

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

<https://www.invivogen.com/raw-lucia-isg-ko-irf3>

For research use only

Version 19K14-MM

RAW-Lucia™ ISG-KO-IRF3 cells were generated from the RAW-Lucia™ ISG cell line through the stable knockout of the interferon regulatory factor 3 (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 (figure 1), sequencing, and Western blot (figure 2). Biological activity has been assessed using the Lucia luciferase reporter assay to monitor IRF induction (figure 3).

PCR amplification

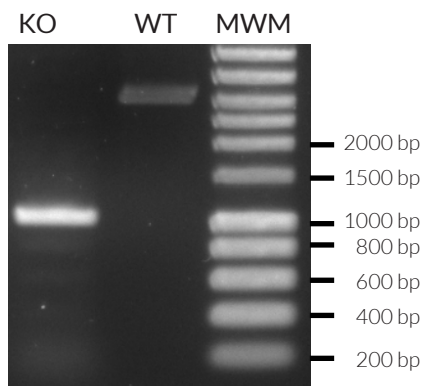


Figure 1: PCR amplification of the targeted region in the RAW-Lucia™ ISG-KO-IRF3 (KO) and RAW-Lucia™ ISG (WT) cells. MWM = molecular weight marker

Western blot

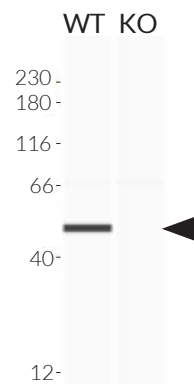


Figure 2: Validation of IRF3 knockout by Western blot (Wes™). Analysis of lysates from the RAW-Lucia™ ISG (WT) and RAW-Lucia™ ISG-KO-IRF3 (KO) cells using Anti-IRF3, followed by an HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the murine IRF3 protein (47 kDa).

IRF INDUCTION (Lucia luciferase reporter)

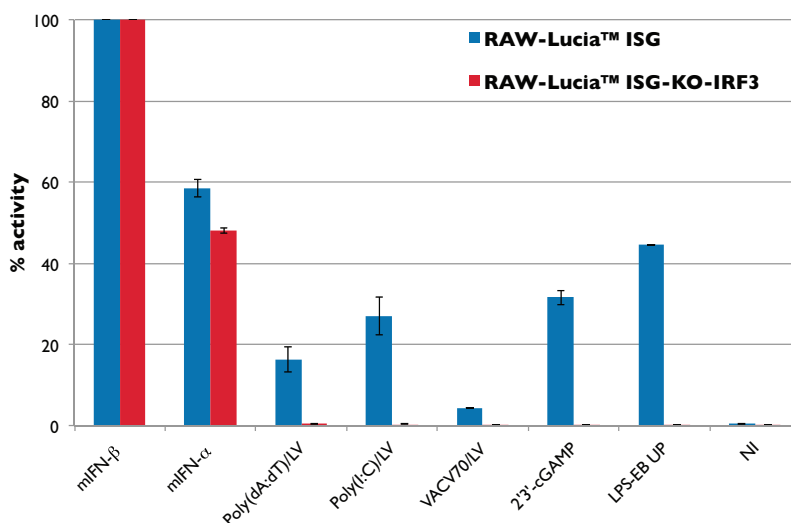


Figure 3: Stimulation of RAW-Lucia™ ISG-KO-IRF3 and RAW-Lucia™ ISG cells (parental cell line) with poly(dA:dT)/LyovVec™ (1 µg/ml), VACV70/LyovVec™ (1 µg/ml), poly(I:C)/LyovVec™ (1 µg/ml), and 2'3'-cGAMP (3 µg/ml). Mouse IFN-α (1x10⁴ U/ml) and IFN-β (1x10⁴ U/ml) serve as positive controls. Non-induced 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 1 x 10⁴ U/ml (taken as 100%).

TECHNICAL SUPPORT

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