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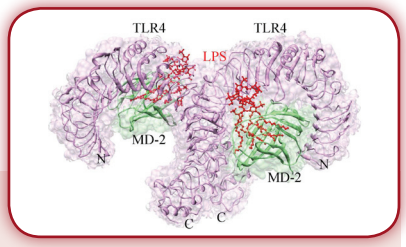
PRODUCT DATA SHEET



Lipid A from E. coli R515 (Re) TLR*pure*™ Sterile Solution

Cat. No.: IAX-100-004 **Date:** 08-Jan-2013

SOURCE:	Lipid A derived from E. *coli* R515 (Re) LPS (biosynthetic).
CONCENTRATION:	1mg/ml (0.5mg/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.
PURITY:	≥99.9 %. No detectable DNA, RNA and protein traces.
PURIFICATION METHOD:	R-type (mutant/rough) LPS was isolated by a 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, from which Lipid A was generated by mild acid hydrolysis.
APPEARANCE:	Colourless opaque aqueous solution.
HANDLING:	Prepare diluted Lipid A working solutions in water just prior to use, keep sterile. Do not pre-dilute in buffer (e.g. PBS) as this will lead to precipitation of Lipid A. To yield a 100µg/ml (1,000-100x) stock solution add 100µl of Lipid A to 900µl endotoxin-free sterile water (not PBS) and mix well. Ready-made solution is cell culture-grade.
ACTIVITY:	Optimal concentration is dependent upon cell type, species, desired activation and analysis: 0.1-1.0µg/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. Monophosphoryl Lipid A (MPLA) represents a detoxified derivative of Lipid A and constitutes an important adjuvant in prophylactic and therapeutic vaccines.

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
[6] *Chemical structure of Escherichia coli lipid A.* Imoto M, et al. Tetrahedron Lett. (1985); 907:908

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E. *coli* TLR*pure*™ Lipid A is a TLR4 specific agonist

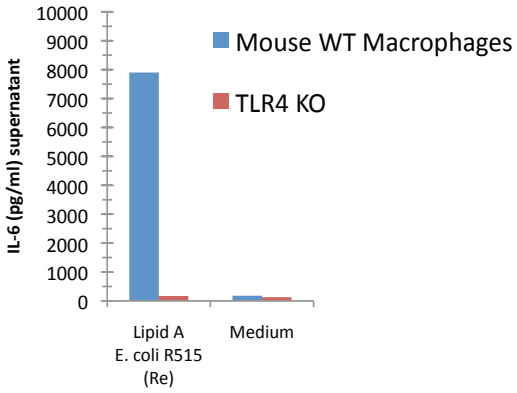

FIGURE:
Macrophages from wild-type (WT) TLR4 expressing or TLR4 deficient (TLR4 KO) mice were stimulated with 1μg/ml TLR*pure*™ Lipid A from E. *coli*.
Cell culture supernatants were analysed by ELISA for IL-6 after 24h.
Optimal concentrations required for activation depend upon bacterial strain and chemotype (R- or S-) LPS, cell species (murine, human, others), cell culture conditions (FCS concentration), sampling time and cytokine.
Recommended range for Lipid A: 0.1-1.0μg/ml.

Product Description:

Lipid A has been generated by mild acid hydrolysis from TLR*pure*™ LPS 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).
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.
All immunological activity of the Lipid A is exclusively dependent upon the presence of TLR4 as determined by the use of the corresponding control cells, where TLR4 has been genetically deleted or missing (from TLR4 deficient also called TLR4 knock-out KO mice).
TLR*pure*™ Lipid A convenient ready-made stabilised solution makes it the reagent of choice for *in vitro* as well as *in vivo* experiments for superior reproducible and comparable results.
Compared to Lipid A derived from conventional (semi-purified) LPS preparations, this product is derived from low yield TLR*pure*™ LPS produced on an industrial fermentation scale under precisely controlled growth conditions to yield large batch sizes, allowing custom formulations/packaging.

Product Specific References:

[1] *A new method for the extraction of R lipopolysaccharides.* Galanos C, et al. Eur. J. Biochem. (1969); 9:245

[2] *Preparation and properties of antisera against the lipid-A component of bacterial lipopolysaccharides.* Galanos C, et al. Eur. J. Biochem. (1971); 24:116

[3] *Endotoxic properties of chemically synthesized lipid A part structures. Comparison of synthetic lipid A precursor and synthetic analogues with biosynthetic lipid A precursor and free lipid A.* Galanos C, et al. Eur. J. Biochem. (1984); 140:221

[4] *Lipopolysaccharides: structural principles and biologic activities.* Lüderitz O, et al. Rev. Infect. Dis. (1984); 6:428

[5] *Synthetic and natural Escherichia coli free lipid A express identical endotoxic activities.* Galanos C, et al. Eur. J. Biochem. (1985); 148:1