

# PGN-SAndi Ultrapure

Insoluble peptidoglycan from *Staphylococcus aureus*; NOD2 ligand

Catalog code: tlr1-sipgn

<https://www.invivogen.com/pgn-sandi>

For research use only

Version 23D06-MM

## PRODUCT INFORMATION

### Contents

- 5 mg of insoluble ultrapure peptidoglycan from *S. aureus* (PGN-SAndi Ultrapure)
- 25 ml of sterile endotoxin-free water

### Storage and stability

- PGN-SAndi Ultrapure is shipped at room temperature.
- Upon receipt, store at -20°C.
- Resuspended PGN-SAndi Ultrapure 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-SAndi Ultrapure is an insoluble ultrapure preparation of peptidoglycan (PGN) from the Gram-positive *Staphylococcus aureus*. 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- $\kappa$ B and TNF- $\alpha$  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<sup>1,2</sup>. PGNS purified by detergent lysis and hydrolysis under basic conditions to eliminate lipophilic constituents lose their ability to activate TLR2.

PGN-SAndi Ultrapure from *S. aureus* 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 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.

## METHODS

### Preparation of stock solution (200 $\mu$ g/ml)

- Add 1 ml of sterile 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  $\mu$ g/ml

### NOD2 activation using PGN-SAndi Ultrapure

PGN-SAndi Ultrapure can be used to activate NOD2 in cells expressing these receptors, such as HEK-Blue™ NOD2 cells. These cells express the human or mouse NOD2 genes and an NF- $\kappa$ 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  $\mu$ l of PGN-SAndi Ultrapure at various concentrations (1-10  $\mu$ 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  $\mu$ l of the cell suspension (~50,000 cells) to each PGN-SAndi-containing well.
- Incubate the plate for 6-24 h at 37°C, 5% CO<sub>2</sub>.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.

## RELATED PRODUCTS

Product	Description	Cat. Code
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-ECndi Ultrapure	Insoluble PGN from <i>E. coli</i> K12	tlrl-kipgn
PGN-ECndss Ultrapure	Soluble PGN from <i>S. aureus</i>	tlrl-ksspgn
PGN-SA	PGN from <i>S. aureus</i>	tlrl-pgns2

### TECHNICAL SUPPORT

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