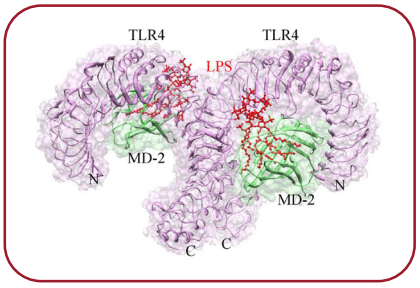
Innaxon
United Kingdom
Email: info@innaxon.com
Website: www.innaxon.co.uk



PRODUCT DATA SHEET

LPS from S. abortus equi (S-form) *Biotin* TLRpure™ Sterile Solution

Cat. No.: IAX-100-009B **Lot. No.:**

Source	Biotinylated Lipopolysaccharide (LPS) from S. abortus equi, S-type (smooth/wild-type) 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 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 a modified phenol-chloroform-petroleum ether method. Semi-purified LPS was subjected to further re-extraction cycles and ultracentrifugation steps, extensively electrodialysed to yield TLRpure™ LPS. TLRpure™ LPS-Biotin was prepared using the biotin reagent biotinamidocaproate N-hydroxysuccinimide ester. Briefly, TLRpure™ LPS at 10mg/ml in distilled water was mixed with biotin reagent in sodium bicarbonate buffer. The reaction mixture was stirred, dialysed extensively against distilled water in the dark, and sterile filtered.
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 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-Biotin to 900µl endotoxin-free and sterile ddWater (Cat. No.: IAX-900-002), 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/staining and analysis: 1-10µg/ml. Does not activate any TLR other than TLR4 as tested up to 10µg/ml in relevant cellular systems (mouse macrophages). LPS binding to monocytes was evaluated by using LPS from S. abortus equi (S-form) Biotin (LPS Biotin) (as described in [1-3]). Briefly, for the kinetic studies, LPS Biotin (5µg/ml) was added to 100µl of heparinised whole human blood and incubated at 37°C in a 5% CO_2 atmosphere for different periods of time. A dose-response curve of LPS Biotin was performed with increasing concentrations of LPS Biotin and an incubation period of 20 min. Samples were incubated with CD14-FITC and HLA-DR PE monoclonal antibodies and with streptavidin-APC for 15 min in the dark, at room temperature. Monocytes were identified with the use of CD14-FITC and side scatter, and LPS Biotin positively stained monocytes with the use of streptavidin-APC.
Shipping	Ambient
Storage	2-8°C (short-term storage). Prepare aliquots and store between -15 and -25°C (shelf-life 2 years). Avoid freeze/thaw cycles.

Document No.: IAX-100-009B | **Version:** 1.9 | **Issue Date:** 01/08/2024

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

LPS from S. abortus equi (S-form) *Biotin* TLRpure™ Sterile Solution

Cat. No.: IAX-100-009B **Lot. No.:**

Stability

- Upon receipt, prepare aliquots into sterile vials and store between -15 and -25°C.

In its unopened original vial, the product is stable for at least 24 months when stored between -15 and -25°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 between -15 and -25°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

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] *Lipopolysaccharide-cell interaction and induced cellular activation in whole blood of septic patients.* Salomao R, et al. J. Endotoxin Res. (2002); 8:371

[2] *Influence of EDTA and heparin on lipopolysaccharide binding and cell activation, evaluated at single-cell level in whole blood.* Brunialti MKC, et al. Cytometry (2002); 50:14

[3] *Peripheral blood mononuclear cell activation induced by Leptospira interrogans glycolipoprotein.* Diament D, et al. Infect. Immun. (2002); 70:677

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

LPS from S. abortus equi (S-form) *Biotin* TLRpure™ Sterile Solution

Cat. No.: IAX-100-009B **Lot. No.:**

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 TLR, 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 true for LPS signaling generally. 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) gramnegative 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. Instead of using TLR4 specific antibodies, the method of using a labelled TLR4 ligand such as LPS for the detection of surface receptor levels capable of binding to LPS offers an attractive alternative detection tool. This is in particular the case, if for certain species other than human TLR4 specific antibodies of sufficient high quality are not readily available. Biotinylated S-type LPS can serve as a useful reagent for evaluating LPS binding and cell activation in white blood cells and may be used to analyse LPS tissue distribution in vivo by immunohistochemistry using streptavidin-conjugates.
- As the biotinylation reagent and standard biotinylation protocol favors protein/peptide (contaminants) about 1,000-fold over sugar (LPS) as a target, it is mandatory to use >99.9% pure LPS to exclude the biotinylation of contaminants. The latter, especially bacterial lipoproteins are usually present in most commercial LPS preparations and would lead to misleading observations of the tracking/staining with those reagents. TLRpure™ fulfills such high purity requirements and the proprietary biotinylation procedure preserves specific TLR4 binding and cellular activation.

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

[6] *Immunoblot analysis of the R-form lipopolysaccharide from Salmonella S forms*. Schlecht S, Freudenberg MA, Galanos C. Zentralbl. Bakteriol. (1992); 277:288