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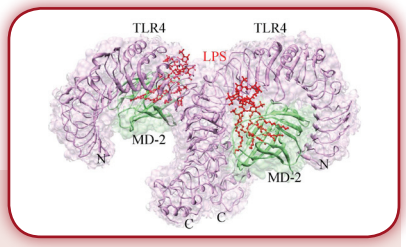
PRODUCT DATA SHEET



LPS from E. coli O8:K27 (S-form) TLR*pure*™ Sterile Solution

Cat. No.: IAX-100-006 **Date:** 08-Jan-2013

SOURCE:	Lipopolysaccharide (LPS) from E. *coli* O8:K27, S-type (smooth/wild-type) LPS.
CONCENTRATION:	1mg/ml stabilised in sterile, double-distilled water (ddWater), without any additives.
TLR*pure*™:	No detectable TLR4 *independent* activity: standardised potent TLR4-specific agonist.
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.
APPEARANCE:	Colourless clear aqueous solution.
HANDLING:	Prepare diluted LPS working solutions just prior to use, keep sterile. Ready-made solution is cell culture-grade. To yield a 100µg/ml (1,000-100x) stock solution add 100µl of LPS 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 and analysis: 0.01-1.0µg/ml. Does not activate any TLR other than TLR4 as tested up to 50µg/ml in relevant cellular systems (macrophages).
SHIPPING:	Ambient.
STORAGE:	4°C. Do not freeze.
STABILITY:	2 years after receipt.

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, synthesized 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 synthesized 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.

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

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E. *coli* TLR*pure*™ O8:K27 LPS is a TLR4 specific agonist

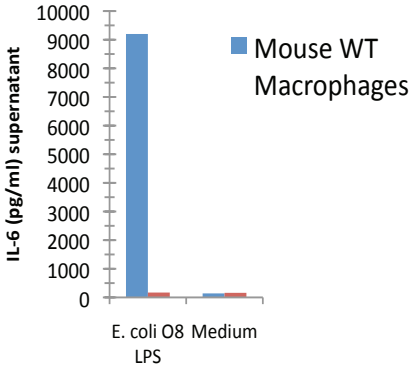

FIGURE:
Macrophages from wild-type (WT) TLR4 expressing or TLR4 deficient (TLR4 KO) mice were stimulated with 1μg/ml E. *coli* O8:K27 S-(smooth) LPS.
Cell culture supernatants were analyzed by ELISA for IL-6 after 24h.
Optimal concentrations required for activation depend upon bacterial strain and chemotype (R- or S-) LPS, cell species (murine, human, others), cell culture conditions (FCS concentration), sampling time and cytokine.
Recommended range for S-type (wild-type) LPS: 0.01-1.0μg/ml.

Product Description:

TLR*pure*™ 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). TLR*pure*™ 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 TLR*pure*™ 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.
TLR*pure*™ 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 (semi-purified) LPS preparations, this low yield TLR*pure*™ 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] *Attachment to erythrocytes of uniform salt forms of lipopolysaccharides from Salmonella abortus-equi and its inhibition by various animal sera.* Praino MD, Galanos C, Neter E. Immunol. Commun. (1979); 8:85
[2] *Preparation and properties of a standardized lipopolysaccharide from salmonella abortus equi*. Galanos C, et al. Zentralbl. Bakteriol. Orig. A. (1979); 243:226
[3] *Large-scale fractionation of S-form lipopolysaccharide from Salmonella abortus equi. Chemical and serological characterization of the fractions*. Galanos C, Jiao BH, Komuro T, Freudenberg MA, Lüderitz O. J. Chromatogr. (1988); 440:397
[4] *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
[5] *Immunoblot analysis of the R-form lipopolysaccharide from Salmonella S forms.* Schlecht S, Freudenberg MA, Galanos C. Zentralbl. Bakteriol. (1992); 277:288