Validation data for HEK-Blue™ hTLR8 cells

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Version 23K27-AK

HEK-Blue™ hTLR8 cells are designed for studying the human Toll-like receptor 8 (hTLR8) signaling by monitoring the activation of NF-κB/AP1. They express the hTLR8 gene, and an NF-κB/AP1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Levels of SEAP produced upon TLR8 activation can be easily determined in real-time with HEK-Blue™ Detection cell culture medium. HEK-Blue™ hTLR8 cells respond in a dose-dependent manner to synthetic base analogs, while they not respond to TLR7-specific base analogs (**Figure 1**). Importantly, HEK-Blue™ hTLR8 cells respond to the TLR8-specific agonist ssRNA40 (a single-stranded RNA sequence from HIV-1) and this response is potentiated by the addition of Poly(dT) (**Figure 2** and data not shown). Of note, there are discrepancies in the functional activities between human and mouse TLR8 (**Figure 2**). As HEK293 cells express endogenous levels of various pattern recognition receptors, HEK-Blue™ hTLR8 cells might respond to the cognate ligangs (**Figure 3**).

Cellular response to synthetic base analogs

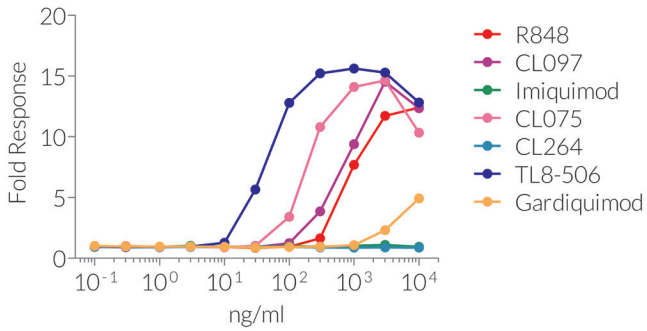

Figure 1. Dose-response of HEK-Blue™ hTLR8 cells to synthetic base analogs. Cells were cultured in HEK-Blue™ Detection medium with increasing concentrations of a TLR8 agonist (TL8-506), various TLR7/8 agonists (R848, CL097, CL075), or TLR7 agonists (CL264, Imiquimod, Gardiquimod). After 24h incubation, TLR8-induced NF-κB/AP1-induced SEAP activity was determined by reading the optical density (OD) at 650 nm. OD fold increase over non-induced cells is shown.

Human and Mouse TLR8-induced responses

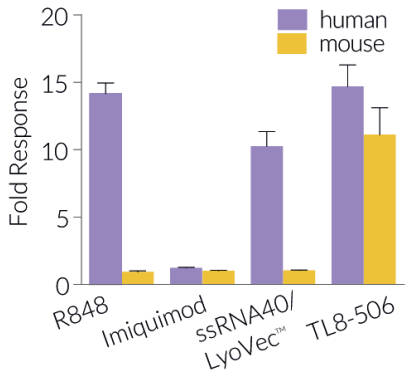

Figure 2. Species-driven TLR8 differential responses. HEK-Blue™ hTLR8 or mTLR8 were cultured in HEK-Blue™ Detection medium with 1 µg/ml R848, 3 µg/ml Imiquimod, 5 µg/ml ssRNA40/LyoVec™ (referred as human TLR8 agonist), or 1 µg/ml TL8-506. After 24h incubation, TLR8-induced NF-κB/AP1 responses were assessed as described before. OD fold increase over non-induced cells is shown (mean ± SEM).

Response to various PRR agonists and cytokines

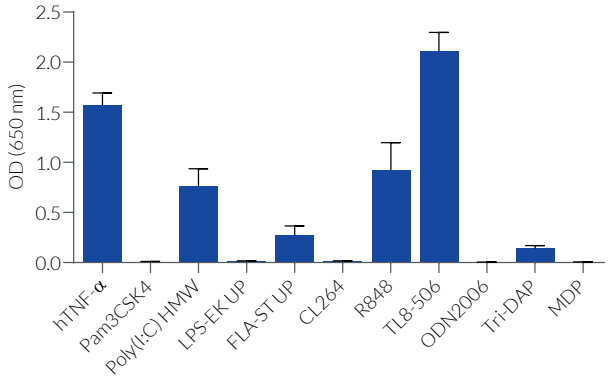

Figure 3. Response of HEK-Blue™ hTLR8 cells to various PRR agonists and cytokines. Cells were cultured in HEK-Blue™ Detection medium and stimulated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 1 ng/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 100 ng/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 10 ng/ml), C264 (TLR7 ligand, 1 µg/ml), R848 (TLR7/8 ligand, 1 µg/ml), ODN 2006 (TLR9 ligand, 10 µg/ml), Tri-DAP (NOD1 ligand, 100 ng/ml), or MDP (NOD2 ligand, 100 ng/ml). After 24h incubation, TLR8-induced NF-κB/AP1 responses were assessed as described before. Data are shown as OD at 650 nm (mean ± SEM).

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