

PGN-SA

Peptidoglycan from *Staphylococcus aureus*; TLR2 ligand

Catalog code: tlrl-pgns2

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

For research use only

Version 23D05-MM

PRODUCT INFORMATION

Contents

- 5 mg of peptidoglycan from *S. aureus* (PGN-SA)
- 25 ml of endotoxin-free water

Storage and stability

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

Quality Control

- The biological activity of PGN-SA has been verified using cellular assays.
- The presence of bacterial endotoxins has been assessed using HEK-Blue™ TLR4 cells.

DESCRIPTION

PGN-SA is a peptidoglycan (PGN) preparation from the Gram-positive bacterium *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- κ B and TNF- α through TLR2, although it has been reported that PGN agonistic 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 species¹⁻³.

PGN-SA is purified by detergent lysis, enzymatic treatment, LiCl/EDTA and acetone cleaning, as previously described⁴. This purification method generates a PGN preparation that activates TLR2.

1. Wolf A.J. & Underhill D.M., 2018. Peptidoglycan recognition by the innate immune system. *Nat. Rev. Immunol.* 18(4):243-254. 2. Dziarski R. & Gupta D., 2005. *Staphylococcus aureus* peptidoglycan is a Toll-Like Receptor 2 activator: a reevaluation. *Infect Immun.* 273(8):5212-6. 8. Müller-Anstett M. et al., 2010. Staphylococcal peptidoglycan co-localizes with Nod2 and TLR2 and activates innate immune response via both receptors in primary murine keratinocytes. *PLoS One.* 5(10):e13153. 4. 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 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 -20°C.

Working concentration: 0.1 - 10 μ g/ml

TLR2 activation using PGN-SA

PGN-SA can be used to activate TLR2 in HEK-Blue™ TLR2 cells, that were designed to study TLR2 stimulation by monitoring NF- κ B activation. Stimulation of HEK-Blue™ TLR2 cells with a TLR2 agonist activates NF- κ B 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-trlr2>.

- Dispense 20 μ l of PGN-SA at various concentrations (0.1 - 10 μ g/ml) 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-SA-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-htrlr2
HEK-Blue™ mTLR2 Cells	Murine TLR2 reporter cells	hkb-mtrlr2
HEK-Blue™ Detection	SEAP detection reagent	hb-det2
FSL-1	Diacylated lipoprotein	tlrl-fsl
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>E. coli</i> K12	tlrl-ksspgn
PGN-SAndi Ultrapure	Insoluble PGN from <i>S. aureus</i>	tlrl-sipgn

TECHNICAL SUPPORT

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