

# CRX-527

Synthetic Lipid A mimic; TLR4 agonist

Catalog Code: tlr1-crx527

<https://www.invivogen.com/crx527>

For research use only

Version 19E14-ED

## PRODUCT INFORMATION

### Contents

- 1 mg CRX-527

### Storage and stability

- CRX-527 is provided as a clear lipidic film and shipped at room temperature. Upon receipt, store product at -20°C.
- Store resuspended product at -20°C. Resuspended product is stable for at least 6 months when properly stored.
- Avoid repeated freeze-thaw cycles.

### Quality control

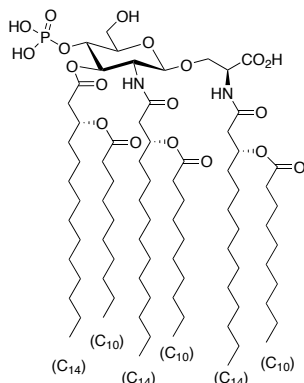
- Activation of TLR4 by CRX-527 has been confirmed using HEK-Blue™ hTLR4 cellular assays.
- Absence of bacterial contamination (e.g. lipoproteins) has been confirmed using HEK-Blue™ hTLR2 cellular assays.

## PRODUCT DESCRIPTION

CRX-527 is a synthetic lipid A mimic belonging to the family of aminoacyl glucoaminide 4-phosphates (AGPs). Specifically, Lipid A is the active component of lipopolysaccharide (LPS) that binds to and activates Toll-like receptor 4 (TLR4) and initiates subsequent pro-inflammatory signaling pathways. AGPs are less toxic than LPS but retain the important immunostimulatory properties and therefore have potential applications as vaccine adjuvants or stand-alone immunostimulants<sup>1</sup>.

CRX-527 is a hexa-acylate compound composed of three myristic and deaconic acyl chains. It is described as the most powerful synthetic lipid A mimic of the AGP family<sup>1,2</sup>. CRX-527 is a highly-specific and potent TLR4 agonist however, unlike other TLR4 ligands, CRX-527 does not require the co-receptor CD14 for its interaction with, and activation of TLR4<sup>2</sup>. The further development of synthetic lipid A mimics like CRX-527 is important in the development of novel vaccine adjuvants and prophylactic protection against Gram-negative bacterial infection<sup>3</sup>.

1. Stover, A.G. *et al.*, 2003. Structure-activity relationship of synthetic Toll-like receptor 4 agonists. *J. Biol. Chem.* 279:4440-4449. 2. Legat, A. *et al.*, 2010. CD14-independent responses induced by a synthetic lipid A mimetic. *Eur. J. Immunol.* 40:792-802. 3. Tan, Z.Y. *et al.*, 2013. Synthetic TLR4 agonists as a potential immunotherapy for melioidosis. *Open J Immunol.* 3: 1-9. doi: 10.4236/oji.2013.31001.



## CHEMICAL PROPERTIES

- CAS Number: 216014-14-1
- Formula: C<sub>81</sub>H<sub>151</sub>N<sub>2</sub>O<sub>19</sub>P
- Molecular weight: 1488.07 g/mol
- Purity: UHPLC >95%
- Endotoxin Level: >5 x 10<sup>4</sup> EU/mg (HEK-Blue™ Detection Kit II)
- Solubility: 1 mg/ml DMSO

## METHODS

### Preparation of stock solution (1 mg/ml)

1. Add 1ml of DMSO containing 0.2% triethylamine (TEA)
2. Sonicate for 10 minutes, until completely dissolved
3. Use immediately or store aliquots at -20°C
4. Dilutions can be prepared using sterile endotoxin-free water

Working concentration range: 100 pg/ml - 10 ng/ml

### Activation of TLR4 by CRX-527

Below is a protocol for using InvivoGen's HEK-Blue™ TLR4 cells, which express an inducible SEAP reporter, to readily measure the activation of TLR4. These cells have been designed to study TLR4 stimulation, with a TLR4 agonist, such as CRX-527, by monitoring NF-κB activation which induces the production of SEAP. Levels of SEAP can be easily determined using HEK-Blue™ Detection, a SEAP detection cell culture medium.

Note: For the full description of the HEK-Blue™ TLR4 cells, please visit <https://www.invivogen.com/hek-blue-htlr4>

1. Add 20 µl of CRX-527 (100 pg/ml - 10 ng/ml final concentration) per well of a flat bottom 96-well plate.
2. Prepare a suspension of HEK-Blue™ hTLR4 cells (~140,000 cells per ml) in HEK-Blue™ Detection medium.
3. Immediately add 180 µl of the cell suspension (~25,000 cells) to each CRX-527-containing well.
4. Incubate the plate at 37°C in a CO<sub>2</sub> incubator for 6-24 hours.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

Note: QUANTI-Blue™ solution, a SEAP detection reagent, can also be used to detect the activation of HEK-Blue™ hTLR4 cells by CRX-527.

## RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ hTLR4 cells	Human TLR4 reporter cells	hkb-htlr4
HEK-Blue™ Detection	SEAP detection medium	hb-det2
Quanti-Blue™ Solution	SEAP detection reagent	rep-qbs

## TECHNICAL SUPPORT

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