Validation data for HEK-Blue™ mTLR7 cells

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Version 22K27-AK

HEK-Blue™ mTLR7 cells are designed for studying the murine Toll-Like Receptor 7 (mTLR7) signaling by monitoring the activation of NF-κB/AP-1. They express the *mTLR7* gene as well as an NF-κB/AP1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Levels of SEAP produced upon TLR7 activation can be easily determined in real-time with HEK-Blue™ Detection cell culture medium. HEK-Blue™ mTLR7 cells respond in a dose-dependent manner to synthetic base analogs such as TLR7/8-specific base analogs including R848 (Resiquimod), CL097, and CL075 (**Figure 1**). Importantly, HEK-Blue™ hTLR7 cells also respond to the TLR8-specific agonists TL8-506 and ssRNA40 (a single-stranded RNA sequence from HIV-1) (**Figure 2**). Of note, there are discrepancies in the functional activities between human and mouse TLR7 (**Figure 2**). Indeed, while HEK-Blue™ hTLR7 cells do not respond to the TLR8 agonists ssRNA40 and TL8-506, HEK-Blue™ mTLR7 cells do respond to these ligands. As HEK293 cells express endogenous levels of various pattern recognition receptors, HEK-Blue™ mTLR7 cells might respond to the cognate ligangs (**Figure 3**).

Cellular response to synthetic base analogs

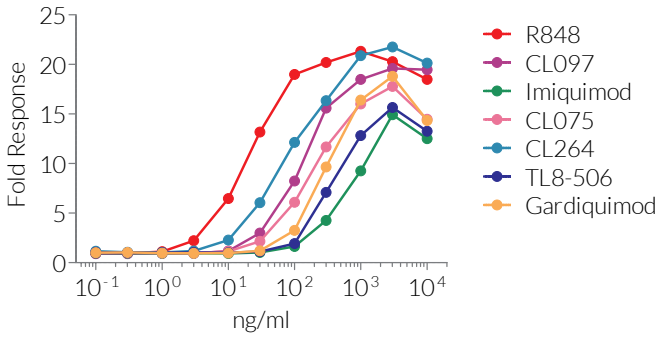

Figure 1. Dose-response of HEK-Blue™ mTLR7 cells to synthetic base analogs. HEK-Blue™ mTLR7 cells were cultured in HEK-Blue™ Detection medium with increasing concentrations of TLR7 agonists (CL264, Imiquimod, Gardiquimod), TLR7/8 agonists (R848, CL097, CL075), or a TLR8 agonist (TL8-506). After 24h incubation, TLR7-induced NF-κB/AP-1 responses were assessed by measuring SEAP levels in the supernatant by reading the optical density (OD) at 650 nm. OD fold increase over non-induced cells is shown.

Human and Mouse TLR7-induced responses

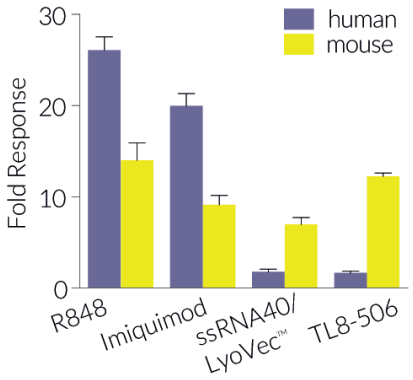

Figure 2. Species-driven TLR7 differential responses. HEK-Blue™ hTLR7 or mTLR7 were cultured in HEK-Blue™ Detection medium with 1 µg/ml R848 ,3 µg/ml Imiquimod, 5 µg/ml ssRNA40/LyoVec™ (referred as human TLR8 agonist), or 1 µg/ml TL8-506. After 24h incubation, TLR8-induced NF-κB/AP1 responses were assessed by measuring SEAP levels in the supernatant and reading the OD at 650 nm. OD fold increase over non-induced cells is shown (mean ± SEM). *Note: The HEK-Blue™ hTLR7 cell line used in this experiment is a different clone than the one on sale.*

Response to various PRR agonists and cytokines

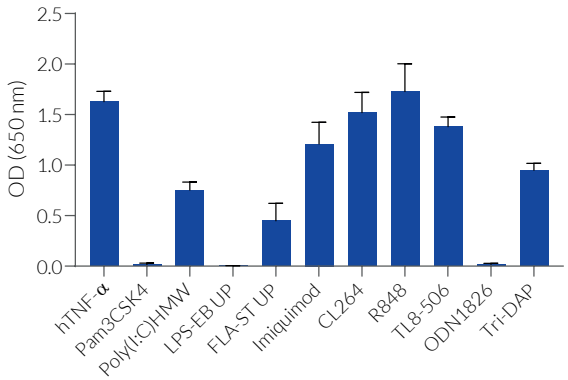

Figure 3. Response of HEK-Blue™ mTLR7 cells to various PRR agonists and cytokines. Cells were cultured in HEK-Blue™ Detection medium and incubated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 1 ng/ml), Pam3CSK4 (TLR2 ligand, 1 µg/ml), Poly(I:C) HMW (TLR3 ligand, 1 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 10 µg/ml), FLA-ST UP (TLR5 ligand, 10 µg/ml), C264 (TLR7 ligand, 1 µg/ml), R848 (TLR7/8 ligand, 1 µg/ml), ODN 2006 (TLR9 ligand, 10 µg/ml), or Tri-DAP (NOD1 ligand, 1 µg/ml). After 24h incubation, TLR8-induced NF-κB/AP1-induced SEAP activity was determined by measuring the SEAP level in the supernatant. Data are shown as OD at 650 nm (mean ± SEM).

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