

BX795

TBK1/IKK ϵ Inhibitor

Catalog # tlr1-bx7

For research use only

Version # 10H30-MM

PRODUCT INFORMATION

Content:

- 5 mg BX795

Storage and stability:

- BX795 is provided as a solid and shipped at room temperature. Store at -20°C. Solid product is stable 1 year at -20°C.
- Upon resuspension, BX795 should be aliquoted and stored at 4°C for short term storage and at -20°C for long term storage. Avoid repeated freeze-thaw cycles.

DESCRIPTION

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. It was recently shown to be a potent inhibitor of the IKK-related kinases, TANK-binding kinase 1 (TBK1) and IKK ϵ , and hence of IRF3 activation and IFN- β production^{2,3}.

1. **Feldman RI. et al., 2005.** Novel Small Molecule Inhibitors of 3-Phosphoinositide-dependent Kinase-1. *J. Biol. Chem.*, 280: 19867 - 19874. 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.

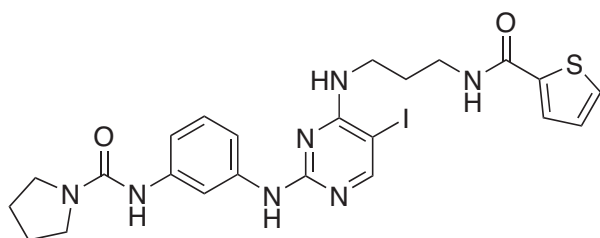
CHEMICAL PROPERTIES

Formula: C₂₃H₂₆N₇O₂S

Molecular weight: 591.5

Solubility: DMSO (10 mg/ml)

Working concentration: 10 nM -1 μ M



METHODS

Preparation of sterile stock solution (10 mM)

Inhibition of TBK1/IKK ϵ can be achieved with 10 nM -1 μ M BX795.

To obtain a 10 mM stock solution:

1. Add 840 μ l DMSO to 5 mg BX795 vial.
2. Vortex until complete solubilization.
3. Aliquote and store at 4°C for short term storage and at -20°C for long term storage.
4. Once BX795 is solubilized, dilutions can be prepared by adding sterile water.

Note: Once diluted with water solution may appear cloudy.

TBK1/IKK ϵ inhibition:

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 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™ (a detection medium that turns purple/blue in the presence of alkaline phosphatase).

- 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 100 nM -10 μ M stock solution to obtain a final concentration of 10 nM -1 μ M. Incubate at 37°C 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™.

- 5- Incubate the plate at 37°C in a 5% CO₂ incubator for 18-24 hours.
- 6- Monitor SEAP production using a SEAP detection assay such as QUANTI-Blue™.

TECHNICAL SUPPORT

Toll free (US): 888-457-5873

Outside US: (+1) 858-457-5873

Europe: +33 562-71-69-39

E-mail: info@invivogen.com

Website: www.invivogen.com



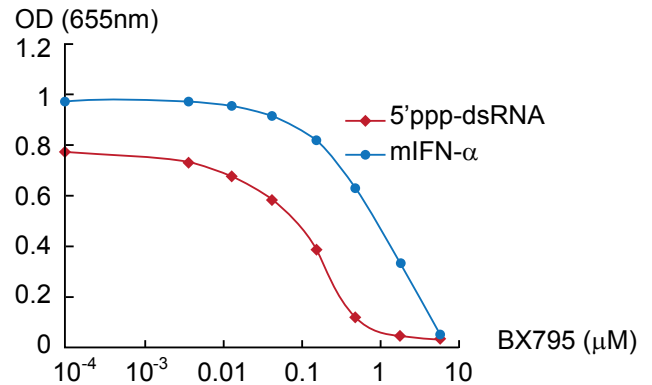
3950 Sorrento Valley Blvd. Suite 100
San Diego, CA 92121 - USA

RELATED PRODUCTS

Product	Catalog Code
B16-Blue™ IFN- α/β	bb-ifnab
5'ppp-dsRNA	tlrl-3prna
5'ppp-dsRNA Control	tlrl-3prnac
Double stranded B DNA (poly(dA:dT)/LyoVec)	tlrl-pat
Poly(I:C)-HMW/LyoVec	tlrl-piclv
Poly(I:C)-HMW	tlrl-pic
Poly(I:C)-LMW/LyoVec	tlrl-picwlv
Poly(I:C)-LMW	tlrl-picw
LyoVec™	lyec-1
QUANTI-Blue™ (5 pouches)	rep-qb1

BX795 inhibition of the TBK1/IKK ϵ pathway in B16-Blue™ IFN- α/β cells

Stimulation of B16-Blue™ IFN- α/β cells with murine IFN- α or type I IFN inducers, such as the RIG-I ligand triphosphate double stranded RNA (5'ppp-dsRNA) delivered intracellularly, triggers the production of SEAP by the activation of the IRF-inducible promoter. Levels of SEAP in the supernatant can be easily determined with QUANTI-Blue™, a medium that turns purple/blue in the presence of SEAP and by reading the OD at 655 nm. Addition of BX795 to stimulated cells resulted in a reduction of the observed signal attributed to the inhibition of the TBK1/IKK ϵ pathway for dsRNA and the inhibition of IKK ϵ /STAT1 pathway for mIFN- α .



B16-Blue™ IFN- α/β cells were incubated for 6 hours with varying concentrations of BX795 prior to overnight stimulation with 1 µg/ml of 5'ppp-dsRNA or 1 x 10⁴ U/ml mIFN- α . Levels of SEAP in the supernatant were measured at O.D. 655nm after incubation with QUANTI-Blue™.

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Toll free (US): 888-457-5873
 Outside US: (+1) 858-457-5873
 Europe: +33 562-71-69-39
 E-mail: info@invivogen.com
 Website: www.invivogen.com