Cell Meter™ Generic Fluorimetric Caspase Binding Assay Kit *Red Fluorescence Optimized for Flow Cytometry*

Catalog number: 22822
Unit size: 100 Tests

Component	Storage	Amount
Component A: 500X TF5-VAD-FMK	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)
Component B: Assay Buffer	Freeze (<-15 °C)	1 bottle (50 mL)
Component C: 500X Green-DCS1	Freeze (<-15 °C), Minimize light exposure	1 vial (100 µL)

OVERVIEW

Our Cell Meter™ assay kits are a set of tools for monitoring cell viability. There are a variety of parameters that can be used for monitoring cell viability. 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 living cells. Caspases activation is widely accepted as a reliable indicator for cell apoptosis. Most caspases have substrate selectivity for the peptide sequence Val-Ala-Asp (VAD). This kit uses TF5-VAD-FMK as a fluorogenic indicator for most caspase activity. TF5-VAD-FMK is cell permeable, nontoxic, and irreversibly binds to activated casepase-1, -3, -4, -5, -6, -7, -8 and -9 in apoptotic cells. Once bound to caspases, the red fluorescent reagent is retained within the cell. The binding event prevents the caspases from further catalysis but will not stop apoptosis from proceeding. The reagent will start to react with active caspase enzymes within 15 minutes of addition to the media. The kit provides all the essential components with an optimized assay protocol. It is used for the quantification of most activated caspases activities in apoptotic cells, or for screening caspases inhibitors. The green label allows for direct detection of activated caspases in apoptotic cells by flow cytometry.

AT A GLANCE

Protocol summary

1. Prepare cells with test compounds at a density of 5×10^5 to 1×10^6 cells/mL
2. Add 1 µL of 500X TF5-VAD-FMK into 0.5 mL of cell solution
3. Incubate the cells in a 37°C, 5% CO_2 incubator for 1 - 4 hours
4. Pellet the cells and resuspend the cells in 0.5 mL of assay buffer or growth medium
5. Analyze cells using flow cytometer with 660/20 nm filter (APC channel)

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

KEY PARAMETERS

Instrument:	Flow cytometer
Excitation:	640 nm laser
Emission:	660/20 nm filter
Instrument specification(s):	APC channel

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. 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.

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.

4. Incubate the cells in a 37°C, 5% CO_2 incubator for 1 - 4 hours.

   Note 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. The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

5. 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.

6. If desired, label the cells with a DNA stain (such as Green-DCS for dead cells, which can be detected with 530/30 nm filter (FITC channel)

7. If desired, fix cells.

8. Monitor the fluorescence intensity using a flow cytometer with 660/20 nm filter (APC channel). Gate on the cells of interest, excluding debris.

EXAMPLE DATA ANALYSIS AND FIGURES

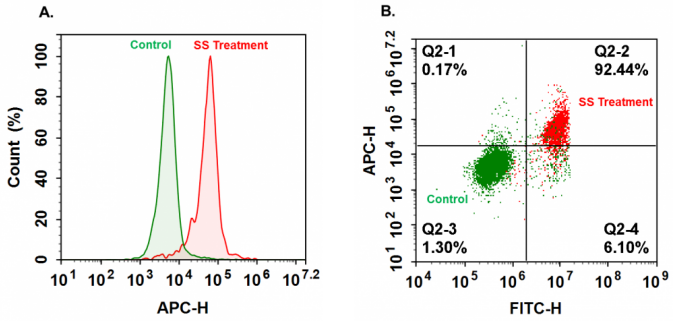

Figure 1.

The increase of caspase is measured by Cell Meter™ Generic Fluorimetric Caspase Binding Assay Kit (Cat# 22822) in Jurkat Cells treated with Staurosporine. Jurkat cells were treated with or without 1 µM of Staurosporine for 4 hours before TF5-VAD-FMK and Nuclear Green DCS1 were added. **A**. Binding of TF5-VAD-FMK to Caspase was measured by NovoCyte at Ex/Em=646/659. **B**. The staining of Nuclear Green DCS1 and TF5-VAD-FMK were both measured by NovoCyte and shown in a dot plot. SS: Staurosporine

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