Cell Meter™ Caspase Multiplexing 3/7, 8 and 9 Activity Assay Kit *Tricolor Fluorescence*

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22820 (3×100 assays)	Keep in freezer and protect from light	Fluorescence microplate readers

Introduction

AAT Bioquest's 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. Caspases activation is widely accepted as a reliable indicator for cell apoptosis. This particular kit is designed to simultaneously monitor four key caspases (caspase-3/7, 8 and 9) activation involved in cell apoptosis using three distinct fluorescent colors in a single assay. The kit uses DEVD-ProRed™, IETD-R110 and LEHD-AMC as fluorogenic indicators for caspase 3/7, 8 and 9 activities respectively. Upon caspase cleavages, these caspase substrates generate three distinct fluorophores: ProRed™ (red fluorescence), R110 (green fluorescence) and AMC (blue fluorescence), which can be readily monitored in a single assay due to their nice spectral separation. The kit can be used to either quantify caspase3/7, 8, and 9 activities in apoptotic cells or screen caspase 3/7, 8, and 9 inhibitors. The kit provides all the essential components with an optimized assay protocol. Using 100 µL of reagents per well in a 96-well format, this kit provides sufficient reagents to perform 100 tests.

Kit Components

Components	Amount
Component A: Caspase 3/7 Substrate (DEVD-ProRed™, 200X)	1 vial (50 µL/vial)
Component B: Caspase 8 Substrate (IETD-R110, 200X)	1 vial (50 µL/vial)
Component C: Caspase 9 Substrate (LEHD-AMC, 200X)	1 vial (50 µL/vial)
Component D: Assay Buffer	30 mL

Assay Protocol (for 96-Well-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 assay solution (100 µL/well/96-well plate or 25 µL/well/384-well plate) → Incubate at room temperature for 30 min to 1 hour → Monitor fluorescence intensity

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 200,000 cells/well/90µL for a 96-well or 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.

1.3 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.

1.4 Incubate the cell plate in a 37 °C, 5% CO_2, incubator for a desired period of time (3-5 hours for Jurkat cells treated with staurosporine) to induce apoptosis.

2. Prepare caspase assay loading solution:

2.1 Thaw kit components at room temperature before use.

2.2 To assay single caspase activity in each well: Make caspase 3/7, caspase 8 or caspase 9 assay loading solution by adding 50 µL of substrate (Component A, B or C) into 10 mL of Assay Buffer (Component D), and mix them well.

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2.3 To assay dual- or tri- caspase activity in the same well: Add 50 µL of each interested caspase substrate into 10 mL of Assay Buffer (Component D) together to make the assay loading solution.
Note: Please prepare the tested substrate solutions and the needed volume proportionally, store the unused substrate stock solution at -20 °C. Avoid repeated freeze/thaw cycles.

3. Run Assay:

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

3.2 Incubate the plate at room temperature for at least 30 to 60 min, protected from light.
Note: If desired, add 1 µL of 1 mM caspase inhibitor to selected samples 10 minutes before adding the assay loading solution at room temperature to confirm the inhibition of caspase activities.

3.3 Monitor the fluorescence intensity as indicated in the table with either top or bottom read mode.

Caspase to be assayed	Ex/Em
Caspase 3/7, Red fluorescence	535/620 nm
Caspase 8, Green fluorescence	490/525 nm
Caspase 9, Blue fluorescence	370/450 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.

Data Analysis

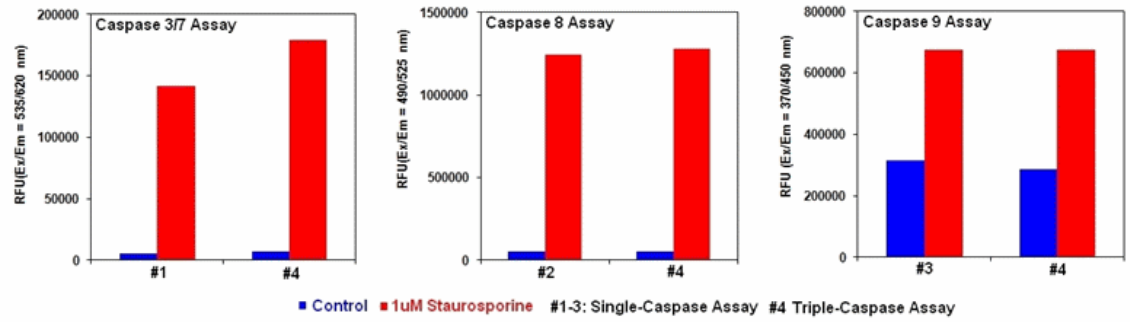

Figure 1. Detection of Caspase Activities in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with staurosporine at the final concentration of 1 µM for 4 hours (Red Bar) while the untreated cells were used as control (Blue Bar). The single-caspase assay loading solution (100 µL/well) was added (in #1 for caspase 3/7, #2 for caspase 8 or #3 for caspase 9) or Triple-caspase assay loading solution (#4 for caspase 3/7, 8 and 9 together) was added, and incubated at room temperature for 1 hour. The fluorescence intensity was measured with FlexStation fluorescence microplate reader at the indicated wavelength. The caspase 3/7, 8 and 9 activities can be detected in a single assay without interferences from other caspases.

References

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2. Abnosi, Mohammad Hussein, and Zahra Jafari Yazdi. "Low Dose and Long Term Toxicity of Sodium Arsenite Caused Caspase Dependent Apoptosis Based on Morphology and Biochemical Character." *Cell* 14.3 (2012): 161-170.
3. Ikner, Aminah, and Avi Ashkenazi. "TWEAK induces apoptosis through a death-signaling complex comprising receptor-interacting protein 1 (RIP1), Fas-associated death domain (FADD), and caspase-8." *Journal of Biological Chemistry* 286.24 (2011): 21546-21554.
4. Wongtongtair, Supim, et al. "Barakol-induced apoptosis in P19 cells through generation of reactive oxygen species and activation of caspase-9." *Journal of ethnopharmacology* 137.2 (2011): 971-978.