

Validation data for HEK-Blue™ mTLR9 cells

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Version 24B05-AK

HEK-Blue™ mTLR9 cells are engineered HEK293 cells that stably co-express the mouse (m)Toll-like receptor 9 (TLR9) and 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. These cells show a strong NF-κB response upon incubation with TLR9 agonists, such as oligonucleotides containing CpG motifs (ODN CpGs). As expected, the mouse-specific ODN 1826 does not activate human (h)TLR9 in HEK-Blue™ hTLR9 cells (**Figure 1**). As HEK293 cells express endogenous levels of TLR3 and TLR5, HEK-Blue™ mTLR9 cells respond to the cognate ligands, Poly(I:C) and flagellin (**Figure 2**).

Human and mouse TLR9-induced responses

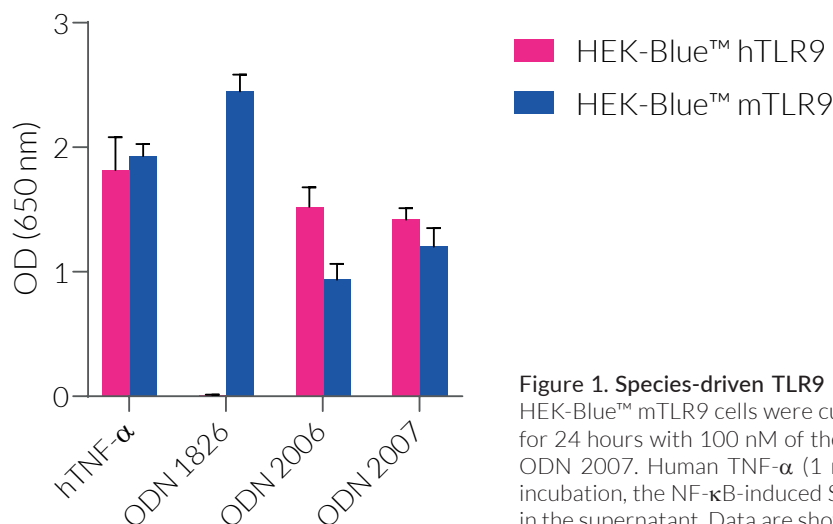


Figure 1. Species-driven TLR9 differential responses. HEK-Blue™ human (h)TLR9 and HEK-Blue™ mTLR9 cells were cultured in HEK-Blue™ Detection reagent and stimulated for 24 hours with 100 nM of the following TLR9 agonists: ODN 1826, ODN 2006, and ODN 2007. Human TNF-α (1 ng/ml) served as an NF-κB-positive 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).

Response of HEK-Blue™ mTLR9 cells to various PRR agonists and cytokines

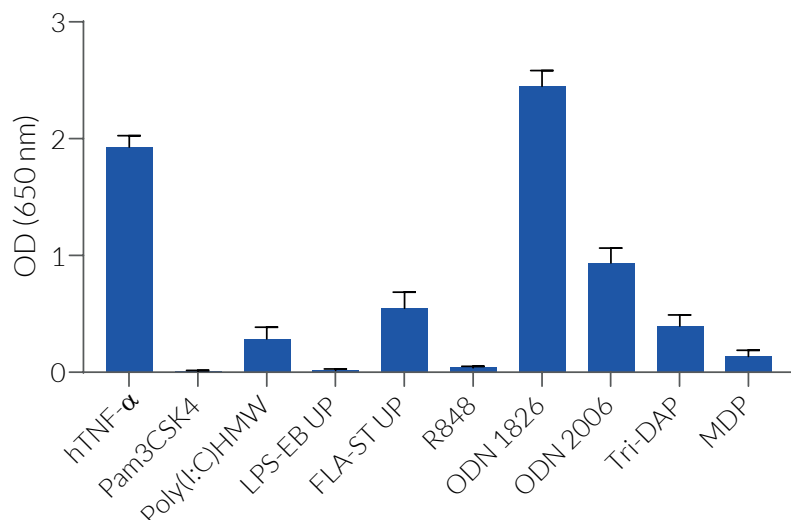


Figure 3. Response of HEK-Blue™ mTLR9 cells to various PRR agonists and cytokines. Cells were cultured in HEK-Blue™ Detection reagent and stimulated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 1 ng/ml), Pam3CSK4 (TLR2 ligand, 1 μg/ml), Poly(I:C) HMW (TLR3 ligand, 100 ng/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 10 μg/ml), FLA-ST UP (TLR5 ligand, 10 μg/ml), R848 (TLR7/8 ligand, 10 μg/ml), ODN 1826, ODN 2006 (TLR9 ligands, 1 μg/ml), Tri-DAP (NOD1 ligand, 1 μg/ml), and MDP (NOD2 ligand, 1 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

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