

Validation data for HEK-Blue™ mTLR8 cells

<https://www.invivogen.com/hek-blue-mtlr8>

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Version 24G17-AK

HEK-Blue™ mTLR8 cells are designed for studying the murine TLR8 (mTLR8) signaling by monitoring the activation of NF-κB/AP1. They express the mTLR8 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. TLR8 was initially thought to be non-functional in mice. This does not hold true when using TL8-506, an analog of the synthetic agonist VX-2337 as HEK-Blue™ mTLR8 cells respond in a dose-dependent manner to this TLR8 specific agonist. These cells do not respond to TLR7-specific base analogs (Figure 1). Importantly, HEK-Blue™ mTLR8 cells do not respond to the TLR8-specific agonist ssRNA40 (a single-stranded RNA sequence from HIV-1), but this response is rescued 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™ mTLR8 cells might respond to the cognate ligands (Figure 3).

Cellular response to synthetic base analogs

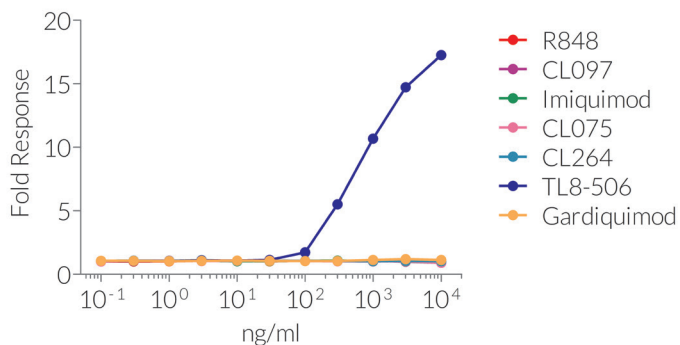


Figure 1: Dose-response of HEK-Blue™ mTLR8 cells to synthetic base analogs. HEK-Blue™ mTLR8 cells were cultured in HEK-Blue™ Detection reagent with increasing concentrations of a TLR8 agonist (TL8-506), various TLR7/8 agonists (R848, CL097, CL075) or TLR7 agonists (CL264, Imiquimod, Gardiquimod). After 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

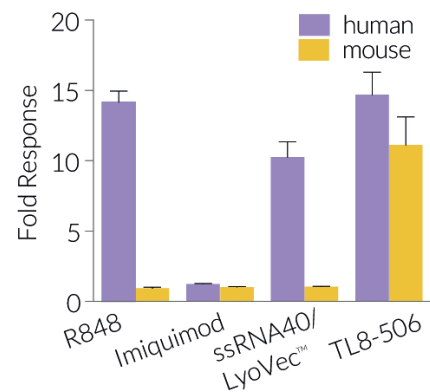


Figure 2. Species-driven TLR8 differential responses. HEK-Blue™ hTLR8 or mTLR8 were cultured in HEK-Blue™ Detection reagent with 1 μg/ml R848, 3 μg/ml Imiquimod, 5 μg/ml ssRNA40/LyoVec™ (referred as human TLR8 agonist), or 1 μg/ml TL8-506. After 24h incubation, TLR8-induced NF-κB/AP1 responses were assessed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

Response to various PRR agonists and cytokines

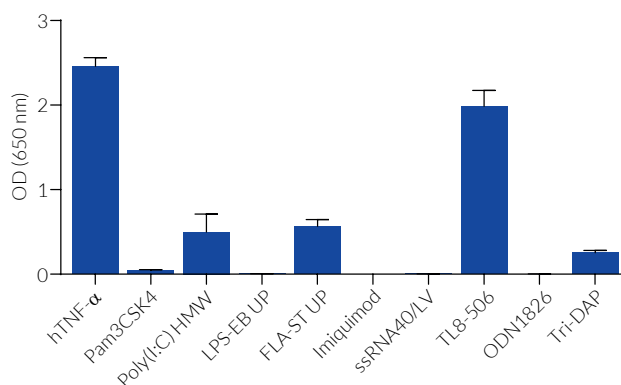


Figure 3. Response of HEK-Blue™ mTLR8 cells to various PRR agonists and cytokines. Cells were cultured in HEK-Blue™ Detection reagent and incubated with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 1 ng/ml), Pam3CSK4 (TLR2 ligand, 1 μg/ml), Poly(I:C) HMW (TLR3 ligand, 100 ng/ml), LPS-EB Ultrapure (UP) (TLR4 ligand, 1 μg/ml), FLA-ST UP (TLR5 ligand, 10 ng/ml), Imiquimod (TLR7 ligand, 10 μg/ml), ssRNA40/LyoVec™ (LV) (TLR8 ligand, 5 μg/ml), TL8-506 (TLR8 ligand, 10 μg/ml), ODN 1826 (TLR9 ligand, 10 μg/ml), or Tri-DAP (NOD1 ligand, 1 μg/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).

TECHNICAL SUPPORT

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