Validation data for RAW-Lucia™ ISG-KO-IRF3 cells

For research use only

Version # 16C15-MM

RAW-Lucia™ ISG-KO-IRF3 cells were generated from the RAW-Lucia™ ISG cell line through the stable knockout of the IRF3 gene. These cells derive from the murine RAW 264.7 macrophage cell line. The knockout of the IRF3 gene in RAW-Lucia™ ISG-KO-IRF3 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).

PCR AMPLIFICATION

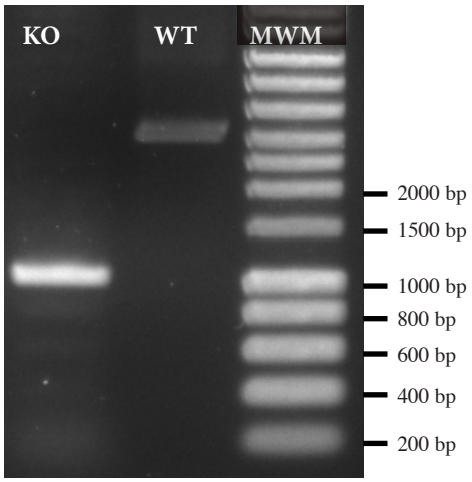

Figure 1: PCR amplification of the targeted region in the RAW-Lucia™ ISG-KO-IRF3 (KO) and RAW-Lucia™ ISG (WT) cells. MWM = molecular weight marker

IRF INDUCTION (Lucia luciferase reporter)

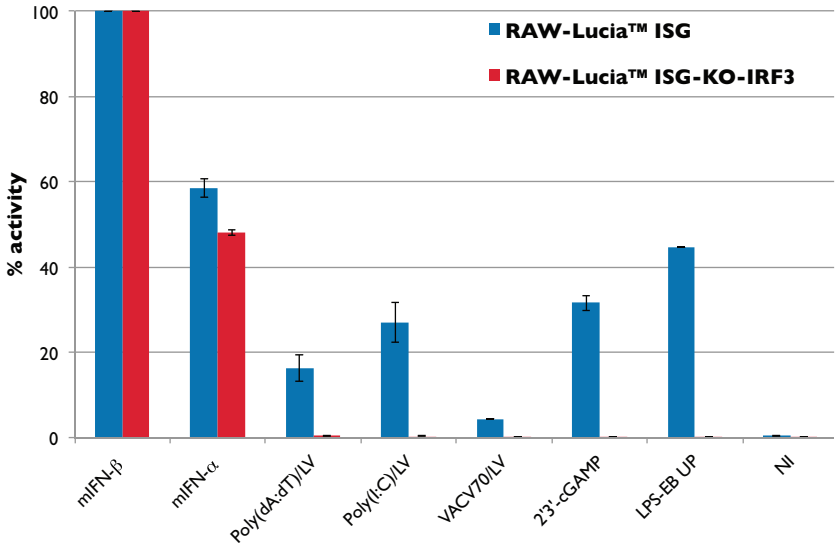

Figure 2: Stimulation of RAW-Lucia™ ISG-KO-IRF3 and RAW-Lucia™ ISG cells (parental cell line) with poly(dA:dT)/LyoVec™ (1 µg/ml), VACV70/LyoVec™ (1 µg/ml), poly(I:C)/LyoVec™ (1 µg/ml), and 2'3'-cGAMP (3 µg/ml). Mouse IFN-α ($1x10^4$ U/ml) and IFN-β ($1x10^4$ U/ml) serve as positive controls. Non-induced cells (NI) have 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 mIFN-β at $1x10^4$ U/ml (taken as 100%).

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