MDP

Muramyl dipeptide, L-D isomer; NOD2 ligand

Catalog code: tlrl-mdp

https://www.invivogen.com/mdp

For research use only

Version 23G26-MM

PRODUCT INFORMATION

Contents

- 5 mg Muramyl dipeptide (MDP)
- 1.5 ml endotoxin-free water

Storage and stability

- MDP is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots of 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™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Muramyl dipeptide (MDP) is a synthetic immunoreactive peptide composed of N-acetylmuramic acid linked by its lactic acid moiety to the N-terminus of an L-alanine D-isoglutamine dipeptide. MDP is the minimal bioactive peptidoglycan motif found in almost all bacteria. It was first identified as an active component in Freund's complete adjuvant[1].

MDP is recognized by the cytosolic receptor NOD2[2, 3]. Ligand-bound NOD2 oligomerizes and signals via the serine/threonine RIP2 (RICK,CARDIAK) kinase through CARD-CARD homophilic interactions[4]. Once activated, RIP2 mediates ubiquitination of NEMO/IKKγ leading to the activation of NF-κB and the production of inflammatory cytokines. Furthermore, poly-ubiquitinated RIP2 recruits TAK1, which leads to IKK complex activation and the activation of MAPKs[5]. This signaling involves the adaptor protein CARD9[6].

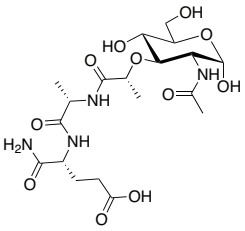
NOD2 recognition of MDP is stereospecific to the L-D isomer, excluding any reaction to D-D or L-L analogs[3]. The potent adjuvant activity of MDP may also be linked to an activation of the NLRP3 inflammasome[7]. Of note, NOD2 mutants associated with susceptibility to Crohn's disease have been found to be deficient in their recognition of MDP[2, 3].

1. Ogawa C. *et al.*, 2011. Muramyl dipeptide and its derivatives: peptide adjuvant in immunological disorders and cancer therapy. Curr Bioact Compd. 7(3):180-97. **2. Girardin S.E. *et al.*, 2003.** Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem. 278(11):8869-72. **3. Inohara N. *et al.*, 2003.** Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem. 278(8):5509-12. **4. Kobayash K. *et al.*, 2002.** RICK/Rip2/CARDIAK mediates signalling for receptors of the innate and adaptive immune systems. Nature 416:194-9. **5. Kobayashi K. *et al.*, 2005.** Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. Science 307: 731-734. **6. Hsu Y. et al., 2007.** The adaptor protein CARD9 is required for innate immune responses to intracellular pathogens. Nat Immunol. 8(2):198-205. **7. Martinon F. *et al.*, 2004.** Identification of bacterial muramyl dipeptide as activator of the NALP3/cryopyrin inflammasome. Curr Biol. 14(21):1929-34..

PRODUCT PROPERTIES

Synonym: N-Acetylmuramyl-L-Alanyl-D-Isoglutamine
CAS number: 53678-77-6
Formula: $C_{19}H_{32}N_4O_{11}$
Molecular weight: 492.5 g/mol
Specificity: NOD2
Solubility: 10 mg/ml in water
Working concentration: 10 ng-10 µg/ml

Chemical structure:



METHODS

Preparation of stock solution (10 mg/ml)

1. Add 500 µl endotoxin-free water (provided) to the vial containing 5 mg of MDP and vortex to solubilize.

NOD2 activation using MDP

MDP can be used to activate NOD2 in cells expressing this receptor, such as HEK-Blue™ NOD2 cells. These cells express the human or murine NOD2 gene and an NF-κB inducible SEAP reporter gene. Levels of SEAP can be easily determined using HEK-Blue™ Detection, a cell culture medium that allows the detection of SEAP as the reporter protein is secreted by the cells. For more information, visit: https://www.invivogen.com/hek-blue-nod.

1. Dispense 20 µl of MDP at various concentrations (10 ng-10 µg/ml) per well of a 96-well plate.
2. Prepare a cell suspension ~280,000 cells per ml in HEK-Blue™ Detection.
3. Add 180 µl of the cell suspension (~50,000 cells) to each MDP-containing well.
4. Incubate the plate for 6-24 h at 37°C, 5% CO_2.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ Detection	SEAP Detection reagent	hb-det2
HEK-Blue™ hNOD2 Cells	Human NOD2 reporter cells	hkb-hnod2
HEK-Blue™ mNOD2 Cells	Murine NOD2 reporter cells	hkb-mnod2
MDP Control	L-L isomer, negative control	tlrl-mdpcl

TECHNICAL SUPPORT
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