BX795

TBK1/IKKε inhibitor - InvitroFit™

Catalog code: tlrl-bx7-2

https://www.invivogen.com/bx795

For research use only

Version 23L08-MM

PRODUCT INFORMATION

Contents

- 2 x 5 mg BX795 - InvitroFit™

Storage and stability

- BX795 is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots of BX795 and store at -20°C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 6 months at -20°C when properly stored.

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.

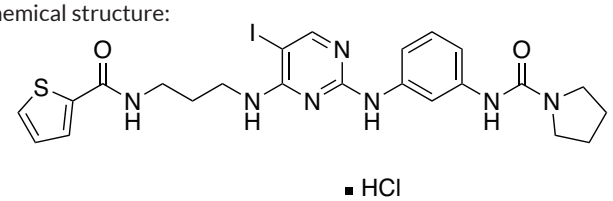
DESCRIPTION

BX795 is a potent inhibitor of the IkB kinases TANK-binding kinase 1 (TBK1) and IkappaB kinase-epsilon (IKKε). TBK1 and IKKε play a central role in the innate immune response. Notably, these noncanonical IkappaB kinase homologs are essential components of the interferon regulatory factor (IRF) signaling pathway. BX795 inhibits the catalytic activity of TBK1/IKKε by blocking their phosphorylation. BX795, an aminopyrimidine compound, was developed as an inhibitor of 3-phosphoinositide-dependent kinase 1 (PDK1)[1]. Studies have demonstrated that BX795 is a potent inhibitor of the IKK-related kinases, TANK-binding kinase 1 (TBK1), and IKKε, and hence of IRF3 activation and interferon-β (IFN-β) production[2, 3].

1. Feldman RL. *et al.*, 2005. Novel Small Molecule Inhibitors of 3-Phosphoinositide- dependent Kinase-1. J. Biol. Chem., 280: 19867- 74. **2. Clark K. *et al.*, 2009.** Use of the Pharmacological Inhibitor BX795 to Study the Regulation and Physiological Roles of TBK1 and I{kappa}B Kinase {epsilon}: a distinct upstream kinase mediates Ser-172 phosphorylation and activation. J. Biol. Chem., 284: 14136 - 14146. **3. Bain J. *et al.*, 2007.** The selectivity of protein kinase inhibitors: a further update, 408: 297-315.

CHEMICAL PROPERTIES

CAS number: 1472611-45-2
Formula: $C_{23}H_{26}IN_7O_2S \cdot HCl$
Molecular weight: 627.93 g/mol
Solubility: 10 mg/ml in DMSO
Working concentration: 100 nM - 10 µM
Chemical structure:



▪ HCl

METHODS

Preparation of stock solution (10 mM)

1. Add 840 µl of DMSO to 5 mg of BX795.
2. Vortex until completely dissolved.
3. Prepare aliquots and store at -20 °C.
4. Once BX795 has been solubilized, dilutions can be prepared by adding sterile water.

Note: Once diluted with water solution may appear cloudy.

TBK1/IKKε inhibition using B16-Blue™ IFN-α/β cells

To assess the role of TBK1/IKKε, pretreat cells, such as B16-Blue™ IFN-α/β cells with BX795, and then incubate at 37 °C with the appropriate ligand, such as 5'ppp-dsRNA delivered intracellularly. Following transfection of 5'ppp-dsRNA with LyoVec™ in B16-Blue™ IFN-α/β cells, recognition by murine RIG-I triggers the secretion of type I interferon (IFN) that results in the production of alkaline phosphatase by activation of an IRF-inducible promoter. Levels of secreted alkaline phosphatase (SEAP) can be easily determined by colorimetric measurement using QUANTI-Blue™ Solution a SEAP detection medium that turns purple/blue in the presence of alkalinephosphatase.
For more information, visit www.invivogen.com/b16-blue-ifnab.

1. Prepare a B16-Blue™ IFN-α/β cell suspension at ~500,000 cells/ml.
2. Add 160 µl of cell suspension (~75,000 cells) per well.
3. Add 20 µl of BX795 to obtain a final concentration of 100 nM-10 µM.
4. Incubate at 37 °C in a 5% CO_2 incubator for 6 hours.
5. Add 20 µl of sample per well of a flat-bottom 96-well plate.

Note: We recommend using a positive control such as 5'ppp-dsRNA delivered intracellularly with LyoVec™.

6. Incubate the plate at 37 °C in a 5% CO_2 incubator for 18-24 hours.
7. Monitor SEAP production using a SEAP detection assay such as QUANTI-Blue™ Solution.

RELATED PRODUCTS

Product	Description	Cat.Code
5'ppp-dsRNA/LyoVec™	RIG-I agonist	tlrl-3prnalv
B16-Blue™ IFN-α/β	Murine IFN-α/β reporter cells	bb-ifnt1
Poly(I:C)-HMW/LyoVec™	RIG-I agonist	tlrl-piclv
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT
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