Validation data for HEK-Dual™ hTLR7 cells

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Version 23K27-AK

HEK-Dual™ hTLR7 cells were generated from the HEK-Dual™ cell line through the stable expression of the human Toll-like receptor 7 (hTLR7) and a mutated (mut) version of the chaperon protein UNC93B1. These cells feature two reporter genes allowing the simultaneous study of NF-κB- and IRF-induced responses, by monitoring the SEAP (secreted embryonic alkaline phosphatase) and Lucia luciferase activities, respectively. Due to the stable expression of hTLR7, these cells show strong NF-κB and IRF responses upon incubation with various TLR7/8- and TLR7-specific ligands, when compared to their parental cells **(Figures 1 & 2)**. Of note, as HEK293 cells express endogenous levels of various PRRs, such as TLR3 and TLR5, HEK-Dual™ -derived cells might respond to the cognate ligand Poly(I:C) and flagellin.

Functional validation of HEK-Dual™-derived cells (NF-κB responses)

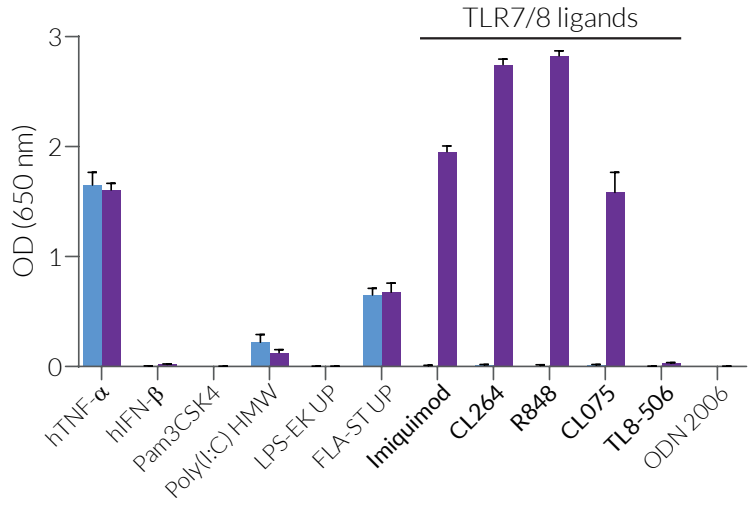

Figure 1. NF-κB responses in HEK-Dual™ -derived cells. HEK-Dual™ and HEK-Dual™ hTLR7 cells were incubated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 1 ng/ml), hIFN-β (IRF-positive control, 1000 U/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 10 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), Imiquimod (TLR7 ligand, 1 µg/ml), CL264 (TLR7 ligand, 1 µg/ml) R848 (TLR7/8 ligand, 1 µg/ml), CL075 (TLR7/8 ligand, 1 µg/ml), TL8-506 (TLR8 ligand, 1 µg/ml), and ODN 2006 (TLR9 ligand, 10 µg/ml). After 24h incubation, the NF-κB-induced SEAP activity was assessed using QUANTI-Blue™. Data are shown as optical density (OD) at 650 nm (mean ± SEM).

Functional validation of HEK-Dual™-derived cells (IRF response)

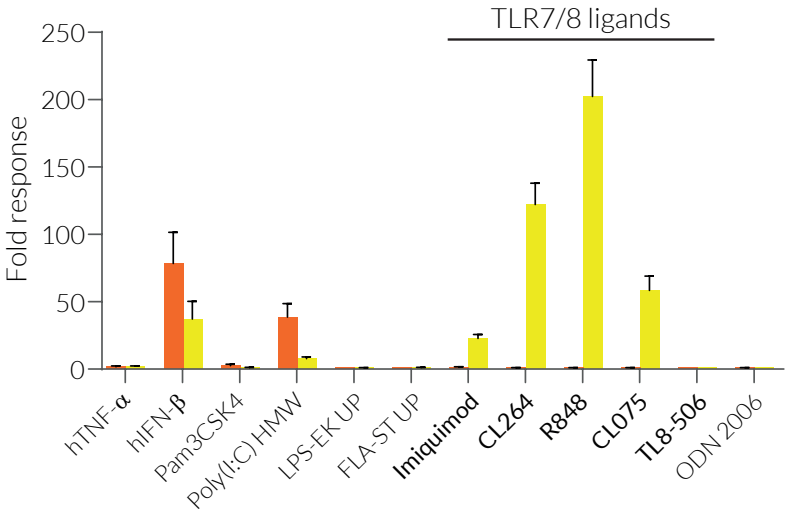

Figure 2. IRF responses in HEK-Dual™ -derived cells. HEK-Dual™ and HEK-Dual™ hTLR7 cells were incubated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 1 ng/ml), hIFN-β (IRF-positive control, 10 U/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 1 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), Imiquimod (TLR7 ligand, 10 µg/ml), CL264 (TLR7 ligand, 10 µg/ml), R848 (TLR7/8 ligand, 1 µg/ml), CL075 (TLR7/8 ligand, 10 µg/ml), TL8-506 (TLR8 ligand, 1 µg/ml), and ODN 2006 (TLR9 ligand, 10 µg/ml). After 24h incubation, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data are shown in fold response over non-induced cells (mean ± SEM).

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