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

**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 Ac-DEVD-AMC as a fluorogenic indicator for caspase-3 activity. Cleavage of AMC peptides by caspase 3 generates strongly fluorescent AMC that is monitored fluorimetrically at 440-460 nm with excitation of 340-350 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 µL of reagents per well in a 96-well format, this kit provides sufficient reagents to perform 200 assays. Using 25 µL 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 = 350/450 nm (Cutoff = 420 nm)

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

**KEY PARAMETERS**

**Instrument:** Fluorescence microplate reader  
**Excitation:** 350 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 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 Components A and B at -20 °C. Avoid repeated freeze/thaw cycles.

**PREPARATION OF CELL SAMPLES**


**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 37°C, 5% CO₂ 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 = 350/450 nm (Cutoff = 420 nm).

**EXAMPLE DATA ANALYSIS AND FIGURES**

![Figure 1: Detection of Caspase 3/7 activity in Jurkat cells with Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit. Jurkat cells were seeded on the same day at 80,000 cells/well (90 µL) in a Costar black wall/clear bottom 96-well plate. The cells were treated with or without 20 µM of camptothecin for 5 hours, and with or without 5 µM of the caspase inhibitor AC-DEVD-CHO for 10 minutes. The caspase 3/7 assay solution (100 µL/well) was added and incubated at room temperature for 1 hour. The fluorescence intensity was measured at Ex/Em = 350/450 nm (Cutoff = 420 nm).](image)