Validation data for HEK-Blue™ hTLR4 cells

https://www.invivogen.com/hek-blue-htlr4

For research use only

Version 24B27-AK

HEK-Blue™ hTLR4 cells are engineered HEK293 cells that feature the stable expression of the human Toll-like receptor 4 (hTLR4), as well as the adapter proteins MD-2 (myeloid differentiation factor 2) and CD14 (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), when compared to their parental cell line HEK-Blue™ Null2 **(Figures 1&2)** or to HEK-Blue™ hMD2-CD14, a cell line that doesn't express TLR4, but MD-2 and CD14 **(Figure 1)**. As HEK293 cells express endogenous levels of various pattern recognition receptors (PRRs), HEK-Blue™ hTLR4 cells respond to their cognate ligands **(Figure 3)**.

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

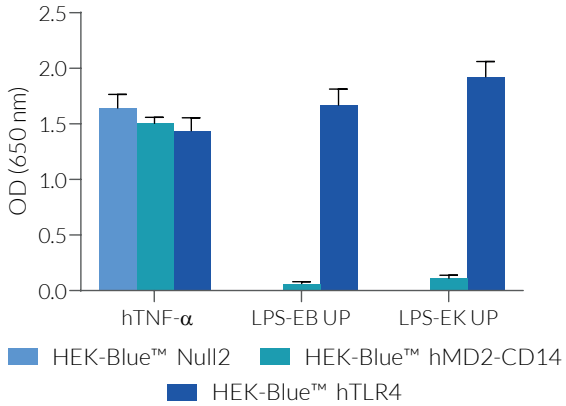

Figure 1. Response of HEK-Blue™-derived cells to TLR4 agonists. HEK-Blue™ Null2, HEK-Blue™ hMD2-CD14, and HEK-Blue™ hTLR4 cells were cultured in HEK-Blue™ Detection reagent and stimulated for 24 hours with 10 ng/ml of the following TLR4 agonists: LPS-EB Ultrapure (UP) and LPS-EK UP. Human TNF-α (1 ng/ml) serves as an NF-κB-positive control. After 24h incubation, the NF-κB-induced SEAP activity was assessed by measuring the SEAP level in the supernatant. Data are shown as optical density (OD) at 650 nm (mean ± SEM).

Dose-response of HEK-Blue™ hTLR4 cells to TLR4 agonists

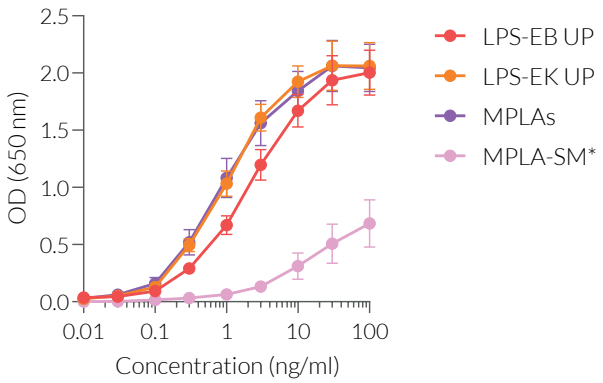

Figure 2. Dose-response of HEK-Blue™ hTLR4 cells to TLR4 agonists. Cells were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of LPS-EB UP, LPS-EK UP, MPLAs (synthetic), and MPLA-SM* (MPLA from *Salmonella minnesota* R595) After 24 hour incubation, the NF-κB-induced SEAP activity was determined by reading the OD at 650 nm (mean ± SEM).

Response of HEK-Blue™ hTLR4 cells to various PRR agonists and cytokines

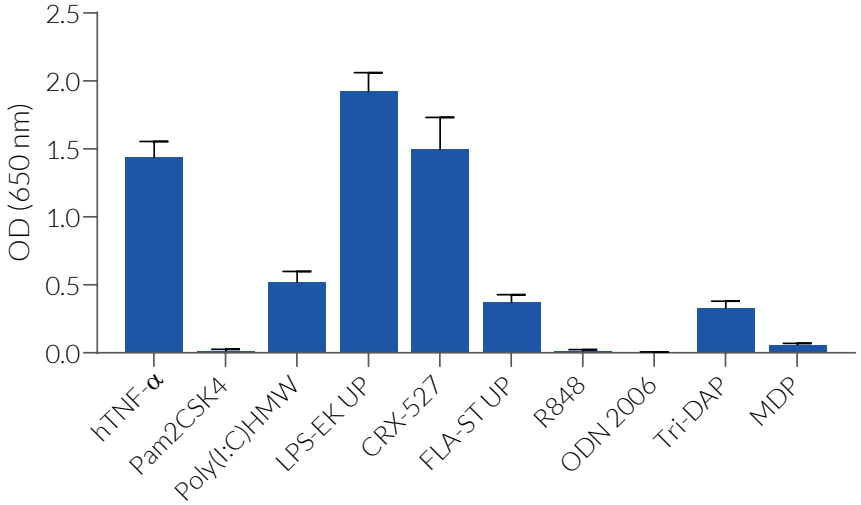

Figure 3. Response of HEK-Blue™ hTLR4 cells to various PRR agonists and cytokines. Cells were cultured in HEK-Blue™ Detection reagent and stimulated for 24 hours with cytokines and various PRR agonists: Human TNF-α (NF-κB-positive control, 1 ng/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 100 ng/ml), LPS-EK UP (TLR4 ligand, 100 ng/ml), CRX-527 (synthetic TLR4 ligand, 10 ng/ml) FLA-ST UP (TLR5 ligand, 10 ng/ml), R848 (TLR7/8 ligand, 10 µg/ml), ODN 2006 (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).

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-34-80
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com