

Validation data for MPLA-SM VacciGrade™

<https://www.invivogen.com/mpla-vaccigrade>

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Version 24H28-NJ

Monophosphoryl Lipid A (MPLA-SM) VacciGrade™ is a pre-clinical grade 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

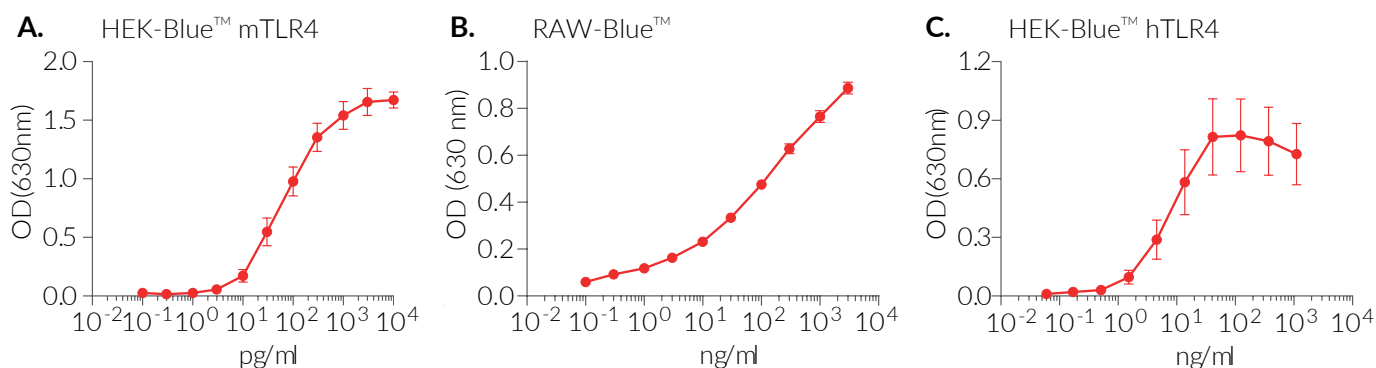


Figure 1. MPLA-SM induces mouse and human TLR4.

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 (\pm SEM). **Note:** MPLA-SM lots that display a higher potency for human TLR4 are denoted with an asterisk (MPLA-SM* VacciGrade™, cat code vac-mpla2).

Absence of TLR2-activating contaminants in MPLA-SM

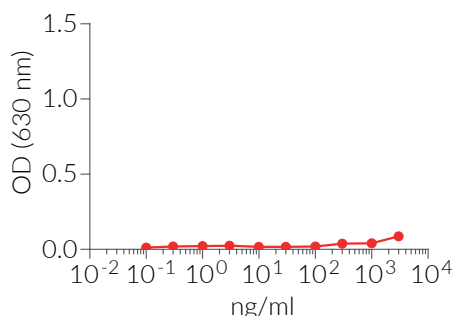


Figure 2. MPLA-SM does not trigger a TLR2 response in HEK-Blue™ hTLR2 reporter cells.

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 (\pm SEM).

TECHNICAL SUPPORT

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