FITC-C6-DEVD-FMK

Dyes and Stains

For detection of active caspase-3 in mammalian cells undergoing apoptosis

Catalog #100-0924 100 µg

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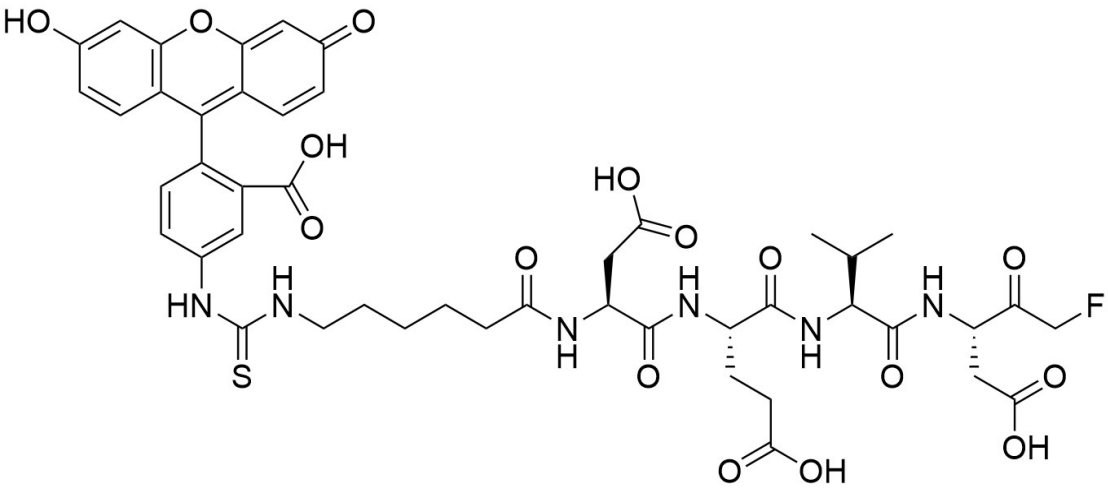
Product Description

Caspases are a family of proteases that play a central role in cellular apoptosis through the cleavage of select proteins, and result in the disassembly of the cell (Thornberry & Lazebnik). This process is switched on by pro-apoptotic signals from death receptors or stimuli such as cytotoxic reagents, which activate the initiator caspases and lead to activation of the effector caspases—caspase-3 and caspase-7 (Thornberry & Lazebnik). Caspase-3 has substrate selectivity for the peptide sequence Asp-Glu-Val-Asp (DEVD). FITC-C6-DEVD-FMK is a non-toxic, cell-permeable fluorescent substrate that irreversibly binds to activated caspase-3 in apoptotic cells. The FITC-labeled substrate can be detected with high sensitivity using flow cytometry. FITC-C6-DEVD-FMK is useful for screening caspase-3 inhibitors and quantifying apoptotic cells containing active caspase-3.

Molecular Weight:	994.99 g/mol
Excitation Wavelength:	491 nm
Emission Wavelength:	516 nm
Extinction Coefficient:	73,000 $cm^{-1}M^{-1}$
Correction Factor:	0.254
Structure:	



Properties

Storage:	Store at -20°C.
Shelf Life:	Stable until expiry date (EXP) on box label. Protect product from prolonged exposure to light.
Format:	Solid

Directions for Use

Please read the entire protocol before proceeding.

Preparation of FITC-C6-DEVD-FMK Working Solution

1. To prepare a stock solution, dissolve FITC-C6-DEVD-FMK in dimethyl sulfoxide (DMSO) at 2 - 5 mM.
   NOTE: If not used immediately, aliquot and store at -20°C. After thawing aliquots, use immediately; do not re-freeze.
2. To prepare a FITC-C6-DEVD-FMK working solution, dilute the stock solution (prepared in step 1) to 20 µM with Hanks' Balanced Salt Solution with 20 mM HEPES (HHBS). Use the working solution immediately; do not store.

Staining Cells

1. To induce apoptosis, treat cells with test compounds and incubate at 37°C and 5% CO_2 for an appropriate length of time.
   Example: Jurkat cells treated with camptothecin require 4 - 6 hours of incubation for apoptosis to occur.
   NOTE: The optimal cell density to induce apoptosis should be determined for different cell lines.
2. Prepare positive and negative controls as follows:
   a. Positive control: camptothecin or staurosporine at 5X or 10X concentration
      NOTE: The optimal concentration should be determined for different cell lines.
   b. Negative control 1: untreated cells
   c. Negative control 2: untreated cells + equal volume of FITC-C6-DEVD-FMK working solution
3. Add an equal volume of the FITC-C6-DEVD-FMK working solution to the cell solution and incubate at 37°C and 5% CO_2 for at least 1 hour.
   NOTE: The optimal incubation time should be determined for different cell lines.
4. Centrifuge cells at 100 - 150 x *g* for 5 minutes. Remove supernatant and wash cells with HHBS at least once.
   NOTE: Centrifugation speed may need to be optimized to ensure apoptotic cells are sufficiently pelleted.
5. Monitor the fluorescence intensity using a flow cytometer or a fluorescence microscope.

References

Thornberry NA & Lazebnik Y. (1998) Caspases: enemies within. Science 281(5381): 1312–6.