Cell Meter™ Live Cell Caspase 8 Binding Assay Kit *Red Fluorescence*

Catalog number: 20116
Unit size: 25 Tests

Component	Storage	Amount
Component A: iFluor 647-LETD-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 Nuclear Green™ DCS1	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 8 Activity Assay Kit is designed to detect cell apoptosis by measuring caspase 8 activation in live cells. It is used for the quantification of activated caspase 8 activities in apoptotic cells, or for screening caspase 8 inhibitors. iFluor 647-LETD-FMK, the red label reagent, allows for direct detection of activated caspase 8 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

1. Prepare cells with test compounds at a density of 5 × 10^5 to 2× 10^6 cells/mL
2. Add iFluor 647-LETD-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. Analyze the cells with flow cytometry using 660/20 nm filter (APC channel)

Important
Thaw all the components at room temperature before use.

KEY PARAMETERS

Flow cytometer

Excitation	640 nm laser
Emission	660/20 nm filter
Instrument specification(s)	APC channel

Fluorescence microscope

Excitation	Cy5 filter set
Emission	Cy5 filter set
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Black wall/clear bottom

Fluorescence microplate reader

Excitation	640 nm
Emission	680 nm
Cutoff	665 nm
Recommended plate	Black wall/clear bottom
Instrument specification(s)	Bottom read mode

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.

iFluor 647-LETD-FMK stock solution (150X)
Make 150 X iFluor 647-LETD-FMK stock solutions by adding 50 µL of DMSO to the vial of iFluor 647-LETD-FMK (Component A).

Note Store the unused iFluor 647-LETD-FMK stock solution at -20°C in single use aliquots in dark place

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 iFluor 647-LETD-FMK stock solution (150X) into the cell solution at a 1:150 ratio, and incubate the cells in a 37 °C, 5% CO_2 incubator or room temperature for 1 hour.

    Note The cells can be concentrated up to ~ 5 X 10^6 cells/mL for iFluor 647-LETD-FMK labeling.

    Note 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 iFluor 647-LETD-FMK.

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

    Note iFluor 647-LETD-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 Nuclear Green DCS1(Ex/Em = 490/520 nm) for dead cells, or Hoechst (Ex/Em = 350/450 nm) for whole population of the cell nucleus stain.

5. Monitor the fluorescence intensity by appropriate application.

    a. For flow cytometry, monitor the fluorescence intensity using 660/20 nm filter (APC channel). Gate on the cells of

interest, excluding debris.

b. 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. Observe cells under a fluorescence microscope using Cy5 filter (FITC channel for Nuclear Green DCS1 staining, DAPI channel for Hoechst staining). Or monitor the fluorescence intensity using Ex/Em = 640/680 nm (cut off at 665 nm) bottom read mode with a fluorescent microplate reader.

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.

EXAMPLE DATA ANALYSIS AND FIGURES

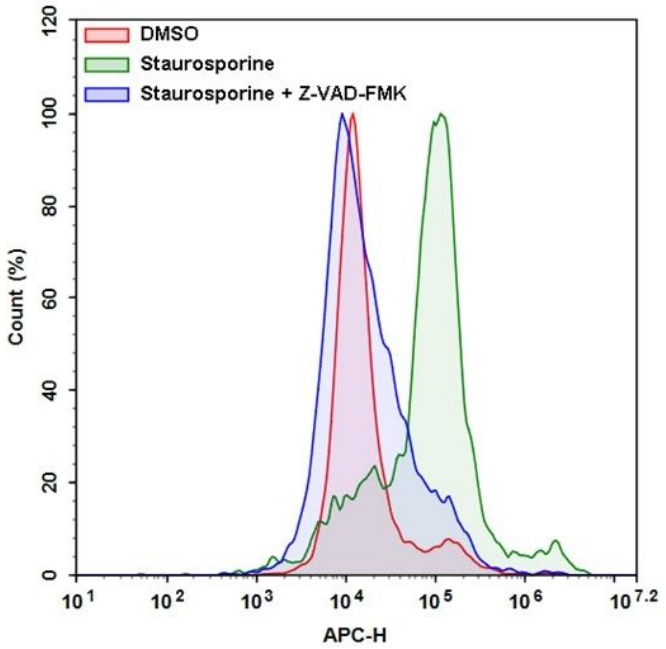

Figure 1. Flow cytometric analysis of active caspase 8 using Cell Meter™ Live Cells Caspase 8 detection kit in Jurkat cells. The cells were treated with 1 µM staurosporine for 5 hours (Green) while untreated cells were used as a control (Red). The staurosporine response was inhibited by Z-VAD-FMK (caspase inhibitor) shown as blue. Cells were incubated with iFluor 647-LETD-FMK for 1 hour at RT. The fluorescent intensity was measured using NovoCyte flow cytometer with 660/20 nm filter (APC channel).

DISCLAIMER

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