Validation data for HEK-Dual™ hTLR3 cells

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Version 23H25-AK

HEK-Dual™ hTLR3 cells were generated from the HEK-Dual™ cell line through the stable expression of the human Toll-like receptor 3 (hTLR3). These cells feature two reporter genes allowing the simultaneous study of 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 hTLR3, these cells show strong NF-κB and IRF responses upon incubation with synthetic double-stranded (ds)RNA analogs, such as Poly(A:U) (polyadenylic–polyuridylic acid) or Poly(I:C) (polyinosinic-polycytidylic acids), when compared to their parental cells **(Figures 1 & 2)**. Of note, as HEK293 cells express endogenous levels of TLR5, HEK-Dual™ -derived cells respond to the cognate ligand flagellin.

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

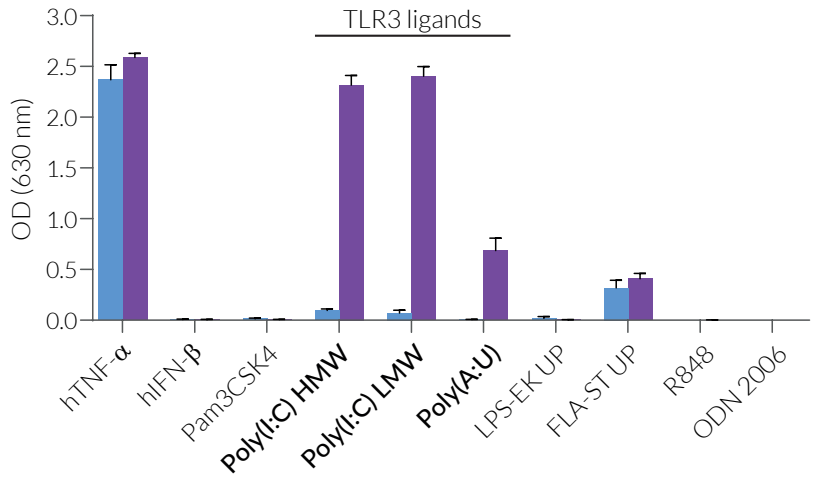

Figure 1. NF-κB responses in HEK-Dual™ -derived cells. HEK-Dual™ and HEK-Dual™ hTLR3 cells were incubated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 10 ng/ml), hIFN-β (IRF-positive control, 1000 U/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) LMW and HMW (TLR3 ligands, 1 µg/ml), Poly(A:U) (TLR3 ligand, 10 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), R848 (TLR7/8 ligand, 10 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 630 nm (mean ± SEM).

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

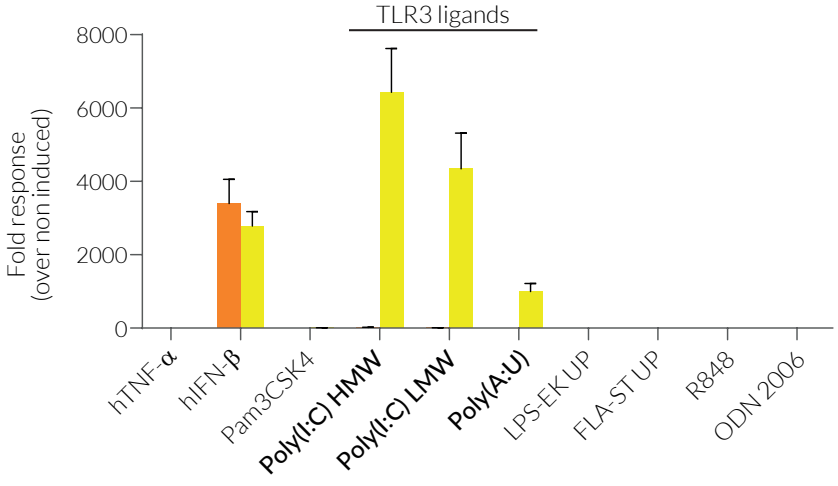

Figure 2. IRF responses in HEK-Dual™ -derived cells. HEK-Dual™ and HEK-Dual™ hTLR3 cells were incubated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 10 ng/ml), hIFN-β (IRF-positive control, 1000 U/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) LMW and HMW (TLR3 ligands, 100 ng/ml), Poly(A:U) (TLR3 ligand, 10 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), R848 (TLR7/8 ligand, 10 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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