

Z-VAD-FMK

Pan-Caspase Inhibitor

Catalog code: tlrl-vad

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

For research use only

Version 21120-MM

PRODUCT INFORMATION

Contents

- 1 mg of Z-VAD-FMK (provided as a translucent film)

Storage and stability:

- Z-VAD-FMK is 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-2³. 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-1 β (IL-1 β) 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 activation⁷.

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 death⁸. 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.** Benzylloxycarbonyl-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 endotoxin 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-1 β 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.

TECHNICAL SUPPORT

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CHEMICAL PROPERTIES

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

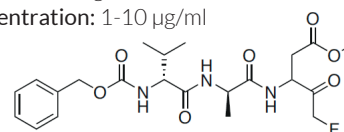
Linear formula: C₂₂H₃₀FN₃O₇

Molecular weight: 467.5 g/mol

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

Working concentration: 1-10 μ g/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-1 β production.

1. Prepare a THP1-Null2 cell suspension and add 3 x 10⁵ cells per well in a 96-well plate.
2. Prime cells by adding 1 μ g/ml LPS-EK for 3 hours at 37°C in 5% CO₂.
3. Gently remove medium and add 180 μ l of fresh test medium.
4. Stimulate cells by adding IL-1 β inducers, such as MSU crystals (100-200 mg/ml) in the presence or absence of Z-VAD-FMK (1-10 μ g/ml).
5. Incubate from 6 hours to overnight at 37°C in 5% CO₂.
6. Determine caspase-1 inhibition by detecting mature IL-1 β with InvivoGen's HEK-Blue™ IL-1 β cells, which are specifically engineered to detect bioactive IL-1 β .

RELATED PRODUCTS

Product	Description	Cat. Code
Ac-YVAD-cmk	Caspase -1 inhibitor	inh-yvad
LPS-EK	LPS from <i>E. coli</i> 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-1 β cells	IL-1 β reporter cells	hkb-il1b
VX-765	Caspase -1 and -4 inhibitor	inh-vx765i-1