

# Validation data for RAW-Lucia™ ISG-KO-IRF7 cells

<https://www.invivogen.com/raw-lucia-isg-ko-irf7>

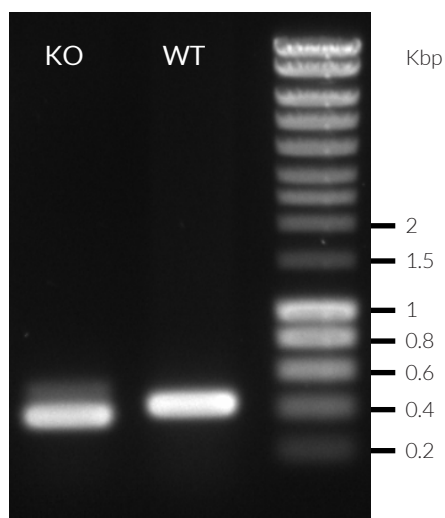
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RAW-Lucia™ ISG-KO-IRF7 cells were generated from the RAW-Lucia™ ISG cell line through the stable knockout of the *IRF7* gene, as verified by PCR (Figure 1) and sequencing. These cells derive from the murine RAW 264.7 macrophage cell line, which has been reported to express many pattern recognition receptors (PRRs), including RIG-I, MDA-5, and several cytosolic DNA sensors (CDSs) including cGAS. RAW-Lucia™ ISG-KO-IRF7 cells feature a reporter gene allowing the study of the interferon regulatory factor (IRF) pathway by monitoring the activity of an IRF-inducible secreted Lucia luciferase.

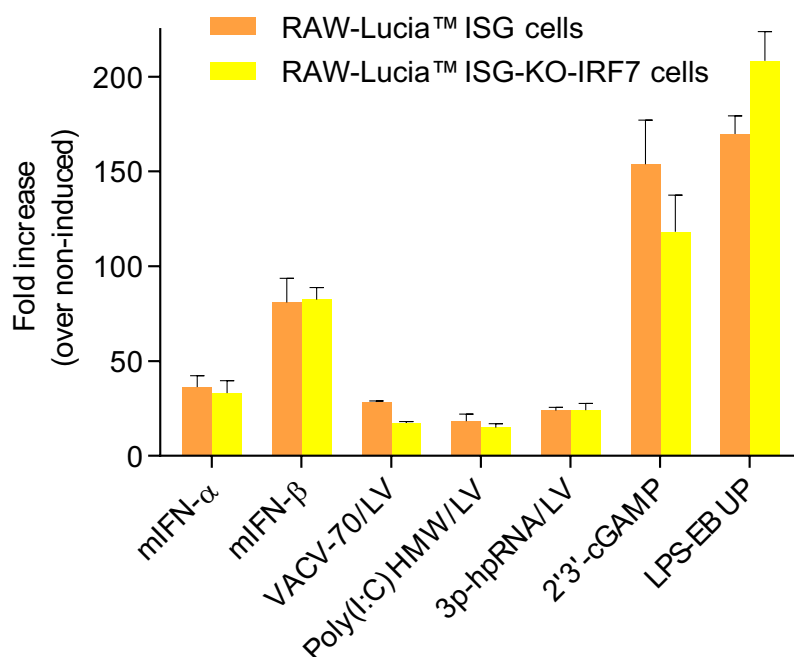
Biological activity in RAW-Lucia™ ISG-KO-IRF7 cells has been assessed using the Lucia luciferase reporter assay to monitor IRF induction (Figure 2). Because IRF7 and other IRF family members bind to closely related and overlapping ISRE motifs in ISG promoters, RAW-Lucia™ ISG-KO-IRF7 cells and their RAW-Lucia™ ISG parental cells display similar induction of the Lucia luciferase reporter upon incubation with several different PPR agonists, such as the RIG-I agonist 3p-hpRNA delivered intracellularly using the cationic lipid LyoVec™ (Figure 2).

## Validation of *IRF7* knockout



**Figure 1: Validation of *IRF7* KO.** The targeted *IRF7* region in RAW-Lucia™ ISG (WT) parental cells and RAW-Lucia™ KO ISG-KO-IRF7 (KO) cells was amplified by PCR. RAW-Lucia™ ISG-KO-IRF7 cells feature a frameshift deletion causing an early stop codon and inactivation of *IRF7*.

## Functional validation of *IRF7* knockout



**Figure 2: IRF response in RAW-Lucia™ ISG-derived cells.** RAW-Lucia™ ISG and RAW-Lucia™ ISG-KO-IRF7 cells were incubated with 1000 U/ml of murine interferon-α (mIFN-α: IRF-Lucia positive control), mIFN-β (IRF-Lucia positive control), 1 µg/ml VACV-70/LyoVec™ (CDS agonist), Poly(I:C)/LyoVec™ (RIG-I/MDA-5 agonist), 3p-hpRNA/LyoVec™ (RIG-I agonist), 10 µg/ml 2'3'-cGAMP (STING agonist), 1 µg/ml LPS-EB Ultrapure (TLR4 agonist). After overnight incubation, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using the QUANTI-Luc™ detection reagent. Activity fold increase over non-induced cells is shown.

### TECHNICAL SUPPORT

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