Cell Meter™ Caspase 8 Activity Apoptosis Assay Kit

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22816 (100 assays)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

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™ IETD blocking peptide residue by Caspase 8 generates strongly red fluorescent ProRed™ that is monitored fluorimetrically at ~620 nm with excitation of ~535 nm. The kit provides all the essential components with an optimized assay protocol. The assay is robust, and can be readily adapted for high-throughput screenings. The kit can be used to either quantify the activated caspase 8activities in apoptotic cells or screen the caspase 8 inhibitors. Using 100 µl of reagents per well in a 96-well format, this kit provides sufficient reagents to perform 100 assays. Using 25 ul of reagents per well in a 384-well format, this kit provides sufficient reagents to perform 400 assays.

Kit Components

Components	**Amount**
Component A: Ac-IETD-ProRed™	1 vial
Component B: Assay Buffer	10 mL

Assay Protocol (for one plate)

Brief Summary

Prepare cells with test compounds (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Add equal volume of caspase 8 assay solution (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Incubate at room temperature for 1 hour → Monitor fluorescence intensity at Ex/Em = 535/620 nm

1. Prepare cells:

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

1.2 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/90µL for a 96-well or 20,000 to 50,000 cells/well/20µL 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.

2. Prepare caspase 8 assay loading solution:

2.1 Thaw both of the kit components at room temperature before use.

2.2 Make 200X Ac-IETD-ProRed™ stock solution by adding 65 µL DMSO into the vial of Component A.

2.3 Make caspase 8 assay loading solution by adding 50 µL of Ac-IETD-ProRed™ Stock solution (200X, from Step 2.2) into 10 mL of Assay Buffer (Component B), and mix them well.
Note 1: Caspasae 8 assay loading solution is not stable, should be used promptly.
Note 2: Aliquot and store unused caspase 8 substrate (Component A) and assay buffer (Component B) at -20 °C. Avoid repeated freeze/thaw cycles.

3. Assay procedures:

3.1 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.

3.2 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.

3.3 Add 100 µL/well/96-wel or 25 µL/well/384-well plate of caspase 8 assay loading solution (from Step 2.3).

3.4 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 the assay loading solution at room temperature to confirm the inhibition of the caspase 8-like activities.

3.5 Monitor the fluorescence intensity at Ex/Em = 535/620 nm (cut off at 610 nm) with either top or bottom read mode.
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.

Data Analysis

The fluorescence in blank wells with the growth medium is subtracted from the values for those wells with the cells. The background fluorescence of the blank wells may vary depending on the sources of the growth media or the microtiter plates.

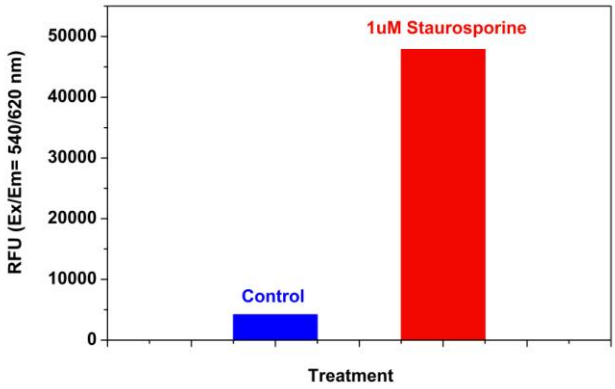

Figure 1. Detection of Caspase 8 Activities in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with staurosporine at the final concentration of 1 µM for 5 hours while the untreated cells were used as control. The caspase 8 assay 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 with FlexStation fluorescence microplate reader (Molecular Devices).

References

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