## Validation data for MPLA-SM\*

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Version 22K24-NJ

Monophosphoryl Lipid A (MPLA-SM) is a TLR4 agonist derived from Lipid A, the immunostimulatory structure of lipopolysaccharide (LPS). This natural compound is extracted from the LPS of *Salmonella minnesota* Re595 (Re mutant). The preparation is a mix of MPLA congeneric forms differing in the number of acyl chains, and possibly responsible for the partial TLR4 agonist function of some preparations. MPLA-SM\* results from an improved process of MPLA-SM extraction. While MPLA-SM\* and MPLA-SM have the same ability to activate murine TLR4 (Figure 1), MPLA-SM\* is more potent than MPLA-SM at inducing human TLR4 responses (Figure 2).

## Mouse TLR4 activation by S. minnesota monophosphoryl lipid A

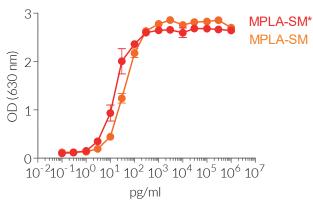


Figure 1. MPLA-SM and MPLA-SM\* induce a similar dose-dependent response in HEK-Blue™ mTLR4 cells.

The cells were incubated with increasing concentrations of two preparations of *S. minnesota* monophosphoryl lipid A, MPLA-SM and MPLA-SM\*. After overnight incubation in HEK-Blue™ detection medium, a SEAP detection growth medium, the activation of mouse (m)TLR4 was assessed by determining the presence of SEAP in the supernatant. Data are expressed as optical density at 630 nm (±SEM).

## Human TLR4 activation by S. minnesota monophosphoryl lipid A

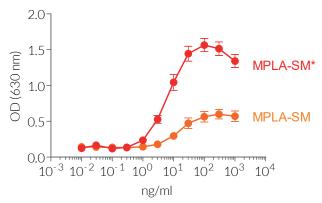


Figure 2. MPLA-SM\* is more potent than MPLA-SM at inducing a dose-dependent response in HEK-Blue™ hTLR4 cells.

The cells were incubated with increasing concentrations of two preparations of *S. minnesota* monophosphoryl lipid A, MPLA-SM and MPLA-SM\*. After overnight incubation in HEK-Blue™ detection medium, a SEAP detection growth medium, the activation of human (h)TLR4 was assessed by determining the presence of SEAP in the supernatant. Data are expressed as optical density at 630 nm (±SEM).



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