

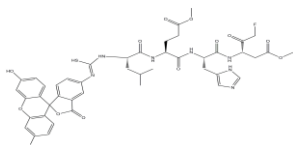
PRODUCT: FITC-LEHD-FMK

ALTERNATE NAMES: FITC-Leu-Glu-His-Asp-fluoromethyl ketone; FITC-L-E(OMe)-HD(OMe)-FMK

CATALOG #: 9534-100

AMOUNT: 100 μ L (100 Assays)

STRUCTURE:



MOLECULAR FORMULA: C₄₅H₄₈FN₇O₁₃S

MOLECULAR WEIGHT: 945.97

CAS#: N/A

APPEARANCE: Liquid

FORMULATION: A 0.6 mM solution in DMSO

PURITY: \geq 98% by HPLC

STORAGE: Store at -20°C. Protect from air and moisture

DESCRIPTION: FITC-LE(OMe)HD(OMe)-FMK is a fluorescein isothiocyanate (FITC) conjugate of the cell-permeable caspase-9 inhibitor LE(OMe)HD(OMe)-FMK that acts as an *in situ* marker for detection of caspase-9 in living cells. FITC-LE(OMe)HD(OMe)-FMK is nontoxic, and irreversibly binds to activated caspase-9 in apoptotic cells. The bound marker is localized by fluorescence detection.

CASPASE-9 ASSAY PROCEDURE:

A. Staining Procedure:

1. Induce apoptosis in cells (1×10^6 /ml) by desired method. Concurrently incubate a control culture *without* induction. An additional control can be prepared by adding the caspase-9 inhibitor LEHD-FMK (**BioVision Cat. No.1074-20C, 100**) at 1 μ /ml to an induced culture to inhibit caspase activation.
2. Aliquot 300 μ l each of the induced and control cultures into eppendorf tubes.
3. Add 1 μ l of FITC-LEHD-FMK into each tube and incubate for 0.5-1 hour at 37°C incubator with 5% CO₂.
4. Centrifuge cells at 3000 rpm for 5 minutes and remove supernatant.
5. Re-suspend cells in 0.5 ml of Wash Buffer (**BioVision Cat. No. 1210**), and centrifuge again.
6. Repeat Step 5.
Proceed to B, C, or D depending on methods of analysis.

B. Quantification by Flow Cytometry:

For flow cytometric analysis, re-suspend cells in 300 μ l of Wash buffer. Put samples on ice. Analyze samples by flow cytometry using the FL-1 channel.

C. Detection by Fluorescence Microscopy:

For fluorescence microscopic analysis, re-suspend cells in 100 μ l Wash buffer. Put one drop of the cell suspension onto a microslide and cover with a coverslip. Observe cells under a fluorescence microscope using FITC filter. Caspase positive cells appear to have brighter green signals, whereas caspase negative control cells show much weaker signal.

- D. Analysis by Fluorescence Plate Reader:** For analysis with fluorescence plate reader, resuspend cells in 100 μ l Wash Buffer and then transfer the cell suspension to each well in the black microtiter plate. Measure the fluorescence intensity at Ex/Em = 485/535 nm. For control, use wells containing unlabeled cells.

RELATED PRODUCTS:

- CaspGLOW™ Fluorescein Active Caspase Staining Kit **(K180)**
- CaspGLOW™ Fluorescein Active Caspase-2 Staining Kit **(K182)**
- CaspGLOW™ Fluorescein Active Caspase-3 Staining Kit **(K183)**
- CaspGLOW™ Fluorescein Active Caspase-8 Staining Kit **(K188)**
- CaspGLOW™ Fluorescein Active Caspase-9 Staining Kit **(K189)**
- CaspGLOW™ Fluorescein Active Caspase-12 Staining Kit **(K172)**
- CaspGLOW™ Red Active Caspase Staining Kit **(K190)**
- CaspGLOW™ Red Active Caspase-3 Staining Kit **(K193)**
- CaspGLOW™ Red Active Caspase-9 Staining Kit **(K199)**
- FITC-YVAD-FMK **(9496)**
- FITC-VAD-FMK **(9497)**
- FITC-VDVAD-FMK **(9498)**
- FITC-DEVD-FMK **(9499)**
- FITC-IETD-FMK **(9533)**
- FITC-LEHD-FMK **(9534)**

FOR RESEARCH USE ONLY! Not to be used on humans.