

FAM-VAD-FMK

Catalog number: 13470, 13484
Unit size: 25 Tests, 100 Tests

Component	Storage	Amount (Cat No. 13470)	Amount (Cat No. 13484)
FAM-VAD-FMK	Freeze (< -15 °C), Minimize light exposure	25 Tests	100 Tests

OVERVIEW

FAM-VAD is a green fluorescent cell-permeable polycaspase inhibitor to target caspases 1, 2, 3, 6, 8, 9, or 10. Once inside the cell, the inhibitor binds covalently to the active caspase, which produces a green fluorescence. When added to a population of cells, the FAM-VAD-FMK probe enters each cell and covalently binds to a reactive cysteine residue that resides on the large subunit of the active caspase heterodimer, thereby inhibiting further enzymatic activity. The bound labeled reagent is retained within the cell, while any unbound reagent will diffuse out of the cell and is washed away. The green fluorescent signal is a direct measure of the amount of active caspase present in the cell at the time the reagent was added. Cells that contain the bound labeled reagent can be analyzed by 96-well plate-based fluorometry, fluorescence microscopy, or flow cytometry. It works in diverse cell lines such as human, rodent and drosophila.

AT A GLANCE
Important Note

It is important to store at <-15 °C and should be stored in cool, dark place.

Following protocol only provides a guideline, and should be modified according to your specific needs.

It can be used within 12 months from the date of receipt.

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

FAM-VAD-FMK Stock Solution

Add 50 µL of DMSO to Cat. #13470 or 200 µL of DMSO to Cat. #13484 make the stock solution.

PREPARATION OF WORKING SOLUTION
FAM-VAD-FMK Working Solution

50 µL of FAM-VAD-FMK stock solution to 5 mL of HHBS buffer.

SAMPLE EXPERIMENTAL PROTOCOL
Cell Caspase Assays Using Cell-Permeable FMK Caspase Probes

1. Treat cells as desired.
2. Add 250 µL of working solution into 250 µL of the cell solution, and incubate the cells in a 37 °C, 5% CO₂ incubator for 1 hour.
3. Treat cells as desired.
4. Monitor the fluorescence intensity by flow cytometer, fluorescence microscopy or fluorescent microplate reader.

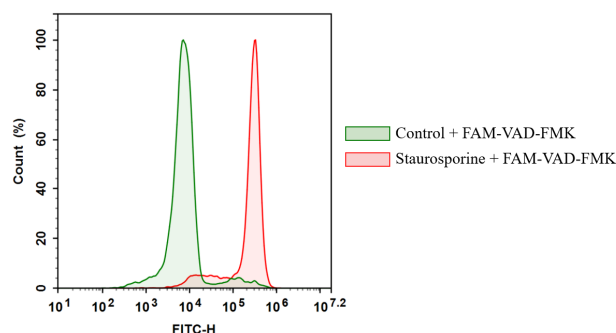
EXAMPLE DATA ANALYSIS AND FIGURES


Figure 1. The increase of caspase is measured by FAM-VAD-FMK in Jurkat Cells treated with Staurosporine. Jurkat cells were treated with or without 1 µM of Staurosporine for 2 hours before FAM-VAD-FMK were added. Cells were treated with FAM-VAD-FMK for 1 hour at 37C, 5% CO₂ incubator. The response was measured with ACEA NovoCyt flow cytometer with FITC channel.

DISCLAIMER

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