AAT Bioquest®

PRODUCT INFORMATION SHEET

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

Catalog number: 22820
Unit size: 100 Tests

Component	Storage	Amount
Component A: Caspase 3/7 Substrate (DEVD-ProRed™, 200X)	Freeze (< -15 °C), Minimize light exposure	1 vial (50 µL/vial)
Component B: Caspase 8 Substrate (IETD-R110, 200X)	Freeze (< -15 °C), Minimize light exposure	1 vial (50 µL/vial)
Component C: Caspase 9 Substrate (LEHD-AMC, 200X)	Freeze (< -15 °C), Minimize light exposure	1 vial (50 µL/vial)
Component D: Assay Buffer	Freeze (< -15 °C)	1 bottle (30 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. Caspases activation is widely accepted as a reliable indicator for cell apoptosis. This particular kit is designed to simultaneously monitorfour key caspases (caspase-3/7, 8 and 9) activation involved in cell apoptosis using three distinct fluorescent colors. This kit uses DEVD-ProRed™, IETD-R110 and LEHD-AMC as fluorogenic indicators for caspase 3/7, 8 and 9 activity respectively. Upon caspase cleavages, DEVD-ProRed™, IETD-R110 and LEHD-AMC 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.

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds
2. Add equal volume of caspase working solution
3. Incubate at room temperature for 30 mins to 1 hour
4. Monitor fluorescence intensity

Important Thaw kit components at room temperature before use.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	See Table 1
Emission	See Table 1
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Use either top or bottom read mode

CELL PREPARATION

For guidelines on cell sample preparation, please visit https://www.aatbio.com/resources/guides/cell-sample-preparation.html

PREPARATION OF WORKING SOLUTION

1. Single caspase activity working solution
Make caspase 3/7, caspase 8 or caspase 9 working solution by adding 50 µL of substrate (Component A, B or C) into 10 mL of Assay Buffer (Component D), and mix them well.

2. Dual- or tri- caspase activity working solution
Add 50 µL of each interested caspase substrate into 10 mL of Assay Buffer (Component D) together to make the working solution.

Note Please prepare the tested substrate solutions and the needed volume proportionally.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Spectral wavelengths for Caspases 3/7, 8, and 9.

Capase	Fluorescence	Excitation	Emission
Caspase 3/7	Red	535 nm	620 nm
Caspase 8	Green	490 nm	525 nm
Caspase 9	Blue	360 nm	470 nm

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.

2. 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.

3. Add 100 µL/well/96-well or 25 µL/well/384-well plate of desired caspase assay working solution.

4. 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.

5. Monitor the fluorescence intensity as indicated in the table with either top or bottom read mode. *Note:* Sometimes, bottom read gives better signal to background ratio, centrifuge cell plate (especially for the nonadherent cells) at 800 rpm for 2 minutes (brake off) if using bottom read mode.

EXAMPLE DATA ANALYSIS AND FIGURES

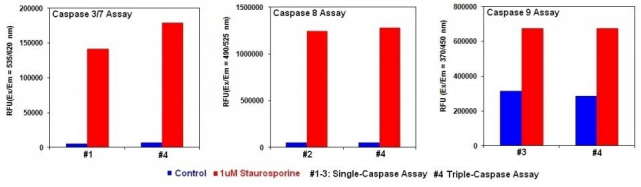

Figure 1. Detection of Caspase Activities in Jurkat cells. Jurkat cells were seeded on the same day at 200,000 cells/well in a Costar black wall/clear bottom 96-well plate. The cells were treated with staurosporine at the final concentration of 1 mM for 4 hours (Red Bar) while the untreated cells were used as control (Blue Bar). The single-caspase assay loading solution (100 uL/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.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.