Cell Meter™ Live Cell Caspase 3/7 and Phosphatidylserine Detection Kit

Triple Fluorescent Color

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22850 (100 assays)	Keep in freezer Avoid light	Fluorescence microscope, flow cytometer and fluorescence microplate reader

Introduction

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 TF3-DEVD-FMK as a fluorescent indicator to detect caspase 3/7 activities. TF3-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. TF3-DEVD-FMK is a red label reagent with Ex/Em = 550/595 nm.

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.

Kit Components

Components	**Amount**
Component A: TF3-DEVD-FMK	1 vial
Component B: 100X Annexin V-iFluor 488™ conjugate	1 vial (200 µL)
Component C: 500X Hoechst stain	1 vial (100 µL)
Component D:500X Propidium iodide	1 vial (100 µL)
Component E: Washing buffer	1 bottle (100 mL)

Assay Protocol for Detached Cells

Brief Summary

Prepare cells with test compounds at a density of 2-3 × 10^6 cells/mL → Add TF3-DEVD-FMK at 1:150 ratio and/or Annexin V-iFluor 488™ into cell solution at 1:100 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 (for caspases), 490/525 nm (for Annexin V)

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 (or not to exceed 3 x 10^5 cells/100 µL/well in a 96-well black clear-bottom plate). At the

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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. For adherent cells, use 2-3 x10^4 cells/well to start.

2. Make 150X TF3-DEVD-FMK DMSO stock solution by adding 200 µL of DMSO to the vial of TF3-DEVD-FMK (Component A).

3. Add TF3-DEVD-FMK at a 1:150 ratio and/or Annexin V-iFluor 488™ (Component B) at 1:100 ratio into each well, 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: 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 (see Refs 1 and 2).
   Note 4: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

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 and wash cells with 1 mL (or 200 µL/well if using 96-well plate) wash buffer (Component E) twice. Resuspend the cells in desired amount of washing buffer.
   Note 1: TF3-DEVD-FMK and Annexin V-iFluor 488™ are 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 6.

6. Monitor the fluorescence intensity by fluorescence microscopy, flow cytometer, or fluorescent 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.

   6.1 For flow cytometry, monitor the fluorescence intensity using the FL1 channel for Annexin V-iFluor 488™, FL2 channel for TF3-DEVD-FMK. Gate on the cells of interest, excluding debris.

   6.2 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.

   6.3 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).

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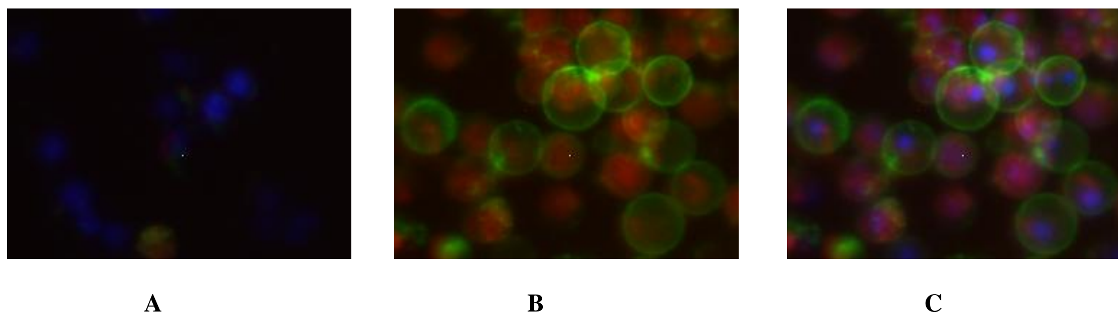
6.4 Monitor the fluorescence intensity using Ex/Em = 490/525 nm (cut off at 515 nm) for TF3-DEVD-FMK, and /or 550/595 nm (cut off 575) for Annexin V-iFluor 488™ bottom read mode for a fluorescent microplate reader.

Data Analysis

1. Fluorescence Microscopy Sample Data:



A **B** **C**

Figure1. 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).

References

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