

For research use only

Version 24G12-MM

PRODUCT INFORMATION

Contents

• 5 mg CL264 provided as a lyophilized powder containing Tris-acetate-EDTA buffer salts

• 10 ml sterile endotoxin-free water

Storage and stability

- CL264 is shipped at room temperature. Upon receipt, store lyophilized product at -20°C.

- Upon resuspension, prepare aliquots of CL264 and store at -20°C. Resuspended product is stable for 6 months at -20°C. Avoid repeated freeze-thaw cycles.

Quality control

• The biological 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.

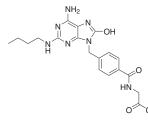
DESCRIPTION

CL264 is a 9-benzyl-8 hydroxyadenine derivative containing a glycine on the benzyl group (in para). Similarly to SM360320, CL264 induces the activation of NF- κ B and the secretion of IFN- α in TLR7-expressing cells¹. CL264 is a TLR7-specific ligand, it does not stimulate TLR8 even at high concentrations (>10 µg/ml). In TLR7-transfected HEK293 cells, CL264 triggers NF- κ B activation at a concentration of 0.1 µM which is 5-10 times less than Imiquimod.

1. Lee J. *et al.*, 2006. Activation of anti-hepatitis C virus responses via Toll-like receptor 7. PNAS 103(6):1828-33.

CHEMICAL PROPERTIES

 $\label{eq:synonym: 2-(4-((6-amino-2-(butylamino)-8-hydroxy-9H_purin_9-yl) methyl)benzamido)acetic acid Formula: C_{19}H_{23}N_7O_4 Molecular weight: 413.43 g/mol Solubility: 1 mg/ml in water Structure:$



METHODS

Preparation of CL264 stock solution (1 mg/ml)

Stimulation of TLR7 can be achieved with 50 ng -10 µg/ml CL264. To obtain a 1 mg/ml stock solution:

- Add 5 ml sterile water (provided) to 5 mg of CL264.

This generates a stock solution at 1 mg/ml with 40 mM Tris, 20 mM acetic acid and 0.25 mM EDTA. Vortex until complete solubilization. Once CL264 is solubilized, prepare aliquots and store at -20°C.

An example of TLR7 stimulation using CL264

CL264 can be used to stimulate TLR7 in HEK-Blue™ TLR7 cells. These cells stably express an NF-κB-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. Add a range of concentrations of CL264 (50 ng -10 µg/ml final concentration) to HEK-Blue™ TLR7 cells (prepare a cell suspension according to data sheet).

2. Incubate cells and CL264 for 6-24 h at 37°C, 5% CO₂.

3. Determine TLR7 stimulation with CL264 by assessing SEAP expression using a SEAP detection reagent, such as QUANTI-Blue™ Solution or HEK-Blue™ Detection.

RELATED PRODUCTS

Product	Description	Cat. Code
Gardiquimod™	TLR7 ligand	tlrl-gdqs-1
HEK-Blue™ hTLR7 cells	Human TLR7 reporter cells	hkb-htlr7v2
HEK-Blue™ mTLR7 cells	Murine TLR7 reporter cells	hkb-mtlr7
HEK-Blue™ Detection	SEAP detection reagent	hb-det2
Imiquimod (R837)	TLR7 ligand	tlrl-imq-1
Loxoribine	TLR7 ligand	tlrl-lox
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

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