PGN-BS

Peptidoglycan from *Bacillus subtilis* - TLR2 ligand

Catalog # tlrl-pgnb3

For research use only

Version # 15D28-MM

PRODUCT INFORMATION

Content

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

Storage

- PGN-BS is shipped at room temperature. Store at -20 °C.
- Resuspended PGN-BS 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.
- The absence of bacterial spores or live bacteria is confirmed using microbiological assays.

DESCRIPTION

PGN-BS is a peptidoglycan (PGN) preparation from the Gram-positive bacterium, *Bacillus subtilis*. 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, including *B. subtilis*[1], 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 bacteria[6].

There are some conflicting results regarding the TLR2 activity of PGN. This discrepancy is attributed to the method of purification[7, 8]. PGN-BS is purified by detergent lysis, enzymatic treatment, LiCl/EDTA and acetone cleaning, as previously described[9]. This purification method generates a PGN preparation that activates both TLR2 and NOD2.

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-BS.

- Add 1 ml 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 provided and homogenize.

Note: The solution remains hazy.

TLR2 activation using PGN-BS

PGN-BS 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-htlr2

- Dispense 20 µl of PGN-BS 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-BS-containing well.
- Incubate the plate for 6-24 h at 37 °C, 5% CO_2.
- Determine SEAP levels using a spectrophotometer at 620 - 655 nm.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR2 Cells (human TLR2)	hkb-htlr2
HEK-Blue™ mTLR2 Cells (mouse TLR2)	hkb-mtlr2
HEK-Blue™ Detection	hb-det2
Other TLR2 Ligands	
FSL-1 (synthetic diacylated lipoprtoein)	tlrl-fsl
HKLM (heat killed *L.monocytogenes*)	tlrl-hklm
PGN-EB (*E.coli* O111:B4)	tlrl-pgnec
PGN-EK (*E.coli* K12)	tlrl-pgnek
PGN-SA (*S.aureus*)	tlrl-pgnsa
NOD2 Ligands	
MDP (muramyl dipeptide)	tlrl-mdp
Murabutide (synthetic derivative of MDP)	tlrl-mbt
NOD1/2 Ligands	
PGN-ECndi Ultrapure (insoluble PGN from *E.coli* K12)	tlrl-kipgn
PGN-ECndss Ultrapure (soluble PGN from *E.coli* K12)	tlrl-ksspgn
PGN-SAndi Ultrapure (insoluble PGN from *S.aureus*)	tlrl-sipgn

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong : +852 3-622-34-80
E-mail: info@invivogen.com