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

Catalog number: 22816
Unit size: 100 Tests

Component	Storage	Amount
Component A: Ac-IETD-ProRed™	Freeze (<-15 °C), Minimize light exposure	1 vial
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 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)-ProRed™ as a fluorogenic indicator for caspase 8 activity. Cleavage of ProRed™ peptides by caspase 8 generates strongly fluorescent ProRed™. 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 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 STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Ac-IETD-ProRed™ stock solution (200X):
Add 65 µL DMSO into the vial of Ac-IETD-ProRed™ (Component A) and mix well to make 200X Ac-IETD-ProRed™ stock solution. Protect from light.

PREPARATION OF WORKING SOLUTION

Add 50 µL of 200X Ac-IETD-ProRed™ stock solution into 10 mL of Assay Buffer (Component B) and mix well to make Caspase 8 Substrate working solution. Protect from light.

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

SAMPLE EXPERIMENTAL PROTOCOL

Prepare cells:

- **For adherent cells**: Plate cells overnight in growth medium at 20,000 cells/well/90 uL for a 96-well or 5,000cells/well/20 uL for a 384-well plate black wall/clear bottom plate.

- **For non-adherent cells**: Centrifuge the cells from the culture medium and then suspend the cell pellet in culture medium at 80,000 to 200,000 cells/well/90 uL for a 96-well or 20,000 to 50,000 cells/well/20 uL for a 384-well black wall/clear bottom plate. Centrifuge the plate at 800 rpm for 2 minutes with brake off prior to the experiments.

  Note Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

Sample 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 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-well or 25 µL/well/384-well plate of Caspase 8 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-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. 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 nonadherent cells) at 800 rpm for 2 minutes (brake off) if using bottom read mode.

EXAMPLE DATA ANALYSIS AND FIGURES

Tel: 408-733-1055 | Fax: 408-733-1304 | Email: support@aatbio.com

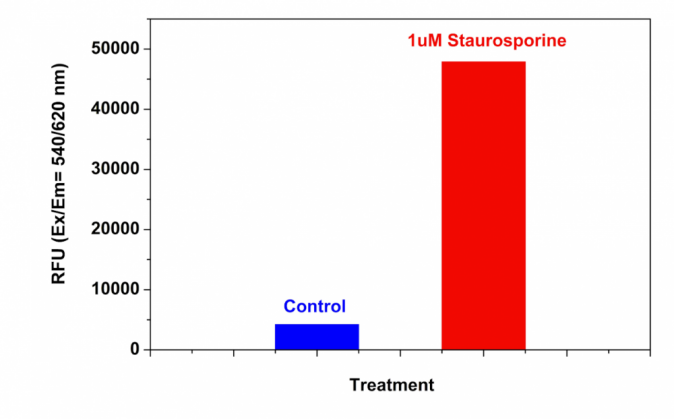

Figure 1. Detection of Caspase 8 Activities in Jurkat cells using Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit *Red Fluorescence*. Jurkat cells were seeded on the same day at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with or without 1 µM of staurosporine for 5 hours. The caspase 8 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).

DISCLAIMER