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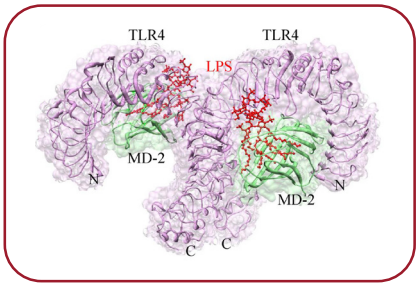

PRODUCT DATA SHEET

LPS from S. minnesota (S-form) TLRpure™ Sterile Solution

Cat. No.: IAX-100-020 **Lot. No.:**

Source	Lipopolysaccharide (LPS) from Salmonella minnesota, S-type (smooth/wild-type) LPS
Concentration	1mg/ml stabilised in sterile, double-distilled water (ddWater), without any additives
TLRpure™	No detectable TLR4 *independent* activity as determined by a mouse macrophage cell culture cytokine secretion assay using TLR4 deficient versus wild-type cells: standardised potent TLR4-specific agonist
Purity	Ultrapure. No detectable DNA, RNA and protein traces.
Purification Method	S-type LPS was isolated by the hot phenol-water method. Semi-purified LPS was subjected to further re-extraction cycles and ultracentrifugation steps, extensively electrodialysed to yield TLRpure™ LPS.
Sterility	Filter method: certified 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) certified according to Ph. Eur. 9. Endotoxin Content: >5,000,000 [EU/ml].
Appearance	Colourless, clear, aqueous solution
Handling	Keep sterile. Prepare aliquots or working dilutions from pre-warmed (~40°C) LPS stock solution just prior to use. Ready-to-use, sterile stock solution is cell culture-grade. No solubilisation required. To yield a 100µg/ml (100x) stock solution, add 100µl of LPS to 900µl endotoxin-free and sterile ddWater (Cat. No.: IAX-900-002), or 0.9% NaCl Solution (Cat. No.: IAX-900-003) or PBS (Cat. No.: IAX-900-001) and mix well.
Activity	Optimal concentration is dependent upon cell type, species, desired activation and analysis: 0.01-1.0µg/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

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S. minnesota TLRpure™ LPS is a TLR4 specific agonist

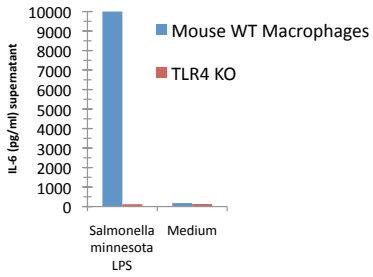

FIGURE:
Macrophages from wild-type (WT) TLR4 expressing or TLR4 deficient (TLR4 KO) mice were stimulated with 1µg/ml TLRpure™ S. minnesota S-LPS. Cell culture supernatants were analysed by ELISA for IL-6 after 24h. Optimal concentrations required for activation depend upon cell species (murine, human, others), cell culture conditions (FCS concentration), sampling time and cytokine analysis. Recommended range for S-type (wild-type) LPS: 0.01-1.0µg/ml.

Product Description

- TLRpure™ LPS has been 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.
- Due to its amphipatic structure and strong tendency to form micelles, the generation of LPS, which is devoid of any non-TLR4 dependent immune modulatory activity, presents a major biochemical purification and analytical challenge. All immunological activity of TLRpure™ LPS is exclusively dependent upon the presence of TLR4 as determined by the use of the corresponding control cells, derived from TLR4 deficient (TLR4 knock-out, KO) mice.
- TLRpure™ LPS convenient ready-made stabilised solution makes it the reagent of choice for *in vitro* and *in vivo* experiments for superior reproducible and comparable results. These unique LPS preparations have been used in numerous publications since 1969. Compared to conventional (semipurified) LPS preparations, this low yield TLRpure™ LPS is produced on an industrial fermentation scale under precisely controlled growth conditions to yield large batch sizes, thus allowing custom formulations/packaging.

