

CLI-095

TLR4 signaling inhibitor - InvitroFit™

Catalog code: tlr4-cli95-4

<https://www.invivogen.com/cli-095>

For research use only

Version 23L08-MM

PRODUCT INFORMATION

Contents

- 4 x 1 mg CLI-095 - InvitroFit™

Storage and stability

- CLI-095 is provided as a solid and shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots of CLI-095 and store at -20°C. Avoid repeated freeze-thaw cycles. Resuspended product is stable for 3 months at -20°C when properly stored.

Quality control

- Purity ≥ 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

CLI-095, also known as resatorvid or TAK-242, is a small-molecule inhibitor of TLR4 signaling. It is a cyclohexene derivative that specifically suppresses TLR4 signaling^{1,2}. TLR4 is a pattern recognition receptor that recognizes bacterial lipopolysaccharide (LPS). Its activation mainly leads to the synthesis of pro-inflammatory cytokines and chemokines. There are two distinct signaling cascades triggered by the dimerization of TLR4; the MyD88-dependent and TRIF-dependent pathways. At the cell surface, activation of TLR4 initiates the MyD88-dependent pathway, ultimately leading to the 'early' activation of NF-κB and the production of a pro-inflammatory response³. Subsequently, the TLR4 complex can be endocytosed into endosomes and result in the 'late' activation of NF-κB as well as the stimulation of interferon regulatory factor (IRF3), which modulates the expression of type I interferons (IFNs)⁴. CLI-095 acts by blocking the signaling mediated by the intracellular domain of TLR4. More precisely, CLI-095 attaches to cysteine 747 in the intracellular sphere of TLR4, thus hindering both MyD88-dependent and TRIF-dependent pathways stimulated by LPS². Interestingly, when tested in animal models, CLI-095 suppressed the progression of atherosclerosis by reducing macrophage foam cell formation⁵.

1. Li M. *et al.*, 2006. A Novel Cyclohexene Derivative, Ethyl (6R)-6-[N-(2-Chloro-4-fluorophenyl)sulfamoyl]cyclohex-1-ene-1-carboxylate (TAK-242). Selectively Inhibits Toll-Like Receptor 4-Mediated Cytokine Production through Suppression of Intracellular Signaling. *Mol. Pharmacol.*, 69:1288-1295. 2. Kawamoto T. *et al.*, 2008. TAK-242 selectively suppresses Toll-like receptor 4-signaling mediated by the intracellular domain. *Eur J Pharmacol.* 584(1):40-8. 3. Kuzmich N.N. *et al.*, 2017. TLR4 Signaling Pathway Modulators as Potential Therapeutics in Inflammation and Sepsis. *Vaccines (Basel)* 5. 4. Marongiu L. *et al.*, 2019. Below the surface: The inner lives of TLR4 and TLR9. *J Leukoc Biol* 106, 147-160. 5. Wang X.Q. *et al.*, 2006. CLI-095 decreases atherosclerosis by modulating foam cell formation in apolipoprotein E-deficient mice. *Mol Med Rep.* 14(1):49-56.

CHEMICAL PROPERTIES

CAS number: 243984-11-4

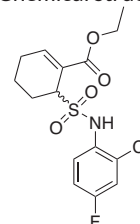
Formula: C₁₅H₁₇ClFNO₄S

Molecular weight: 361.82 g/mol

Solubility: 1 mg/ml in DMSO or methanol

Working concentration: 50 nM-5 μM

Chemical structure:



METHODS

Preparation of CLI-095 stock solution (100 μg/ml)

1. Add 1 ml DMSO to 1 mg CLI-095 vial (1 mg/ml).
2. Vortex until completely dissolved.
3. Take 100 μl of solubilized CLI-095 (1 mg/ml), add 900 μl sterile culture media to obtain a stock solution of 100 μg/ml.
4. Prepare aliquots and store stock solution at -20 °C.

TLR4 signaling using HEK-Blue™ TLR4 cells:

HEK-Blue™ TLR4 cells are engineered HEK293 cells that stably express an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene, and the TLR4, MD2 and CD14 genes. LPS-induced TLR4 activation in HEK-Blue™ TLR4 cells induces the activation of NF-κB and the subsequent release of SEAP which can be easily determined using QUANTI-Blue™ Solution, a SEAP detection reagent. For more information, visit www.invivogen.com/hek-blue-trl4.

1. Prepare a HEK-Blue™ hTLR4 cell suspension in culture medium.
2. Add 20 μl of CLI-095 (50 nM-5 μM final concentration) in a well of a 96-well plate.
3. Add 160 μl of cell suspension (~50,000 cells) per well.
4. Incubate the plate at 37°C in a 5% CO₂ incubator for 3 h.
5. Add 20 μl of LPS-EK Ultrapure (10 ng/ml final concentration) per well.
6. Incubate the plate at 37°C in a 5% CO₂ incubator for 18-24 h.
7. Monitor SEAP production using a SEAP detection assay such as QUANTI-Blue™ Solution.

RELATED PRODUCTS

Product	Description	Cat.Code
HEK-Blue™ hTLR4 Cells	Human TLR4 reporter cells	hkb-htlr4
HEK-Blue™ mTLR4 Cells	Murine TLR4 reporter cells	hkb-mtlr4
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
LPS-EK Ultrapure	TLR4 agonist	tlr4-peklps

TECHNICAL SUPPORT

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