Validation data for HEK-Blue™ mTLR8 cells

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

For research use only

Version 23K27-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 ligangs (**Figure 3**).

Cellular response to synthetic base analogs

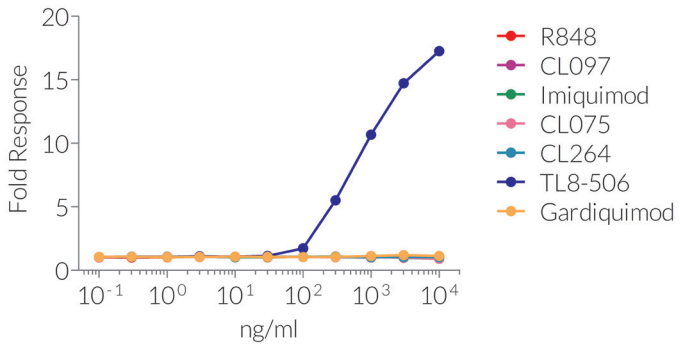

Figure 1: Dose-response of HEK-Blue™ mTLR8 cells to synthetic base analogs. HEK-Blue™ mTLR8 cells were cultured in HEK-Blue™ Detection reagent with increasing concentrations of a TLR8 agonist (TL8-506), various TLR7/8 agonists (R848, CL097, CL075) or TLR7 agonists (CL264, Imiquimod, Gardiquimod). After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

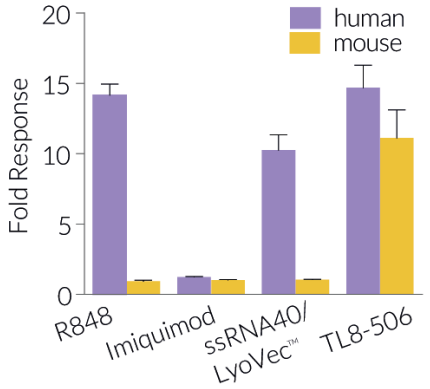

Figure 2. Species-driven TLR8 differential responses. HEK-Blue™ hTLR8 or mTLR8 were cultured in HEK-Blue™ Detection reagent with 1 µg/ml R848, 3 µg/ml Imiquimod, 5 µg/ml ssRNA40/LyoVec™ (referred as human TLR8 agonist), or 1 µg/ml TL8-506. After 24h incubation, TLR8-induced NF-κB/AP1 responses were assessed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

Response to various PRR agonists and cytokines

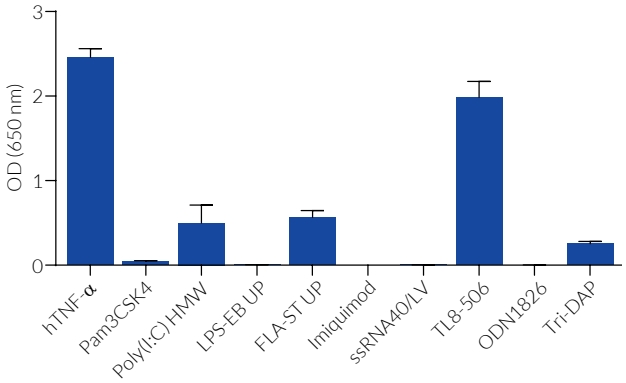

Figure 3. Response of HEK-Blue™ mTLR8 cells to various PRR agonists and cytokines. Cells were cultured in HEK-Blue™ Detection reagent and incubated with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 1 ng/ml), Pam3CSK4 (TLR2 ligand, 1 µg/ml), Poly(I:C) HMW (TLR3 ligand, 100 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), 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
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com