CL429

Dual TLR2 and NOD2 ligand

Catalog code: tlrl-c429

https://www.invivogen.com/cl429

For research use only

Version 23A27-MM

PRODUCT INFORMATION

Contents

- 5 mg of CL429

Note: CL429 is sterile filtered prior to lyophilization.

- 1.5 ml of endotoxin-free water

Storage and stability

- CL429 is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, store at 4°C. Resuspended product is stable for 6 months at 4 °C. Do **not** store resuspended product in plastic tubes.

Quality Control:

- TLR2 and NOD2 activity has been confirmed using cellular assays.
- The absence of bacterial contamination (e.g. endotoxins) has been confirmed using HEK-Blue™ TLR4 cells.

DESCRIPTION

CL429 is a chimeric compound that stimulates TLR2 and NOD2[1]. This compound is composed of murabutide (NOD2 ligand) covalently linked to Pam2C (TLR2 ligand) via a spacer. Murabutide is derived from muramyl dipeptide (MDP), the smallest bioactive unit of bacterial peptidoglycan. Murabutide is recognized by the cytosolic sensor NOD2, which induces the activation of NF-κB through the adaptor RIP2[2]. Pam2C is the lipid moiety of Pam2CSK4, which is recognized by the cell surface receptor TLR2 leading to the activation of NF-κB through the adaptor MyD88[3]. NOD2 and TLR2 have been shown to exert synergistic effects on the production of cytokines[4-6]. CL429 was reported to possess a very potent adjuvant activity, with no apparent toxicity[1].

1. Pavot V. *et al.*, 2014. Cutting edge: New chimeric NOD2/TLR2 adjuvant drastically increases vaccine immunogenicity. J Immunol. 193(12):5781-5. **2. Jakopin Ž., 2013.** Murabutide revisited: a review of its pleiotropic biological effects. Curr Med Chem. 20(16):2068-79. **3. Buwitt-Beckmann U. *et al.*, 2005.** Toll-like receptor 6-independent signaling by diacylated lipopeptides. Eur J Immunol. 35(1):282-9. **4. Moreira L.O. *et al.*, 2008.** The TLR2-MyD88-NOD2-RIPK2 signalling axis regulates a balanced proinflammatory and IL-10-mediated anti-inflammatory cytokine response to Gram-positive cell walls. Cell Microbiol. 10(10):2067-77. **5. Jeong Y.J. *et al.*, 2014.** Nod2 and Rip2 contribute to innate immune responses in mouse neutrophils. Immunology. 143(2):269-76. **6. Trinchieri G. & Sher A., 2007.** Cooperation of Toll-like receptor signals in innate immune defence. Nat Rev Immunol. 7(3):179-90.

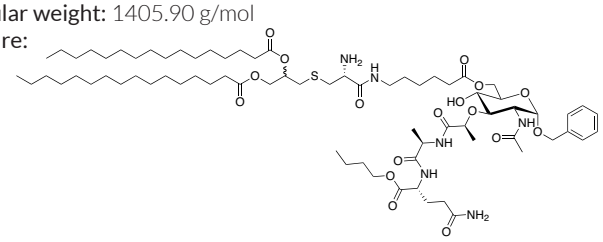
CHEMICAL PROPERTIES

Synonym: 6-O-[S-((2,3-bis(palmitoyloxy))-((2RS)propyl)-(R)-cysteinyl)-(6-aminocaproyl)]-1-O-Benzyl-N-Acetyl-muramyl-LAlanyl-D-Glutamin-n-butyl-ester, Pam2C-Aca-Benzyl-Murabutide

Formula: $C_{74}H_{128}N_6O_{17}S$

Molecular weight: 1405.90 g/mol

Structure:



METHODS

Preparation of stock solution (5 mg/ml)

1. Add 1 ml of DMSO to 5 mg of CL429.
2. Vortex until completely dissolved. Do **not** store resuspended product in plastic tubes.

Working concentrations: 1 ng - 10 µg/ml

TLR2 and NOD2 stimulation using HEK-Blue™ cells

CL429 can be used to stimulate TLR2 in HEK-Blue™ TLR2 cells and NOD2 in HEK-Blue™ NOD2 cells. These cells stably express an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) and overexpress the appropriate gene(s) of interest.
For more information visit: https://www.invivogen.com/hek-293.

1. Stimulate HEK-Blue™ TLR2 cells or HEK-Blue™ NOD2 cells with 1 ng - 10 µg/ml of CL429.
2. Incubate for 6 - 24 h at 37 °C, 5% CO_2.
3. Determine TLR stimulation using a SEAP detection medium, such as QUANTI-Blue™ Solution or HEK-Blue™ Detection or by assessing cytokine expression using an ELISA.

RELATED PRODUCTS

Product	Description	Cat.Code
HEK-Blue™ Detection	SEAP detection medium	hb-det2
HEK-Blue™ hTLR2 Cells	hTLR2 reporter cells	hkb-htlr2
HEK-Blue™ hNOD2 Cells	hNOD2 reporter cells	hkb-hnod2
Murabutide	NOD2 ligand	tlrl-mbt
Pam2CSK4	TLR7 ligand	tlrl-pm2s-1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT
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