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

Catalog number: 22799
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)2-R110 as a fluorogenic indicator for caspase 9 activity. Cleavage of R110 peptides by caspase 9 generates strongly fluorescent rhodamine 110 (R110)which is monitored at the emission between 520 nm and 530 nm with the excitation between 480 nm and 500 nm. 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
2. Add equal volume of Caspase 9 Substrate working solution
3. Incubate at room temperature for 30 min to 1 hour
4. Monitor the fluorescence at Ex/Em = 490/525 nm

Important Thaw all the kit components to room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 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 them well.

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 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 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 the assay 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 at Ex/Em = 490/525 nm (Cutoff=515 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

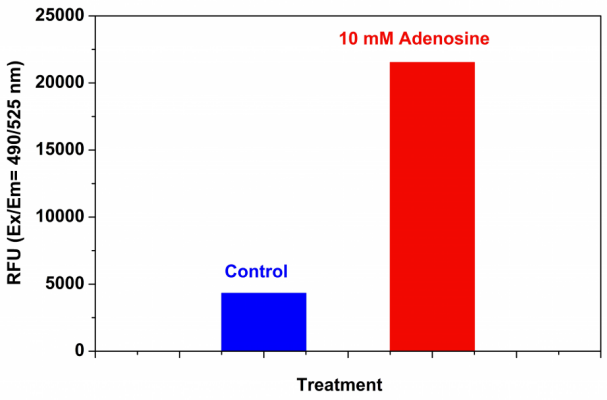

Figure 1. Detection of caspase 9 activities using Cell Meter™ Caspase 9 Activity Apoptosis Assay Kit in Jurkat cells. Jurkat cells were seeded at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with 10 mM adenosine for 48 hours while the untreated cells were used as control. 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 = 490/525 nm with a FlexStation™ microplate reader (Molecular Devices).

DISCLAIMER