

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

Catalog number: 20100

Unit size: 25 Tests

Component	Storage	Amount
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 at room temperature for 1 hour
4. Pellet the cells, wash and resuspend the cells with buffer or growth medium
5. Optional: label the cells with DNA stain Propidium Iodide or Hoechst 33342
6. Analyze the cells at Ex/Em = 490/525 nm

Important Thaw all the components at room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	See Table 1
Instrument:	Flow cytometer
Instrument specification(s):	See Table 1
Instrument:	Fluorescence microplate reader
Recommended plate:	Solid black
Instrument specification(s):	See Table 2

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.

1. **FAM-DEVD-FMK DMSO stock solution (150X):**
Add 50 µL of DMSO to the vial of FAM-DEVD-FMK (Component A).

PREPARATION OF CELL SAMPLES

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

SAMPLE EXPERIMENTAL PROTOCOL

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 $\sim 5 \times 10^6$ cells/mL for FAM-DEVD-FMK labeling. The appropriate incubation time depends on the individual cell type and cell concentration used.

3. Spin down the cells at $\sim 200g$ 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 $2 - 5 \times 10^5$ cells/100 µL aliquot per microtiter plate well.

Table 1. Fluorescence intensity monitoring for flow cytometry and fluorescence 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	

EXAMPLE DATA ANALYSIS AND FIGURES

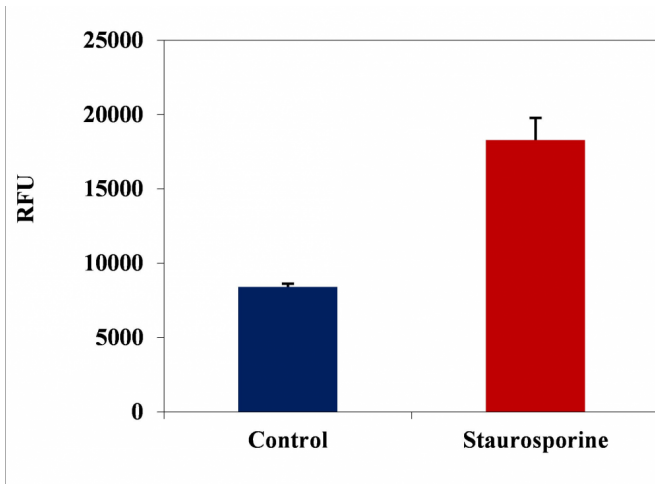


Figure 1.

FAM-DEVD-FMK fluorometric detection of active caspases 3/7 using Kit #20100 in Jurkat cells. The cells were treated with 1 μ M 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 μ L/well) was measured at Ex/Em = 490/525 nm (cut off at 515 nm) with a FlexStation microplate reader using bottom read mode.

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