Validation data for HEK-Dual™ hTLR4 cells

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Version 24C19-AK

HEK-Dual™ hTLR4 cells were generated from the HEK-Dual™ cell line through the stable expression of the human Toll-like receptor 4 (hTLR4), as well as the adapter proteins myeloid differentiation factor 2 (MD-2), and cluster of differentiation 14 (CD14). 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. These cells show strong NF-κB and IRF responses upon incubation with TLR4 ligands, such as Lipopolysaccharide (LPS) and monophosphoryl Lipid A (MPLA), when compared to their parental cells HEK-Dual™ **(Figures 1 & 2)**. Of note, as HEK293 cells express endogenous levels of TLR3 and TLR5, HEK-Dual™ - derived cells respond to their cognate ligands. Using CLI-095, a small-molecule inhibitor of TLR4 signaling, completely abrogates NF-κB and IRF responses in HEK-Dual™ hTLR4 cells **(Figure 3)**.

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

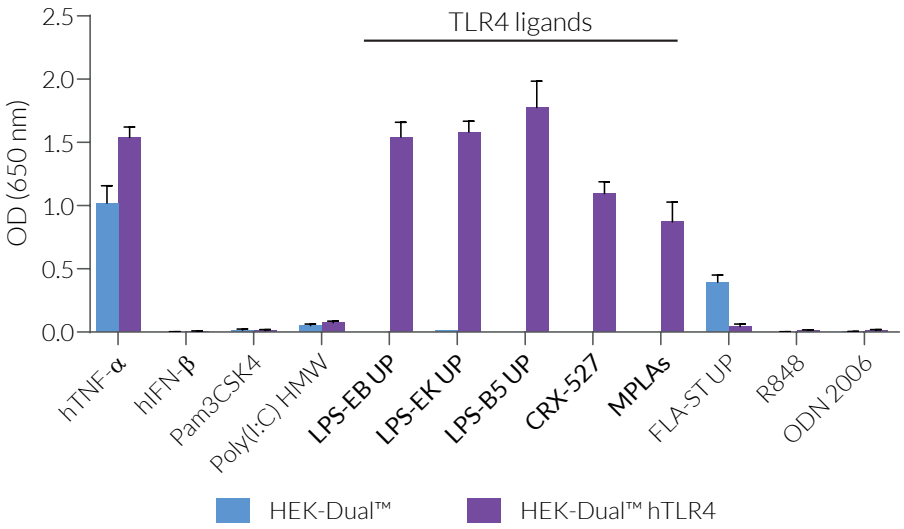

Figure 1. NF-κB responses in HEK-Dual™ -derived cells. HEK-Dual™ and HEK-Dual™ hTLR4 cells were incubated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 1 ng/ml), hIFN-β (IRF-positive control, 1000 U/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 1 µg/ml), LPS-EB Ultrapure (UP) (TLR4 ligand, 1 ng/ml), LPS-EK UP (TLR4 ligand, 1 ng/ml), LPS-B5 UP (TLR4 ligand, 100 pg/ml), CRX-527 (TLR4 ligand, 1 ng/ml), MPLAs (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), R848 (TLR7/8 ligand, 10 µg/ml), or 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 responses)

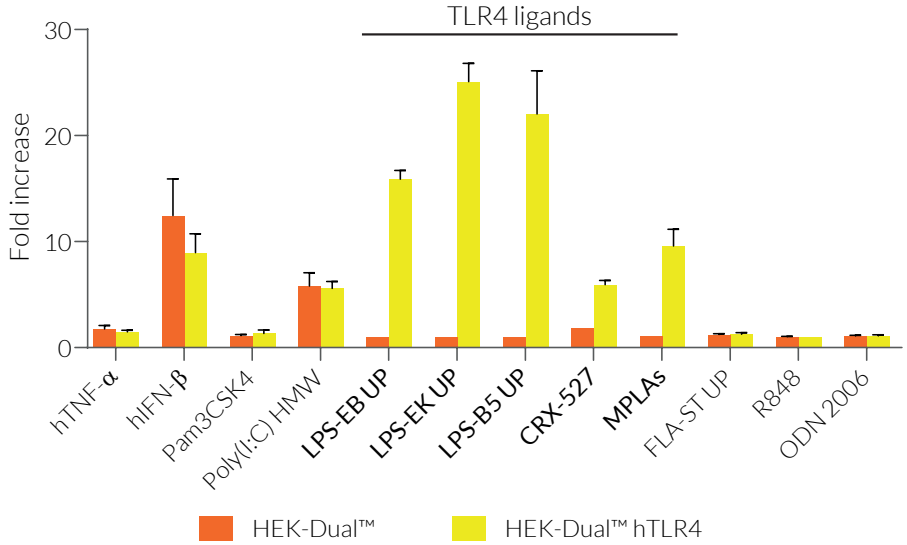

Figure 2. IRF responses in HEK-Dual™ -derived cells. HEK-Dual™ and HEK-Dual™ hTLR4 cells were incubated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 10 ng/ml), hIFN-β (IRF-positive control, 10 U/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 100 ng/ml), LPS-EB Ultrapure (UP) (TLR4 ligand, 10 ng/ml), LPS-EK UP (TLR4 ligand, 10 ng/ml), LPS-B5 UP (TLR4 ligand, 1 ng/ml), CRX-527 (TLR4 ligand, 10 ng/ml), MPLAs (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), R848 (TLR7/8 ligand, 10 µg/ml), or 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).

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com

Inhibition of TLR4-dependent NF-κB and IRF responses

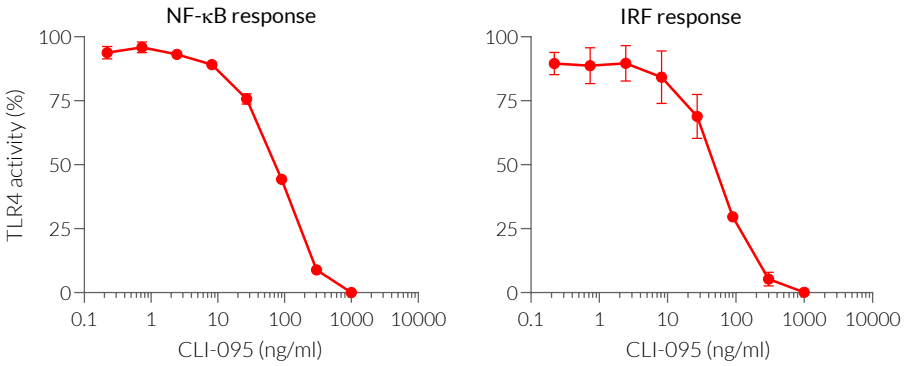

Figure 3. Inhibition of hTLR4 signaling pathway using CLI-095. HEK-Dual™ hTLR4 cells were incubated in the presence of increasing concentrations of CLI-095 for 3 hours before adding 10 ng/ml LPS-EK Ultrapure (TLR4 agonist). After overnight incubation, the inhibitory effect of CLI-095 on the NF-κB and IRF pathways was determined by measuring the reduction of SEAP and Lucia production in the supernatant using the QUANTI-Blue™ and QUANTI-Lucia™ detection reagents, respectively. Data are shown as a percentage (%) of maximal TLR4 activation (without inhibitor).

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E-mail: info@invivogen.com