

# PGN-EK

Peptidoglycan from *E. coli* K12; TLR2 and TLR4 ligand

Catalog code: tlr1-pgnek

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

For research use only

Version 20J29-MM

## PRODUCT INFORMATION

### Contents

- 1 mg *E. coli* K12 peptidoglycan (PGN-EK)
- 10 ml endotoxin-free water

### Storage and stability

- PGN-EK is shipped at room temperature.
- Upon receipt, store -20°C for up to 2 years.
- Resuspended PGN-EK can be stored at -20°C for 1 year. Avoid repeated freeze-thaw cycles.

### Quality Control

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

## DESCRIPTION

PGN-EK is a peptidoglycan (PGN) preparation from the Gram-negative *Escherichia coli* K12. PGN, a major component of the bacterial cell wall, is a large polymeric molecule made of glycan strands connected by short peptides. It is mainly single-layered in Gram-negative bacteria (e.g. *E. coli*) and multi-layered in Gram-positive bacteria (e.g. *B. subtilis*). The structure of PGN varies among bacterial species at both the level of sugar polymers and of cross-linked amino-acids. In Gram-positive bacteria, the amino-acid side chain structure consists of L-alanine, D-glutamate, L-lysine, and D-alanine, whereas in Gram-negative bacteria, the L-Lysine is replaced by meso-diaminopimelic acid (mDAP or meso-DAP)<sup>1</sup>.

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-κB 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 species<sup>1</sup>.

1. Wolf A.J. & Underhill D.M., 2018. Peptidoglycan recognition by the innate immune system. *Nat. Rev. Immunol.* 18(4):243-254. 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.

## 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 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-EK

PGN-EK 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: <https://www.invivogen.com/hek-blue-trl2>.

- Dispense 20 µl of PGN-EK at various concentrations (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-EK-containing well.
- Incubate the plate for 6-24 h at 37°C, 5% CO<sub>2</sub>.
- 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-htrl2
HEK-Blue™ mTLR2 Cells	Murine TLR2 reporter cells	hkb-mtrl2
HEK-Blue™ Detection	SEAP detection reagent	hb-det2
<b>Other TLR2 ligands</b>		
FSL-1	Diacylated lipoprotein	tlrl-fsl
HKLM	Heat killed <i>L. monocytogenes</i>	tlrl-hklm
PGN-B5	PGN from <i>B. subtilis</i>	tlrl-pgnb3
PGN-SA	PGN from <i>S. aureus</i>	tlrl-pgnsa

## TECHNICAL SUPPORT

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