

N-Glycolyl-MDP

N-glycolylated muramyl dipeptide; NOD2 agonist

Catalog code: tlrl-gmdp

<https://www.invivogen.com/n-glycolyl-mdp>

For research use only

Version 23J16-MM

PRODUCT INFORMATION

Contents

- 5 mg N-Glycolyl-MDP
- 1.5 ml sterile endotoxin-free water

Storage and stability

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

N-Glycolyl-MDP is a synthetic N-glycolylated form of muramyl dipeptide (MDP). It is a potent agonist of the cytosolic receptor NOD2^{1,2}. MDP is the minimal bioactive peptidoglycan (PGN) motif present in almost all bacteria. MDP was first identified as an active component in Freund's complete adjuvant³.

Most bacteria produce N-acetylated MDP in contrast to Mycobacteria which produce N-glycolylated MDP. In Mycobacterial PGN the N-acetyl group at the carbon 2 of the muramic acid is preferentially hydroxylated to an N-glycolyl group through action of the enzyme N-acetyl muramic acid hydroxylase (NamH). The cell wall of mycobacteria is known to be extremely immunogenic. This potent activity is attributed to their N-glycolylated MDP.

NOD2 activation with N-Glycolyl-MDP causes pro-inflammatory cytokine release through the mitogen-activated protein kinase (MAPK) and NF-κB activation, thus contributing to host defense^{1,2}.

1. Coulombe F. *et al.*, 2009. Increased NOD2-mediated recognition of N-glycolyl muramyl dipeptide. *J Exp Med.* 206(8):1709-16.
2. Hansen J.M. *et al.*, 2014. N-glycolylated peptidoglycan contributes to the immunogenicity but not pathogenicity of *Mycobacterium tuberculosis*. *C Infect Dis.* 209(7):1045-54.
3. 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.
4. Raymond J.B. *et al.*, 2005. Identification of the *namH* gene, encoding the hydroxylase responsible for the N-glycolylation of the mycobacterial peptidoglycan. *J Biol Chem.* 280(1):326-33.

CHEMICAL PROPERTIES

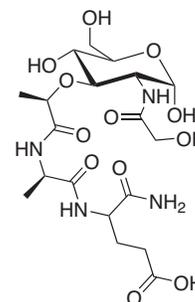
Structure:

Formula: C₁₉H₃₂N₄O₁₂

Molecular weight: 508.48 g/mol

Working concentration: 100 ng/ml - 10 µg/ml

Solubility: 5 mg/ml in water



METHODS

Preparation of stock solution (5 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 N-Glycolyl-MDP

N-Glycolyl-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 N-Glycolyl-MDP at 100 ng-10 µg/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 N-Glycolyl-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	Description	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

TECHNICAL SUPPORT

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