Cell Meter™ Live Cell Caspase 3/7 and Phosphatidylserine Detection Kit *Triple Fluorescence Colors*

Catalog number: 22850
Unit size: 100 Tests

Component	Storage	Amount
Component A: TF3-DEVD-FMK	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: 100X Annexin V-iFluor 488™ conjugate	Freeze (<-15 °C), Minimize light exposure	1 vial (200 µL)
Component C: 500X Hoechst Stain	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)
Component D: 500X Propidium Iodide	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)
Component E: Washing Buffer	Freeze (<-15 °C), Minimize light exposure	1 bottle (100 mL)

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cellular functions. In the process of apoptosis, one of key events is the activation of caspases. The activation of caspase 3/7 is an 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 SR-DEVD-FMK as a fluorescent indicator to detect caspase 3/7 activities. SR-DEVD-FMK is cell permeable and nontoxic, once bound to caspases, the fluorescent reagent is retained inside the cell. The binding event prevents the caspases from further catalysis but will not stop apoptosis from proceeding. SR-DEVD-FMK is a red label reagent. Annexins are a family of proteins that bind to phospholipid membranes in the presence of calcium. Annexin V is used to detect apoptotic cells that express phosphatidylserine (PS) on the cell surface. The appearance of PS on the cell surface is a universal indicator of the initial/intermediate stages of cell apoptosis. Annexin V-dye conjugates monitor cell apoptosis through measuring the translocation of PS. The Annexin V-iFluor 488™ used in this kit is a green labeling reagent, with Ex/Em = 490/525 nm. The kit is designed to detect apoptosis by simultaneously monitoring Caspase 3/7 and Annexin V activities in mammalian cells. The kit also provides a Hoechst dye for labeling the nucleus of the whole population of the cells, and propidium iodide dye for staining necrosis cells. This kit is applicable for fluorescence microscope, flow cytometer, and fluorescence 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 2×10^6 cells/mL
2. Add TF3-DEVD-FMK at 1:150 ratio and/or Annexin V-iFluor 488™ into cell solution at 1:100 ratio
3. Incubate the cells in a 37°C, 5% CO_2 incubator 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) and/or 550/595 nm (Cutoff = 570 nm), fluorescence microscope with FITC and TRITC filters, or flow cytometer with FL1 and FL2 channels for Annexin V-iFluor 488™ and TF3-DEVD-FMK respectively

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

KEY PARAMETERS

Instrument:	Fluorescence microscope
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	FITC channel for Annexin V-iFluor 488™, TRITC channel for TF3-DEVD-FMK,
Instrument:	Flow cytometer
Excitation:	488 nm laser
Emission:	530/30 nm, 575/26 nm, 610/20 nm filter
Instrument specification(s):	FITC, PE, PE-Texas Red channel
Instrument:	Fluorescence microplate reader
Excitation:	490 nm, 550 nm
Emission:	525 nm, 595 nm
Cutoff:	515 nm, 570 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.

1. TF3-DEVD-FMK stock solution (150X):
Add 200 µL of DMSO into the vial of TF3-DEVD-FMK (Component A) to make 150X TF3-DEVD-FMK stock solution.

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. Culture cells to a density optimal for apoptosis induction according to your specific induction protocol, but not to exceed 2×10^6 cells/ mL (or not to exceed 3×10^5 cells/100 µL/well in a 96-well black clear-bottom plate). 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 TF3-DEVD-FMK stock solution at a 1:150 ratio and/or Annexin V-iFluor 488™ (Component B) at 1:100 ratio into each well.

3. Incubate the cells in a 37°C, 5% CO2 incubator for 1 hour.

   Note The cells can be concentrated up to ~ 5×10^6 cells/mL for TF3-DEVD-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 TF3 -DEVD-FMK. The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation

time for each experiment. Annexin V flow cytometric analysis on adherent cells is not routinely tested since specific membrane damage may occur during cell detachment or harvesting. However, methods for utilizing Annexin V for flow cytometry on adherent cell types have been previously reported by Casiola-Rosen et al. and van Engelend et al.

4. If desired, label the cells with a DNA stain (such as Hoechst for whole population of the cell nucleus stain, or propidium iodide for dead cells if the cells label with Annexin V-iFluor 488™ only).

5. Spin down the cells at ~200g for 2 minutes and wash cells with 1 mL (or 200 µL/well if using 96-well plate) Washing Buffer (Component E) twice. Resuspend the cells in desired amount of washing buffer.

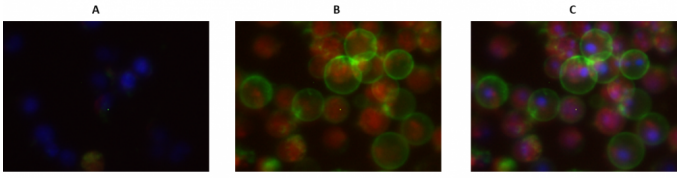
   Note TF3-DEVD-FMK and Annexin V-iFluor 488™ are fluorescent, thus it is important to wash out any unbound reagent to eliminate the background. For detached cells, the concentration of cells should be adjusted to 2-5 X 10^5 cells/100 µL aliquot per microtiter plate well.

6. Monitor the fluorescence intensity by fluorescence microscope, flow cytometer, or fluorescence microplate reader at Ex/Em = 550/595 nm for TF3-DEVD-FMK, 490/525 for Annexin V-iFluor 488™, 350/461 nm for Hoechst stain, and 535/635 for propidium iodide.
   For flow cytometry: Monitor the fluorescence intensity using FL1 channel for Annexin V-iFluor 488™, FL2 channel for TF3-DEVD-FMK. 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 TRITC channel for TF3-DEVD-FMK, and/or FITC channel for Annexin V-iFluor 488™ (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 microplate reader at Ex/Em = 490/525 nm (Cutoff = 515 nm) for Annexin V-iFluor 488™, and/or 550/595 nm (Cutoff = 570 nn) for TF3-DEVD-FMK.

   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



A: Non-induced control cells;
B: Doublestaining of staurosporine-induced cells for caspase 3/7 (red) and Annexin V (green);
C. Triple staining of staurosporine-induced cells for caspase 3/7(red), Annexin V (green) and nuclear (blue).

Figure 1. The fluorescence image analysis indicated the increased expression of caspase 3/7 (red, stained by TF3- DEVD-FMK) and Annexin V (Green, stained by Annexin V-iFluor 488™) in Jurkat cells induced by 1 µM staurosporine for 3 hour. The fluorescence images of the cells (300,000 cells/ well) were taken with Olympus fluorescence microscope through the DAPI, FITC, and TRITC channel respectively. Individual images taken from each channel from the same cell population were merged as shown above. A: Non-induced control cells; B: Doublestaining of staurosporine-induced cells for caspase 3/7 (red) and Annexin V (green); C: Triple staining of staurosporine-induced cells for caspase 3/7(red), Annexin V (green) and nuclear (blue).

DISCLAIMER