Validation data for RAW-Lucia™ ISG-KO-TRIF cells

For research use only

Version # 16A20-MM

RAW-Lucia™ ISG-KO-TRIF cells were generated from the RAW-Lucia™ ISG cell line through the stable knockout of the TRIF gene. These cells derive from the murine RAW 264.7 macrophage cell line, which has been reported to express many pattern recognition receptors (PRRs), including the Toll-like receptors TLR3 and TLR4, and their signaling partners. Both TLR3 and TLR4 signal through the adapter protein TRIF to induce the activation of interferon regulatory factor (IRF) and the subsequent production of type I interferons (IFNs). The knockout of the TRIF gene in these cells has been confirmed by PCR (see figure 1) and sequencing. Biological activity has been assessed using the Lucia luciferase reporter assay to monitor IRF induction (see figure 2). The response of RAW-Lucia™ ISG-KO-TRIF cells to the TLR3 ligand Poly(I:C) and the TLR4 ligand lipopolysaccharide (LPS) is significantly reduced when compared to the RAW-Lucia™ ISG cells. The response of RAW-Lucia™ ISG-KO-TRIF cells to murine type I IFNs is unaffected by the TRIF gene knockout.

PCR AMPLIFICATION

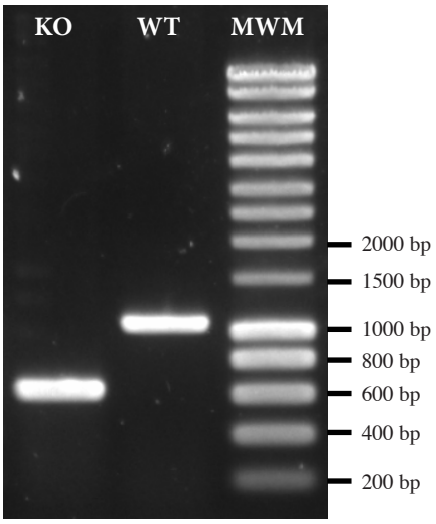

Figure 1: PCR amplification of the targeted region in the RAW-Lucia™ ISG-KO-TRIF (KO) and RAW-Lucia™ ISG (WT) cells. MWM = molecular weight marker

IRF INDUCTION (Lucia luciferase reporter)

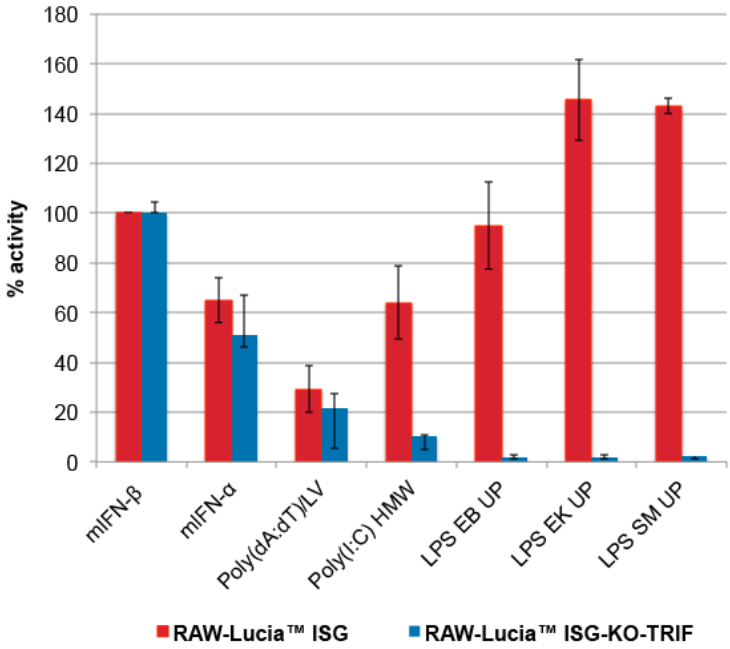

Figure 2: Stimulation of RAW-Lucia™ ISG and RAW-Lucia™ ISG-KO-TRIF cells with poly(dA:dT)/LyoVec™ (1 µg/ml), poly(I:C) HMW (1 µg/ml), LPS-EB Ultrapure (100 ng/ml), LPS-EK Ultrapure (100 ng/ml), and LPS-SM Ultrapure (100 ng/ml). Mouse IFN-α ($1x10^4$ U/ml) and IFN-β ($1x10^4$ U/ml) serve as positive controls. 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 mIFN-β at $1x10^4$ U/ml (taken as 100%).

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