Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Blue Fluorescence*

Catalog number: 22812
Unit size: 200 tests

Component	Storage	Amount
Component A: Caspase 8 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 to monitor cell apoptosis. This particular kit is designed to monitor cell apoptosis by measuring caspase 8 activity. Caspase 8 is a caspase protein, encoded by the CASP8 gene. Caspase 8 also plays an important role in neurodegenerative diseases such as Huntington disease. Caspase 8 is proven to have substrate selectivity for the peptide sequence Ile-Glu-Thr-Asp (IETD). This kit uses (Ac-IETD)-AMC as a fluorogenic indicator for caspase 8 activity. Cleavage of AMC peptides by caspase 8 generates strongly fluorescent AMC. This spectral feature makes the kit compatible with the DAPI filter set. The kit provides all the essential components with an optimized assay protocol. The assay can be readily adapted for high throughput screenings. It can be used to either quantify the activated caspase 8 activities in apoptotic cells or screen the caspase 8 inhibitors.

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 8 Substrate 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 fluorescence increase at Ex/Em = 370/450 nm (Cutoff = 420 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	370 nm
Emission:	450 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 8 Substrate (Component A) into 10 mL of Assay Buffer (Component B) and mix well to make Caspase 8 Substrate working solution. Protect from light.

Note Caspase 8 Substrate working solution is not stable, use it promptly.

Note Aliquot and store unused Caspase 8 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 8 Substrate working solution.

4. Incubate the Caspase 8 Substrate working solution plate at room temperature for 30 to 60 minutes, protected from light.

   Note If desired, add 1 µL of the 1 mM Ac-IETD-CHO caspase 8 inhibitor to selected samples 10 minutes before adding Caspase 8 Substrate working solution at room temperature to confirm the inhibition of the caspase 8-like activities.

5. Centrifuge cell plate (especially for the non-adherent cells) at 800 rpm for 2 minutes (brake off).

6. Monitor the fluorescence increase with a fluorescence microplate reader at Ex/Em = 370/450 nm (Cutoff = 420 nm).

EXAMPLE DATA ANALYSIS AND FIGURES

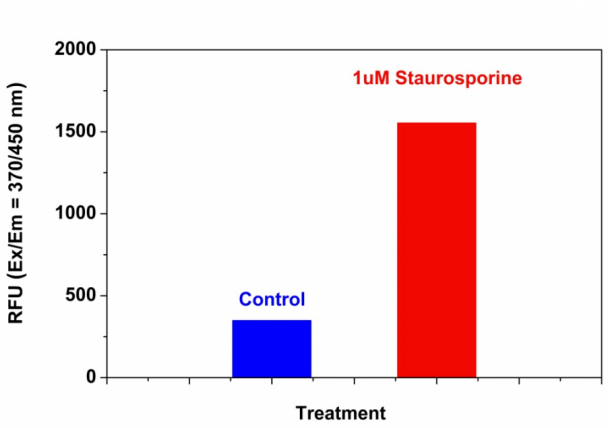

Figure 1. Detection of caspase 8 activity in Jurkat cells using Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Blue Fluorescence*. Jurkat cells were seeded on the same day at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with or without 1 µM of staurosporine for 5 hours. The caspase 8 working solution (100 µL/well) was added and incubated at room temperature for 30 minutes. The fluorescence intensity was measured at Ex/Em = 370/450 nm (Cutoff = 420 nm) with a FlexStation™ microplate reader (Molecular Devices).

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