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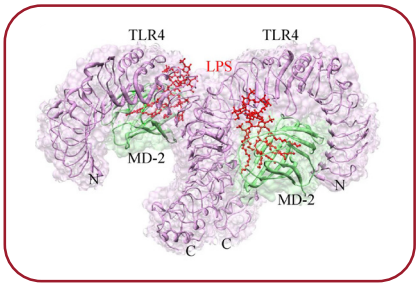

PRODUCT DATA SHEET

MPLA from E. coli R515 (Re) TLRpure™ Sterile Solution

Cat. No.: IAX-100-003 **Lot. No.:**

Source	Monophosphoryl Lipid A [MPLA] derived from E. coli R515 (Re) 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: standardised potent TLR4-specific agonist
Purity	Ultrapure. 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 to yield LPS, from which MPLA was generated by acid hydrolysis.
Sterility	Filter method: 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) according to Ph. Eur. 9. Endotoxin Content: >1,000,000 [EU/ml].
Appearance	Colourless, clear to opaque aqueous solution
Handling	Keep sterile. Prepare working dilutions from pre-warmed (~40°C) MPLA stock solution just prior to use. Ready-to-use, sterile stock solution is cell culture-grade. No solubilisation required. Do not pre-dilute in buffer (e.g. PBS) as this will lead to precipitation of MPLA. To yield a 100µg/ml (1,000-100x) stock solution add 100µl of MPLA to 900µl endotoxin-free and sterile ddWater (Cat. No.: IAX-900-002) (not PBS) and mix well.
Activity	Optimal concentration is dependent upon cell type, species, desired activation and analysis: 0.1-1.0µg/ml *in vitro* and 5-15mg/kg *in vivo* in animal rodent models. Does not activate any TLR other than TLR4 as tested up to 1µg/ml in relevant cellular systems (mouse macrophages).
Shipping	Ambient
Storage	2-8°C
Stability	• Upon receipt, store product at 2-8°C. • Do not freeze. In its unopened original vial, the product is stable for at least 24 months when stored at 2-8°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 at 2-8°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

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Product Information

- MPLA has been generated by acid hydrolysis from TLRpure™ 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).
- 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.
- All immunological activity of the MPLA 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).
- TLRpure™ MPLA 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 MPLA derived from conventional (semi-purified) LPS preparations, this product is derived from low yield TLRpure™ 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

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

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

[7] *Purification and structural determination of nontoxic lipid A obtained from the lipopolysaccharide of Salmonella typhimurium*. Qureshi N, Takayama K, Ribi E. J. Biol. Chem. (1982); 257:11808