Cell Meter™ Live Cell Caspase 9 Binding Assay Kit *Green Fluorescence*

Catalog number: 20117
Unit size: 25 Tests

Component	Storage	Amount
Component A: FAM-LEHD-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 9 Activity Assay Kit is designed to detect cell apoptosis by measuring caspase 9 activation in live cells. It is used for the quantification of activated caspase 9 activities in apoptotic cells, or for screening caspase 9 inhibitors. FAM-LEHD-FMK, the green label reagent, allows for direct detection of activated caspase 9 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-LEHD-FMK into cell solution at 1:150 ratio
3. Incubate at room 37°C for 1 hour
4. Pellet the cells, wash and resuspend the cells with buffer or growth medium
5. Optional: label the cells iwth DNA stain Propidium Iodide or Hoechst 33342
6. Analyze the cells with flow cytometer using 530/30 nm filter (FiTC channel), fluorescence microscope using FITC filter or fluorescence micro plate reader at 490/525 nm (Cutoff=515 nm)

Important Thaw all the components at room temperature before use.

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 2
Emission:	See Table 2
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-LEHD-FMK DMSO stock solution (150X):
Add 50 µL of DMSO to the vial of FAM-LEHD-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-LEHD-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-LEHD-FMK labeling. The appropriate incubation time depends on the individual cell type and cell concentration used.

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-LEHD-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 X 10^5 cells/100 µL aliquot per microtiter plate well.

Table 1. Fluorescence intensity monitoring for flow cytometry and fluoresence microscopes.

	Flow Cytometry	Fluorescence Microscope
FAM-LEHD-FMK	530/30 nm filter (FITC channel)	FITC channel
Propidium Iodide	610/20 nm filter (PE-Texas Red channel)	TRITC channel
Hoechst Dye	450/40 nm filter (Pacific Blue channel)	DAPI channel

Table 2. Fluorescence intensity monitoring for fluorescence microplate readers.

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	Excitation	Emission	Cut Off
FAM-LEHD-FMK	490 nm	525 nm	515 nm
Propidium Iodide	535 nm	635 nm	
Hoechst Dye	350 nm	461 nm	

EXAMPLE DATA ANALYSIS AND FIGURES

Fluorescence Microscopy Sample Data:

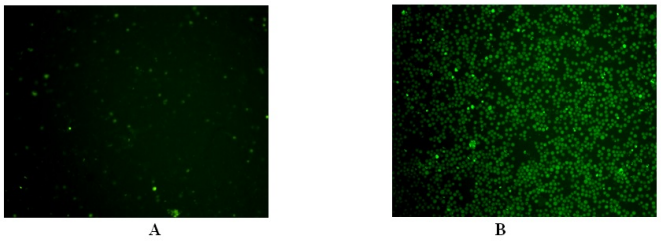

Figure 1.

The fluorescent microscopy showing the increase in FAM-LEHD-FMK fluorescence intensity with the addition of 1 µM staurosporin in Jurkat cells. Cells were incubated with FAM-LEHD-FMK for 1 hour at 37°C. The fluorescent intensity of the cells (300,000 cells/100 µL/well) was viewed under a fluorescence microscope with a FITC channel.

DISCLAIMER