Cell Meter™ Caspase 3/7 Activity Apoptosis Assay Kit

Green Fluorescence Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22823 (100 assays)	Keep in freezer and avoid exposure to light	Flow Cytometer

Introduction

The activation of caspase 3 (CPP32/apopain) 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 TF2-DEVD-FMK as a fluorescent indicator for caspase 3/7 activity. TF2-DEVD-FMK, which is cell permeable and nontoxic, 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 prevents the caspase 3/7 from further catalysis but will not stop apoptosis from proceeding. Within 15 minutes after being added into the medium, the reagent will start to react with active caspase 3/7 enzymes.

There are a variety of parameters that can be used for monitoring cell apoptosis. This Cell Meter™ 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. TF2-DEVD-FMK, the green label reagent, allows for direct detection of activated caspase 3/7 in apoptotic cells by flow cytometry. The kit provides all the essential components with an optimized assay protocol.

Kit Key Features

Non-Radioactive:	No special requirements for waste treatment.
Convenient and Robust:	Formulated to have minimal hands-on time.
Optimized Performance:	Provide optimal conditions for the detection of caspase 3/7 activity.
Enhanced Value:	Less expensive than the sum of individual components.

Kit Components

Components	**Amount**
Component A: 500X TF2-DEVD-FMK	1 vial (100 µL)
Component B: Assay Buffer	1 bottle (50 mL)
Component C: 500X Propidium Iodide	1 vial (100 µL)

Assay Protocol for Flow Cytometer

Brief Summary

Prepare cells with test compounds at a density of 5×10^5 to 1×10^6 cells/mL → Add 1 µL of 500X TF2-DEVD-FMK into 0.5 mL of cell solution → Incubate at 37 °C, 5% CO_2 incubator for 1-4 hours → Pellet the cells, and resuspend the cells in 0.5 mL of assay buffer or growth medium → Analyze with a flow cytometer

Note: Thaw all the components at room temperature before use.

1. For each sample, prepare cells in 0.5 mL warm medium or buffer of your choice at a density of 5×10^5 to 1×10^6 cells/mL.

   Note: Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

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2. Treat cells with test compounds for a desired period of time to induce apoptosis, and create positive and negative controls.

3. Add 1 µL of 500X TF2-DEVD-FMK (Component A), and incubate the cells in a 37 °C, 5% CO_2 incubator for 1-4 hours.

Note 1: 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 TF2-DEVD-FMK.
Note 2: The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

4. Wash and spin the cells twice. Resuspend the cells in 0.5 mL of assay buffer or growth medium.

Note: TF2-DEVD-FMK is fluorescent, thus it is important to wash out any unbound reagent to remove the background.

5. If desired, label the cells with a DNA stain (such as propidium iodide or 7-AAD for dead cells).

6. If desired, fix cells.

7. Monitor the fluorescence intensity with a flow cytometer using the FL1 channel (Ex/Em = 490/525 nm). Gate on the cells of interest, excluding debris.

Data Analysis

In live non-apoptotic cells, TF2-DEVD-FMK detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells, TF2-DEVD-FMK binds to active caspases 3/7 resulted in increased stain intensity.

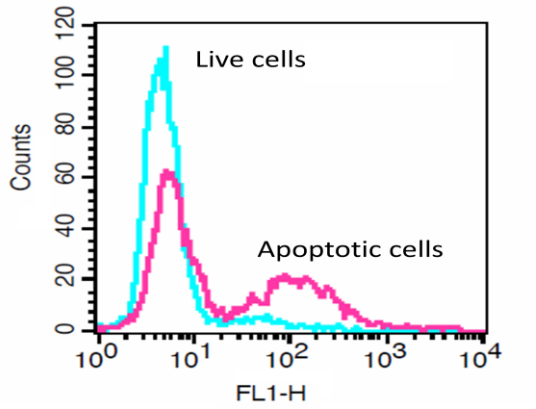

Figure 1. The increase in TF2-DEVD-FMK fluorescence intensity with the addition of Camptothecin in Jurkat cells. Jurkat cells were treated without (Blue) or with 20 µM camptothecin (Pink) in a 37 °C, 5% CO_2 incubator for 4-5 hours, and then dye loaded with TF2-DEVD-FMK for 1 hour.

References

1. Li JN, Song DQ, Jiang JD. (2004) [Antitumor mechanism of 3-bromopropionylamino benzoylurea on leukemia and lymphoma]. Yao Xue Xue Bao, 39, 491.
2. Thrane C, Kaufmann U, Stummann BM, Olsson S. (2004) Activation of caspase-like activity and poly (ADP-ribose) polymerase degradation during sporulation in Aspergillus nidulans. Fungal Genet Biol, 41, 361.
3. Pandey S, Smith B, Walker PR, Sikorska M. (2000) Caspase-dependent and independent cell death in rat hepatoma 5123tc cells. Apoptosis, 5, 265.