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 NOD22, 3. Ligand-bound NOD2 oligomerizes and signals via the serine/threonine RIP2 (RICK,CARDIAK) kinase through CARD-CARD homophilic interactions. Once activated, RIP2 mediates ubiquitination of NEMO/IkKβ 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.5 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.8-10

METHODS
Preparation of stock solution (10 mg/ml)
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) 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. Add 500 μl endotoxin-free water (provided) to the vial containing 5 mg of MDP and vortex to solubilize.

2. Prepare a cell suspension (~50,000 cells) to each MDP-containing well of a 96-well plate.

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% CO2.

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

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