L₁₈-MDP

Synthetic lipophilic derivative of muramyl dipeptide; NOD2 agonist

Catalog code: tlrl-lmdp https://www.invivogen.com/l18-mdp

For research use only

Version 23J16-MM

PRODUCT INFORMATION

Contents

- 1 mg L18-MDP
- 1.5 ml sterile endotoxin-free water

Storage and stability

- L18-MDP is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots of L18-MDP 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

- Activation of NOD2 has been confirmed using HEK-Blue™ NOD2 cells.
- The absence of NOD1 activity has been confirmed using HEK-Blue™ NOD1 cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue $^{\text{\tiny M}}$ TLR2 and HEK-Blue $^{\text{\tiny M}}$ TLR4 cells.

DESCRIPTION

L18-MDP is a potent agonist of the cytosolic receptor NOD2^{1,2}. It is a lipophilic derivative of muramyl dipeptide (MDP) in which the OH group of the C6 position is esterified with stearic acid. MDP is the minimal bioactive peptidoglycan motif present in almost all bacteria. NOD2 acts as a general sensor of bacterial invasion through MDP detection³.

In an attempt to enhance the protective activity of MDP, numerous derivatives were synthesized. Among them, L18-MDP, a 6-O-acyl derivative with a stearoyl fatty acid, showed the highest activity⁴. This modification results in enhanced uptake of MDP and hydrolysis of the ester bond within the cell, releasing MDP in the cytoplasm. NOD2 activation with L18-MDP causes pro-inflammatory cytokine release through the mitogen-activated protein kinase (MAPK) and NF- κ B activation, thus contributing to host defense 5 .

1. Stafford C.A. et al., 2022. Phosphorylation of muramyl peptides by NAGK is required for NOD2 activation. Nature 609(7927):590-6. 2. Hespen C.W. et al., 2022. Membrane targeting enhances muramyl dipeptide binding to NOD2 and Arf6-GTPase in mammalian cells. Chem Commun (Camb). 58(46):6598-601. 3. Girardin SE. et al., 2003. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem. 278(11):8869-72. 4. Matsumoto K. et al., 1981. Stimulation of nonspecific resistance to infection induced by 6-O-acyl muramyl dipeptide analogs in mice. Infect Immun. 32(2):748-58. 5. Fujimura T. et al., 2011. A synthetic NOD2 agonist, muramyl dipeptide (MDP)-Lys (L18) and IFN-β synergistically induce dendritic cell maturation with augmented IL-12 production and suppress melanoma growth. J Dermatol Sci. 62(2):107-15

CHEMICAL PROPERTIES

Synonym: 6-O-stearoyl-N-Acetyl-muramyl-L-alanyl-D-isoglutamine

Formula: C₃₇H₆₆N₄O₁₂ Molecular weight: 758.94 g/mol Working concentration: 1-100 ng/ml

Solubility: 1 mg/ml in water

Structure:

METHODS

Preparation of stock solution (1 mg/ml)

- 1. Add 1 ml endotoxin-free water (provided) and vortex until completely dissolved.
- 2. Prepare aliquots and store at -20°C.

NOD2 stimulation with L18-MDP

L18-MDP can be used to activate NOD2 in cells expressing this receptor, such as HEK-Blue™ NOD2 cells. These cells express the human or mouse NOD2 gene and an NF-κB inducible SEAP reporter gene. Levels of SEAP can be determined using HEK-Blue™ Detection, a cell culture medium that allows the detection of SEAP as it is secreted.

For more information visit: https://www.invivogen.com/hek-blue-nod.

- 1. Add 20 μl of L18-MDP at 1-100 ng/ml per well of a 96-well plate.
- 2. Prepare a cell suspension as described on the technical data sheet in HEK-Blue™ Detection medium and immediately add 180 µl of the cell suspension to each well containing L18-MDP.
- 3. Incubate the plate for 6-24 h at 37 °C, 5% CO₂.
- 4. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Desciption	Cat.Code
HEK-Blue™ Detection	SEAP detection medium	hb-det2
HEK-Blue™ hNOD2 Cells	Human NOD2 reporter cells	hkb-hnod2
HEK-Blue™ mNOD2 Cells	Murine NOD2 reporter cells	hkb-mnod2
MDP	NOD2 agonist	tlrl-mdp
Murabutide	NOD2 agonist	tlrl-mbt



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