Z-VAD-FMK

Pan-Caspase Inhibitor - InvitroFit™

Catalog code: tlrl-vad

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

For research use only

Version 23I07-MM

PRODUCT INFORMATION

Contents

• 1 mg of Z-VAD-FMK - InvitroFit™

Storage and stability:

- Z-VAD-FMK is provided as a translucent film and shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots of Z-VAD-FMK and store at -20°C. Resuspended product is stable for at least 6 months at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- Purity ≥ 95% (UHPLC)
- The inhibitory activity has been validated using 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-VAD-FMK is a pan-caspase inhibitor that irreversibly binds to the catalytic site of caspases[1,2]. It potently inhibits caspase-1 to -11 with the exception of caspase-2[3]. It inhibits murine caspases, notably caspase-1, caspase-3 and caspase-11, the ortholog of human caspase-4 and -5[4,5]. The caspase enzymes are a family of cytosolic proteases involved in the regulation of inflammation and cell death. They can be divided into inflammatory caspases (such as caspase-1, -4, -5, -11 and -12) and apoptotic caspases (such as caspase-2, -3, -6, -7, -8, -9, and -10)[6].

Of note, caspase-1 plays a crucial role in the control of inflammation. Its activity is regulated by a multi-protein complex known as the inflammasome. Upon activation, caspase-1 processes interleukin-1β (IL-1β) and IL-18, and Gasdermin-D, promoting inflammation and pyroptosis, a form of cell death. Indeed, Z-VAD-FMK is widely-used to investigate inflammasome activation[7].

Through its inhibitory activity, Z-VAD-FMK can reduce inflammation, block the induction of caspase-mediated apoptosis and trigger necroptosis[2,7]. Interestingly, studies in L929 cells have shown that Z-VAD-FMK can induce autophagic cell death[8]. To conclude, this broad-spectrum inhibitor is a useful tool for studying the role of caspases in inflammation and cell death.

1. Slee EA. *et al.*, 1996. Benzyloxycarbonyl-Val-Ala-Asp (OMe) fluoromethylketone (Z-VAD.FMK) inhibits apoptosis by blocking the processing of CPP32. Biochem J. 315 (Pt 1):21-4. **2. Li X. *et al.*, 2019.** The caspase inhibitor Z-VAD-FMK alleviates endotoxic shock via inducing macrophages necroptosis and promoting MDSCs-mediated inhibition of macrophages activation. Front Immunol. 10:1824. **3. Chauvier D. *et al.*, 2007.** Broad-spectrum caspase inhibitors: from myth to reality? Cell Death Differ. 14:387-91. **4. Guey B. *et al.*, 2014.** Caspase-1 autoproteolysis is differentially required for NLRP1b and NLRP3 inflammasome function. PNAS 11(48):17254-9. **5. Py B.F. *et al.*, 2014.** Caspase-11 controls interleukin-1β release through degradation of TRPC1. Cell Rep. 6: 1122–8. **6. Shalini M. *et al.*, 2015.** Old, new and emerging functions of caspases. Cell Death Differ. 22:526-39. **7. Dostert C. *et al.*, 2009.** Malarial hemozoin is a Nalp3 inflammasome activating danger signal. PLoS One. 4(8):e6510. **8. Chen SY. *et al.*, 2011.** zVAD-induced autophagic cell death requires c-Src-dependent ERK and JNK activation and reactive oxygen species generation. Autophagy.7(2):217-28.

CHEMICAL PROPERTIES

Synonym: Carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone

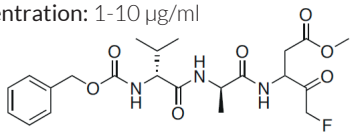
Linear formula: $C_{22}H_{30}FN_3O_7$

Molecular weight: 467.5 g/mol

Solubility: 20 mM (10 mg/ml) in DMSO

Working concentration: 1-10 µg/ml

Structure:



METHODS

Preparation of 1 mg/ml stock solution:

Note: Spin briefly the vial before opening the cap.

- Add 1 ml of DMSO to the vial and mix by vortexing.
- Prepare further dilutions using sterile endotoxin-free water.

***In vitro* inhibition of caspase-1:**

The following protocol describes the monitoring of caspase-1 inhibition in human THP1-Null2 cells by assessing the inhibition of IL-1β production.

1. Prepare a THP1-Null2 cell suspension and add 3 x 10^5 cells per well in a 96-well plate.
2. Prime cells by adding 1 µg/ml LPS-EK for 3 hours at 37°C in 5% CO_2.
3. Gently remove medium and add 180 µl of fresh test medium.
4. Stimulate cells by adding IL-1β inducers, such as MSU crystals (100-200 mg/ml) in the presence or absence of Z-VAD-FMK (1-10 µg/ml).
5. Incubate from 6 hours to overnight at 37°C in 5% CO_2.
6. Determine caspase-1 inhibition by detecting mature IL-1β with InvivoGen's HEK-Blue™ IL-1β cells, which are specifically engineered to detect bioactive IL-1β.

RELATED PRODUCTS

Product	Description	Cat. Code
Ac-YVAD-cmk	Caspase -1 inhibitor	inh-yvad
LPS-EK	LPS from *E. coli* K12	tlrl-eklps
Nigericin	Inflammasome inducer	tlrl-nig
MSU Crystals	Inflammasome inducer	tlrl-msu
Poly(dA:dT)	Inflammasome inducer	tlrl-patn
THP1-Null2 Cells	Human monocytes	thp-nullz
HEK-Blue™ IL-1β cells	IL-1β reporter cells	hkb-il1bv2
VX-765	Caspase -1 and -4 inhibitor	inh-vx765i-1

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