Z-IETD-FMK

Inhibitor of Caspase-8

Catalog code: inh-ietd

https://www.invivogen.com/z-ietd-fmk

For research use only

Version 21G30-MM

PRODUCT INFORMATION

Contents

- 1 mg Z-IETD-FMK (provided as a powder)

Storage and stability

- Z-IETD-FMK is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots and store at -20 °C. Resuspended product is stable for 6 months at -20 °C when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- Purity: ≥95% (UHPLC)
- The inhibitory activity has been confirmed using in-house cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Z-IETD-FMK is a potent small-molecule inhibitor of Caspase-8 (CASP8)[1,2]. CASP8 is a key actor of apoptosis initiated by the engagement of TNF family death domain receptors at the cell surface ('extrinsic' apoptosis), or by a developmental signal or a genotoxic agent ('intrinsic' apoptosis)[2-4]. When CASP-8 is absent or inactivated, the death signals trigger necroptosis, a type of regulated necrosis[4]. The tetrapeptide IETD is suited to bind preferentially to the active site of CASP8[1,2]. The addition of a benzylocarboxonyl group (Z), O-methyl side chains, and a fluoromethyl ketone (FMK) group enhance Z-IETD-FMK cell permeability with no additional cytotoxic effects.

1. Thornberry N.A. *et al.*, 1997. A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. J Biol Chem. 272(29):17907-11. **2. Concha, N.O. & Abdel-Meguid, S.S. 2002.** Controlling apoptosis by inhibition of caspases. Curr Med Chem. 9(6):713-26. **3. Barnhart B.C. & Peter M.E. 2003.** The TNF receptor 1: a split personality complex. Cell. 114(2):148-150. **4. Bertheloot D. *et al.*, 2021.** Necroptosis, pyroptosis and apoptosis: an intricate game of cell death. Cellular & Molecular Immunology. 18:1106-1121.

CHEMICAL PROPERTIES

CAS number: 210344-98-2

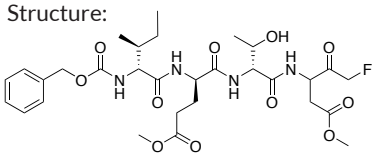
Synonym: Z-Ile-Glu(O-ME)-Thr-Asp(O-Me) fluoromethyl ketone

Formula: $C_{30}H_{43}FN_4O_{11}$

Molecular weight: 654.68 g/mol

Solubility: 68.7 mM (45 mg/ml) in DMSO

Structure:



METHODS

Preparation of stock solution (5 mM)

1. Add 306 µl DMSO to 1 mg Z-IETD-FMK vial.
2. Vortex until completely resuspended.
3. Prepare aliquots of Z-IETD-FMK and store at -20°C.
4. Once Z-IETD-FMK is resuspended, further dilutions can be prepared using sterile aqueous buffers.

Working concentration range: 6-20 µM for cell culture assays

PROTOCOL

Below is a protocol for measuring cell death using THP1-HMGB1-Lucia™ cells. This assay relies on the luminescence quantification of the HMGB1::Lucia fusion protein released in the supernatant upon pyroptosis or necroptosis. For more information, visit: https://www.invivogen.com/thp1-hmgb1-lucia.

Necroptosis assay

It is recommended to perform the assay with test medium which does not contain Normocin™ nor Zeocin™.

1. Add 20 µl of a caspase inhibitor such as Z-IETD-FMK (20 µM final concentration) per well of a flat-bottom 96-well plate.
2. Prepare a THP1-HMGB1-Lucia™ suspension at ~2.5 x 10^6 cells/ml.
3. Dispense 120 µl of cell suspension (~300,000 cells) per well.
4. Incubate at 37 °C in 5% CO_2 for 1 h.
5. Add 20 µl of a cIAP inhibitor such as BV6 (5 µM final concentration) and recombinant hTNF-α (100 ng/ml final concentration) per well.
6. Incubate the plate at 37 °C in a CO_2 incubator for 8-24 h. Proceed to detection of HMGB1::Lucia using QUANTI-Luc™ as described on the next page.

TECHNICAL SUPPORT
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Detection of HMGB1::Lucia

Below is a protocol for end-point readings using a luminometer. This protocol can be adapted for use with kinetic measurements.

1. Prepare the QUANTI-Luc™ assay solution following the instructions on the enclosed data sheet.
2. Transfer 10 µl of THP1-HMGB1-Lucia™ stimulated cell supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of QUANTI-Luc™.
4. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
Necrostatin-1	RIPK1 inhibitor	inh-ncst1
BV6	IAP inhibitor	inh-bv6
Z-VAD-FMK	Pan-caspase inhibitor	tlrl-vad
Recombinant hTNF-α	Recombinant cytokine	rcyc-htnfa
THP1-HMGB1-Lucia™	Reporter cell line	thp-gb1lc
QUANTI-Luc™	Detection reagent	rep-qlc1