Validation data for HEK-Dual™ cells

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Version 23E03-AK

HEK-Dual™ cells are derived from the human embryonic kidney 293 (HEK293) cell line through the stable integration of two inducible reporter genes for SEAP (secreted embryonic alkaline phosphatase) and Lucia luciferase. As a result, these cells allow the simultaneous study of the NF-κB pathway, by monitoring the activity of SEAP, and the IRF pathway, by assessing the activity of a secreted luciferase. Levels of SEAP and Lucia luciferase are readily measurable in the cell culture supernatant using QUANTI-Blue™ Solution and QUANTI-Luc™ 4 Lucia/Gaussia, respectively. HEK293 cells express endogenous levels of various pattern recognition receptors (PRRs), including TLR3, TLR5, NOD1, RIG-I-like receptors (RLR), and STING. Therefore, HEK-Dual™ cells might respond to their cognate ligands **(Figures 1 & 2)**.

NF-κB response of HEK-Dual™ cells to various PRR agonists and cytokines

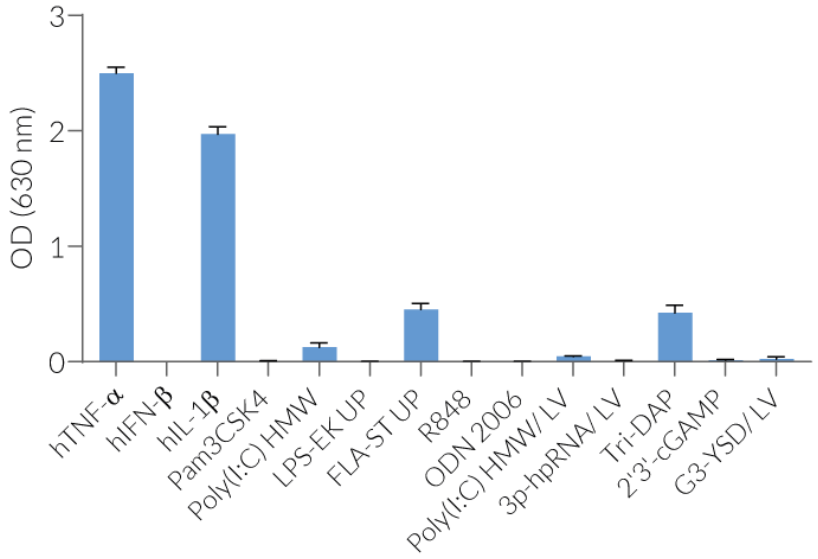

Figure 1. NF-κB response of HEK-Dual™ cells to various PRR agonists and cytokines. HEK-Dual™ cells were stimulated for 24h with various cytokines and PRR agonists: Human (h)TNF-α (NF-κB-positive control, 10 ng/ml), hIFN-β (IRF-positive control, 1000 U/ml), hIL-1β (NF-κB-positive control, 1 ng/ml), Pam3CSK4 (TLR2 ligand, 1 µg/ml), Poly(I:C) HMW (TLR3 ligand, 10 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 1 µg/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), R848 (TLR7/8 ligand, 10 µg/ml) ODN 2006 (TLR9 ligand, 10 µg/ml), Poly(I:C) HMW, complexed with LyoVec™ (LV) (RLR ligand, 1 µg/ml), 3p-hpRNA/LV (RLR ligand, 1 µg/ml), Tri-DAP (NOD1 ligand, 10 µg/ml), 2'3'-cGAMP (STING ligand, 10 µg/ml), and G3-YSD/LV (cGAS ligand, 1 µg/ml). The NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ (1h incubation). Data are shown as optical density (OD) at 630 nm (mean ± SEM).

IRF response of HEK-Dual™ cells to various PRR agonists and cytokines

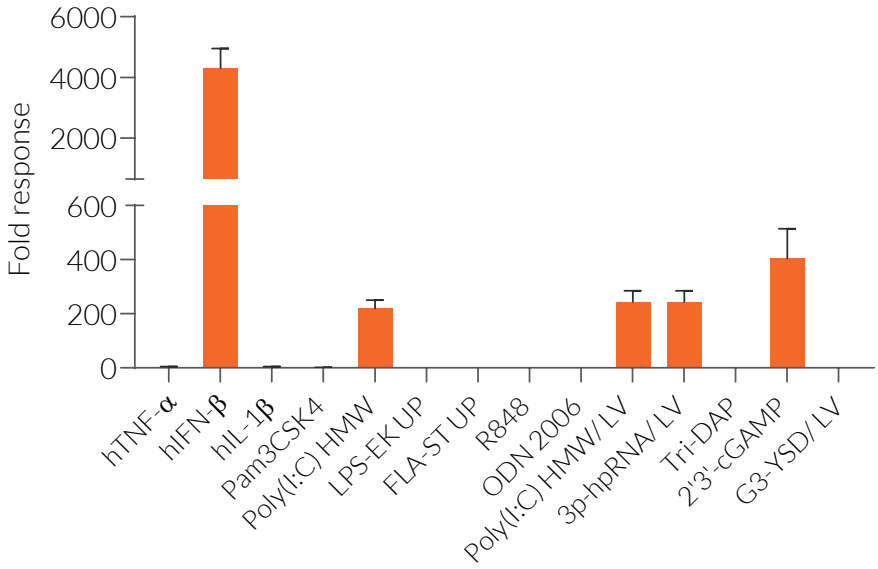

Figure 2. IRF response of HEK-Dual™ cells to various PRR agonists and cytokines. HEK-Dual™ cells were were treated as described above. The IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data are shown in fold response over non-induced cells (mean ± SEM).

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