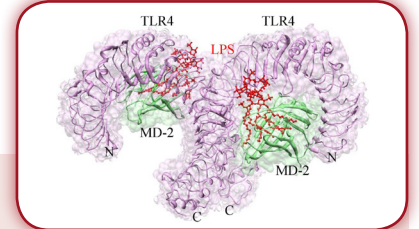


## PRODUCT DATA SHEET

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

Cat. No.: IAX-100-003

Date: 14-Dec-2014



|                             |   |
|-----------------------------|---|
| <b>SOURCE:</b>              | Monophosphoryl Lipid A [MPLA] derived from <i>E. coli</i> R515 (Re) LPS (biosynthetic).   |
| <b>CONCENTRATION:</b>       | 1mg/ml (0.5mg/ml for 250µg size) stabilised in sterile, double-distilled water (ddWater), without any additives.  |
| <b>TLRpure™:</b>            | No detectable TLR4 <i>independent</i> activity: standardised potent TLR4-specific agonist.  |
| <b>PURITY:</b>              | ≥99.9 %. No detectable DNA, RNA and protein traces.   |
| <b>PURIFICATION METHOD:</b> | 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 electro dialysed before converted to its uniform sodium salt form to yield LPS, from which MPLA was generated by acid hydrolysis and detoxification by alkali treatment. |
| <b>APPEARANCE:</b>          | Colourless opaque aqueous solution.   |
| <b>HANDLING:</b>            | Prepare diluted MPLA 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 MPLA. To yield a 100µg/ml (1,000-100x) stock solution add 100µl of MPLA to 900µl endotoxin-free sterile water (not PBS) and mix well. Ready-made solution is cell culture-grade.                            |
| <b>ACTIVITY:</b>            | 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).  |
| <b>SHIPPING:</b>            | Ambient.  |
| <b>STORAGE:</b>             | 4°C. Do not freeze.   |
| <b>STABILITY:</b>           | 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- $\alpha$  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:

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

**DISCLAIMER:** THIS PRODUCT IS NOT INTENDED OR APPROVED FOR HUMAN, DIAGNOSTICS OR VETERINARY USE. USE OF THIS PRODUCT FOR HUMAN OR ANIMAL TESTING MAY BE EXTREMELY HAZARDOUS AND MAY RESULT IN DISEASE, SEVERE INJURY, OR DEATH. THIS PRODUCT IS FOR RESEARCH USE ONLY (RUO).

**MATERIAL SAFETY DATA:** This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, inhale or get into the blood stream. Do not get in eyes, on skin, or clothing. Wash thoroughly after handling. This information contains some, but not all, of the information required for the safe and proper use of this material. Access to this material must be restricted to personnel, who is appropriately experienced, qualified, competent and properly trained to use it. Material Safety Data Sheet is available upon request.