Validation data for RAW-Lucia™ ISG-KO-RIG-I cells

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

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Version 19K14-MM

RAW-Lucia™ ISG-KO-RIG-I cells were generated from the RAW-Lucia™ ISG cell line, which is derived from the murine RAW 264.7 macrophage cell line, through the stable knockout of the RIG-I gene which has been confirmed by Western blot (figure 1). RAW-Lucia™ ISG-KO-RIG-I and RAW-Lucia™ ISG cells express a secreted reporter gene, Lucia luciferase, under the control of the I-ISG54 (interferon-stimulated genes 54) promoter which is comprised of the interferon (IFN)-inducible ISG54 promoter enhanced by a multimeric ISRE (IFN-stimulated response elements). As a result, RAW-Lucia™ ISG-KO-RIG-I cells can be used to study the RIG-I signaling pathway through the monitoring of IRF (interferon regulatory factor) activation by determining the activity of Lucia luciferase (figure 2). The response of RAW-Lucia™ ISG-KO-RIG-I cells to murine type I IFNs is unaffected by the knockout of the RIG-I gene. As expected, the ability of RAW-Lucia™ ISG-KO-RIG-I cells to respond to transfected RNA is greatly reduced. Furthermore, when compared to the parental cell line, RAW-Lucia™ ISG-KO-RIG-I cells respond weakly to cyclic dinucelotides.

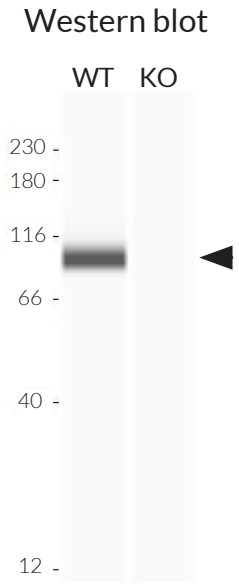

Figure 1: Validation of RIG-I knockout by Western blot (Wes™). Analysis of lysates from the RAW-Lucia™ (WT) and RAW-Lucia™ ISG-KO-RIG-I (KO) cells using Anti-RIG-I, followed by an HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the murine RIG-I protein (102 KDa).

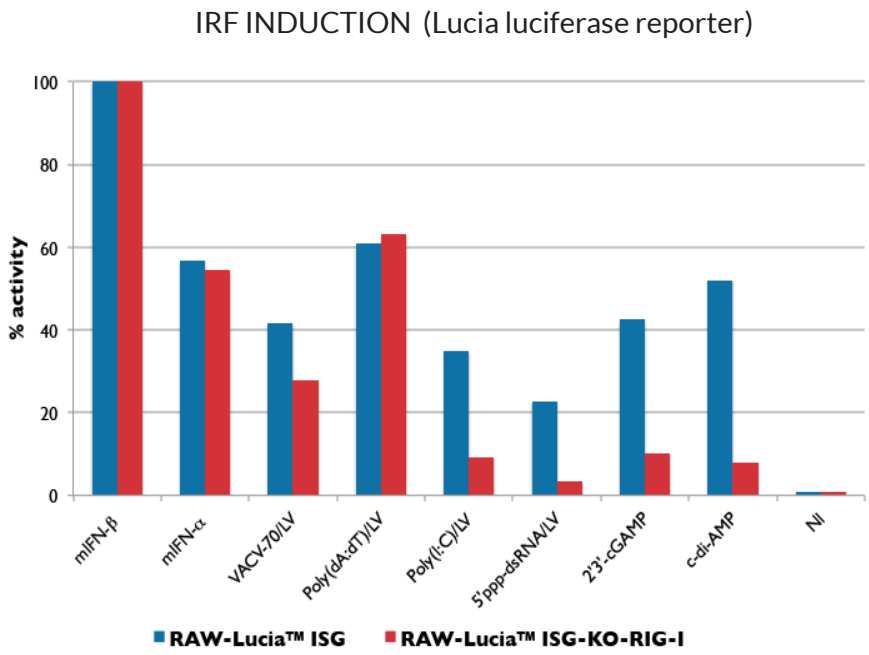

Figure 2: Response of RAW-Lucia™ ISG-KO-RIG-I cells to various stimuli. RAW-Lucia™ ISG-KO-RIG-I and RAW-Lucia™ ISG cells (parental cell line) were incubated with VACV70/LyoVec™ (1 µg/ml), poly(dA:dT)/LyoVec™ (1 µg/ml), poly(I:C) HMW/LyoVec™ (1 µg/ml), 5'ppp-dsRNA/LyoVec™ (1 µg/ml), 2'3'-cGAMP (3 µg/ml) 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. 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%).

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com