Validation data for HEK-Dual™ RNA-hRIG-I cells

https://www.invivogen.com/hek-dual-rna-sensor-rigi

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Version 25A21-AK

HEK-Dual™ RNA-hRIG-I cells are derived from the HEK-Dual™ RNA-Null, expressing two reporter proteins, an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter and an interferon regulatory factor (IRF)-inducible Lucia® luciferase reporter. They lack three important dsRNA sensors Toll-like receptor 3 (TLR3), Retinoic acid-inducible protein 1 (RIG-I), and Melanoma differentiation-associated gene 5 (MDA5). In HEK-Dual™ RNA-hRIG-I cells, the gene for the human (*h*) *RIG-I* was re-introduced and overexpressed, as verified by Western blot **(Figure 1)**. Upon stimulation with RIG-I ligands, such as 3p-hpRNA complexed with LyoVec™ (LV), a strong IRF response can be observed in HEK-Dual™ RNA-hRIG-I cells when compared to their parental cell line HEK-Dual™ RNA-Null **(Figures 2-3)**. Since RIG-I does not signal via NF-κB, no SEAP induction is observed **(Figure 4)**.

Validation of *RIG-I* overexpression (Western blot)

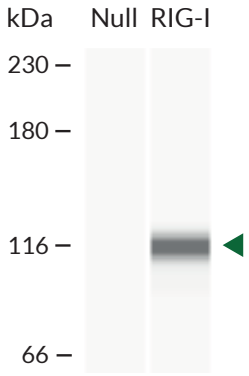

Figure 1. Human RIG-I expression in HEK-Dual™-derived cells. Lysates from HEK-Dual™ RNA-Null (Null) and HEK-Dual™ RNA-hRIG-I cells (RIG-I) were analyzed using an anti-human RIG-I antibody, followed by an HRP-conjugated secondary antibody (WESS™).

IRF Dose responses of HEK-Dual™ RNA-hRIG-I cells

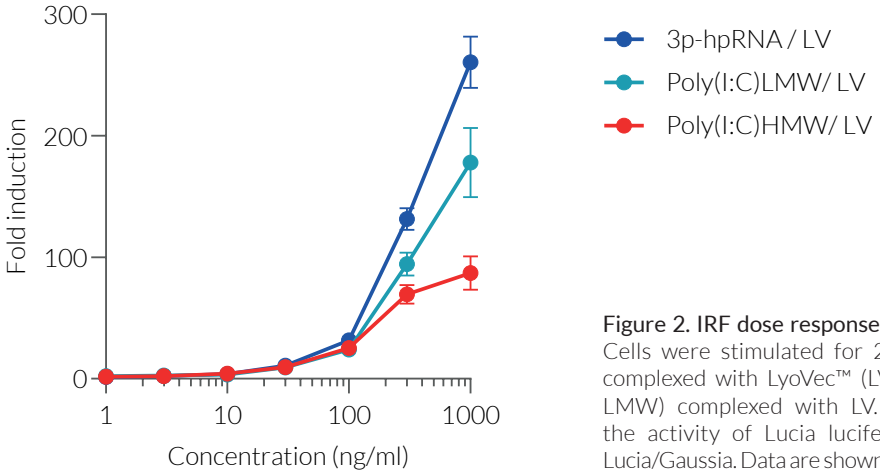

Figure 2. IRF dose responses of HEK-Dual™ RNA-hRIG-I cells to RIG-I ligands. Cells were stimulated for 24h with increasing concentrations of 3p-hpRNA, complexed with LyoVec™ (LV) or Poly(I:C) high/low molecular weight (HMW/LMW) complexed with LV. The IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™ 4 Lucia/Gaussia. Data are shown in fold response over non-induced cells (mean ± SEM).

