

For research use only Version 20E15-MM

PRODUCT INFORMATION

Contents:

- 500 µg CL307 provided as a lyophilized powder
- 1.5 ml endotoxin-free water

Storage and stability

- CL307 is shipped at room temperature. Store lyophilized product at -20°C.

- Upon resuspension, prepare aliquots of CL307 and store at -20°C. Resuspended product is stable for 6 months at -20°C. Avoid repeated freeze-thaw cycles.

Quality control

• Purity: ≥95% (UHPLC)

• Activation of TLR7 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

CL307 is a potent and specific Toll-like receptor 7 (TLR7) agonist. It was generated by covalently linking a spermine to the adenine analog CL264, a TLR7 agonist developed by InvivoGen. Coupling with spermine enhances cellular uptake by endocytosis^{1,2} and the subsequent delivery to TLR7.

TLR7 is an innate immune sensor expressed on the surface of endosomes. It recognizes viral single-stranded ribonucleic acid (ssRNA) as its natural ligand and also small synthetic molecules such as imidazoquinolines and nucleoside analogs³. Activation of TLR7 leads to NF-kB/AP1- and interferon (IFN) regulatory factor-mediated production of type I IFNs and pro-inflammatory cytokines³.

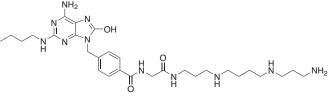
Notably, titration experiments with InvivoGen's HEK-Blue[™] hTLR7 cells, which stably express an NF-kB-inducible SEAP reporter gene and human TLR7, demonstrated that CL307 induces robust NF-kB activation at concentrations as low as 20 nM (10 ng/ml). Furthermore, it has been reported in the literature that CL307 induces the production of the pro-inflammatory cytokine, interleukin-6, by peripheral blood mononuclear cells¹. Importantly, CL307 is a TLR7-specific ligand that does not activate TLR8 even at high concentrations (>10 µg/ml).

1. Järver P. *et al.*, **2018**. Single-stranded nucleic acids regulate TLR3/4/7 activation through interference with Clathrin-mediated endocytosis. Sci Rep. 8(1):15841. **2.** Soulet D. *et al.*, **2002**. Role of endocytosis in the internalization of spermidine-C(2)-BODIPY, a highly fluorescent probe of polyamine transport. Biochem. J. 367:347-57. **3.** Georg P. & Sander L.E., **2019**. Innate sensors that regulate vaccine responses. Curr. Op. Immunol. 59:31.

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CHEMICAL PROPERTIES

Synonym: N1-glycinyl[4-((6-amino-2-(butylamino)-8-hydroxy-9H-purin-9-yl)methyl) benzoyl] spermine Formula: $C_{29}H_{47}N_{11}O_3$ Molecular weight: 597 g/mol Solubility: 2 mg/ml in water Working cncentration: 5 ng - 1 µg/ml (~10 nM - 2 µM) Structure:



METHODS

Preparation of CL307 stock solution (1 mg/ml)

To obtain a 1 mg/ml stock solution:

- Add 500 µl water to 500 µg CL307. Vortex until completely dissolved.

- Prepare aliquots and store at -20°C.

TLR stimulation with CL307 using HEK-Blue™ TLR7cells

CL307 can be used to stimulate TLR7 in HEK-Blue^M TLR7 cells. These cells stably express an NF- κ B-inducible secreted embryonic alkaline phosphatase (SEAP) and overexpress the TLR7 gene. For more information visit <u>https://www.invivogen.com/hek-blue-tlr7</u>.

1. Dispense 20 μl of CL307 (5 ng -1 $\mu g/ml$ final concentration) per well of a 96-well plate.

2. Prepare a suspension of HEK-Blue™ TLR7 cells in HEK-Blue™ Detection medium.

3. Immediately add 180 μI of the cell suspension to each CL307-containing well.

4. Incubate the plate at 37° C in a CO₂ incubator for 6-24 hours.

5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ hTLR7 Cells	Human TLR7 reporter cells	hkb-htlr7
HEK-Blue™ mTLR7 Cells	Murine TLR7 reporter cells	hkb-mtlr7
HEK-Blue™ Detection	SEAP detection medium	hb-det2
CL264	Adenine analog	tlrl-c264e

