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

Catalog number: 22796
Unit size: 200 Tests

| Component | Storage | Amount (Cat No. 22796) |
|---|---|---|
| Component A: Caspase 3/7 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 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-Rh 110-DVED-Z as a fluorogenic indicator for caspase-3 activity. Cleavage of Rh 110 peptides by caspase 3 generates strongly fluorescent Rh 110 that is monitored fluorimetrically at 520-530 nm with excitation of 480-500 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 200 assays. Using 25 uL of reagents per well in a 384-well format, this kit provides sufficient reagents to perform 800 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
3. Incubate at room temperature for 1 hour
4. Monitor fluorescence intensity at Ex/Em = 490/525 nm (Cutoff = 515 nm)

**Important Note**

Thaw one vial of each kit component at room temperature before starting the experiment.

## KEY PARAMETERS

**Fluorescence microplate reader**

| | |
|---|---|
| Cutoff | 515 nm |
| Emission | 525 nm |
| Excitation | 490 nm |
| Recommended plate | Black wall/clear bottom |
| Instrument specification(s) | Top/Bottom read mode |

## PREPARATION OF WORKING SOLUTION

Add 50 µL of Caspase 3/7 Substrate (Component A) 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 Caspase 3/7 Substrate (Components A) and Assay Buffer (Component B) at -20 $^{O}$C. Avoid repeated freeze/thaw cycles

## SAMPLE EXPERIMENTAL PROTOCOL

**Cells preparation:**

- **For adherent cells:** Plate cells overnight in growth medium at 20,000 cells/well/90 uL for a 96-well plate or 5,000 cells/well/20 uL for a 384-well plate.
- **For non-adherent cells:** Centrifuge the cells from the culture medium and then suspend the cell pellet in culture medium at 80,000 cells/well/90 uL for a 96-well poly-D lysine plate or 20,000 cells/well/20 uL for a 384-well poly-D lysine 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 (4 - 6 hours for Jurkat cells treated with camptothecin) to induce apoptosis.

3. Add 100 µL/well (96-well plate) 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 into 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. Centrifuge cell plate (especially for the non-adherent cells) at 800 rpm for 2 minutes (brake off).

6. Monitor the fluorescence intensity with a fluorescence microplate reader at Ex/Em = 490/525 nm (Cutoff = 515 nm).

## EXAMPLE DATA ANALYSIS AND FIGURES

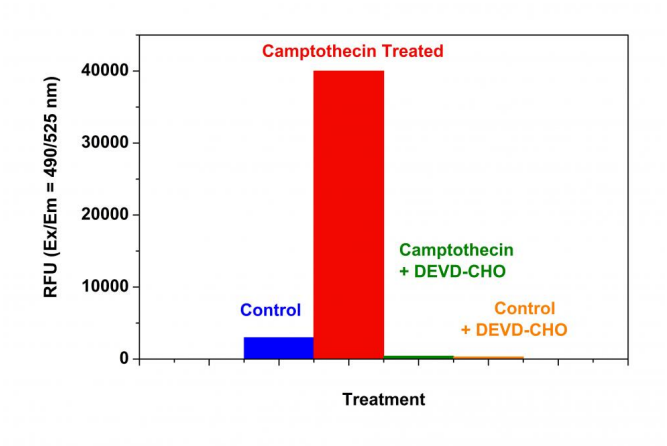


**Figure 1.** Detection of caspase 3/7 Activity in Jurkat cells. Jurkat cells were seeded on the same day at 80,000 cells/well/90 µL in a black wall/clear bottom 96-well costar plate. The cells were treated with or without 20 µM of camptothecin for 5 hours, and/or 5 µM caspase 3/7 inhibitor AC-DEVD-CHO for 10 minutes. 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 = 490/525 nm (Cutoff = 515 nm) using the NOVOstar instrument (BMG Labtech).

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