# **MDP Control**

L-L isomer; Negative control for muramyl dipeptide (MDP)

Catalog code: tlrl-mdpcl https://www.invivogen.com/mdp-control

## For research use only

Version 23H01-MM

## PRODUCT INFORMATION

Contents

- 5 mg Muramyl dipeptide (MDP) Control, L-L isomer

- 1.5 ml endotoxin-free water

#### Storage and stability

- MDP Control is shipped at room temperature. Upon receipt, store at -20°C.

- Upon resuspension, prepare aliquots of MDP Control 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 absence of NOD2 activity 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^ $\rm M$  TLR2 and HEK-Blue^ $\rm TLR4$  cells.

## DESCRIPTION

MDP Control, the negative control for muramyl dipeptide (MDP), is an L-L isomer that does not activate NOD2. MDP is the minimal bioactive peptidoglycan motif present in almost all bacteria. MDP was first identified as an active component in Freund's complete adjuvant<sup>1</sup>. It is recognized by the cytosolic receptor NOD2<sup>2.3</sup>.

This recognition of MDP is stereospecific to the L-D isomer. The core structure required for recognition of NOD2 is MurNAc attached to L-Ala and D-isoGln. Replacement of L-Ala for D-Ala (or D-isoGln for L-isoGln) eliminates the ability of muramyl dipeptide to stimulate NOD2<sup>3</sup>. MDP Control contains L-Ala and L-isoGln, and hence does not activate NOD2.

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.

## PRODUCT PROPERTIES

Synonym: N-Acetylmuramyl-L-Alanyl-L-Isoglutamine Formula:  $C_{19}H_{32}N_4O_{11}$ Molecular weight: 492.5 g/mol Solubility: 10 mg/ml in water Chemical structure:



### METHODS

#### Preparation of stock solution (10 mg/ml)

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

#### NOD2 activation

MDP Control can be used as a negative control to study the stimulatory effect of MDP on NOD2 in HEK-Blue<sup>™</sup> 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<sup>™</sup> 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.

Below is a protocol for determining NOD2 stimulation MDP. Note: Use MDP Control at the same concentration as MDP.

1. Dispense 20  $\mu$ l of MDP or MDP Control at various concentrations (10 ng-10  $\mu$ g/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  $\mu l$  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<sub>2</sub>.

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



