Cell Meter™ Live Cell Caspase 3/7 Binding Assay Kit

Green Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 20100 (25 assays)	Keep in freezer Avoid light	Fluorescent microscopy, flow cytometer, and fluorescent microplate reader

Introduction

Our Cell Meter™ live cell caspases activity assay kits are based on fluorescent 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.

Kit Components

Components	**Amount**
Component A: FAM-DEVD-FMK	1 vial
Component B: Washing Buffer	1 bottle (100 mL)
Component C:500X Propidium Iodide	1 vial (100 µL)
Component D: 500X Hoechst 33342	1 vial (100 µL)

Assay Protocol for Detached Cells

Brief Summary

Prepare cells with test compounds at a density of 5 × 10^5 to 2× 10^6 cells/mL → Add FAM-DEVD-FMK into cell solution at 1:150 ratio → Incubate at room temperature for 1 hour → Pellet the cells, wash and resuspend the cells with buffer or growth medium → Analyze the cells at Ex/Em = 490/525 nm

Note: Thaw all the components at room temperature before use.

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:

1) Treating Jurkat cells with 2 μg/ml camptothecin for 3 hours.
2) Treating Jurkat cells with 1 μM staurosporine for 3 hours.
3) Treating HL-60 cells with 4 μg/ml camptothecin for 4 hours.
4) 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. Make 150X FAM-DEVD-FMK DMSO stock solution by adding 50 μL of DMSO to the vial of FAM-DEVD-FMK (Component A). Add 150 X FAM-DEVD-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 1: The cells can be concentrated up to ~ 5 X 10^6 cells/mL for FAM-DEVD-FMK labeling. The unused 150X FAM-DEVD-FMK DMSO stock solution should be divided as single use aliquot and stored at -20 °C.
   Note 2: 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 -DEVD-FMK.
   Note 3: 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 1: FAM-DEVD-FMK is fluorescent, thus it is important to wash out any unbound reagent to eliminate the background.
   Note 2: For detached cells, the concentration of cells should be adjusted to 2-5 X 10^5 cells/100 μL aliquot per microtiter plate well for use in step 5.

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

   5.1 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.

   5.2 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: 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.

   5.3 Observe cells under a fluorescence microscope using FITC channel (TRITC channel for propidium iodide staining, DAPI channel for Hoechst staining).

   5.4 Monitor the fluorescence intensity using Ex/Em = 490/525 nm (cut off at 515 nm) bottom read mode using a fluorescent microplate reader.

Data Analysis

1. 96-Well Fluorescence Plate Reader Sample Data:

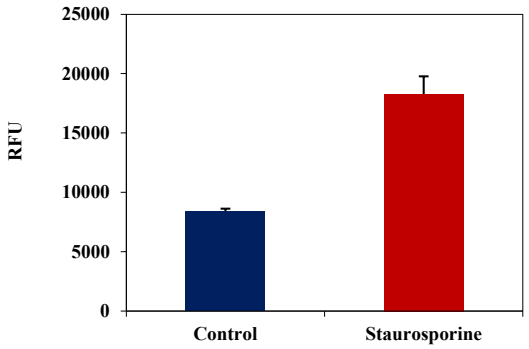

Figure 1. FAM-DEVD-FMK fluorometric detection of active caspases 3/7 using Kit #20100 in Jurkat cells. The cells were treated with 1 µM staurosporine for 3 hours (Red) while untreated cells were used as a control (Blue). Cells were incubated with FAM-DEVD-FMK for 1 hour at 37°C. The Fluorescent intensity (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.

2. Fluorescence Microscopy Sample Data:

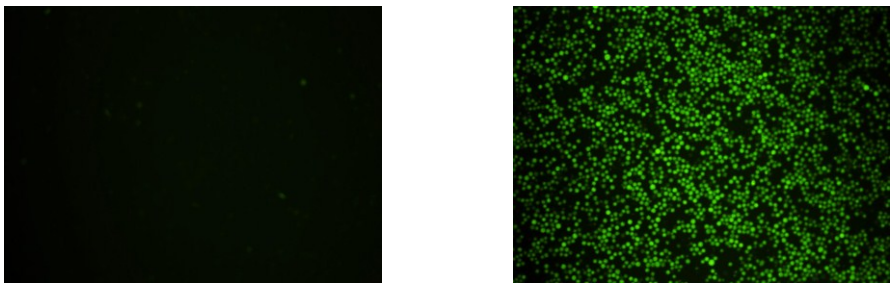

Figure 2. The Fluorescent Microscopy showing the increase in FAM-DEVD-FMK fluorescence intensity with the addition of 1 µM Staurosporin in Jurkat cells. Cells were incubated with FAM-DEVD-FMK for 1 hour at 37°C. The Fluorescent intensity of the cells (200,000 cells/100 µL/well) was viewed under a fluorescence microscope with a FITC channel.

References

1. Slee, E. A., C. Adrain, and S. J. Maritin. 1999. Serial Killers: ordering caspase activation events in apoptosis. *Cell Death and Differ.* 6:1067-1074.
2. Walker, N. P., R. V. Talanian, K. D. Brady, L. C. Dang, N. J. Bump, C. R. Ferenz, S. Franklin, T. Ghayur, M. C. Hackett and L. D. Hammill. 1994. Crystal Structure of the Cysteine Protease Interleukin-1β-Converting Enzyme: A $(p20/p10)_2$ Homodimer. *Cell* 78:343-352.
3. Wilson, K. P., J. F. Black, J. A. Thomson, E. E. Kim, J. P. Griffith, M. A. Navia, M. A. Murcko, S. P. Chambers, R. A. Aldape, S. A. Raybuck, and D. J. Livingston. 1994. Structure and mechanism of interleukin-1 beta converting enzyme. *Nature* 370: 270-275.
4. Rotonda, J., D. W. Nicholson, K. M. Fazil, M. Gallant, Y. Gareau, M. Labelle, E. P. Peterson, D. M. Rasper, R. Ruel, J. P. Vaillancourt, N. A. Thornberry and J. W. Becker. 1996. The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. *Nature Struct. Biol.* 3(7): 619-625.