Validation data for HEK-Blue™ mTLR4 cells

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Version 24B29-AK

HEK-Blue™ mTLR4 cells are engineered HEK293 cells that feature the stable expression of the mouse Toll-like receptor 4 (mTLR4), as well as the adapter proteins mouse (m)MD-2 (myeloid differentiation factor 2) and mCD14 (cluster of differentiation 14). They also express an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. These cells are highly responsive to TLR4 ligands, such as Lipopolysaccharide (LPS) and monophosphoryl Lipid A (MPLA) **(Figure 1)**, when compared to their parental cell line HEK-Blue™ Null-1v **(Figure 2).** As HEK293 cells express endogenous levels of various pattern recognition receptors (PRRs), HEK-Blue™ mTLR4 cells respond to their cognate ligands **(Figure 3)**.

Dose-response of HEK-Blue™ mTLR4 cells to TLR4 agonists

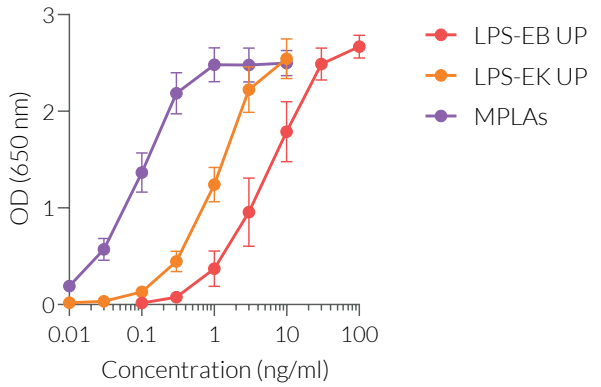

Figure 1. Dose-response of HEK-Blue™ mTLR4 cells to TLR4 agonists. Cells were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of LPS-EB Ultrapure (UP), LPS-EK UP, and MPLAs (synthetic). After 24 hour incubation, the NF-κB-induced SEAP activity was determined by reading the OD at 650 nm (mean ± SEM).

Response of HEK-Blue™-derived cells to TLR4 agonists

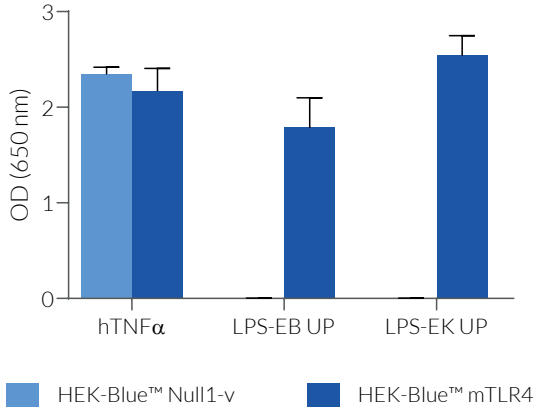

Figure 2. Response of HEK-Blue™-derived cells to TLR4 agonists. HEK-Blue™ Null1-v and HEK-Blue™ mTLR4 cells were cultured in HEK-Blue™ Detection reagent and incubated with 10 ng/ml of the TLR4 agonists LPS-EB Ultrapure (UP) and LPS-EK UP. Human TNF-α, (10 ng/ml) serves as an NF-κB-positive control. After 24h incubation, the NF-κB-induced SEAP activity was assessed by reading the OD at 650 nm (mean ± SEM).

Response of HEK-Blue™ mTLR4 cells to various PRR agonists and cytokines

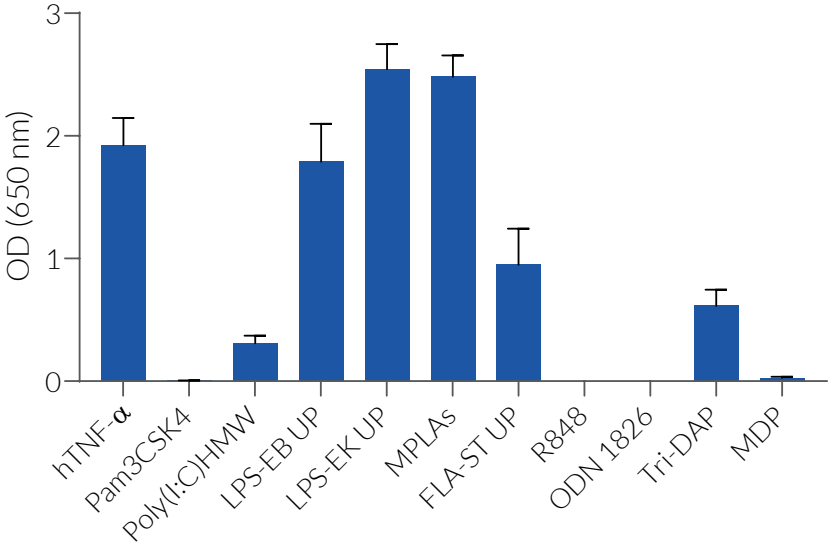

Figure 3. Response of HEK-Blue™ mTLR4 cells to various PRR agonists and cytokines. Cells were cultured in HEK-Blue™ Detection reagent and stimulated for 24 hours with cytokines and various PRR agonists: Human TNF-α (NF-κB-positive control, 10 ng/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 1 µg/ml), LPS-EB UP, LPS-EK UP (TLR4 ligands, 10 ng/ml), MPLAs (synthetic TLR4 ligand, 1 ng/ml) FLA-ST UP (TLR5 ligand, 30 ng/ml), R848 (TLR7/8 ligand, 10 µg/ml), ODN 1826 (TLR9 ligand, 10 µg/ml), Tri-DAP (NOD1 ligand, 10 µg/ml), and MDP (NOD2 ligand, 10 µg/ml). After 24h incubation, the NF-κB-induced SEAP activity was assessed by measuring the SEAP level in the supernatant. Data are shown as OD at 650 nm (mean ± SEM).

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