

BX795

TBK1/IKK ϵ inhibitor - InvitroFit™

Catalog code: tlr1-bx7, tlr1-bx7-2

<https://www.invivogen.com/bx795>

For research use only

Version 25A31-MM

PRODUCT INFORMATION

Contents: BX795 - InvitroFit™ is available in two quantities:

- tlr1-bx7: 5 mg
- tlr1-bx7-2: 10 mg (2 x 5 mg)

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 \geq 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 I κ B kinases TANK-binding kinase 1 (TBK1) and I κ B kinase-epsilon (IKK ϵ). TBK1 and IKK ϵ play a central role in the innate immune response. Notably, these noncanonical I κ B kinase homologs are essential components of the interferon regulatory factor (IRF) signaling pathway. BX795 inhibits the catalytic activity of TBK1/IKK ϵ 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 IKK ϵ , 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 κ 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

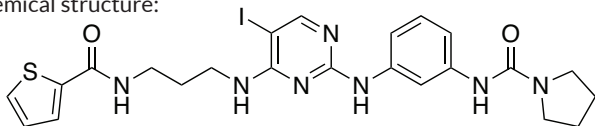
Formula: C₂₃H₂₆IN₇O₂S •HCl

Molecular weight: 627.93 g/mol

Solubility: 10 mg/ml in DMSO

Working concentration: 100 nM - 10 μ M

Chemical structure:



■ HCl

METHODS

Preparation of stock solution (10 mM)

1. Add 840 μ l 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/IKK ϵ inhibition using B16-Blue™ IFN- α/β cells

To assess the role of TBK1/IKK ϵ , 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 alkaline phosphatase.

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 μ l of cell suspension (~75,000 cells) per well.
3. Add 20 μ l of BX795 to obtain a final concentration of 100 nM-10 μ M.
4. Incubate at 37 °C in a 5% CO₂ incubator for 6 hours.
5. Add 20 μ l 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% CO₂ 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	tlr1-3prnalv
B16-Blue™ IFN- α/β	Murine IFN- α/β reporter cells	bb-ifnt1
Poly(I:C)-HMW/LyoVec™	RIG-I agonist	tlr1-piclv
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT

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