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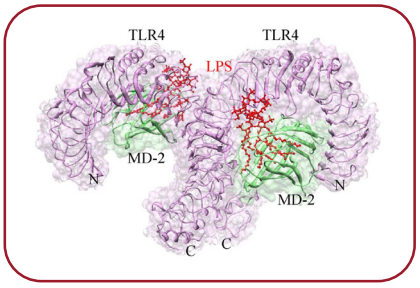
United Kingdom
Email: info@innaxon.com
Website: www.innaxon.co.uk



PRODUCT DATA SHEET

Lipid A from S. minnesota R595 (Re) TLRpure™ Sterile Solution

Cat. No.: IAX-100-001 **Lot. No.:**

Source	Lipid A derived from S. minnesota R595 (Re) TLRpure™ LPS
Concentration	1mg/ml (0.5mg/ml for 250µg size) stabilised in sterile, double-distilled water (ddWater) without any additives
TLRpure™	No detectable TLR4 *independent* activity: standardised potent TLR4-specific agonist
Purity	Ultrapure. No detectable DNA, RNA and protein traces.
Purification Method	R-type (mutant/rough) LPS was isolated by a phenol-chloroform-petroleum-ether method. Semi-purified LPS was subjected to further re-extraction cycles and ultracentrifugation steps, extensively electrodialysed to yield TLRpure™ LPS, from which Lipid A was generated by mild acid hydrolysis.
Sterility	Filter method: according to Ph. Eur. 9. Passed according to specification: • No growth in Thioglycolate medium at 30-35°C after 14 days. • No growth in Soybean Casein Digest Broth (TSB) at 20-25°C after 14 days.
Endotoxin Content	Bacterial Endotoxin Test (kinetic turbidimetric LAL method) according to Ph. Eur. 9. Endotoxin Content: >5,000,000 [EU/ml].
Appearance	Colourless, clear, aqueous solution
Handling	Keep sterile. Prepare working dilutions from pre-warmed (~40°C) Lipid A stock solution just prior to use. Ready-to-use, sterile stock solution is cell culture-grade. No solubilisation required. Do not pre-dilute in buffer (e.g. PBS) as this will lead to precipitation of Lipid A. To yield a 100µg/ml (1,000-100x) stock solution add 100µl of Lipid A to 900µl endotoxin-free and sterile ddWater (Cat. No.: IAX-900-002) (not PBS) and mix well.
Activity	Optimal concentration is dependent upon cell type, species, desired activation and analysis: 0.1-1.0µg/ml *in vitro* and 5-15mg/kg *in vivo* in animal rodent models. Does not activate any TLR other than TLR4 as tested up to 1µg/ml in relevant cellular systems (mouse macrophages).
Shipping	Ambient
Storage	2-8°C
Stability	• Upon receipt, store product at 2-8°C. • Do not freeze. In its unopened original vial, the product is stable for at least 24 months when stored at 2-8°C. Once the glass vial is opened, or if aliquoted into sterile vials under sterile conditions, the product remains stable for an additional 12 months at 2-8°C. Pre-diluted, sterile aqueous solutions (e.g., 10-100µg/ml) are stable for a maximum of 12 hours when stored at 2-8°C due to vial surface effects on diluted solutions.
MSDS	Available on request

Document No.: IAX-100-001 | **Version:** 2.1 | **Issue Date:** 26/07/2024

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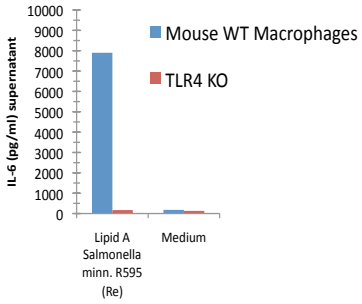

Figure:
Macrophages from wild-type (WT) TLR4 expressing or TLR4 deficient (TLR4 KO) mice were stimulated with 1μg/ml TLR*pure*™ Lipid A from S. *minnesota*. Cell culture supernatants were analysed by ELISA for IL-6 after 24h. Optimal concentrations required for activation depend upon cell species (murine, human, others), cell culture conditions (FCS concentration), sampling time and cytokine analysis. Recommended range for Lipid A: 0.1-1.0μg/ml.

