C₁₄-Tri-LAN-Gly

NOD1 ligand

Catalog code: tlrl-ctlg http://www.invivogen.com/c14-tri-lan-gly

For research use only

Version # 18D05-NJ

PRODUCT INFORMATION

Contents

- 1 mg C₁₄-Tri-LAN-Gly
- 1.5 ml sterile endotoxin-free water

Storage and stability:

- C_{14} -Tri-LAN-Gly is provided lyophilized and shipped at room temperature. Upon receipt, store at -20 $^{\circ}$ C.
- Upon resuspension, prepare aliquots of C_{14} -Tri-LAN-Gly 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:

- Purity: ≥95% (UHPLC)
- The biological activity has been validated using HEK-Blue™ NOD1 cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-BlueTM TLR2 and HEK-BlueTM TLR4 cells.

DESCRIPTION

NOD1 is a pattern recognition receptor that senses Gram⁻ bacterial peptidoglycan (PGN), and more specifically the minimal motif γ-glutamyl-*meso*-diaminopimelic acid (iE-DAP)¹. Synthetic agonists of NOD1 have been developed in various ways to enhance their potency and intracellular delivery: NOD1 preferentially detects diaminopimelic tri-peptides (tri-DAP) with a N-terminal lipophilic chain, such as FK-565 (Heptanoyl-γ-D-glutamyl-L-*meso*-diaminopimelyl-D-Alanine)^{1,2}.

InvivoGen has developed C₁₄-Tri-LAN-Gly, an FK-565 analog which contains *meso*-lanthionine instead of the *meso*-DAP moiety, a myristic lipophilic chain, and three peptides (Ala, Glu, Gly)³. *Meso*-lanthionine is a diamino-type amino acid specific to PGN of some Gram⁻ bacteria such as *Fusobacterium nucleatum*⁴. C₁₄-Tri-LAN-Gly offers the advantage of being a single diastereoisomer, as opposed to other synthetic NOD1 agonists which are a mixture of L and D isomers.

Recognition of C_{14} -Tri-LAN-Gly by NOD1 induces the activation of the NF- κ B signaling pathway. While it activates both human and murine NOD1, it is the agonist of choice for murine NOD1 as it exhibits a \sim 10-fold better potency than the C_{12} -iE-DAP and a \sim 100-fold better potency than the Tri-DAP.

1. Girardin SE. et al., 2003. Nod1 detects a unique muropeptide from Gram-negative bacterial peptidoglycan. Science. 300: 1584-87. 2. Nielsen AE. et al., 2017. Synthetic agonists of NOD-like, RIG-like, and C-type lectin receptors for probing the inflammatory immune response. Future Med Chem. (in press). 3. Kingston Mills, Patrick Kelly, Jean-Gerard Tiraby, Thierry Lioux, Eric Perouzel, Patent online 2016, Novel compounds, WO 2016008946 A1, https://encrypted.google.com/patents/WO2016008946A1?cl=und 4. Uehara A. et al., 2006. Meso-diaminopimelic acid and meso-lanthionine, amino acids specific to bacterial peptidoglycans, activate human epithelial cells through NOD1. J Immunol. 177(3):1796-804.

CHEMICAL PROPERTIES

Synonym: Myristic-Alanine-D-iso-Glutamine-ε-meso-Lanthionine-

Glycine

Source: Synthetic

CAS number: 1863978-69-1 Formula: C₃₀H₅₃N₅O₁₀S Molecular weight: 675.84 Solubility: 2 mg/ml in water

Working concentration: 1 ng/ml -1 µg/ml

Structure:

METHODS

Preparation of stock solution (2 mg/ml)

- Add 500 µl endotoxin-free water (provided) and vortex until completely dissolved.
- Prepare aliquots of C₁₄-Tri-LAN-Gly and store at -20 °C.

NOD1 activation using C₁₄-Tri-LAN-Gly

 C_{14} -Tri-LAN-Gly can be used to activate NOD1 in cells expressing this receptor, such as HEK-BlueTM NOD1 cells. These cells express the human or mouse NOD1 gene and an NF- κ B inducible SEAP reporter gene. Levels of SEAP can be determined using HEK-BlueTM Detection, a cell culture medium that allows the detection of SEAP as it is secreted. For more information visit: www.invivogen.com/hek-blue-nod

- Add 20 μl of $C_{14}\text{-Tri-LAN-Gly}$ at 1 ng/ml -1 $\mu g/ml$ per well of a 96-well plate.
- Prepare a cell suspension as described on the technical data sheet in HEK-BlueTM Detection medium and immediately add 180 μ l of the cell suspension to each well containing C₁₄-Tri-LAN-Gly.
- Incubate the plate for 6-24 h at 37 °C, 5% CO₂.
- Determine SEAP levels using a spectrophotometer at 620-655 nm

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hNOD1 cells	hkb-hnod1
HEK-Blue™ mNOD1 cells	hkb-mnod1
C ₁₂ -iE-DAP	tlrl-c12dap
Tri-DAP	tlrl-tdap

