MPLA-SM

Monophosphoryl Lipid A from *S. minnesota* R595; TLR4 ligand

Catalog code: tlrl-mpla

https://www.invivogen.com/mpla

For research use only

Version 18L19-MM

PRODUCT INFORMATION

Contents

- 1 mg Monophosphoryl Lipid A (MPLA-SM)

Storage

- MPLA-SM is provided lyophilized and shipped at room temperature. Store at -20°C. Lyophilized product is stable for 1 year when properly stored.
- Upon resuspension, prepare aliquots of MPLA-SM and store at -20°C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- Biological activity has been tested using HEK-Blue™ TLR4 cells.
- The absence of bacterial contamination (e.g. lipoproteins) has been confirmed using HEK-Blue™ TLR2 cells.

DESCRIPTION

Monophosphoryl Lipid A (MPLA-SM) is extracted from lipopolysaccharide (LPS or endotoxin) produced by the Re mutant of a rough strain *Salmonella minnesota* R595. LPS synthesized by rough strain mutants of Gram-negative bacteria lacks the O-polysaccharide chain, one of the three structural regions of LPS. The most extreme mutants are the Re mutants, which produce an LPS that is composed of Lipid A and 2-keto-3-deoxyoctonate (KDO)[1]. Lipid A, a disaccharide with fatty acid side chains recognized by TLR4 receptors, is the component responsible for the endotoxic activity of LPS[2,3]. Removal of one phosphate group from Lipid A produces MPLA which has reduced toxicity while retaining the ability to stimulate the immune system[4,5]. MPLA-SM is a potent activator of TLR4 with negligible TLR2 activity. MPLA-SM was extracted from LPS using treatment with acid and heat followed by chromatography. Preparations of natural MPLA, such as MPLA-SM, contain a mixture of 5, 6, and 7 acyl lipid A[6].

Note: *Due to the intrinsic structural complexity of lipid A, some batch-to-batch variation may occur.*

1. Raetz CR. 1990. Biochemistry of endotoxins. Annu. Rev. Biochem. 59, 129-70. **2. Martin M. *et al.*, 2003.** Role of innate immune factors in the adjuvant activity of monophosphoryl lipid A. Infect Immun. 71:2498-507. **3. Ogawa T. *et al.*, 2002.** Cell activation by *Porphyromonas gingivalis* lipid A molecule through Toll-like receptor 4- and myeloid differentiation factor 88-dependent signaling pathway. Int Immunol. 14:1325-32. **4. Qureshi N. *et al.*, 1982.** Purification and structural determination of nontoxic lipid A obtained from the lipopolysaccharide of *Salmonella typhimurium*. J. Biol. Chem., 257:11808-15. **5. Romero CD. *et al.*, 2011.** The Toll-Like Receptor 4 agonist monophosphoryl Lipid A augments innate host resistance to systemic bacterial infection. Infect Immun. 79: 3576–3587. **6. Qureshi N. *et al.*, 1985.** Monophosphoryl lipid A obtained from lipopolysaccharides of *Salmonella minnesota* R595. Purification of the dimethyl derivative by high performance liquid chromatography and complete structural determination. J. Biol. Chem. 260:5271–8.

PRODUCT PROPERTIES

Specificity: TLR4 agonist
Working concentration: 10 ng -1 µg/ml
Endotoxin level: > 0.5 x 10^6 EU/mg
Appearance: Clear lipidic film
Solubility: 1 mg/ml in DMSO

METHODS

Preparation of stock suspension (1 mg/ml)

TLR4 activation can be achieved with 10 ng -1 µg/ml MPLA-SM.

- Add 1 ml of DMSO and vortex until completely resuspended.
- Prepare aliquots of stock solution and store at -20°C. Further dilutions can be prepared using water.

Notes:

- *The suspension may appear to contain floating fine particles, sonication may help to disperse these particles.*
- *Alternatively, MPLA-SM can be resuspended in DMSO containing 0.2% triethylamine.*

TLR4 activation using MPLA-SM

MPLA-SM can be used to activate TLR4 in HEK-Blue™ TLR4 cells, that were designed to study TLR4 stimulation by monitoring NF-κB activation. Stimulation of HEK-Blue™ TLR4 cells with a TLR4 agonist activates NF-κB which induces the production of SEAP (secreted embryonic alkaline phosphatase). Levels of SEAP can be easily determined using a SEAP detection medium, such as QUANTI-Blue™.
For more information visit: https://www.invivogen.com/hek-blue-tlr4.

- Add 20 µl of MPLA-SM at various concentrations (10 ng -1 µg/ml) in a well of a 96-well plate.
- Add 180 µl of HEK-Blue™ TLR4 cell suspension per well.
- Incubate the plate for 16-24 h at 37°C, 5% CO_2.
- Collect 20 µl of supernatant and add to a well of a 96-well plate containing 180 µl of QUANTI-Blue™.
- Incubate the plate at 37°C for 1-3 h.
- Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR4 Cells (human TLR4)	hkb-htlr4
HEK-Blue™ mTLR4 Cells (mouse TLR4)	hkb-mtlr4
LPS-EB Ultrapure (LPS from *E. coli 0111:B4*)	tlrl-3pelps
LPS-EK Ultrapure (LPS from *E. coli K12*)	tlrl-peklps
MPLAs (synthetic MPLA)	tlrl-mpls
QUANTI-Blue™	rep-qb1

TECHNICAL SUPPORT
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