Val-boroPro

NLRP1 inflammasome inducer

Catalog code: tlrl-vbp-10, tlrl-vbp-50

https://www.invivogen.com/valboropro

For research use only

Version 22H03-AK

PRODUCT INFORMATION

Contents

Val-boroPro is provided as a dried powder:

- **10 mg:** tlrl-vbp-10
- **50 mg:** tlrl-vbp-50

Storage and stability

- Val-boroPro is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, Val-boroPro can be stored at 4°C or at -20°C.
- Resuspended product is stable for 1 month at -20°C and 6 month at -80°C.

Avoid repeated freeze-thaw cycles.

Quality control

- Purity: ≥ 95% (HPLC)
- The biological activity has been confirmed 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

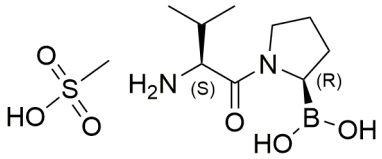
Val-boroPro (VbP, also known as Talabostat, PT-100 or L-valinyl-L-boroproline) was first described as a nonselective inhibitor of various dipeptidyl peptidases (DPPs)[1]. Recent studies have elucidated the immunostimulatory mechanism of VbP by interacting with inflammatory response initiators like pro-caspase-1, CARD8 and NLRP1[2]. VbP weakens the inhibitory complex between DPP9 and NLRP1 by direct competition for DPP9's catalytic residues. As a result, NLRP1 is displaced from the interaction, which leads to auto-proteolysis of its FIIND domain and to proteasomal degradation of the N-terminal domain[3]. The bioactive C-terminal CARD domain is released, activates Caspase-1 via oligomerization with ACS proteins and subsequently, triggers pyroptosis[1-4].

In addition to this, VbP exhibits potent antitumor activity *in vivo* through the induction of cytokines and chemokines[1,4]. It also increases the effect of antitumor antibodies like rituximab and trastuzumab[4].

1. Okondo MC *et al.*, 2017. DPP8 and DPP9 inhibition induces pro-caspase-1-dependent monocyte and macrophage pyroptosis. Nat Chem Biol. 13(1):46-53. **2. Sato T, *et al.*, 2019.** Hatano R, Iwao N, Ohnuma K, Morimoto C. DPP8 is a novel therapeutic target for multiple myeloma. Sci Rep. 2;9(1):18094. **3. Hollingsworth LR *et al.* 2021,** DPP9 sequesters the C terminus of NLRP1 to repress inflammasome activation. Nature. 592(7856):778-783. **4. Adams et al., 2004.** PT-100, a Small Molecule Dipeptidyl Peptidase Inhibitor, Has Potent Antitumor Effects and Augments Antibody-Mediated Cytotoxicity via a Novel Immune Mechanism, Cancer Res 64 (15): 5471–5480.

CHEMICAL PROPERTIES

CAS Number: 150080-09-4
Synonym: Talabostat, PT-100
Linear formula: $C_9H_{19}BN_2O_3.CH_3SO_3H$
Molecular weight: 310.18 g/mol
Solubility: 40 mg/ml in H_2O or 30 mg/ml in DMSO
Chemical structure:



METHODS

Preparation of stock solution (5 mg/ml):

Note: Spin the vial briefly before opening the cap.

1. Add 2 ml DMSO or H_2O to 10 mg Val-boroPro vial.
2. Vortex until completely resuspended.
3. Prepare aliquots and store at -80°C.
4. Once Val-boroPro is resuspended, further dilutions can be prepared using sterile aqueous buffers.

Working concentration range: 10 - 100 µM for cell culture assays.

ACTIVATION OF A549-ASC-NLRP1 CELLS

Below is a protocol to assess A549-ASC-NLRP1 cell activation. *For more information, please visit https://www.invivogen.com/a549-ascg-nlrp1*. It is recommended to perform the assay with test medium which does not contain Normocin™, nor Blasticidin.

Day 1: Cell preparation

1. Prepare a suspension of A549-ASC-NLRP1 cells and A549-ASC control cells in test medium at 2.5 x 10^5 cells/ml.
2. Add 200 µl of the cell suspension per well of a flat-bottom 96-well plate (~5.0 x 10^4 cells/well).
3. Incubate overnight at 37°C in 5% CO_2.

Day 2: Induction of ASC::GFP expression

1. Carefully remove cell supernatant.
2. Add 180 µl of freshly made test medium.
3. Add 20 µl of NF-κB-inducer, such as hTNF-α (final conc. 4 ng/ml), per well.
4. Incubate overnight at 37°C in 5% CO_2.

TECHNICAL SUPPORT
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Day 3: Induction and visualization of ASC speck formation

1. Carefully remove cell supernatant.
2. Add 180 µl of freshly made test medium.
3. Add 20 µl of the NLRP1 activator Val-boroPro (final conc. 10 µM), per well.
4. Incubate for 8 hours at 37°C in 5% CO_2.
5. Monitor fluorescent ASC signal in real-time using fluorescence microscopy and normal FITC filter sets.

Spectral properties of GFP

Excitation λ max: 480 nm
Emission λ max: 505 nlm

RELATED PRODUCTS

Product	Cat. Code
A549-ASC cells	a549-ascg
A549-ASC-NLRP1 cells	a549-ascg-nlrp1
A549-ASCoV2-NLRP1 cells	a549-ascg-cov-nlrp1
hTNF-α	rcyc-htnfa
Blasticidin	ant-bl-05