

PGN-ECndi Ultrapure

Insoluble peptidoglycan from *Escherichia coli* K12; NOD1/NOD2 ligand

Catalog code: tlr1-kipgn

<https://www.invivogen.com/png-ecndi>

For research use only

Version 23D05-MM

PRODUCT INFORMATION

Contents

- 5 mg of insoluble peptidoglycan from *E. coli* K12 (PGN-ECndi)
- 25 ml of sterile endotoxin-free water

Storage and stability

- PGN-ECndi is shipped at room temperature.
- Upon receipt, store at -20°C.
- Resuspended PGN-ECndi can be stored at 4°C for 1 month or at -20°C for 1 year. Avoid repeated freeze-thaw cycles.

Quality Control

- The biological activity has been verified using cellular assays.
- The absence of bacterial contaminants has been confirmed using HEK-Blue™ TLR2 cells (for lipoproteins) and HEK-Blue™ TLR4 cells (for endotoxins).

DESCRIPTION

PGN-ECndi is an insoluble ultrapure preparation of peptidoglycan (PGN) from the Gram-negative *Escherichia coli* K12. PGN, either intact or fragmented, is sensed by the innate immune system through different PRRs (pattern recognition receptors). PGN is known to be a potent activator of NF- κ B and TNF- α through TLR2, although it has been reported that PGN agonist activity for TLR2 is likely relying on other commonly co-purified molecules, such as cell wall lipoproteins and lipoteichoic acids (LTAs). The role of TLR2 in the direct recognition of PGN remains controversial, and the discrepancies among studies may arise from the purification methods as well as from the variation in PGN structure in the different bacterial species^{1,4}. PGNs purified by detergent lysis and hydrolysis under basic conditions to eliminate lipophilic constituents lose their ability to activate TLR2.

Soluble PGN-ECndi from *E. coli* K12 is an insoluble preparation of PGN purified by detergent lysis and hydrolysis under basic conditions to eliminate lipophilic constituents. This PGN preparation does not activate TLR2-transfected HEK293 cells, but it still activates NOD1- and NOD2-transfected cells.

1. Wolf A.J. & Underhill D.M., 2018. Peptidoglycan recognition by the innate immune system. *Nat. Rev. Immunol.* 18(4):243-254. 2. Travassos L.H. et al., 2004. Toll-like receptor 2-dependent bacterial sensing does not occur via peptidoglycan recognition. *EMBO Rep.* 5(10):1000-1006. 3. Girardin S.E et al., 2003. Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. *J Biol Chem.* 278(43):41702-8. 4. Dziarski R., 2004. Peptidoglycan recognition proteins (PGRPs). *Mol Immunol.* 40(12):877-86.

METHODS

Preparation of stock solution (200 μ g/ml)

- Add 1 ml of endotoxin-free water (provided) and vortex to homogenize.
- Transfer this solution in a sterile non-pyrogenic 50 ml bottle.
- Add 24 ml of endotoxin-free water and homogenize.

Note: The solution remains hazy.

- Prepare aliquots and store at 4°C for 1 month or at -20°C for 1 year.

Working Concentration: 1 - 10 μ g/ml

NOD1/NOD2 activation using PGN-ECndi

PGN-ECndi can be used to activate NOD1 or NOD2 in cells expressing these receptors, such as HEK-Blue™ NOD1 and HEK-Blue™ NOD2 cells. These cells express the human or mouse NOD1 or NOD2 genes and an NF- κ B 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 it is secreted by the cells.

For more information visit: <https://www.invivogen.com/nod-cell-lines>.

- Add 20 μ l of PGN-ECndi at various concentrations (1-10 μ g/ml final concentration) per well of a 96-well plate.
- Prepare a cell suspension (~280,000 cells per ml) in HEK-Blue™ Detection medium and immediately add 180 μ l of the cell suspension (~50,000 cells) to each PGN-ECndi-containing well.
- Incubate the plate for 6-24 h at 37°C, 5% CO₂.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ hNOD1 Cells	Human NOD1 reporter cells	hkb-hnod1
HEK-Blue™ mNOD1 Cells	Murine NOD1 reporter cells	hkb-mnod1
HEK-Blue™ hNOD2 Cells	Human NOD2 reporter cells	hkb-hnod2
HEK-Blue™ mNOD2 Cells	Murine NOD2 reporter cells	hkb-mnod2
HEK-Blue™ Detection	SEAP detection reagent	hb-det2
PGN-BS	PGN from <i>Bacillus subtilis</i>	tlrl-pgnb3
PGN-ECndss Ultrapure	Soluble PGN from <i>S. aureus</i>	tlrl-ksspgn
PGN-SA	PGN from <i>S. aureus</i>	tlrl-pgns2
PGN-SAndi Ultrapure	Insoluble PGN from <i>S. aureus</i>	tlrl-sipgn

TECHNICAL SUPPORT

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