Human and mouse NOD1/2 Agonist Kit

Set of known agonists for human and mouse NOD1/2

Catalog # tlrl-nodkit2

For research use only

Version # 12E03-MM

PRODUCT INFORMATION

Content:

- NOD1 agonist - **C12-iE-DAP** (25 µg)
- NOD1 agonist - **iE-DAP** (100 µg)
- NOD2 agonist - **L18-MDP** (25 µg)
- NOD2 agonist - **MDP** (100 µg)
- NOD1/2 agonist - **M-Tri$_{DAP}$** (25 µg)
- NOD2 agonist - **M-Tri$_{LYS}$** (25 µg)
- NOD2 agonist - **Murabutide** (100 µg)
- NOD1/2 agonist - **PGN-ECndi ultra-pure** (100 µg)
- NOD1/2 agonist - **PGN-SAndi ultra-pure** (100 µg)
- NOD1 agonist - **Tri-DAP** (25 µg)
- 2x 2 ml endotoxin-free water

Storage and stability:

- All agonists are provided in a powdered form. Products are shipped at room temperature. Store lyophilized product at -20˚C.
- Resuspended products should be aliquoted and then stored at -20˚C.
- Lyophilized and resuspended products are stable 6 months when properly stored. Avoid repeated freeze-thaw cycles.

DESCRIPTION

• C12-iE-DAP - NOD1 agonist

C12-iE-DAP is an acylated derivative of iE-DAP. It was generated by addition of a lauroyl (C_{12}) group to the glutamic residue of iE-DAP. C12-iE-DAP stimulates specifically NOD1 at concentrations 100- to 1000-fold lower than the original iE-DAP.

• iE-DAP - NOD1 agonist

iE-DAP (D-γ-Glu-mDAP) is a dipeptide present in the peptidoglycan (PGN) of a subset of bacteria that include Gram-negative bacilli and particular Gram-positive bacteri such as *Bacillus subtilis* and *Listeria monocytogenes*[1]. iE-DAP is the minimal motif recognized by NOD1.

• MDP - NOD2 agonist

MDP (MurNAc-L-Ala-D-isoGln, also known as murmyl dipeptide), is the minimal bioactive PGN motif common to all bateria and the essential structure required for adjuvant activity in vaccines. MDP has been shown to be recognized by NOD2, but not TLR2, nor TLR2/1 or TLR2/6 associations[2,3]. This recognition is highly stereospecific of the L-D isomer, excluding any reaction to the D-D or L-L analogs[3,4].

• L18-MDP - NOD2 agonist

L18-MDP is a synthetic derivative of MDP. L18-MDP has been shown to display a higher adjuvant activity than MDP[5].

• M-Tri$_{DAP}$ - NOD1/2 agonist

M-Tri$_{DAP}$ (MurNAc-L-Ala-D-γ-Glu-mDAP, also known as DAP-containing muramyl tripeptide) is a PGN degradation product found mostly in Gram-negative bacteria. M-Tri$_{DAP}$ is recognized by NOD1 and to a lesser extend NOD2. M-Tri$_{DAP}$ induces the activation of NF-κB at similar levels to Tri-DAP[6].

• M-Tri$_{LYS}$ - NOD2 agonist

M-Tri$_{LYS}$ (MurNAc-Ala-D-isoGln-Lys) is a muropeptide released by *Lactobacillus salivarus* after digestion of their PGN. The chemically synthesized M-Tri$_{LYS}$ is sensed by NOD2 and induces the activation of NF-κB[7]. M-Tri$_{LYS}$ has been shown to produce the anti-inflammatory cytokine IL-10 and protect mice from colitis[7].

• Murabutide - NOD2 agonist

Murabutide (MurNAc-L-Ala-D-GlnOBu) is a safe synthetic immunomodulator derived from muramyl dipeptide (MDP). In contrast to MDP, murabutide is devoid of pyrogenic activity[8] and lacks somnogenic activity[9]. Murabutide is recognized by the intracellular receptor NOD2 inducing the activation of NF-κB.

• PGN-ECndi and PGN-SAndi ultra pure - NOD1/2 agonist

PGN-ECndi from *E. coli K12* and PGN-SAndi from *S. aureus* are insoluble preparations of PGNs purified by detergent lysis and hydrolysis under basic conditions to eliminate lipophilic constituents. These PGN preparations have lost their ability to activate TLR2-transfected HEK293 cells but can still activate NOD2-transfected cells. PGN-ECndi activates also NOD1-transfected cells.

