

CL429

TLR2 & NOD2 ligand

Catalog # tlrl-c429

For research use only. Not for use in humans.

Version # 16H31-MM

PRODUCT INFORMATION

Content:

- 5 mg CL429

Note: CL429 is sterile filtered prior to lyophilization.

- 1.5 ml endotoxin-free water

Storage and stability

- CL429 is provided as a white lyophilized powder and shipped at room temperature. Store at -20°C. Lyophilized product is stable for 1 year at -20°C.

- Upon resuspension, prepare aliquots of CL429 and store at -20°C. Resuspended product is stable for 6 months at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- The TLR2 and NOD2 biological activities have been validated using HEK-Blue™ TLR2 and HEK-Blue™ NOD2 cells, respectively.

- The absence of bacterial contamination (e.g. endotoxins) has been confirmed using HEK-Blue™ TLR4 cells.

DESCRIPTION

CL429 is a chimeric compound that stimulates both TLR2 and NOD2¹. 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 RIP2². 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 MyD88³. 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 toxicity¹.

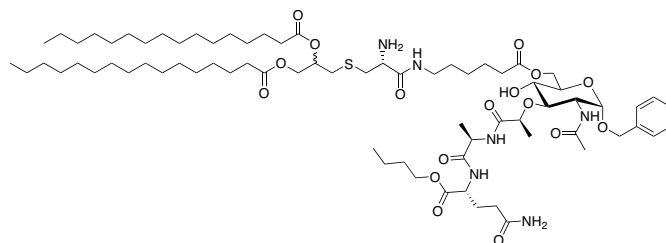
1. Pavot V. et al., 2014. Cutting edge: New chimeric NOD2/TLR2 adjuvant drastically increases vaccine immunogenicity. *J Immunol.* 193(12):5781-5. **2. Jakopin Z., 2013.** Murabutide revisited: a review of its pleiotropic biological effects. *Curr Med Chem.* 20(16):2068-79. **3. Buwitt-Beckmann U. et 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 pro-inflammatory 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

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

Formula: C₇₄H₁₂₈N₆O₁₇S

Molecular weight: 1405.90



METHODS

Preparation of stock solution (5 mg/ml)

- Add 1 ml of DMSO to the 5 mg CL429 vial to obtain a solution at 5 mg/ml and vortex to homogenize.

- Prepare aliquots of stock solution and store at -20°C.

- Dilutions can be prepared using endotoxin-free water (provided).

TLR2 and NOD2 activation using CL429

CL429 can be used to activate TLR2 and NOD2 in cells expressing these receptors, such as RAW-Blue™ cells. These mouse macrophages express an NF-κB inducible SEAP reporter gene to conveniently monitor the activation of TLR2- and NOD2-induced NF-κB activation, using QUANTI-Blue, a SEAP detection reagent.

For more information visit: www.invivogen.com/raw-blue

Day 1:

- Add 20 µl of CL429 at various concentrations (1 ng - 10 µg/ml) per well of a 96-well plate.

- Prepare a RAW-Blue™ cell suspension (~550,000 cells per ml) in growth medium containing heat-inactivated serum and immediately add 180 µl of the cell suspension (~100,000 cells) per well.

- Incubate the plate for 18-24 h at 37°C, 5% CO₂.

Day 2:

1- Prepare QUANTI-Blue™ following the instructions on the pouch.

2- Add 150 µl of resuspended QUANTI-Blue™ per well of a flat-bottom 96-well plate.

3- Add 50 µl of induced RAW-Blue™ cells supernatant.

4- Incubate the plate at 37°C for 30 min to 6 h.

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

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3-622-34-80

E-mail: info@invivogen.com

RELATED PRODUCTS

Product	Description	Catalog Code
CL429 VacciGrade™	Preclinical grade CL429	vac-c429
Pam2CSK4	TLR2 agonist	tlrl-pm2s-1
Murabutide	NOD2 agonist	tlrl-mbt
RAW-Blue™ cells	NF-κB-SEAP reporter mouse macrophages	raw-sp
HEK-Blue™ hTLR2 cells	Human TLR2 reporter cells	hkb-htlr2
HEK-Blue™ hNOD2 cells	Human NOD2 reporter cells	hkb-hnod2
QUANTI-Blue™	SEAP detection reagent	rep-qb1

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