Validation data for HEK-Blue™ hMD2-CD14 cells

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Version 24B27-AK

HEK-Blue™ hMD2-CD14 cells are engineered HEK293 cells that feature the stable expression of 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. The following data were obtained using the QUANTI-Blue™ or HEK-Blue™ Detection assays. HEK-Blue™ hMD2-CD14 cells are not responsive to TLR4 ligands, such as Lipopolysaccharide (LPS), when compared to HEK-Blue™ hTLR4, a cell line that does express human Toll-like receptor 4 (hTLR4), MD-2, and CD14 **(Figure 1)**. They strongly respond to LPS after transient transfection of the hTLR4 **(Figure 2)**. As HEK293 cells express endogenous levels of various pattern recognition receptors (PRRs), HEK-Blue™ hMD2-CD14 cells respond to their cognate ligands **(Figure 3)**.

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

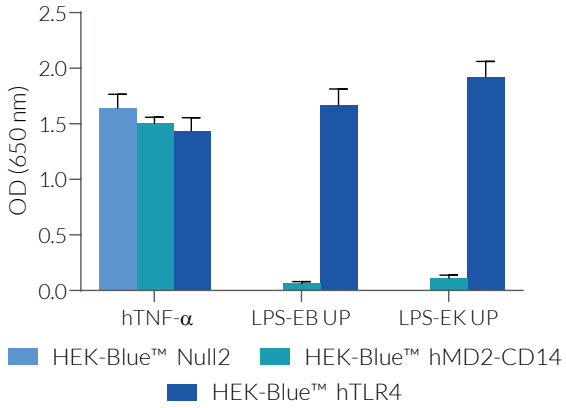

Figure 1. Response of HEK-Blue™-derived cells to TLR4 agonists. HEK-Blue™ Null2, HEK-Blue™ hMD2-CD14, and HEK-Blue™ hTLR4 cells were cultured in HEK-Blue™ Detection reagent and stimulated for 24 hours with 10 ng/ml of the following TLR4 agonists: LPS-EB Ultrapure (UP) and LPS-EK UP. Human TNF-α (1 ng/ml) 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).

Transient hTLR4 expression in HEK-Blue™ hMD2-CD14 cells

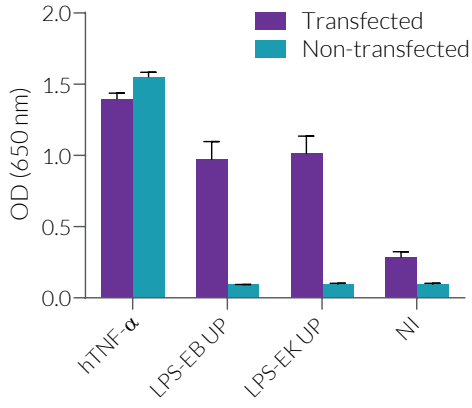

Figure 2. Response of HEK-Blue™ hMD2-CD14 cells to TLR4 agonists after transient transfection of hTLR4. Cells were transiently transfected with a hTLR4A plasmid and stimulated with human TNF-α (NF-κB-positive control, 10 ng/ml), LPS-EB UP (1 µg/ml), and LPS-EK UP (1 µg/ml). After overnight incubation, the activation of NF-κB was assessed by measuring the activity of SEAP in the supernatant using QUANTI-Blue™ Solution. Data are shown as optical density (OD) at 650 nm (mean ± SEM).

Response of HEK-Blue™ hMD2-CD14 cells to various PRR agonists and cytokines

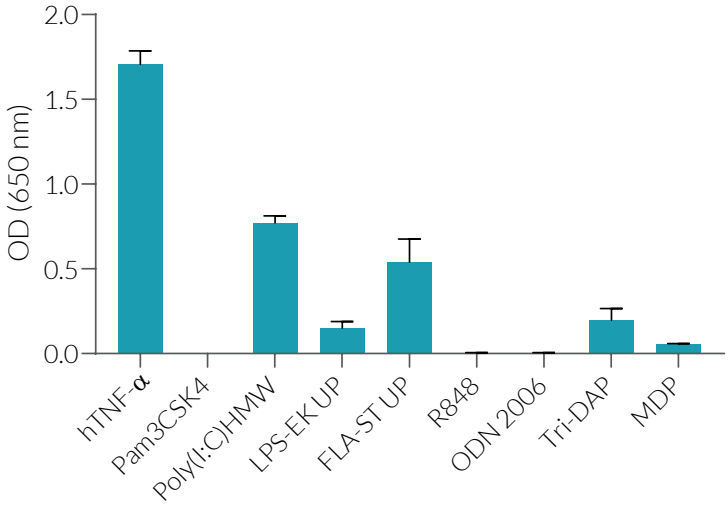

Figure 3. Response of HEK-Blue™ hMD2-CD14 cells to various PRR agonists and cytokines. Cells were cultured in HEK-Blue™ Detection reagent and stimulated for 24 hours with cytokines and various PRR agonists: Human TNF-α (NF-κB-positive control, 10 ng/ml), Pam3CSK4 (TLR2 ligand, 1 µg/ml), Poly(I:C) HMW (TLR3 ligand, 1 µg/ml), LPS-EK UP (TLR4 ligand, 1 µg/ml), FLA-ST UP (TLR5 ligand, 1 µg/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).

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