

PGN-SA

Peptidoglycan from *Staphylococcus aureus*; TLR2 ligand

Catalog # tlr1-pgns2

For research use only

Version # 15H30-MM

PRODUCT INFORMATION

Content:

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

Storage :

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

Quality control

- The presence of bacterial contamination (e.g. endotoxins) is assessed using HEK-Blue™ TLR4 cells.

DESCRIPTION

PGN-SA is a peptidoglycan (PGN) preparation from the Gram-positive bacterium, *Staphylococcus aureus*. PGN is a major surface component of Gram-positive bacteria. It is embedded in a relatively thick cell wall with other polymers, such as lipoteichoic acids (LTAs). PGNS from diverse bacteria are known to be potent activators of NF-κB and TNF-α through TLR2^{2,3}.

PGN is also recognized by the intracellular pattern recognition receptors, NOD1 and NOD2. NOD1 senses the D-γ-glutamyl-meso-DAP dipeptide (iE-DAP), which is found in PGN of all Gram-negative and certain Gram-positive bacteria^{4,5} whereas NOD2 recognizes the muramyl dipeptide (MDP) structure found in almost all bacteria⁶.

There are some conflicting results regarding the TLR2 activity of PGN. This discrepancy is attributed to the method of purification^{7,8}. PGN-SA is purified by detergent lysis, enzymatic treatment, LiCl/EDTA and acetone cleaning, as previously described⁹. This purification method generates a PGN preparation that activates TLR2.

1. **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. 2. **Schwandner R. et al., 1999.** Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. J Biol Chem. 274(25):17406-9. 3. **Takeuchi O. et al., 1999.** Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity 11(4):443-51. 4. **Chamaillard M. et al., 2003.** An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat. Immunol. 4: 702-707. 5. **Girardin SE. et al., 2003.** Nod1 detects a unique muropeptide from Gram-negative bacterial peptidoglycan. Science 300: 1584-1587. 6. **Girardin SE. et al., 2003.** Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem. 278(11):8869-72. 7. **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. 9. **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)

- Stimulation of TLR2 can be achieved with 0.1 - 10 µg/ml PGN-SA.
- Add 1 ml endotoxin-free water (provided) and vortex to homogenize.
 - Transfer this solution to a sterile non-pyrogenic 50 ml bottle.
 - Add 24 ml of endotoxin-free and homogenize.

Note: The solution remains hazy.

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-κB activation. Stimulation of HEK-Blue™ TLR2 cells with a TLR2 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: www.invivogen.com/hek-blue-hltr2

- Dispense 20 µl of PGN-SA at various concentrations (0.1 - 10 µg/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 µl 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	Catalog Code
HEK-Blue™ hTLR2 Cells (human TLR2)	hkb-hltr2
HEK-Blue™ mTLR2 Cells (mouse TLR2)	hkb-mtlr2
HEK-Blue™ Detection	hb-det2
Other TLR2 ligands	
FSL-1 (synthetic diacylated lipoprotein)	tlr1-fsl1
HKLM (heat killed <i>L.monocytogenes</i>)	tlr1-hklm
PGN-BS (<i>B.subtilis</i>)	tlr1-pgnb3
PGN-EB (<i>E.coli</i> O111:B4)	tlr1-pgnc
PGN-EK (<i>E.coli</i> K12)	tlr1-pgnek
NOD2 ligands (without TLR2 activity):	
MDP (muramyl dipeptide)	tlr1-mdp
Murabutide (synthetic derivative of MDP)	tlr1-mbt
NOD1/2 ligands (without TLR2 activity):	
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

TECHNICAL SUPPORT

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