SRB-VAD-FMK [Sulforhodamine B-VAD-FMK]

Catalog number: 13472
Unit size: 25 Tests

Component	Storage	Amount
SRB-VAD-FMK [Sulforhodamine B-VAD-FMK]	Freeze (<-15 °C), Minimize light exposure	25 Tests

OVERVIEW

SRB-VAD is a red 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 SRB-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. The probe has the spectral properties similar to Cy3® and TRITC, thus the filter set of Cy3® and TRITC can be conveniently used with a flow cytometer or microscope.

AT A GLANCE

Important notes

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

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

SAMPLE EXPERIMENTAL PROTOCOL

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

General Solution Caspase Assays Using AMC, AFC, pNA, R110 and ProRed Substrates

1. Prepare a 10 mM stock solution in DMSO.
2. Prepare a 2X caspase substrate (50 µM) assay solution as the following: 50 µL substrate stock solution, 100 µL DTT (1M), 400 µL EDTA (100 mM), 10 mL Tris Buffer (20 mM), pH =7.4.
3. Mix equal volume of the caspase standards or samples with 2X caspase substrate assay solution, and incubate the solutions at room temperature for at least 1 hour.
4. Monitor the fluorescence using a fluorescence microplate reader, or absorbance using an absorbance microplate reader.

Cell Caspase Assays Using Cell-Permeable FMK Caspase Probes

1. Prepare a 2-5 mM stock solution in DMSO.
2. Treat cells as desired.
3. Prepare a 2X permeable caspase substrate (20 µM) assay solution by diluting the DMSO stock solution (from Step 2.1) in Hanks with 20 mM Hepes buffer (HHBS).
4. Mix equal volume of the treated cells with 2X caspase substrate assay solution (from Step 2.3), and incubate the cells in a 37°C, 5% CO_2 incubator for at least1 hour.
5. Wash the cells with HHBS for at least once.
6. Monitor the fluorescence intensity by a flow cytometer, a fluorescence microscope or a fluorescence microplate reader.

Cell Caspase Assays Using Cell-Permeable FMK Caspase Probes (For #13470-13476 only)

1. Prepare a 250X stock solution by adding 50 µL DMSO into the vial.
2. Treat cells as desired.
3. Add 250 X DMSO stock solution into the cell solution at a 1:250 ratio (such as 2 µL to 500 µL cells), and incubate the cells in a 37°C, 5% CO2 incubator for 1 hour.
4. Wash the cells with HHBS for at least once.
5. Monitor the fluorescence intensity by flow cytometer, fluorescence microscopy or fluorescent microplate reader.

EXAMPLE DATA ANALYSIS AND FIGURES

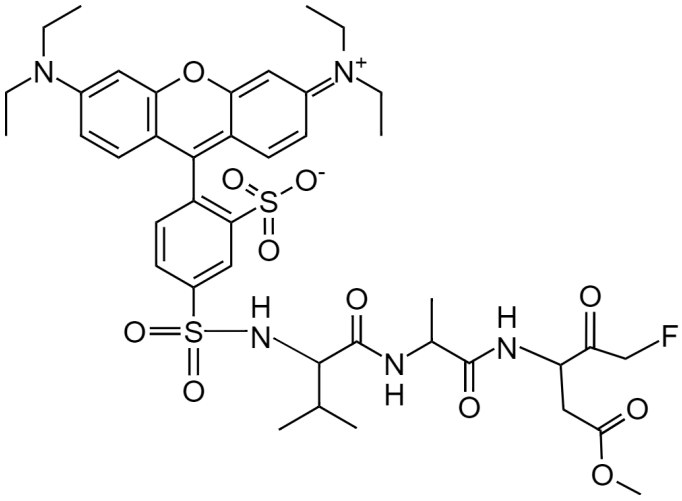

Figure 1. Chemical structure for SRB-VAD-FMK [Sulforhodamine B-VAD-FMK]

DISCLAIMER

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