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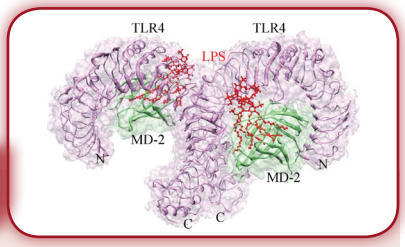
PRODUCT DATA SHEET



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

Cat. No.: IAX-100-009B **Date:** 08-Jan-2013

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.
TLR*pure*™:	No detectable TLR4 *independent* activity: standardised potent TLR4-specific agonist and ligand.
PURITY:	≥99.9 %. No detectable DNA, RNA and protein traces.
PURIFICATION METHOD:	S-type LPS was isolated by the hot phenol-water method followed by a modified phenol-chloroform-petroleum-ether method. Semi-purified LPS was subjected to further re-extraction cycles and ultracentrifugation steps, extensively electrodialysed before converted to its uniform sodium salt form to yield TLR*pure*™ LPS. TLR*pure*™ LPS-Biotin was prepared using the biotin reagent biotinamidocaproate N-hydroxysuccinimide ester. Briefly, TLR*pure*™ 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.
APPEARANCE:	Colourless clear aqueous solution.
HANDLING:	Prepare diluted LPS-Biotin working solutions just prior to use, keep sterile. Ready-made solution is cell culture-grade. 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.
SHIPPING:	Ambient.
STORAGE:	4°C. Prepare aliquots and store at -20°C (shelf-life 2 years). Avoid freeze/thaw cycles.
STABILITY:	12 months after receipt as supplied.

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-α 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.

TLR*pure*™ fulfills such high purity requirements and the proprietary biotinylation procedure preserves specific TLR4 binding and cellular activation.

References:

[1] *Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene*. Poltorak A, et al. Science (1998); 282:2085

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

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

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