

PGN-EB

Peptidoglycan from *E. coli* 0111:B4; TLR2 ligand

Catalog # tlr1-pgneb

For research use only

Version # 16D08-MM

PRODUCT INFORMATION

Content:

- 1 mg of peptidoglycan from *Escherichia coli* 0111:B4 (PGN-EB)
- 10 ml endotoxin-free water

Storage:

- PGN-EB is shipped at room temperature and should be stored at -20 °C. Lyophilized product is stable for 1 year at -20 °C.
- Resuspended product should be stored at -20 °C. It is stable for 1 year at -20 °C when properly stored. Avoid repeated freeze-thaw cycles.

QUALITY CONTROL

- TLR2 activity has been confirmed using HEK-Blue™ TLR2 cells.
- TLR4 activity has been assessed using HEK-Blue™ TLR4 cells.

DESCRIPTION

PGN-EB is a peptidoglycan (PGN) preparation from the Gram-negative bacterium, *Escherichia coli* 0111:B4. PGN is a major surface component of Gram-positive bacteria. It is embedded in a relatively thick cell wall with other polymers, such as lipopolysaccharide (LPS) and lipoteichoic acids (LTAs). PGNs are known to be potent activators of NF-κB and TNF-α through TLR2^{1,2}, but also activates TLR4³ presumably due to the presence of LPS.

There are some conflicting results regarding the TLR2 activity of PGN. This discrepancy is attributed to the method of purification^{3,4}. PGN-EB is purified by detergent lysis, enzymatic treatment, LiCl/EDTA and acetone cleaning. This purification method generates a PGN-EB preparation that is a potent stimulator of TLR2, and has a weak stimulatory effect on TLR4.

1. 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. 2. 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. 3. Dziarski R. & Gupta D., 2005. Staphylococcus aureus peptidoglycan is a Toll-Like Receptor 2 activator: a reevaluation. *Infect Immun.* 73(8):5212-6. 4. 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.

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 25 ml bottle.
- Add 4 ml of sterile endotoxin-free water and homogenize.

Note: The solution remains hazy.

- Prepare aliquots and store at -20 °C for long term storage.

TLR2 activation using PGN-EB

PGN-EB 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

- Dispense 20 µl of PGN-EB at various concentrations (10 ng-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-EB-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-htlr2
HEK-Blue™ mTLR2 Cells (mouse TLR2)	hkb-mtlr2
HEK-Blue™ Detection	hb-det2
Other TLR2 ligands	
FSL-1 (synthetic diacylated lipoprotein)	tlr1-fsl
HKLM (heat killed <i>L. monocytogenes</i>)	tlr1-hklm
PGN-BS (<i>B. subtilis</i>)	tlr1-pgnb3
PGN-EK (<i>E. coli</i> K12)	tlr1-pgnek
PGN-SA (<i>S. aureus</i>)	tlr1-pgnsa

TECHNICAL SUPPORT

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