Validation data for RAW-Lucia™ ISG-KO-TREX1 cells

https://www.invivogen.com/raw-lucia-isg-ko-trex1

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Version 19K29-MM

RAW-Lucia™ ISG-KO-TREX1 cells were generated from the RAW-Lucia™ ISG cell line through the stable knockout of the TREX1 gene. These cells derive from the murine RAW 264.7 macrophage cell line. The knockout of the TREX1 gene in RAW-Lucia™ ISG-KO-TREX1 cells has been confirmed by sequencing, PCR (figure 1A), Western blot (figure 1B) and functional assays (figure 2). Biological activity has been assessed using the Lucia luciferase reporter assay to monitor IRF induction (figure 2). The response of RAW-Lucia™ ISG-KO-TREX1 cells to murine type I interferons (IFNs) is unaffected by the knockout of the TREX1 gene. The knockout of the TREX1 gene appears to alter the response to various stimuli, such as VACV70/LyoVec™ (transfected double-stranded DNA).

Validation of TREX1 Knockout (KO)

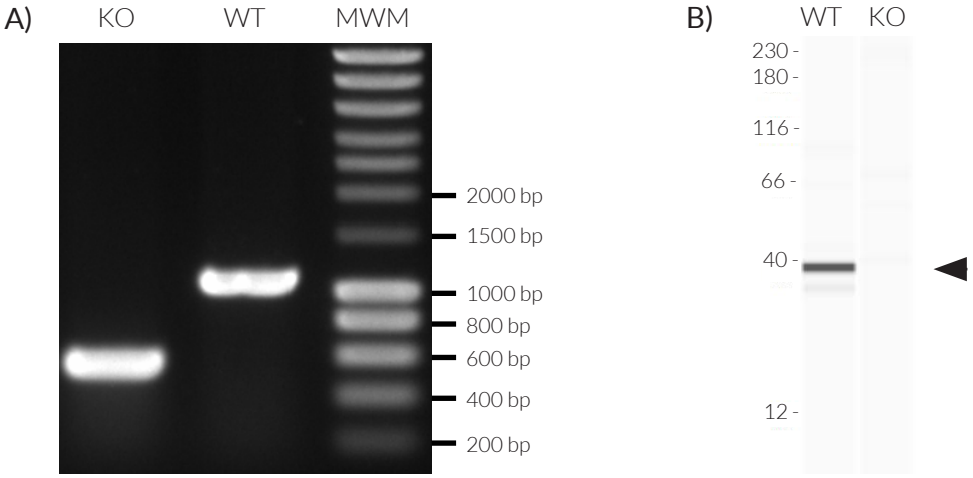

Figure 1: Validation of TREX1 knockout in RAW-Lucia™ ISG-KO-TREX1 cells. **(A)** The targeted TREX1 region in RAW-Lucia™ ISG (WT) and RAW-Lucia™ ISG KO-TREX1(KO) cells was amplified by PCR. RAW-Lucia™ ISG-KO-TREX1 cells feature a biallelic deletion (arrow). MWM = molecular weight marker. **(B)** Lysates from RAW-Lucia™ ISG (WT) and RAW-Lucia™ ISG KO-TREX1 (KO) cells were analyzed by Western blot (Wes™) using an anti-murine TREX1 antibody, followed by an HRP conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the TREX1 protein (34 kDa).

Functional validation of RAW-Lucia™ ISG-KO-TREX1 cells

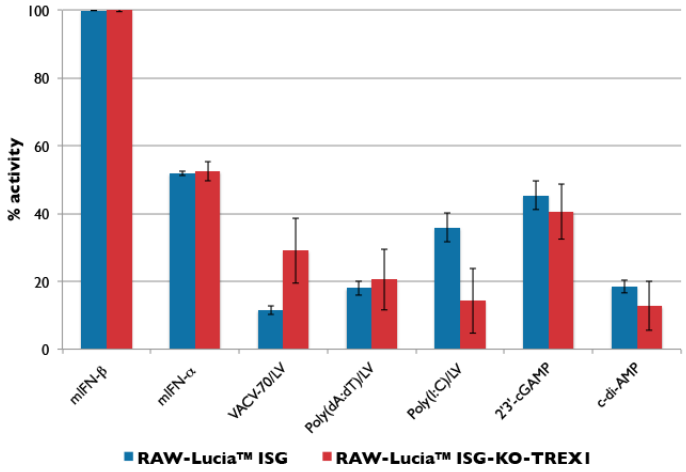

Figure 2: RAW-Lucia™ ISG-KO-TREX1 and RAW-Lucia™ ISG cells (parental cell line) were incubated with VACV70/LyoVec™ (1 µg/ml), poly(dA:dT)/LyoVec™ (1 µg/ml), poly(I:C) HMW/LyoVec™ (1 µg/ml), 5'ppp-dsRNA/LyoVec™ (1 µg/ml), 2'3'-cGAMP (3 µg/ml) and c-di-AMP (3 µg/ml). Mouse IFN-α (1x10^4 U/ml) and IFN-β (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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