Validation data for MPLA-SM

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Version 24H28-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). MPLA-SM induces both mouse and human TLR4 responses as validated using HEK-Blue™ mTLR4, RAW-Blue™, and HEK-Blue™ hTLR4 cells **(Figure 1)**. Of note, MPLA-SM displays varying activity on human TLR4. The natural extraction of MPLA-SM from LPS generates congeneric forms differing in the number of acyl chains, and responsible for different TLR4 agonist potency of each preparation. Importantly, MPLA-SM is free of TLR2 contaminants as validated using HEK-Blue™ hTLR2 cells **(Figure 2)**.

Activation of mouse and human TLR4 by MPLA-SM

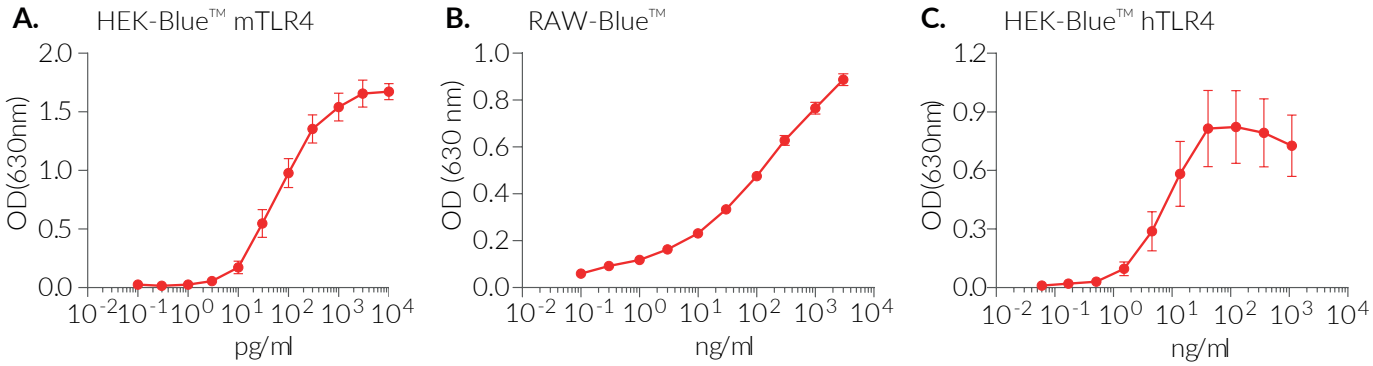

Figure 1. MPLA-SM induces mouse and human TLR4.
HEK-Blue™ mTLR4 cells stably expressing mouse TLR4 **(A)**, RAW-Blue™ cells expressing endogenous mouse TLR4 **(B)**, and HEK-Blue™ hTLR4 cells stably expressing human TLR4 **(C)** were incubated overnight with increasing concentrations of *S. minnesota* monophosphoryl lipid A, MPLA-SM. The activation of human and mouse TLR4 was assessed by determining the presence of SEAP in the supernatant, using HEK-Blue™ detection medium **(A, C)** or QUANTI-Blue™ **(B)**. Data are compiled with multiple lots of MPLA-SM and MPLA-SM*. Data are expressed as optical density at 630 nm (±SEM). ***Note:*** *MPLA-SM lots that display a higher potency for human TLR4 are denoted with an asterisk (MPLA-SM*, cat code tlrl-mpla2).*

Absence of TLR2-activating contaminants in MPLA-SM

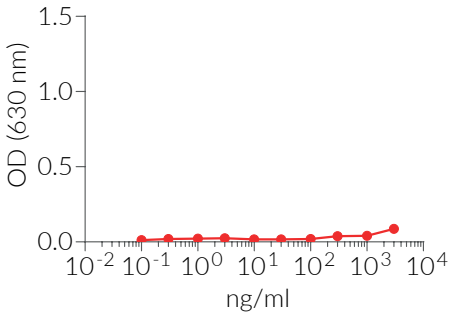

Figure 2. MPLA-SM does not trigger a TLR2 response in HEK-Blue™ hTLR2 reporter cells.
The cells were incubated with increasing concentrations of *S. minnesota* monophosphoryl lipid A, MPLA-SM. After overnight incubation in HEK-Blue™ detection medium, a SEAP detection growth medium, the activation of human (h)TLR2 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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