

# Amlexanox

TBK1 & IKK $\epsilon$  inhibitor - InvitroFit™  
Catalog code: inh-amx  
<https://www.invivogen.com/amlexanox>

For research use only  
Version 23118-MM

## PRODUCT INFORMATION

### Contents

- 50 mg Amlexanox - InvitroFit™

### Storage and stability

- Amlexanox is provided as a solid and shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots of Amlexanox 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  $\geq$  97% (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

Amlexanox is a specific inhibitor of the noncanonical I $\kappa$ B kinases IKK $\epsilon$  and TANK-binding kinase 1 (TBK1)<sup>1</sup>. At the concentrations that block IKK $\epsilon$  and TBK1, it has no effect on the canonical I $\kappa$ B kinases IKK $\alpha$  and IKK $\beta$ , or a large panel of other kinases. Amlexanox inhibits IKK $\epsilon$  and TBK1 by competing for ATP-binding to the enzyme. IKK $\epsilon$  and TBK1 are essential players in the coordination of interferon regulatory factor 3 (IRF3)- and NF- $\kappa$ B-mediated inflammatory signaling pathways. Indeed, elevated IKK $\epsilon$  and TBK1 activity has been associated with several inflammatory diseases<sup>2,3</sup>. Amlexanox has been approved for the treatment of a variety of conditions, including asthma and allergic rhinitis, due to its anti-inflammatory and anti-allergic properties. In addition, Amlexanox is being investigated as a novel therapeutic for type II diabetes and obesity<sup>1</sup>, as increased IKK $\epsilon$  and TBK1 activity has been linked to low-grade chronic inflammation associated with insulin resistance and metabolic disorders<sup>1,3,4</sup>.

1. Reilly SM. *et al.*, 2013. An inhibitor of the protein kinases TBK1 and IKK- $\epsilon$  improves obesity-related metabolic dysfunctions in mice. *Nat Med.* 19(3):313-21. 2. Yu J. *et al.*, 2015. Regulation of T-cell activation and migration by the kinase TBK1 during neuroinflammation. *Nat Commun.* 6:6074. 3. Niederberger E. *et al.*, 2013. The non-canonical I $\kappa$ B kinases IKK $\epsilon$  and TBK1 as potential targets for the development of novel therapeutic drugs. *Curr Mol Med.* 13(7):1089-97. 4. Chiang SH. *et al.*, 2009. The protein kinase IKKepsilon regulates energy balance in obese mice. *Cell.* 138(5):961-75.

## CHEMICAL PROPERTIES

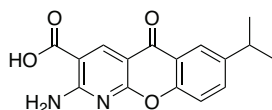
CAS number: 68302-57-8

Formula: C<sub>16</sub>H<sub>14</sub>N<sub>2</sub>O<sub>4</sub>

Molecular weight: 298.3 g/mol

Solubility: 10 mg/ml in DMSO

Chemical structure:



## METHODS

### Preparation of 10 mg/ml (33.5 mM) stock solution

1. Weigh 10 mg of Amlexanox
2. Add 1 ml of DMSO to 10 mg Amlexanox. Mix by vortexing.
3. Prepare further dilutions using endotoxin-free water.

**Working concentration:** 10-300  $\mu$ g/ml for cell culture assays

### TBK1/IKK $\epsilon$ inhibition

Amlexanox can be used to assess the role of TBK1/IKK $\epsilon$  using cellular assays, as described below in [B16-Blue™ ISG cells](#).

1. Prepare a B16-Blue™ ISG cell suspension at ~500,000 cells/ml.
2. Add 160  $\mu$ l of cell suspension (~75,000 cells) per well.
3. Add 20  $\mu$ l of Amlexanox 30-300  $\mu$ g/ml (final concentration) and incubate at 37 °C for 1 hour.

4. Add 20  $\mu$ 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% CO<sub>2</sub> incubator for 18-24 hours.
6. Monitor SEAP production using a SEAP detection assay such as [QUANTI-Blue™ Solution](#).

## PROTOCOLS

For reference only; as described in the indicated publications.

### Cell Culture Assay<sup>1</sup>(supplementary data)

Cells: 3T3-L1 adipocytes and RAW 264.7 macrophages

Working concentration: 10–50  $\mu$ M (3–15  $\mu$ g/ml)

Incubation time: 24 hours

Method: promoter reporter assay of NF- $\kappa$ B activity and in vitro wound-healing assay

### Animal Study<sup>1</sup>

Animal model: C57Bl/6 mice

Dose: 25–100 mg/kg daily for 12 weeks

Administration: Oral gavage

### Animal Study<sup>2</sup>

Animal model: B6.129 mice

Dose: 25 mg/kg daily for 14 days

Administration: Intraperitoneal injection

## RELATED PRODUCTS

| Product               | Description                   | Cat.Code  |
|-----------------------|-------------------------------|-----------|
| B16-Blue™ ISG cells   | IRF reporter cells            | bb-ifnabg |
| BX795                 | TBK1/IKK $\epsilon$ inhibitor | tlrl-bx7  |
| QUANTI-Blue™ Solution | SEAP detection reagent        | rep-qbs   |

## TECHNICAL SUPPORT

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