Validation data for HEK-Blue™ mTLR8 cells

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Version 20B05-NJ

HEK-Blue™ mTLR8 cells are designed for studying the murine TLR8 (mTLR8) signaling by monitoring the activation of NF-κB/AP1. These cells are derived from the human embryonic kidney HEK293 cell line. 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 which offers a one-step colorimetric monitoring of SEAP levels. 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**).

Cellular response to synthetic base analogs

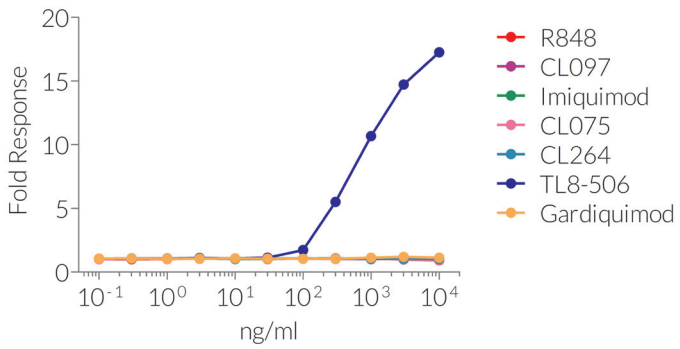

Figure 1: Dose-response of HEK-Blue™ mTLR8 cells to synthetic base analogs. HEK-Blue™ mTLR8 cells were cultured in HEK-Blue™ Detection medium with increasing concentrations of a TLR8 agonist (TL8-506), various TLR7/8 agonists (R848, CL097, CL075) or TLR7 agonists (CL264, Imiquimod, Gardiquimod). After 24h incubation, TLR8-induced NF-κB/AP1 responses were assessed by measuring SEAP levels in the supernatant by reading the OD at 630 nm. OD fold increase over non-induced cells is shown.

Human and Mouse TLR8-induced responses

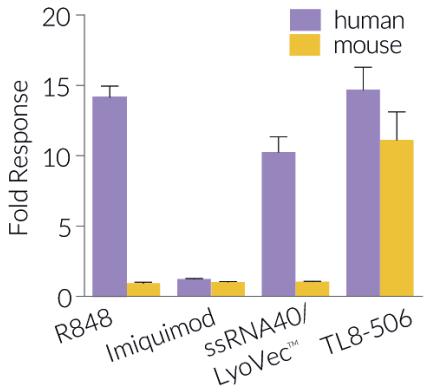

Figure 2: Species-driven TLR8 differential responses. HEK-Blue™ hTLR8 or mTLR8 were cultured in HEK-Blue™ Detection medium with 1 µg/ml R848 (TLR7/8 agonist), 3 µg/ml Imiquimod (TLR7 agonist), 5 µg/ml ssRNA40/LyoVec™ (referred as human TLR8 agonist), or 1 µg/ml TL8-506 (TLR8 agonist, VTX-2337 analog). After 24h incubation, TLR8-induced NF-κB/AP1 responses were assessed by measuring SEAP levels in the supernatant by reading the OD at 630 nm. OD fold increase over non-induced cells is shown.

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