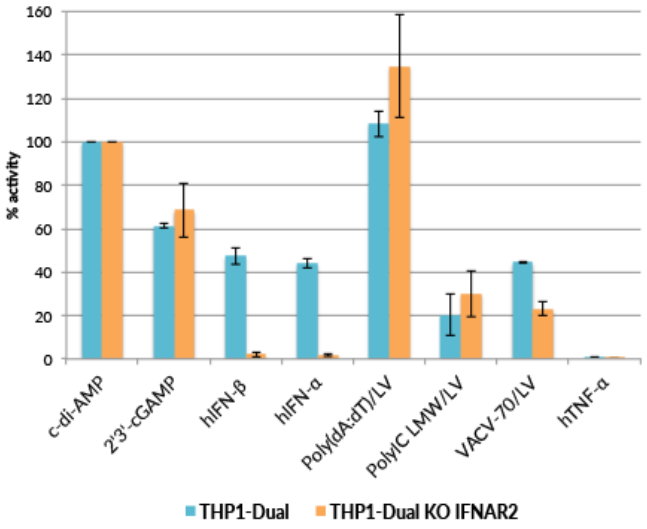
Validation data for THP1-Dual™ KO-IFNAR2 cells

For research use only

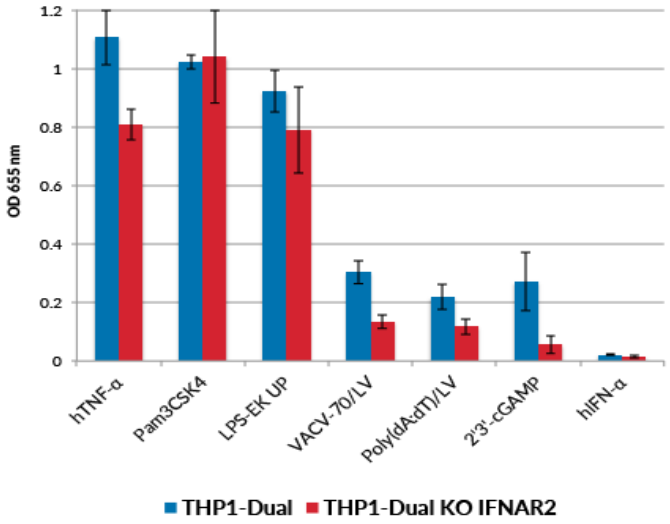
Version # 16I02-MM

THP1-Dual™ KO-IFNAR2 cells were generated from THP1-Dual™ cells by stable knockout of the interferon (IFN)-α receptor 2 (IFNAR2) gene. These cells derive from the human monocytic cell line THP-1. THP1-Dual™ and THP1-Dual™ KO-IFNAR2 cells can be used to study type I interferon signaling. Both cell lines express two inducible reporter constructs that enable the simultaneous study of the NF-κB pathway, by monitoring the activity of SEAP, and the IRF pathway, by assessing the activity of the secreted Lucia luciferase. The IRF induction response of these cells to different ligands has been assessed (see figure 1). RAW-Lucia™ ISG-KO-IFNAR2 cells respond to cyclic dinucelotides (e.g. c-di-AMP and 2'3'-cGAMP) and transfected DNA (e.g. VACV70/LyoVec™ and poly(dA:dT)/LyoVec™). However, as expected, they do not respond to human type I interferons (e.g. IFN-α and IFN-β). The NF-κB response of THP1-Dual™ KO-IFNAR2 cells to different ligands has been assessed (see figure 2). The knockout of the IFNAR2 gene in these cells has been confirmed by PCR (see figure 3) and sequencing.

IRF INDUCTION (Lucia luciferase reporter)



NF-κB INDUCTION (SEAP reporter)



PCR AMPLIFICATION

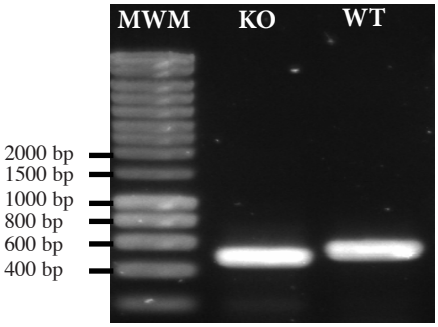


Figure 1 (top left): Stimulation of THP1-Dual™ (parental cell line) and THP1-Dual™ KO-IFNAR cells with c-di-AMP (30 μg/ml), 2'3'-cGAMP (3 μg/ml), hIFN-β (1x10⁴ U/ml), hIFN-α (1x10⁴ U/ml), poly(dA:dT)/LyoVec™ (1 μg/ml), poly(I:C)/LyoVec™ (1 μg/ml), and VACV70/LyoVec™ (1 μg/ml). Human TNF-α (10 ng/ml) has been included as a negative control. After a 24h incubation, IRF activation was determined by measuring the relative light units (RLUs) in a luminometer using QUANTI-Luc™, a Lucia luciferase detection reagent. The IRF induction of each ligand is expressed relative to that of c-di-AMP at 30 μg/ml (taken as 100%).

Figure 2 (top right): THP1-Dual™ and THP1-Dual™ KO-IFNAR2 cells were incubated with human TNF-α (0.1 ng/ml), Pam3CSK4 (0.1 ng/ml; TLR1/2 ligand), LPS-EB Ultrapure (100 ng/ml; TLR4 ligand), VACV70/LyoVec™ (1 μg/ml), poly(dA:dT)/LyoVec™ (100 ng/ml), 2'3'-cGAMP (3 μg/ml) and hIFN-α (1x10⁴ U/ml). After a 24h incubation, NF-κB activation was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm.

Figure 3 (bottom left): PCR amplification of the targeted region in the THP1-Dual™ KO-IFNAR2 (KO) and THP1-Dual™ (WT) cells. MWM = molecular weight marker

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