

Z-VAD-FMK

Pan-Caspase Inhibitor

Catalog # t1rl-vad

For research use only

Version # 09J28-MT

PRODUCT INFORMATION

Content:

• 1 mg of Z-VAD-FMK (carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]- fluoromethylketone)

Storage and stability:

- Z-VAD-FMK is provided as a white solid and shipped at room temperature. Store at -20°C. Solid product is stable 1 year at -20°C.
- Upon resuspension, Z-VAD-FMK should be aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 6-8 months at -20°C when properly stored.

DESCRIPTION

Z-VAD-FMK is a cell-permeable pan-caspase inhibitor that irreversibly binds to the catalytic site of caspase proteases¹. The peptide is O-methylated in the P1 position on aspartic acid, providing enhanced stability and increased cell permeability. Z-VAD-FMK is used in apoptosis studies and also in inflammasome studies. It is a potent inhibitor of caspase-1 activation in NLRP3-induced cells².

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. Dostert C. *et al.*, 2009. Malarial hemozoin is a Nalp3 inflammasome activating danger signal. *PLoS One.* 4(8):e6510.

CHEMICAL PROPERTIES

Linear formula: C₂₂H₃₀FN₃O₇

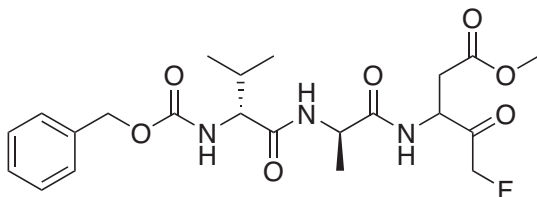
Molecular weight: 467.5

Appearance: White solid

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

Purity: 95%

Working concentration: 20 μM/10 μg/ml



METHODS

Preparation of 10 mg/ml stock solution:

Note: Spin briefly the vial before opening the cap.

- Add 100 μl of DMSO to the vial and mix by vortexing.

- Prepare further dilutions by adding the appropriate amount of H₂O.

Inhibition of NLRP3-induced caspase-1:

The following protocol describes the inhibition of NLRP3-induced caspase-1 in THP-1 cells, a human monocytic cell line used in many studies on the inflammasome. The cells are grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine and antibacterial antibiotics such as penicillin/streptomycin or Normocin™. THP-1 cells are grown in suspension to a density of 1.0x10⁶ cells/ml in tissue culture flasks.

1- Inoculate a 96-well plate with THP-1 cells at a density of 2.0x10⁵ cells/well.

2- Prime cells with 1 μg/ml LPS for 3 hours at 37°C in 5% CO₂.

3- Wash cells gently with PBS and add fresh culture medium.

4- Stimulate cells by adding ATP (5 mM) or MSU crystals (100-200 μg/ml) in the presence or absence of 10 μg/ml Z-VAD-FMK.

5- Incubate from 6 hours to overnight at 37°C in 5% CO₂.

Detection of IL-1β in the supernatant of THP-1 cells

Detection of mature IL-1β in the supernatant of THP-1 cells can be determined by Western blot, ELISA or by using InvivoGen's HEK-Blue™ IL-1β cells. These cells are specifically engineered to detect bioactive IL-1β.

RELATED PRODUCTS

Product	Catalog Code
ATP	t1rl-atp
HEK-Blue™ IL-1β cells	hkb-il1b
LPS (<i>E. coli</i> lipopolysaccharide)	t1rl-eklps
MSU crystals	t1rl-msu
Normocin™	ant-nr-1

TECHNICAL SUPPORT

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