MRT67307

Kinase inhibitor; TBK1 and IKKε inhibitor - InvitroFit™

Catalog code: inh-mrt, inh-mrt-3

https://www.invivogen.com/mrt67307

For research use only

Version 24A02-MM

PRODUCT INFORMATION

Contents MRT67307 (hydrochloride) is available in two quantities:

- **inh-mrt:** 10 mg MRT67307 - InvitroFit™
- **inh-mrt-3:** 3 x 10 mg MRT67307 - InvitroFit™

Storage and stability

- MRT67307 is provided as a dried powder and shipped at room temperature. Upon receipt, store product at -20 °C.
- Upon resuspension of MRT67307 prepare aliquots and store at -20 °C. Resuspended product is stable for at least 3 months when properly stored.
- Avoid repeated freeze-thaw cycles.

Quality control

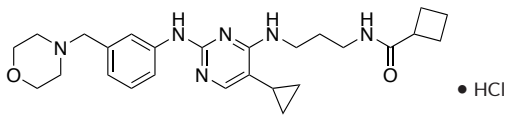
- Purity: ≥95% (UHPLC)
- Inhibition of TBK1/IKKε by MRT67307 has been confirmed using cellular assays.
- Absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ hTLR2 and HEK-Blue™ hTLR4 cells.

PRODUCT DESCRIPTION

MRT67307 is a potent, reversible kinase inhibitor, and a derivative of BX795[1]. MRT67307 is a dual inhibitor specifically blocking the function of two IKK-related kinases: IKKε (I-kappa-B kinase (IKK) epsilon or IKBKE), and TBK1 (TANK binding kinase 1), with reported IC_{50} values of 160 nM and 19 nM, respectively[1]. MRT67307 prevents the phosphorylation of IRF3 (interferon regulatory factor 3) by TBK1/IKKε, and thereby blocks expression of interferon stimulated genes (ISGs), such as IFN-β.

Importantly, MRT67307 has no noted effect on the canonical IKKs, IKKα or IKKβ, that are involved in the activation of NF-κB. Therefore, MRT67307 does not affect the NF-κB pathway[1]. However, MRT67307 has been shown to enhance IL-1-stimulated activation of NF-κB-dependent gene expression, suggesting a negative regulation of the canonical IKKs by TBK1/IKKε.

Additionally, MRT67307 is a highly potent inhibitor of ULK1, a key component of the autophagy pathway. Hence, the targeting of autophagy by small molecule inhibitors such as MRT67307 has been proposed as a possible novel therapeutic strategy in autophagy-related diseases, such as cancer[2].



1.Clark, K. *et al.* 2011. Novel cross-talk within the IKK family controls innate immunity. Biochem J 434, 93-104. 2.Petherick K.J. *et al.* 2015. Pharmacological inhibition of ULK1 kinase blocks mammalian target of rapamycin (mTOR)-dependent autophagy. J Biol Chem 290, 11376-11383.

CHEMICAL PROPERTIES

CAS Number: 1190378-57-4 (free base)

Formula: $C_{26}H_{36}N_6O_2$. x HCl

Molecular weight: 464.60 g/mol (free base)

Solubility: 15 mg/ml H_2O

METHODS

Preparation of stock solution (10 mg/ml)

1. Add 1 ml of endotoxin-free H_2O to 10 mg of MRT67307.
2. Use immediately or store aliquots at -20 °C.
3. Prepare dilutions using sterile endotoxin-free water.

Working concentration range: 1 - 20 µM (for cell culture assays)

Inhibition of TBK1/IKKε by MRT67307 in a cellular assay

Below is a protocol using InvivoGen's THP1-Dual™ cells for studying specific inhibition of the IRF pathway by MRT67307. These cells express both an inducible Lucia luciferase reporter and an inducible secreted embryonic alkaline phosphatase (SEAP) reporter to measure the activation of the IRF or NF-κB pathways, respectively. Changes in the Lucia expression levels upon inhibition can be readily assessed by measuring the luciferase activity using QUANTI-Luc™ 4 Lucia/Gaussia detection reagent. *Note: For the full description of the THP1-Dual™ cells, please visit https://www.invivogen.com/thp1-dual*

1. Add 20 µl MRT67307 (10 - 200 µM) per well of a flat bottom 96-well plate.
2. Prepare a suspension of THP1-Dual™ cells (~500,000 cells per ml) in culture medium.
3. Add 180 µl of the cell suspension (~100,000 cells) to each well.
4. Incubate the plate at 37°C in a CO_2 incubator for 1 hour.
5. Add an inducer of the IRF pathway (such as 2'3'-cGAMP) and incubate the plate at 37°C in a CO_2 incubator for 24 hours.
6. Prepare QUANTI-Luc™ and carry out the measurements following the instructions on the data sheet.

Note: NF-κB-induced SEAP expression in THP1-Dual™ cells can be monitored using QUANTI-Blue™ Solution, a SEAP detection reagent.

RELATED PRODUCTS

Product	Description	Cat. Code
2'3'-cGAMP	STING agonist	tlrl-nacga23
BX795	TBK1/IKKε Inhibitor	tlrl-bx7-2
THP1™ Dual cells	Human reporter cells	thpd-nfis
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
QUANTI-Luc™ 4 Lucia/Gaussia	Luciferase detection reagent	rep-qlc4lg1

TECHNICAL SUPPORT
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