Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit *Red Fluorescence*

Catalog number: 22797
Unit size: 100 Tests

Component	Storage	Amount
Component A: Z-DEVD-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 cell viability. There are a variety of parameters that can be used for monitoring cell viability. This particular kit is designed to monitor cell apoptosis through measuring Caspase 3 activation. Caspase 3 is widely accepted as a reliable indicator for cell apoptosis since the activation of caspase-3 (CPP32/apopain) is important for the initiation of apoptosis. Caspase 3 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). This kit uses Z-DEVD-ProRed™ as the fluorogenic indicator for caspase-3 activity. Cleavage of ProRed™ DEVD blocking peptide residue by caspase 3 generates strongly red fluorescent ProRed™ that is monitored fluorimetrically at ~620 nm with excitation of ~530 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 assays. Using 100 uL 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.

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 3/7 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
Instrument specification(s):	Top or bottom read mode
Recommended plate:	Black wall/clear bottom

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. Z-DEVD-ProRed™ stock solution (200X):
Add 65 µL of DMSO into the vial of Z-DEVD-ProRed™ (Component A) to make 200X Z-DEVD-ProRed™ stock solution. Protect from light.

PREPARATION OF WORKING SOLUTION

Add 50 µL of 200X Z-DEVD-ProRed™ stock solution into 10 mL of Assay Buffer (Component B) and mix well to make Caspase 3/7 Substrate working solution.

Note Aliquot and store the unused Z-DEVD-ProRed™ Stock solution (from Step 2.2) and Assay Buffer (Component B) at -20 °C. Avoid repeated freeze/thaw cycles.

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 3/7 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-DEVD-CHO caspase 3/7 inhibitor to selected samples 10 minutes before adding Caspase 3/7 working solution at room temperature to confirm the inhibition of the caspase 3/7-like activities.

5. Monitor the fluorescence intensity with a fluorescence microplate reader (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

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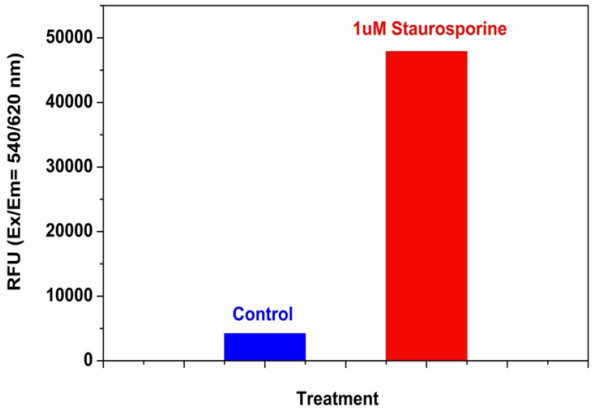

Figure 1. Detection of Caspase 3/7 Activities in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells/90 µL/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with or without 1 µM of staurosporine for 5 hours. The caspase 3/7 working solution (100 µL/well) 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