

# Validation data for BX795

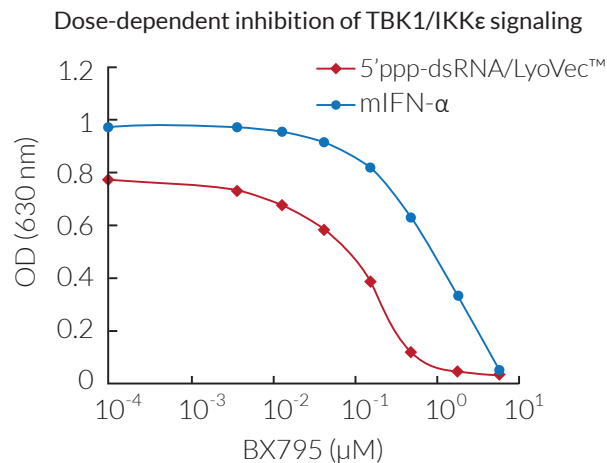
<https://www.invivogen.com/bx795>

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BX795 is a potent inhibitor of the I $\kappa$ B kinases TANK-binding kinase 1 (TBK1) and I $\kappa$ B kinase-epsilon (IKK $\epsilon$ ). TBK1 and IKK $\epsilon$  play a central role in the innate immune response. Notably, these noncanonical I $\kappa$ B kinase homologs are essential components of the interferon regulatory factor (IRF) signaling pathway.

Stimulation of B16-Blue™ IFN- $\alpha$ / $\beta$  cells with murine IFN- $\alpha$  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™ Solution, a medium that turns purple/blue in the presence of SEAP and by reading the optical density (OD) at 630 nm. Addition of BX795 to stimulated cells resulted in a reduction of the observed signal attributed to the inhibition of the TBK1/IKK $\epsilon$  pathway for dsRNA and the inhibition of IKK $\epsilon$ /STAT1 pathway for mIFN- $\alpha$ . (Figure 1).



**Figure 1: BX795 inhibits TBK1/IKK $\epsilon$  signaling in a dose-dependent manner.** B16-Blue™ IFN- $\alpha$ / $\beta$  cells were incubated for 6 hours with varying concentrations of BX795 prior to overnight stimulation with 1  $\mu$ g/ml of 5'ppp-dsRNA/LyoVec™ or 1x10<sup>4</sup> U/ml mIFN- $\alpha$ . Levels of SEAP in the supernatant were measured after incubation with QUANTI-Blue™ Solution by reading the optical density (OD) at 630 nm.

## TECHNICAL SUPPORT

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