Product Information

- Lipid A has been generated by mild acid hydrolysis from TLRpure™ LPS purified according to an optimised and proprietary extraction and purification protocol, but based upon the methods published by Galanos, et al. (laboratory of Westphal and Lüderitz, Freiburg, Germany).
- TLRpure™ LPS lacks any detectable bacterial, (lipo-)protein, RNA or DNA or other TLR-stimulating activity due to its ultra-purified formulation. Its unique potency and purity are quality controlled using a physiological system of primary innate immune cells and a relevant biological cytokine expression read-out.
- All immunological activity of the Lipid A is exclusively dependent upon the presence of TLR4 as determined by the use of the corresponding control cells, where TLR4 has been genetically deleted or missing (from TLR4 deficient also called TLR4 knock-out KO mice).
- TLRpure™ Lipid A convenient ready-made stabilised solution makes it the reagent of choice for in vitro as well as in vivo experiments for superior reproducible and comparable results.
- Compared to Lipid A derived from conventional (semi-purified) LPS preparations, this product is derived from low yield TLRpure™ LPS produced on an industrial fermentation scale under precisely controlled growth conditions to yield large batch sizes, allowing custom formulations/packaging.

Product Specific References

[1] *A new method for the extraction of R lipopolysaccharides*. Galanos C, et al. Eur. J. Biochem. (1969); 9:245

[2] *Endotoxic properties of chemically synthesized lipid A part structures. Comparison of synthetic lipid A precursor and synthetic analogues with biosynthetic lipid A precursor and free lipid A.* Galanos C, et al. Eur. J. Biochem. (1984); 140:221

[3] *Guanylate-Binding Proteins (GBPs) convert cytosolic bacteria into caspase-4 signaling platforms.* Wandel MP, et al. Nat. Immunol. 2020; 21: 880–891

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General Information

- Activation of cells by LPS is mediated by the Toll-like receptor 4 (TLR4), a member of the highly conserved protein family of TLRs, which are specialised in the recognition of microbial components. In mice, defects in TLR4 result in LPS unresponsiveness.
- For optimal interaction with LPS, TLR4 requires association with myeloid differentiation protein 2 (MD-2). According to current consensus activation of TLR4 is preceded by the transfer of LPS to membrane-bound (m) or soluble (s) CD14 by LPS-binding protein (LBP). This mechanism is believed to be generally true for LPS signaling. Re-form LPS and lipid A, but not S-form LPS, are capable of inducing TNF-α responses also in the absence of CD14.
- LPS, synthesised by most wild-type (WT) Gram-negative bacteria (S-form LPS), consists of three regions, the O-polysaccharide chain, which is made up of repeating oligosaccharide units, the core oligosaccharide and the lipid A, which harbors the endotoxic activity of the entire molecule. R-form LPS synthesised by the so-called rough (R) mutants of Gram-negative bacteria lacks the O-specific chain. Furthermore, the core-oligosaccharide may be present in different degrees of completion, depending on the class (Ra to Re) to which the mutant belongs. Monophosphoryl Lipid A (MPLA) represents a detoxified derivative of Lipid A and constitutes an important adjuvant in prophylactic and therapeutic vaccines.

References

[1] *R-form LPS, the master key to the activation of TLR4/MD-2-positive cells*. Huber M, et al. Eur. J. Immunol. (2006); 36:701

[2] *CD14 is required for MyD88-independent LPS signaling*. Jiang Z, Georgel P, Du X, Shamel L, Sovath S, Mudd S, Huber M, Kalis C, Keck S, Galanos C, Freudenberg M, Beutler B. Nat. Immunol. (2005); 6:565

[3] *Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene*. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Science (1998); 282:2085

[4] *Structural relationship of Salmonella 0 and R antigens*. Lüderitz O, Galanos C, et al. Ann. N.Y. Acad. Sci. (1966); 133:349

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[6] *Lipid A: chemical structure and biological activity*. Lüderitz O, Galanos C, et al. J. Infect. Dis. (1973); 128:17

[7] *Purification and structural determination of nontoxic lipid A obtained from the lipopolysaccharide of Salmonella typhimurium*. Qureshi N, Takayama K, Ribi E. J. Biol. Chem. (1982); 257:11808