• Tri-DAP - NOD1 agonist

Tri-DAP (L-Ala-γ-D-Glu-mDAP) comprises the iE-DAP dipeptide and an L-Ala residue. Similarly to iE-DAP, this tripeptide is specifically recognized by NOD1 but exhibits a ~3-fold higher ability to activate NF-κB than iE-DAP[6].

1. Chamaillard M. *et al.*, 2003. An esssential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat. Immunol. 4:702-7. **2. Girardin SE. *et al.*, 2003**. NOD2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J. Biol. Chem. 278:8869-72. **3. Inohara N. *et al.*, 2003.** Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J. Biol. Chem. 278:5509-12. **4. Traub S. *et al.*, 2004.**Structural requirement of syntheti muropeptides to synergize with lipopolysaccharide in cytokine induction. J. Biol. Chem. 279:8694-700. **5. Ishihara C. *et al.*, 1985.** Effect of muramyl dipeptide and its stearoyl derivatives on resistance to Sendai virus infection in mice. Vaccine 3:370-4. **6. Girardin SE. *et al.*, 2003.** Peptidoglycan molecular requirement allowing detection by NOD1 and NOD2. J. Biol. Chem. 278:41702-8. **7. Macho Fernandez E. *et al.*, 2011.** Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide, Gut 60:1050-9. **8. Chedid LA. *et al.*, 1982.** Biological activity of a new synthetic muramyl peptide adjuvant tripeptide devoid of pyrogenicity. Infect. Immun. 35:417-24. **9. Krueger JM. *et al.*, 1984**. Muramyl peptides. Variations of somnogenic activity with structure. J. Exp. Med.159:68-76.

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METHODS

Preparation of NOD agonist stock solutions:

Product	Molecular weight	Working concentration	Volume of solvent to obtain 1 mg/ml
C12-iE-DAP	501.61	1 ng-1 µg/ml	25 µl DMSO
iE-DAP	319.31	1-100 µg/ml	100 µl H_2O
L18-MDP	772.97	1-100 ng/ml	25 µl H_2O
MDP	492.5	10 ng-10 µg/ml	100 µl H_2O
M-Tri$_{DAP}$	665.64	1-100 µg/ml	25 µl H_2O
M-Tri$_{LYS}$	734.75	100 ng-10 µg/ml	25 µl H_2O
Murabutide	548.58	10 ng-1 µg/ml	100 µl H_2O
PGN-ECndi ultra pure	NA	1-5 µg/ml	100 µl H_2O
PGN-SAndi ultra pure	NA	1-5 µg/ml	100 µl H_2O
Tri-DAP	390.39	100 ng-10 µg/ml	25 µl H_2O

NOD1/2 stimulation

- Transfect your cell line with an NF-κB-inducible reporter plasmid, i.e. a plasmid carrying a reporter gene, such as SEAP or luciferase, under the control of an NF-κB-inducible ELAM-1 (E-selectin) promoter.

Note: InvivoGen provides pNiFty, a family of NF-κB-inducible reporter plasmids that can be transfected transiently (pNiFty) or stably (pNiFty2). pNiFty plasmids are available either with the SEAP or luciferase reporter genes.

If your cell line does not naturally express NOD1/2, cotransfect with a plasmid expressing NOD1/2 gene, such as the pUNO plasmid family.

Note: Alternatively, evaluate NOD activation using reporter cells, such as InvivoGen's HEK-Blue™ NOD cells which express the human or mouse NOD and SEAP reporter genes. NF-κB production in these cells can be easily quantified using a SEAP detection medium, such as QUANTI-Blue™ or HEK-Blue™ Detection.

- Twenty-four to forty-eight hours after transfection, stimulate cells with the NOD1/2 agonists for 6 hours to 24 hours.
- Determine NOD1/2 stimulation by assessing reporter gene expression using the appropriate detection system, such as QUANTI-Blue™ for cells transfected with pNiFty-SEAP plasmids.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hNOD1 cells	hkb-hnod1
HEK-Blue™ mNOD1 cells	hkb-mnod1
HEK-Blue™ hNOD2 cells	hkb-hnod2
HEK-Blue™ mNOD2 cells	hkb-mnod2
QUANTI-Blue™	rep-qb1
HEK-Blue™ Detection	hb-det2
pUNO-hNOD1	puno-hnod1
pUNO-mNOD1	puno-mnod1
pUNO-hNOD2	puno-hnod2a
pUNO-mNOD2	puno-mnod2a
pNiFty-SEAP (Amp^R)	pnifty-seap
pNiFty2-SEAP (Zeo^R)	pnifty2-seap

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