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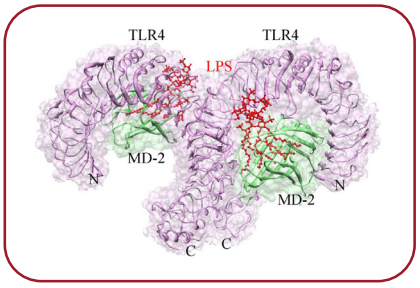




**PRODUCT DATA SHEET** 

# 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 (0.5 mg/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** | S-type LPS was isolated by a modified phenol-chloroform-petroleum ether method. Semi-purified LPS was sujected to further re-extraction cycles and ultracentrifugation steps, extensively electrodialysed before converted to its uniform sodium salt form 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: according to Ph. Eur. 9.<br>Passed according to specification:<br>• No growth in Thioglycolate medium at 30-35°C after 14 days.<br>• 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.<br>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-made solution is cell culturegrade. 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: 1-10 µg/ml. Does not activate any TLR other than TLR4 as tested up to 10µg/ml in relevant cellular systems (mouse macrophages). |
| **Shipping** | Ambient |
| **Storage** | 2-8°C. Prepare aliquots and store between -15 and -25°C (shelf-life 2 years). Avoid freeze/thaw cycles. |
| **Stability** | 12 months after receipt (unopened and as supplied). Diluted solutions are stable for 12 hours at 2-8°C. |
| **MSDS** | Available on request |

**Document No.:** IAX-100-009B | **Version:** 1.6 | **Issue Date:** 24/01/2023

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

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

**Product Description**

- TLRpure™ LPS has been purified according to an optimized 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

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

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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 TLR, which are specialized 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, synthesized 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