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

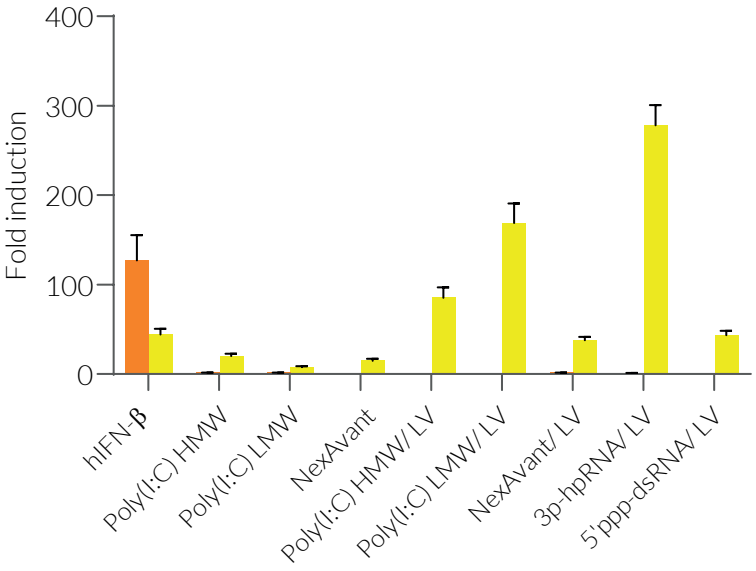

Figure 3. IRF responses of HEK-Dual™ RNA-Null and HEK-Dual™ RNA-hRIG-I cells to PRR agonists and cytokines. Cells were stimulated for 24h with various cytokines and PRR agonists: Human (h)IFN-β (IRF-positive control, 30 ng/ml), Poly(I:C) HMW or LMW, NexaVant® (TLR3 ligands, 10 µg/ml), Poly(I:C) HMW or LMW complexed with LyoVec™ (LV) (RLR ligands, 1 µg/ml), 3p-hpRNA/LV, or 5'ppp-dsRNA/LV (RIG-I ligands, 1 µg/ml). After 24h incubation, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™ 4 Lucia/Gaussia. Data are shown in fold response over non-induced cells (mean ± SEM).

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

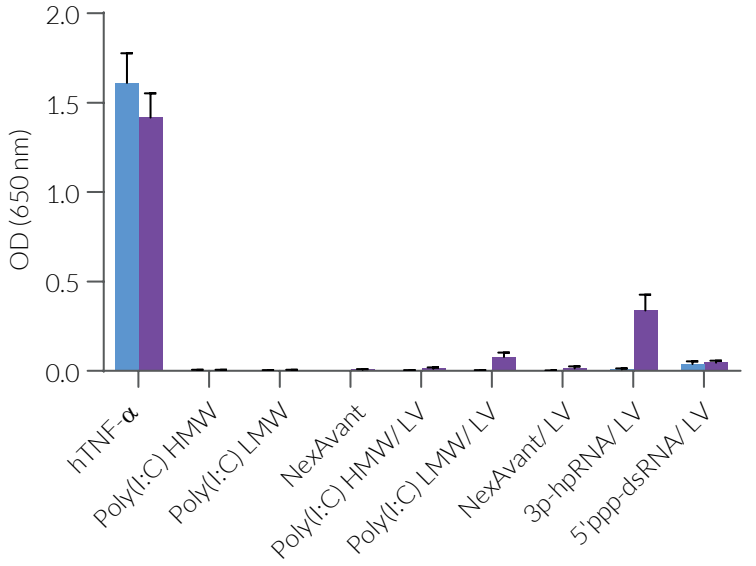

Figure 4. NF-κB responses of HEK-Dual™ RNA-Null and HEK-Dual™ RNA-hRIG-I cells to PRR agonists and cytokines. Cells were treated as described above. Human (h)TNF-α (1 ng/ml) was used as an NF-κB-positive control. After 24h incubation, the NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ (1h incubation). Data are shown as optical density (OD) at 650 nm (mean ± SEM).

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