

# Polymyxin B

An inhibitor of LPS-induced activation of TLR4 - InvitroFit™

Catalog code: tlrl-pmb

<https://www.invivogen.com/polymyxin-b>

For research use only

Version 24B05-MM

## PRODUCT INFORMATION

### Contents

- 100 mg Polymyxin B sulfate - InvitroFit™

### Storage and stability

- Polymyxin B is shipped at room temperature. Upon receipt, store at 4°C.
- Upon resuspension, store at 4°C. Resuspended product is stable for 1 month at 4°C when properly stored.

### Quality control

- The inhibitory activity has been validated using cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

## DESCRIPTION

Polymyxin B is a cyclic cationic polypeptide antibiotic produced by the soil bacterium *Paenibacillus polymyxa*. It blocks the biological effects of Gram-negative lipopolysaccharide (LPS, also known as endotoxin) through binding to lipid A, the toxic component of LPS, which is negatively charged<sup>1,2</sup>.

LPS binds to Toll-like receptor 4 (TLR4) triggering an increase in transcription factor NF-κB, activator protein 1 (AP-1), and pro-inflammatory cytokines such as interleukin-6 (IL-6). The neutralizing effect of Polymyxin B on LPS is dose-related and specific for LPS<sup>3</sup>.

Of note, Polymyxin B is widely used to eliminate the effects of endotoxin contamination, both *in vitro* and *in vivo*. Indeed, Polymyxin B was commonly used in clinical treatment until the 1980s, when its nephrotoxicity led to a decline in its use. More recently, polymyxin B has resurged as a last-resort antibiotics against multi-drug resistant Gram-negative bacteria<sup>4</sup>.

1. Goode A. *et al.*, 2021. Interactions of polymyxin B with lipopolysaccharide-containing membranes. *Faraday Discuss.* 232(0):317-329. 2. Velkov T. *et al.*, 2013. Pharmacology of polymyxins: new insights into an 'old' class of antibiotics. *Future Microbiol.* 8(6):711-24. 3. Alipour M. *et al.*, 2008. Antimicrobial effectiveness of liposomal polymyxin B against resistant Gram-negative bacterial strains. *Int J Pharm.* 355(1-2):293-8. 4. Trimble M.J. *et al.*, 2016. Polymyxin: Alternative Mechanisms of Action and Resistance. *Cold Spring Harb Perspect Med.* 6(10):a025288.

## CHEMICAL PROPERTIES

CAS number: 1405-20-5

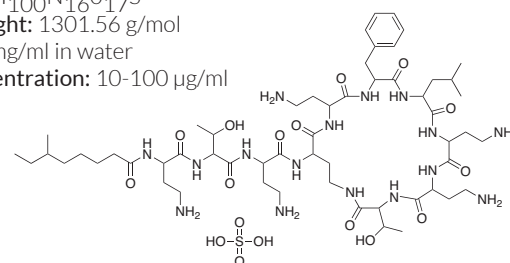
Formula: C<sub>56</sub>H<sub>100</sub>N<sub>16</sub>O<sub>17</sub>S

Molecular weight: 1301.56 g/mol

Solubility: 50 mg/ml in water

Working concentration: 10-100 µg/ml

Chemical structure:



## METHODS

### Preparation of Polymyxin B stock solution (50 mg/ml)

1. Add 2 ml of water to 100 mg.
2. Vortex until completely dissolved.
3. Filter sterilize and store at 4°C.

### TLR4 signaling using HEK-Blue™ TLR4 cells:

HEK-Blue™ TLR4 cells are engineered HEK293 cells that stably express an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene, and the TLR4, MD2 and CD14 genes. LPS-induced TLR4 activation in HEK-Blue™ TLR4 cells induces the activation of NF-κB and the subsequent release of SEAP which can be easily determined using QUANTI-Blue™ Solution, a SEAP detection reagent. For more information, visit [www.invivogen.com/hek-blue-trl4](http://www.invivogen.com/hek-blue-trl4).

1. Prepare a HEK-Blue™ hTLR4 cell suspension at ~150,000 cells/ml in culture medium.
2. Add 20 µl of Polymyxin B (10-100 µg/ml final concentration) in each well.
3. Add 160 µl of cell suspension (~25,000 cells) per well.
4. Add 20 µl of LPS-EK Ultrapure (10 ng/ml final concentration) per well of a flat-bottom 96-well plate.
5. Incubate the plate at 37°C in a 5% CO<sub>2</sub> incubator for 18-24 h.
6. Monitor SEAP production using a SEAP detection assay such as QUANTI-Blue™ Solution.

## RELATED PRODUCTS

Product	Description	Cat.Code
HEK-Blue™ hTLR4 Cells	Human TLR4 reporter cells	hkb-htlr4
HEK-Blue™ mTLR4 Cells	Murine TLR4 reporter cells	hkb-mtlr4
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
LPS-EK Ultrapure	TLR4 agonist	tlrl-peklps

## TECHNICAL SUPPORT

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