CU-T12-9

Synthetic TLR2-TLR1 agonist

Catalog Code: tlrl-cut129

https://www.invivogen.com/cut129

For research use only

Version 20J30-MM

PRODUCT INFORMATION

Contents

- 10 mg CU-T12-9

Storage and stability

- CU-T12-9 is provided as a dried powder and shipped at room temperature. Upon receipt, store product at -20 °C.
- Store resuspended product at -20 °C. Resuspended product is stable for at least 6 months when properly stored.
- Avoid repeated freeze-thaw cycles.

Quality control

- Purity: UHPLC ≥95%
- Specific activation of hTLR2-TLR1 heterodimer by CU-T12-9 has been confirmed using HEK-Blue™ hTLR2 cellular assays.
- Absence of bacterial contamination (e.g. endotoxins) has been confirmed using HEK-Blue™ hTLR4 cellular assays.

PRODUCT DESCRIPTION

CU-T12-9 is a synthetic small molecule that activates Toll-like receptor 2 (TLR2). CU-T12-9 derives from a TLR2-activating molecule identified in the screening of a synthetic compound library[2]. Minor changes to this molecule had a profound effect on its agonistic activity and led to the optimized immune stimulant[1].

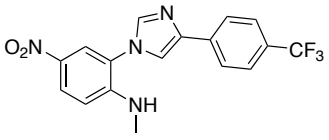
Specifically, CU-T12-9 binds to and activates TLR2-TLR1, one of the essential TLR2-heterodimers required for downstream signaling[1]. CU-T12-9 has been shown to activate NF-κB-dependent signaling, i.e. expression of TNF-α, IL-10, and iNOS[1].

The binding site of CU-T12-9 has been suggested to be on the interface of the heterodimer, supporting its selective affinity for TLR2-TLR1. Importantly, even at high concentrations CU-T12-9 maintains its selective agonistic activity. Furthermore, it has minimal cytotoxicity at concentrations up to 100 µM.

1. Cheng, K. *et al.* 2015. Specific activation of the TLR1-TLR2 heterodimer by small-molecule agonists. Sci Adv 1. **2. Guan, Y. *et al.* 2010.** Identification of novel synthetic toll-like receptor 2 agonists by high throughput screening. J Biol Chem 285, 23755-23762.

CHEMICAL PROPERTIES

- **CAS Number:** 1821387-73-8
- **Formula:** $C_{17}H_{13}F_3N_4O_2$
- **Molecular weight:** 362.31 g/mol
- **Solubility:** 100 mM (~36 mg/ml) in DMSO



METHODS

Preparation of a 20 mM stock solution of CU-T12-9

1. Resuspend CU-T12-9 in 1.4 ml of DMSO.
2. Mix well by vortexing.
3. Use immediately or store aliquots at -20 °C.
4. Subsequent 1:100 dilutions can be directly prepared using sterile endotoxin-free water or culture medium, such as DMEM.

Note: Dilutions in water or medium at <1:100 may cause the product to precipitate.

Working concentration range: 10 nM - 10 µM

Activation of TLR2 by CU-T12-9

Below is a protocol for using InvivoGen's HEK-Blue™ TLR2 cells. These cells express TLR2, its co-receptors TLR1 and TLR6, and an inducible SEAP reporter to monitor NF-κB activation following TLR2 stimulation. Levels of SEAP can be easily determined using HEK-Blue™ Detection, a SEAP detection cell culture medium.

Note: For more information, visit https://www.invivogen.com/hek-blue-htlr2

1. Add 20 µl of CU-T12-9 (10x final concentration) per well of a flat bottom 96-well plate.
2. Add 20 µl of a positive control (i.e. FSL-1) to another well.
3. Prepare a suspension of HEK-Blue™ hTLR2 cells (~280,000 cells per ml) in HEK-Blue™ Detection medium.
4. Immediately add 180 µl of the cell suspension (~50,000 cells) to each well.
5. Incubate the plate at 37°C in a CO_2 incubator for 6-24 hours.
6. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Note: QUANTI-Blue™ solution, a SEAP detection reagent, can also be used to detect the activation of HEK-Blue™ hTLR2 cells by CU-T12-9

InvivoGen offers a HEK-Blue™ TLR2 cell collection which includes HEK-Blue™ hTLR2-TLR1 cells, a convenient tool to specifically study the TLR2-TLR1 signaling pathway, in the absence of TLR6. For information, visit: https://www.invivogen.com/hek-blue-htlr2tlr1

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ hTLR2 cells	TLR2 reporter cells	hkb-htlr2
HEK-Blue™ hTLR2/1 cells	TLR2/1 reporter cells	hkb-htlr21
FSL-1	TLR2-TLR6 agonist	tlrl-fsl
HEK-Blue™ Detection	SEAP detection medium	hb-det2
Quanti-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT
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