Cell Meter™ Live Cell Caspase 3/7 Binding Assay Kit *Green Fluorescence*

Catalog number: 20100
Unit size: 25 Tests

Component	Storage	Amount (Cat No. 20100)
Component A: FAM-DEVD-FMK	Freeze (< -15 °C), Minimize light exposure	1 vial
Component B: Washing Buffer	Freeze (< -15 °C), Minimize light exposure	1 bottle (100 mL)
Component C: 500X Propidium Iodide	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)
Component D: 500X Hoechst 33342	Freeze (< -15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

Our Cell Meter™ live cell caspases activity assay kits are based on fluorescent FMK inhibitors of caspases. These inhibitors are cell permeable and non-cytotoxic. Once inside the cell, the caspase inhibitors bind covalently to the active caspases. The activation of caspase 3/7 is important for the initiation of apoptosis. It has been proven that caspase 3/7 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). This kit uses FAM-DEVD-FMK as a fluorescent indicator for caspase 3/7 activity. FAM-DEVD-FMK irreversibly binds to activated caspase 3/7 in apoptotic cells. Once bound to caspase 3/7, the fluorescent reagent is retained inside the cell. The binding event inhibits caspase 3/7 but will not stop apoptosis from proceeding. There are a variety of parameters that can be used for monitoring cell apoptosis. This Cell Meter™ Live Cell Caspase 3/7 Activity Assay Kit is designed to detect cell apoptosis by measuring caspase 3/7 activation in live cells. It is used for the quantification of activated caspase 3/7 activities in apoptotic cells, or for screening caspase 3/7 inhibitors. FAM-DEVD-FMK, the green label reagent, allows for direct detection of activated caspase 3/7 in apoptotic cells by fluorescence microscopy, flow cytometer, or fluorescent microplate reader. The kit provides all the essential components with an optimized assay protocol.

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds at a density of 5 × 10^5 to 2 × 10^6 cells/mL
2. Add FAM-DEVD-FMK into cell solution at 1:150 ratio
3. Incubate the cells in a 37°C, 5% CO2 incubator for 1 hour
4. Pellet the cells, wash and resuspend the cells with buffer or growth medium
5. Optional: label the cells iwth DNA stain Propidium Iodide or Hoechst 33342
6. Analyze the cells at Ex/Em = 490/525 nm

Important Note

Thaw all the components at room temperature before use.

KEY PARAMETERS

Fluorescence microscope

Emission	
Excitation	
Recommended plate	Black wall/clear bottom
Instrument specification(s)	See Table 1

Flow cytometer

Emission	
Excitation	
Instrument specification(s)	See Table 1

Fluorescence microplate reader

Cutoff	
Emission	
Excitation	
Recommended plate	Solid black
Instrument specification(s)	See Table 2

CELL PREPARATION

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

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

FAM-DEVD-FMK DMSO stock solution (150X)

Add 50 µL of DMSO to the vial of FAM-DEVD-FMK (Component A).

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Fluorescence intensity monitoring for flow cytometry and fluoresence microscopes.

	Flow Cytometry	Fluorescence Microscope
FAM-DEVD-FMK	FL1 channel	FITC channel
Propidium Iodide	FL2 channel	TRITC channel
Hoechst Dye	Violet Laser	DAPI channel

Table 2. Fluorescence intensity monitoring for fluorescence microplate readers.

	Excitation	Emission	Cut Off
FAM-DEVD-FMK	490 nm	525 nm	515 nm
Propidium Iodide	535 nm	635 nm	
Hoechst Dye	350 nm	461 nm	

1. Examples for inducing apoptosis in suspension culture:

Treat Jurkat cells with 2 µg/ml camptothecin for 3 hours

Treat Jurkat cells with 1 µM staurosporine for 3 hours

Treat HL-60 cells with 4 µg/ml camptothecin for 4 hours

Treat HL-60 cells with 1 µM staurosporine for 4 hours.

Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction

2. Add 150 X FAM-DEVD-FMK into the cell solution at a 1:150 ratio, and incubate the cells in a 37°C, 5% CO2 incubator for 1 hour.

   Note: The cells can be concentrated up to ~5 X 106 cells/mL for FAM-DEVDFMK labeling. The appropriate incubation time depends on the individual cell type and cell concentration used.

3. Spin down the cells at ~200g for 5 minutes, and wash cells with 1 mL washing buffer (Component B) twice. Resuspend the cells in desired amount of washing buffer.

   Note: FAM-DEVD-FMK is fluorescent, thus it is important to wash out any unbound reagent to eliminate the background.

4. If desired, label the cells with a DNA stain (such as propidium iodide for dead cells, or Hoechst for whole population of the cell nucleus stain).

5. Monitor the fluorescence intensity by fluorescence microscopy, flow cytometer, or fluorescent microplate reader according to table 1 or table 2. For fluorescence microscopy and fluorescent microplate reader, place 100 µL of the cell suspensions into each of wells of a 96-well black microtiter plate.

   Note: For detached cells, the concentration of cells should be adjusted to 2 - 5 X 10^5 cells/100 µL aliquot per microtiter plate well.

EXAMPLE DATA ANALYSIS AND FIGURES

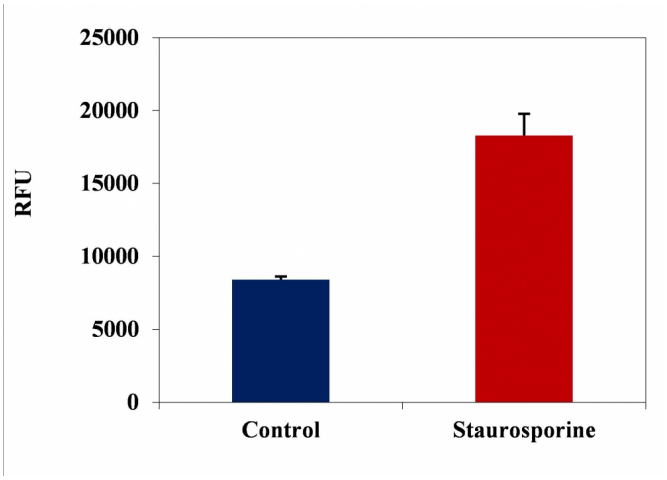

Figure 1.

FAM-DEVD-FMK fluorometric detection of active caspases 3/7 using Kit #20100 in Jurkat cells. The cells were treated with 1 µM staurosporine for 3 hours (Red) while untreated cells were used as a control (Blue). Cells were incubated with FAM-DEVD-FMK for 1 hour at 37°C. The Fluorescent intensity (300, 000 cells/100 µL/well) was measured at Ex/Em = 490/525 nm (cut off at 515 nm) with a FlexStation microplate reader using bottom read mode.

DISCLAIMER