

Validation data for HEK-Dual™ hTLR8 cells

<https://www.invivogen.com/hek-dual-htlr8>

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Version 23K27-AK

HEK-Dual™ hTLR8 cells were generated from the HEK-Dual™ cell line through the stable expression of the human Toll-like receptor 8 (hTLR8). These cells feature two reporter genes allowing the simultaneous study of NF- κ B- and IRF-induced responses, by monitoring the SEAP (secreted embryonic alkaline phosphatase) and Lucia luciferase activities, respectively. Due to the stable expression of hTLR8, these cells show strong NF- κ B and IRF responses upon incubation with various TLR7/8- and TLR8-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- κ B responses)

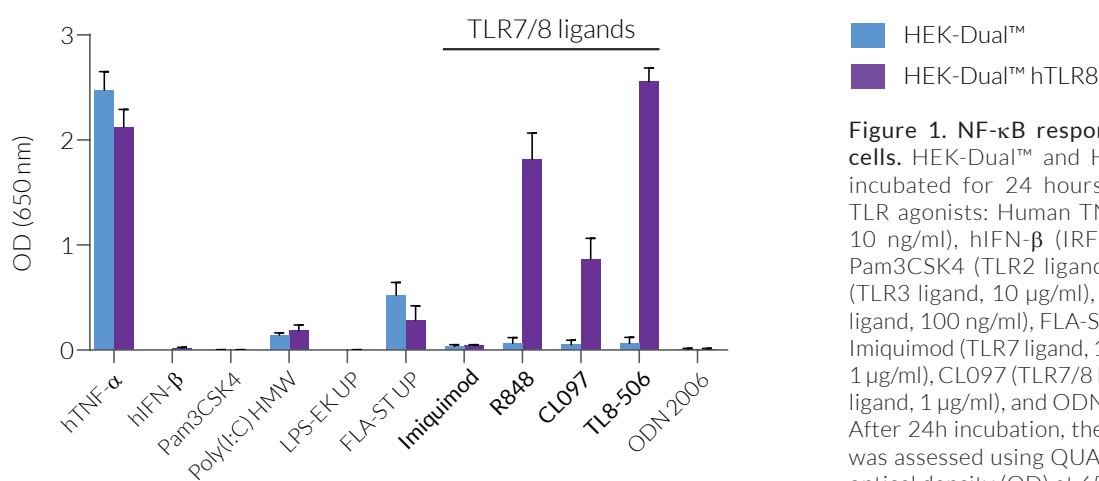


Figure 1. NF- κ B responses in HEK-Dual™ -derived cells. HEK-Dual™ and HEK-Dual™ hTLR8 cells were incubated for 24 hours with cytokines and various TLR agonists: Human TNF- α (NF- κ B-positive control, 10 ng/ml), hIFN- β (IRF-positive control, 100 U/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 10 μ g/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), Imiquimod (TLR7 ligand, 10 μ g/ml), R848 (TLR7/8 ligand, 1 μ g/ml), CL097 (TLR7/8 ligand, 1 μ g/ml), TL8-506 (TLR8 ligand, 1 μ g/ml), and ODN 2006 (TLR9 ligand, 10 μ g/ml). After 24h incubation, the NF- κ B-induced SEAP activity was assessed using QUANTI-Blue™. Data are shown as optical density (OD) at 650 nm (mean \pm SEM).

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

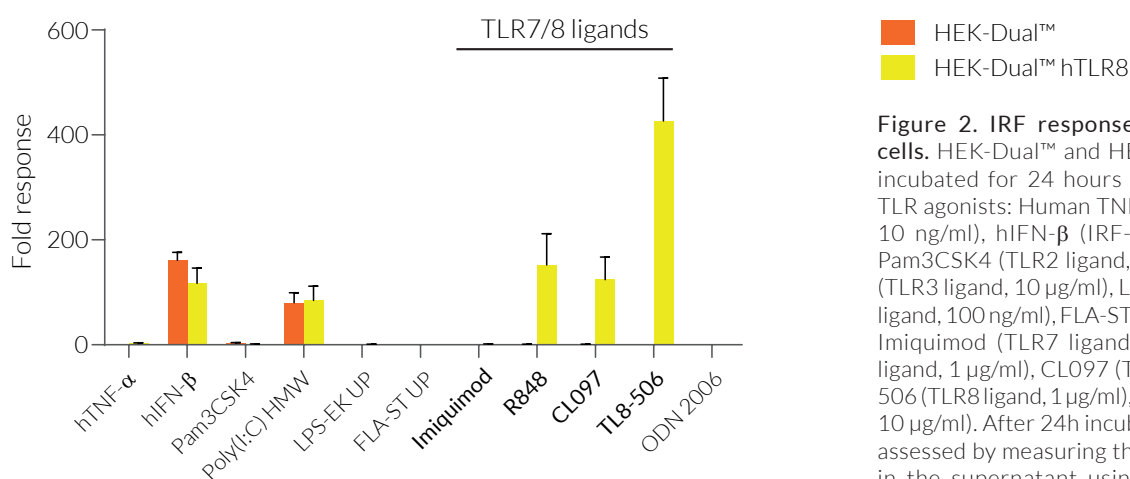


Figure 2. IRF responses in HEK-Dual™ -derived cells. HEK-Dual™ and HEK-Dual™ hTLR8 cells were incubated for 24 hours with cytokines and various TLR agonists: Human TNF- α (NF- κ B-positive control, 10 ng/ml), hIFN- β (IRF-positive control, 10 U/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 10 μ g/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), Imiquimod (TLR7 ligand, 10 μ g/ml), R848 (TLR7/8 ligand, 1 μ g/ml), CL097 (TLR7/8 ligand, 1 μ g/ml), TL8-506 (TLR8 ligand, 1 μ g/ml), and ODN 2006 (TLR9 ligand, 10 μ g/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 \pm SEM).

TECHNICAL SUPPORT

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