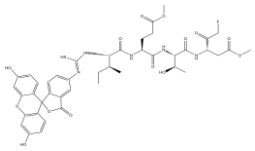
PRODUCT: FITC-IETD-FMK

ALTERNATE NAMES: FITC-Ile-Glu-Thr-Asp-fluoromethyl ketone; FITC-I-E(OMe)-TD(OMe)-FMK

CATALOG #: 9533-100

AMOUNT: 100 µl (100 Assays)

STRUCTURE:



MOLECULAR FORMULA: $C_{43}H_{48}FN_5O_{14}S$

MOLECULAR WEIGHT: 909.93

CAS#: N/A

APPEARANCE: Liquid

FORMULATION: A 0.6 mM solution in DMSO

PURITY: ≥98% by HPLC

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

DESCRIPTION: FITC-IE(OMe)TD(OMe)-FMK is a fluorescein isothiocyanate (FITC) conjugate of the cell- permeable caspase-8 inhibitor IE(OMe)TD(OMe)-FMK that acts as an *in situ* marker for detection of caspase-8 in living cells. FITC-IE(OMe)TD(OMe)-FMK is nontoxic, and irreversibly binds to activated caspase-8 in apoptotic cells. The bound marker is localized by fluorescence detection.

CASPASE-8 ASSAY PROCEDURE:

A. Staining Procedure:

1. Induce apoptosis in cells (1 x 10^6/ml) by desired method. Concurrently incubate a control culture *without* induction. An additional control can be prepared by adding the caspase-8 inhibitor IETD-FMK **(BioVision Cat. No.1064-20C, 100)** at 1 µl/ml to an induced culture to inhibit caspase activation.
2. Aliquot 300 µl each of the induced and control cultures into eppendorf tubes.
3. Add 1 µl of FITC-IETD-FMK into each tube and incubate for 0.5-1 hour at 37˚C incubator with 5% CO_2.
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 µl 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 µl 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 µl 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.