Validation data for HEK-Blue™ hTLR7 cells

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Version 23B23-AK

HEK-Blue™ hTLR7 cells are designed to study the human Toll-Like Receptor 7 (hTLR7) signaling by monitoring the activation of NF-κB/AP-1. These cells are derived from the human embryonic kidney HEK293 cell line. They express the *hTLR7* gene, the mutated UNC93B1 chaperone protein, and an NF-κB/AP-1-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™ hTLR7 cells respond in a dose dependent manner to synthetic base analogs, such as the TLR7-specific CL307 & Imiquimod, or the TLR7/8 specific R848 (Resiquimod). Importantly, these cells do not respond to the TLR8-specific agonist TL8-506 **(Figure 1&2)**. Of note, as HEK293 cells express endogenous levels of TLR3, TLR5 and NOD [in-house data], HEK-Blue™ hTLR7 cells respond to the cognate ligands such as Poly(I:C) HMW, flagellin and Tri-DAP, respectively **(Figure 2)**.

Dose-response of HEK-Blue™ hTLR7 cells to synthetic base analogs

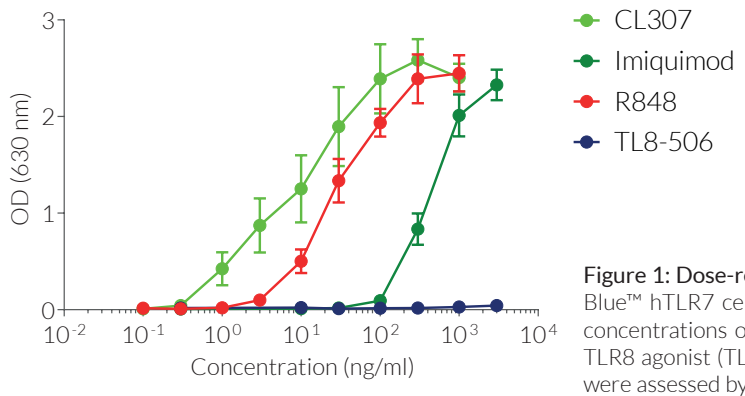

Figure 1: Dose-response of HEK-Blue™ hTLR7 cells to synthetic base analogs. HEK-Blue™ hTLR7 cells were cultured in HEK-Blue™ Detection medium with increasing concentrations of TLR7 agonists (CL307, Imiquimod), TLR7/8 agonists (R848), or a TLR8 agonist (TL8-506). After 24h incubation, TLR7-induced NF-κB/AP-1 responses were assessed by measuring SEAP levels in the supernatant. Data are shown as optical density (OD) at 630 nm.

NF-κB response of HEK-Blue™ hTLR7 cells to various TLR/NOD agonists

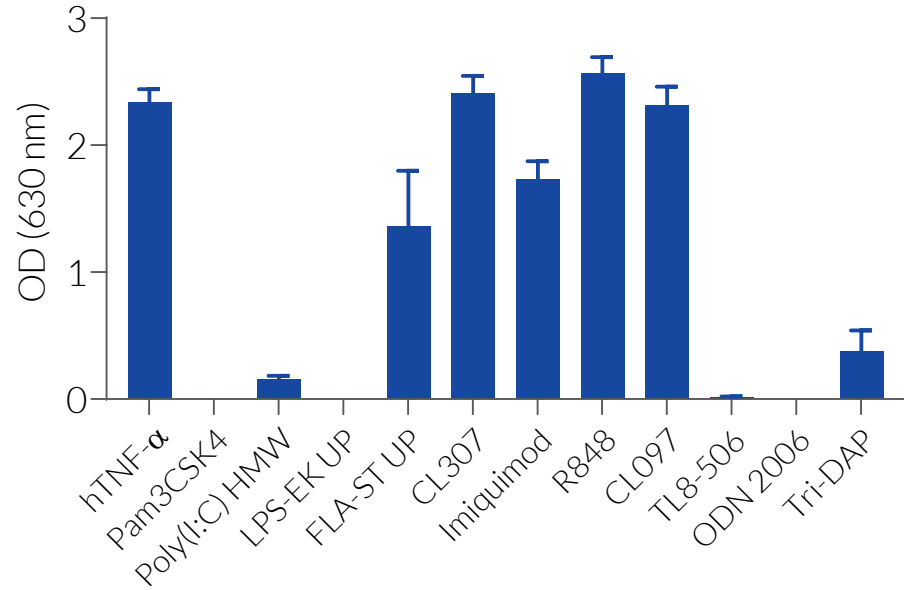

Figure 2: NF-κB response of HEK-Blue™ hTLR7 cells to various TLR/NOD agonists. HEK-Blue™ hTLR7 cells were cultured in HEK-Blue™ Detection medium and stimulated with various TLR and NOD agonists: Pam3CSK4 (TLR2 ligand, 10 ng/ml), Poly(I:C) HMW (TLR3 ligand, 10 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 10 ng/ml), FLA-ST UP (TLR5 ligand, 1 µg/ml), CL307 (TLR7 ligand, 1 µg/ml), Imiquimod (TLR7 ligand, 10 µg/ml), R848 (TLR7/8 ligand, 1 µg/ml) CL097 (TLR7/8 ligand, 1 µg/ml), TL8-506 (TLR8 ligand, 1 µg/ml), ODN 2006 (TLR9 ligand, 10 µg/ml), and Tri-DAP (NOD1 ligand, 10 µg/ml). Human TNF-α (10 ng/ml) was used as a NF-κB-positive control. After 24h incubation, the NF-κB-induced SEAP activity was assessed. Data are shown as OD at 630 nm (mean ± SEM).

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