BV6

Inhibitor of IAPs (inhibitor of apoptosis proteins) - InvitroFit™

Catalog code: inh-bv6

https://www.invivogen.com/bv6

For research use only

Version 23I18-MM

PRODUCT INFORMATION

Contents

- 5 mg BV6 - InvitroFit™

Storage and stability

- BV6 is provided as a powder and 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

BV6 is a potent and specific antagonist of three inhibitor of apoptosis proteins (IAPs), namely cIAP1, cIAP2, and XIAP[1]. IAPs exert their anti-apoptotic actions through ubiquitylation of RIPK1 (receptor-interacting serine/threonine-protein kinase 1), which results in RIPK1 proteasomal degradation[2]. The apoptosis initiator caspase, Caspase-8 (CASP-8), can no longer associate with RIPK1 and RIPK3, and thus remains non-activated[3].

Smac/DIABLO (second mitochondria-derived activator of caspases/direct IAP-binding protein with low isoelectric point) is a natural antagonist of IAPs, which is released in the cytosol in response to pro-apoptotic stimuli. BV6 acts as a Smac mimetic. Upon binding to select BIR (baculovirus IAP repeat) domains, BV6 induces auto-ubiquitination and rapid proteasomal degradation of cIAP, thus allowing for TNF-α-induced apoptosis or necroptosis[1,4]. Restoring and promoting cancer cell death using Smac mimetics are promising therapeutic strategies[4,5].

1. Varfolomeev E. *et al.*, 2007. IAP antagonists induce autoubiquitination of c-IAPs, NF-kB Activation, and TNFα-dependent apoptosis. Cell. 131(4):669-681. **2. Annibaldi A. *et al.*, 2018.** Ubiquitin-mediated regulation of RIPK1 kinase activity independent of IKK and MK2. Molecular Cell. 69:566-580. **3. Choi M.E. *et al.*, 2019.** Necroptosis: a crucial pathogenic mediator of human disease. JCI Insight. 4(15):e128834. **4. Li W. *et al.*, 2011.** BV6, an IAP antagonist, activates apoptosis and enhances radiosensitization of non-small cell lung carcinoma in vitro. J. Thorac. Oncol. 6(11), 1801-1809. **3. Schirmer M. *et al.*, 2016.** Intrinsic and chemo-sensitizing activity of SMAC-mimetics on high-risk childhood acute lymphoblastic leukemia. Cell Death Dis. 7:e2052.

CHEMICAL PROPERTIES

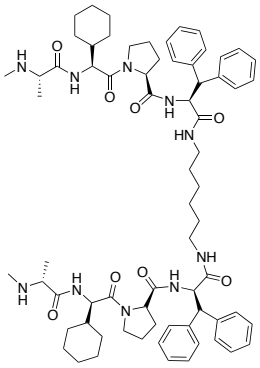
CAS number: 1001600-56-1

Synonyms: BV-6; N,N'-(hexane-1,6-diyl)bis(1-{(2S)-2-cyclohexyl-2-[(N-methyl-L-alanyl)amino]acetyl}-L-prolyl-beta-phenyl-L-phenylalaninamide)

Formula: $C_{70}H_{96}N_{10}O_8$ **Structure:**

Molecular weight: 1205.6 g/mol

Solubility: 10 mg/ml (8.33 mM) in DMSO



METHODS

Preparation of stock solution (5 mM)

1. Add 833 µl DMSO to 5 mg BV6 vial.
2. Vortex until completely resuspended.
3. Prepare aliquots of BV6 and store at -20°C.
4. Once BV6 is resuspended, further dilutions can be prepared using sterile aqueous buffers.

Working concentration range: 0.3-5 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-VAD-FMK (25 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™ 4 Lucia/Gaussia 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™ 4 Lucia/Gaussia 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 Lucia/Gaussia.
4. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
Necrostatin-1	RIPK1 inhibitor	inh-ncst1
Z-IETD-FMK	Caspase-8 inhibitor	inh-ietd
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™ 4 Lucia/Gaussia	Detection reagent	rep-qlc4lg1