22817
Unit size: 100 Tests

Component	Storage	Amount
Component A: Ac-LEHD-ProRed™	Freeze (<-15 °C), Minimize light exposure	1 vial (50 µL, 200X)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (10 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-ProRed™ as a fluorogenic indicator for caspase 9 activity. Cleavage of ProRed™ by caspase 9 generates strongly fluorescent ProRed™. 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 Substrate working solution (100 µL/well/96-well plate or 25 µL/well/384-well plate)
3. Incubate at room temperature for 1 hour
4. Monitor fluorescence intensity (top or bottom read mode) at Ex/Em = 540/620 nm (Cutoff = 610 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	620 nm
Cutoff:	610 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Top or bottom read mode

PREPARATION OF WORKING SOLUTION

Add 5 µL of 200X Ac-LEHD-ProRed™ stock solution (Component A) into 1 mL of Assay Buffer (Component B) and mix well to make Caspase 9 Substrate working solution. Protect from light.

Note Caspase 9 working solution is not stable, use it promptly. 1 mL of Caspase 9 Substrate working solution is enough for 10 assays.

Note Aliquot and store 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- 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 37 °C, 5% CO_2, incubator for a desired period of time (3-4 hours for Jurkat cells treated with staurosporine) to induce apoptosis.

3. Add 100 µL/well/96-wel or 25 µL/well/384-well plate of Caspase 9 Substrate working solution.

4. Incubate the 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 Substarte working solution at room temperature to confirm the inhibition of the caspase 9-like activities.

5. Monitor the fluorescence intensity with a fluorescence microplate reader (either top or bottom read mode) at Ex/Em = 540/620 nm (Cutoff = 610 nm).

   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.

EXAMPLE DATA ANALYSIS AND FIGURES

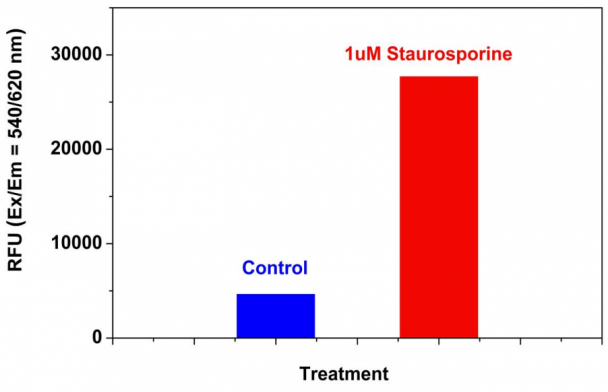

Figure 1. Detection of Caspase 9 Activities in Jurkat cells using Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit *Red Fluorescence*. Jurkat cells were seeded on the same day at 300,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with or without 1 µM 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 = 540/620 nm (Cutoff = 610 nm) with FlexStation fluorescence microplate reader (Molecular Devices).

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