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

Red Fluorescence

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 20101 (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 TF3-DEVD-FMK as a fluorescent indicator for caspase 3/7 activity. TF3-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. TF3-DEVD-FMK, the red 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: TF3-DEVD-FMK	1 vial
Component B: Washing Buffer	1 bottle (100 mL)
Component C: 500X Nuclear Green™ DCS1	1 vial (100 µL)
Component D: 500X Hoechst	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 TF3-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 = 550/595 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 TF3-DEVD-FMK DMSO stock solution by adding 50 μL of DMSO to the vial of TF3-DEVD-FMK (Component A). Add 150 X TF3-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 TF3-DEVD-FMK labeling. The unused 150X TF3-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 TF3 -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: TF3-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 Nuclear Green™ DCS1for 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 = 550/595 nm (for Nuclear Green™ DCS1, Ex/Em = 490/525 nm, for Hoechst dyes, Ex/Em = 350/461 nm)

   5.1 For flow cytometry, monitor the fluorescence intensity using the channel with Ex/Em = 550/595 nm (FL1 channel for Nuclear Green™ DCS1staining). 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 TRITC channel (FITC channel for Nuclear Green™ DCS1staining, DAPI channel for Hoechst staining).

   5.4 Monitor the fluorescence intensity using Ex/Em = 550/595 nm (cut off at 570 nm) bottom read mode using a fluorescent microplate reader.

Data Analysis

1. 96-Well Fluorescence Plate Reader Sample Data:

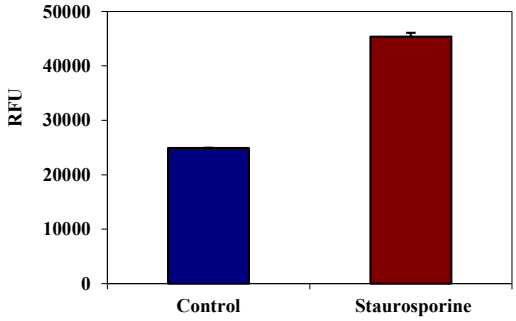
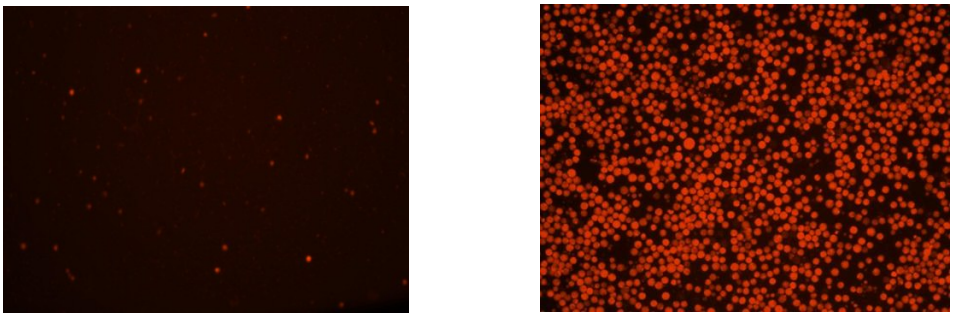


Figure 1. TF3-DEVD-FMK fluorometric detection of active caspases 3/7 using Kit #20101 in Jurkat cells. The cells were treated with 1 µM staurosporine for 3 hours (Red) while untreated cells were used as a control (Blue). Cells were incubated with TF3-DEVD-FMK for 1 hour at 37°C. The Fluorescent intensity (300, 000 cells/ 100 µL/well) was measured at Ex/Em = 550/595 nm (cut off at 515 nm) with a FlexStation microplate reader using bottom read mode.

2. Fluorescence Microscopy Sample Data:



A **B**

Figure 2. The Fluorescent Microscopy showing the increase in TF3-DEVD-FMK fluorescence intensity with the addition of 1 µM Staurosporin in Jurkat cells. Cells were incubated with TF3-DEVD-FMK for 1 hour at 37°C. The fluorescent intensity of the cells (200,000 cells/ 100 µL per well) was viewed under a fluorescence microscope with a TRITC channel.

References

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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.
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