Cell Meter™ Live Cell Caspase 2 Binding Assay Kit *Green Fluorescence*

Catalog number: 20111
Unit size: 25 Tests

Component	Storage	Amount
Component A: FAM-VDVAD-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	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. This Cell Meter™ Live Cell Caspase 2 Activity Assay Kit is designed to detect cell apoptosis by measuring caspase 2 activation in live cells. It is used for the quantification of activated caspase 2 activities in apoptotic cells, or for screening caspase 2 inhibitors. FAM- VDVAD-FMK, the green label reagent, allows for direct detection of activated caspase 2 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-VDVAD-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. Monitor fluorescence intensity (bottom read mode) at Ex/Em = 490/525 nm (Cutoff = 515 nm), fluorescence microscope with FITC filter, or flow cytometer with FL1 channel

Important Thaw all the components at room temperature before starting the experiment.

KEY PARAMETERS

Instrument:	Fluorescence microscope
Excitation:	See Table 1
Emission:	See Table 1
Recommended plate:	Black wall/clear bottom
Instrument:	Flow cytometer
Excitation:	See Table 1
Emission:	See Table 1
Instrument:	Fluorescence microplate reader
Excitation:	See Table 1
Emission:	See Table 1
Instrument specification(s):	Bottom read mode
Recommended plate:	Black wall/clear bottom

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-VDVAD-FMK stock solution (150X):
Add 50 µL of DMSO into the vial of FAM-VDVAD-FMK (Component A) to make 150X FAM-VDVAD-FMK stock solution.

SAMPLE EXPERIMENTAL PROTOCOL

1. Culture cells to a density optimal for apoptosis induction according to your specific induction protocol, but not to exceed 2 x 10^6 cells/ mL. At the same time, culture a non-induced negative control cell population at the same density as the induced population for every labeling condition. Here are a few examples for inducing apoptosis in suspension culture:

   a. Treating Jurkat cells with 2 µg/ml camptothecin for 3 hours.

   b. Treating Jurkat cells with 1 µM staurosporine for 3 hours.

   c. Treating HL-60 cells with 4 µg/ml camptothecin for 4 hours.

   d. Treating 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 150X FAM-VDVAD-FMK into the cell solution at a 1:150 ratio, and incubate the cells in a 37°C, 5% CO_2 incubator for 1 hour.

   Note The cells can be concentrated up to ~ 5 X 10^6 cells/mL for FAM-VDVAD-FMK labeling. For adherent cells, gently lift the cells with 0.5 mM EDTA to keep the cells intact, and wash the cells once with serum-containing media prior to incubation with FAM-VDVAD-FMK. The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

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-VDVAD-FMK is fluorescent, thus it is important to wash out any unbound reagent to eliminate the background. For detached cells, the concentration of cells should be adjusted to 2 - 5 X 10^5 cells/100 µL aliquot per microtiter plate well.

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 fluorescence microplate reader at Ex/Em = 490/525 nm (for propidium iodide, Ex/Em = 535/635 nm; for Hoechst dyes, Ex/Em = 350/461 nm).

   For flow cytometry: Monitor the fluorescence intensity using the FL1 channel (FL2 channel for propidium iodide staining). Gate on the cells of interest, excluding debris.

   For fluorescence microscope: Place 100 µL of the cell suspensions into each of wells of a 96-well black microtiter plate. Observe cells under a fluorescence microscope using FITC channel (TRITC channel for propidium iodide staining, DAPI channel for Hoechst staining).

   For fluorescence microplate reader: Place 100 µL of the cell suspensions into

each of wells of a 96-well black microtiter plate. Monitor the fluorescence intensity (bottom read mode) with a fluorescence plate reader at Ex/Em = 490/525 nm (Cutoff = 515 nm).

Note If it is necessary to equilibrate the cell concentrations, adjust the suspension volume for the induced cells to approximate the cell density of the non-induced population. This adjustment step is optional if your cell treatment does not result in a dramatic loss in stimulated cell population numbers.

Table 1. Spectral information for measuring fluorescence intensity.

	FAM-VDVAD-FMK	Propidium Iodide	Hoechst Dye
Flow Cytometer	FL1 channel	FL2 channel	FL1 channel
Fluorescence Microscope	FITC channel	TRITC channel	DAPI channel
Fluorescence Microplate	490/525 nm	535/635 nm	350/461 nm

EXAMPLE DATA ANALYSIS AND FIGURES

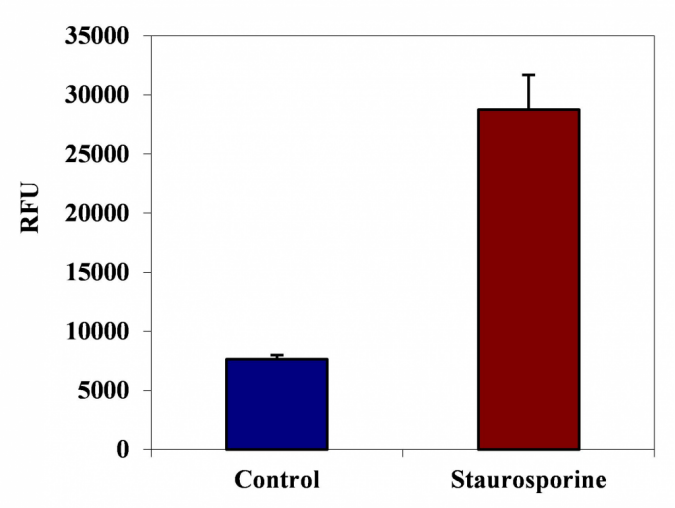

Figure 1.

FAM-VDVAD-FMK fluorometric detection of active caspase 2 using Kit # 20111 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-VDVAD-FMK for 1 hour at 37°C. The fluorescent intensity of the cells (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