Product Specific References

[1] *Induction of human granulocyte chemiluminescence by bacterial lipopolysaccharides.* Kapp A, Freudenberg M, Galanos C. Infect. Immun. (1987); 55:758

[2] *Differential clearance and induction of host responses by various administered or released lipopolysaccharides.* Hasunuma R, Morita H, Tanaka S, Ryll R, Freudenberg MA, Galanos C, Kumazawa Y. J. Endotoxin Res. (2001); 7:421

[3] *Synthetic Glycolipids as Molecular Vaccine Adjuvants: Mechanism of Action in Human Cells and In Vivo Activity.* Facchini F A, Minotti A, Pirianov G, Peri F, et al. J. Med. Chem. (2021); 64:12261-12272

[4] *Synthetic glycolipid-based TLR4 antagonists negatively regulate TRIF-dependent TLR4 signalling in human macrophages.* Palmer C, Facchini F A, Jones R PO, Neumann F, Peri F, Pirianov G. Innate Immunity (2021); 27:275-284

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[5] *Overcoming Challenges in Chemical Glycosylation to Achieve Innovative Vaccine Adjuvants Possessing Enhanced TLR4 Activity.* Romerio A, Franco A R, Peri F, et al. ACS Omega (2023); 8:36412-36417

[6] *New Glucosamine-Based TLR4 Agonists: Design, Synthesis, Mechanism of Action, and In Vivo Activity as Vaccine Adjuvants.* Romerio A, Peri F, et al. J. Med. Chem. (2023); 66:3010-3029

General Information

- The innate immune response to lipopolysaccharide is essential for host defense against Gram-negative bacteria. In response to bacterial infection, the TLR4/MD-2 complex that is expressed on the surface of macrophages, monocytes, dendritic, and epithelial cells senses picomolar concentrations of endotoxic LPS and triggers the production of various pro-inflammatory mediators.
- Activation of cells by LPS is mediated on the plasma membrane 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-a 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. Notably, LPS from WT bacteria are always highly heterogeneous mixtures of S-form LPS molecules containing 1 to over 50 repeating oligosaccharide units and contain ubiquitously a varying proportion of R-form molecules lacking the O-specific chain. LPS are amphipathic molecules whose hydrophobicity decreases with increasing length of the sugar part.
- Based upon these differences, S- and R-form LPS show marked differences in the kinetics of their blood clearance and cellular uptake as well as in the ability to induce oxidative burst in human granulocytes and to activate the host complement system.
- In addition, LPS from extracellular bacteria which is either endocytosed or transfected into the cytosol of host cells or cytosolic LPS produced by intracellular bacteria is recognised by cytosolic proteases caspase-4/11 and hosts guanylate binding proteins that are involved in the assembly and activation of the NLRP3 inflammasome.
- One of the plausible mechanisms for LPS internalisation and intracellular delivery involves LPS binding by high-mobility group box 1 (HMGB1) - an alarmin which can efficiently transport LPS into the cytoplasm through receptor for advanced glycation end products (RAGE)-mediated endocytosis. Through internalisation of HMGB1-LPS complexes mediated by RAGE, HMGB1 induces destabilisation of lysosomes for cytosolic LPS delivery.
- It has been also suggested that outer membrane vesicles (OMVs) — the naturally secreted products of Gram-negative bacteria — can function as cytosolic LPS delivery vehicles.

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References

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

[2] *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

[3] *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

[4] *Defective immunogenic cell death of HMGB1-deficient tumors: compensatory therapy with TLR4 agonists*. Yamazaki T, et al. Cell Death and Differentiation (2014); 21:69

[5] *Lipopolysaccharide Recognition in the Crossroads of TLR4 and Caspase-4/11 Mediated Inflammatory Pathways*. Zamyatina A , Heine H. Front Immunol. (2020); 11: 585146