# CERTIFICATE OF ANALYSIS – TECHNICAL DATA SHEET

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| **Product name** | Monosphosphoryl Lipid A (MPL-A) from E.coli, R515 (Re) | | |
| **Catalog number** | HC4050 | | |
| **Lot number** | - | **Expiry date** | - |
| **Volume** | 0.5 ml | **Activity** | N.A. |
| **Formulation** | Ready-to-use in aqueous solution in ultra-pure endotoxin-free double-distilled sterile water. | **Amount** | 0.5 mg |
| **Host Species** | *Escherichia coli (E.coli)* MPL-A generated from Lipid A from R515 LPS (Re), cell culture grade. | **Concentration** | 1 mg/ml |
| **Endotoxin** | N.A. | **Purification** | N.A. |
| **Storage** | 4°C | **Purity** | ≥99.9% |

## Application notes

| | IHC-F | IHC-P | IF | FC | FS | IA | IP | W |
|---|---|---|---|---|---|---|---|---|
| Reference # | | | | | | | | |
| Yes | | | | | • | | | |
| No | | | | | | | | |
| N.D. | • | • | • | • | | • | • | • |

*N.D.= Not Determined; IHC = Immuno histochemistry; F = Frozen sections; P = Paraffin sections; IF = Immuno Fluorescence; FC = Flow Cytometry; FS = Functional Studies; IA = Immuno Assays; IP = Immuno Precipitation; W = Western blot*

- Do not pre-dilute in buffer, e.g. PBS, as this will lead to precipitation of MPL-A. Prepare diluted MPLA working solutions just prior to use, keep sterile. Ready-made solution is cell culture-grade. Optimal concentration is dependent upon cell type, species, desired activation and analysis: 0.1 -1.0 µg/ml. Does not activate any TLR other than TLR4 as tested in relevant biological systems.
- The purity is ≥99.9 %. No detectable TLR4 independent activity: potent standardized TLR4-specific agonist.

## General Information

**Description**

Humans as well as other vertebrates are often exposed to lipopolysaccharide (LPS), for instance via enterobacteria. LPS responses are mediated via Toll-like receptor 4 (TLR4). TLRs are conserved pattern recognition receptors which recognize and respond to molecules derived from bacterial, viral and fungal pathogens, such as LPS from the outer membrane of Gram negative bacteria. Recognition of LPS occurs largely by the TLR4/MD2/CD14 complex, expressed among others by macrophages and dendritic cells. The acute phase LPS-binding protein (LBP) recognizes the lipid A part of LPS and catalyses the monomeric LPS transfer to CD14. This facilitates the LPS transfer to TLR4/MD2. All immunological activity of LPS is exclusively dependent upon the presence of TLR4 as determined by the usage of the corresponding control cells, where TLR4 is missing. Recognition of LPS triggers a cascade of adverse systemic responses and organ failure (septic shock). LPS is a key component of the cell wall of gram negative bacteria (S-form LPS). The molecule consist of three structural regions: the O-polysaccharide chain made up of repeating oligosaccharide units, the core oligosaccharide and Lipid A. The latter is responsible for 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. LPS from wild type bacteria are always a highly heterogeneous mixtures of S-form LPS molecules containing 1 to over 50 repeating oligosaccharide units and contain a varying proportion of R-form molecules. R-form LPS and lipid A, but not S-form LPS, are capable of inducing TNF-α responses also in the absence of CD14. 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. In mice, defects in TLR4 result in LPS unresponsiveness. According to current consensus, activation of TLR4 is preceded by the transfer of LPS to membrane-bound or soluble CD14 by LPS-binding protein (LBP). This mechanism is believed to be true for LPS signaling in general. Re-form LPS and lipid A, but not S-form LPS, are capable of inducing TNF-a responses also in the absence of CD14. LPS is an amphipathic molecule whose hydrophobicity decreases when the length of the sugar part of LPS increases. 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. MPL-A is a detoxified derivative of Lipid A that is the active endotoxic component of LPS. MPL-A represents an important adjuvant in vaccines.

**Storage&stability**

Product should be stored at 4°C. Do not freeze. Under recommended storage conditions, product is stable for at least one year.

**Precautions**

For research use only. Not for use in or on humans or animals or for diagnostics. Use of this product for human or animal testing is extremely hazardous and may result in disease, severe injury, or death.
It is the responsibility of the user to comply with all local/state and federal rules in the use of this product. Hycult Biotech is not responsible for any patent infringements that might result from the use or derivation of this product.

Material Safety Data Sheet: This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, inhale or get in the blood stream.

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We hereby certify that the above-stated information is correct and that this product has been successfully tested by the Quality Control Department. This product was released for sale according to the existing specifications. This document has been produced electronically and is valid without a signature.

| Approved by Manager of QC | Date |
|---|---|
| Robbert Zwinkels | 29/03/2018 |

Do you have any questions or comments regarding this product? Please contact us via support@hycultbiotech.com.