Cell Meter™ Generic Fluorometric Caspase Activity Assay Kit

Red Fluorescence Optimized for Flow Cytometry

Ordering Information	*Storage Conditions*	*Instrument Platform*
Product Number: 22822 (100 assays)	Keep in freezer and avoid exposure to light	Flow Cytometer

Introduction

Our Cell Meter™ assay kits are a set of tools for monitoring cellular functions. The activation of caspase is widely accepted as a reliable indicator for cell apoptosis. Most caspases have substrate selectivity for the peptide sequence Val-Ala-Asp (VAD).

This particular kit is designed to monitor cell apoptosis through measuring generic caspases (caspase-1, -3, -4, -5, -6, -7, -8 and -9) activation in live cells with our red fluorescent TF5-VAD-FMK probe. Most caspases have substrate selectivity for the peptide sequence Val-Ala-Asp (VAD). The cell permeable and nontoxic TF5-VAD-FMK irreversibly binds to activated casepase-1, -3, -4, -5, -6, -7, -8 and -9 in apoptotic cells. Once bound to caspases, the fluorescent reagent is retained inside the cell. The binding event prevents the caspases from further activation but will not stop apoptosis from proceeding.

With the spectral properties almost identical to those of Cy5® or Alexa Fluor® 647 (Ex/Em = ~647/660 nm), TF5-VAD-FMK can be conveniently used with the common fluorescence instruments equipped with the light sources and filters for Cy5® or Alexa Fluor® 647 (Cy5® or Alexa Fluor® 647 are the trademarks of GE Healthcare and Invitrogen respectively). The red label allows for direct detection of activated caspases in apoptotic cells by a flow cytometer. It can be used for the quantification of most activated caspases activities in apoptotic cells, or for the screening of caspases inhibitors. The kit provides all the essential components.

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 many caspase activities.
Enhanced Value:	Less expensive than the sum of individual components.

Kit Components

Components	Amount
Component A: 500 X TF5-VAD-FMK	1 vial (100 µL)
Component B: Assay Buffer	1 bottle (50 mL)
Component C: 500 X Green-DCS1	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 TF5-VAD-FMK into 0.5 mL of cell solution → Incubate at room temperature 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 kit 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 TF5-VAD-FMK (Component A) into the treated cells (from Step 2), 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 TF5-VAD-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 (Component B) or growth medium.

Note: TF5-VAD-FMK is fluorescent; therefore 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 Green-DCS for dead cells, which can be detected in the FL1 channel (Ex/Em = 490/525 nm)].

6. If desired, fix cells.

7. Monitor the fluorescence intensity with a flow cytometer using the FL4 channel (Ex/Em = 650/670 nm). Gate on the cells of interest, excluding debris.

Data Analysis

In live non-apoptotic cells, TF5-VAD-FMK detects innate apoptosis in non-induced cells, which is typically 2-6% of all cells. In apoptotic cells, TF5-VAD-FMK binds to active caspases resulted in increased staining intensity.

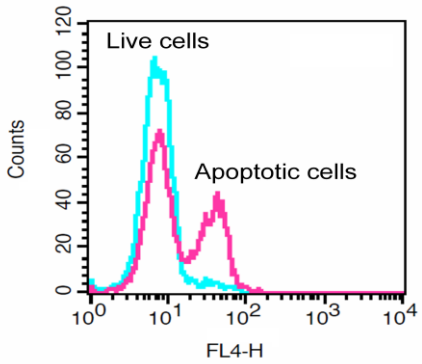

Figure 1. The increase in TF5-VAD-FMK fluorescence intensity with the addition of Camptothecin in Jurkat cells. Jurkat cells were treated without (Blue) or with 20 µM camptothecin (Red) in a 37 °C, 5% CO_2 incubator for 4-5 hours, and then dye loaded with TF5-VAD-FMK for 1 hour.

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.