

PGN-BS

Peptidoglycan from *Bacillus subtilis*; TLR2 and NOD2 ligand

Catalog code: tlr1-pgnb3

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

For research use only

Version 23D06-MM

PRODUCT INFORMATION

Contents

- 5 mg of peptidoglycan from *B. subtilis* (PGN-BS)
- 25 ml of endotoxin-free water

Storage and stability

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

Quality Control

- The biological activity of PGN-BS has been verified using cellular assays.
- The absence of bacterial endotoxins has been assessed using HEK-Blue™ TLR4 cells.
- The absence of bacterial spores or live bacteria has been confirmed using microbiological assays.

DESCRIPTION

PGN-BS is a peptidoglycan (PGN) preparation from the Gram-positive bacterium, *Bacillus subtilis*. PGN, either intact or fragmented, is sensed by the innate immune system through different PRRs (pattern recognition receptors). PGN from diverse bacteria, including *B. subtilis*², are known to be potent activators of NF-κB and TNF-α through TLR2, although it has been reported that PGN agonist activity for TLR2 may rely 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 species¹⁻⁵.

PGN-BS is purified by detergent lysis, enzymatic treatment, LiCl/EDTA and acetone cleaning, as previously described⁶. This purification method generates a PGN preparation that activates TLR2 and NOD2.

1. Wolf A.J. & Underhill D.M., 2018. Peptidoglycan recognition by the innate immune system. *Nat. Rev. Immunol.* 18(4):243-254. 2. Shah S. et al., 2012. Peripheral blood mononuclear cells of Murrah Buffalo (*Bubalus Bubalis*) on TLR2 induction by *B. Subtilis* peptidoglycan. *Asian-Aust. J. Anim. Sci.* 25(7):1021-8. 3. 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. 4. Girardin S.E. et al., 2003. Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. *J Biol Chem.* 278(43):41702-8. 5. Dziarski R., 2004. Peptidoglycan recognition proteins (PGRPs). *Mol Immunol.* 40(12): 877-86. 6. de Jonge B. et al., 1992. Peptidoglycan composition of a highly methicillin-resistant *Staphylococcus aureus* strain. The role of penicillin binding protein 2A. *J Biol Chem.* 267(16):11248-54.

METHODS

Preparation of stock solution (200 µg/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 -20°C.

Working concentrations:

- 30 ng/ml - 10 µg/ml PGN-BS for human TLR2 in cell culture assays
- 3 - 10 µg/ml PGN-BS for human NOD2 in cell culture assays

TLR2 and/or NOD2 activation using PGN-BS

PGN-BS can be used to activate TLR2 and NOD2 in HEK-Blue™ TLR2 cells and HEK-Blue™ NOD2 cells respectively, that were designed to study TLR2 and NOD2 stimulation by monitoring NF-κB activation. Stimulation of these cells with the appropriate agonist activates NF-κB which induces the production of SEAP (secreted embryonic alkaline phosphatase). Levels of SEAP can be easily determined using HEK-Blue™ 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-tnfr2>.

- Dispense 20 µl of PGN-BS at various concentrations (30 ng-10 µg/ml for TLR2 and 3 - 10 µg/ml for NOD2 activation) 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 µl of the cell suspension (~50,000 cells) to each PGN-BS-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™ hTLR2 Cells	Human TLR2 reporter cells	hkb-htlr2
HEK-Blue™ mTLR2 Cells	Murine TLR2 reporter cells	hkb-mtlr2
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
FSL-1	Diacylated lipoprotein	tlr1-fsl
PGN-SA	PGN from <i>S. aureus</i>	tlr1-pgns2
PGN-ECndi Ultrapure	Insoluble PGN from <i>E. coli</i> K12	tlr1-kipgn
PGN-ECndss Ultrapure	Soluble PGN from <i>E. coli</i> K12	tlr1-ksspgn
PGN-SAndi Ultrapure	Insoluble PGN from <i>S. aureus</i>	tlr1-sipgn
MDP	Muramyl dipeptide	tlr1-mdp

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