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

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

For research use only

Version 19K10-MM

RAW-Lucia™ ISG-KO-STING 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 STING gene which has been confirmed by sequencing and Western blot (figure 1). RAW-Lucia™ ISG and RAW-Lucia™ ISG-KO-STING cells express a secreted reporter gene, Lucia luciferase, under the control of the I-ISG54 promoter which is comprised of the IFN-inducible ISG54 promoter enhanced by a multimeric ISRE. As a result, RAW-Lucia™ ISG and RAW-Lucia™ ISG-KO-STING cells can be used to study the STING signaling pathway through the monitoring of IRF activation by determining the activity of Lucia luciferase (figure 2). The response of RAW-Lucia™ ISG-KO-STING cells to murine type I interferons (IFNs) is unaffected by the knockout of the STING gene. As expected, RAW-Lucia™ ISG-KO-STING cells do not respond to cyclic dinucleotides.

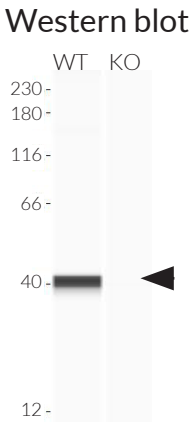

Figure 1: Validation of STING knockout by Western blot (Wes™). Analysis of lysates from the RAW-Lucia™ ISG (WT) and RAW-Lucia™ ISG-KO-STING (KO) cells using Anti-STING, followed by an HRP-conjugated anti-rabbit secondary antibody. The arrow indicates the expected band for the murine STING protein (43 kDa).

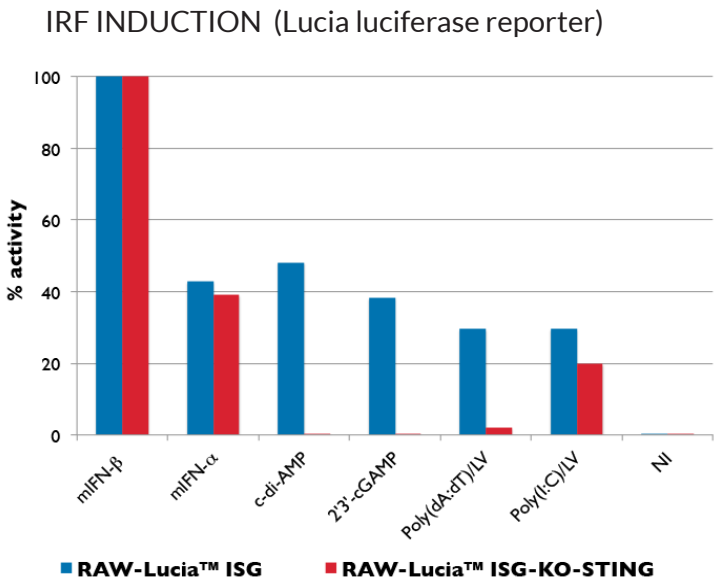

Figure 2: Response of RAW-Lucia™ ISG-KO-STING cells to various stimuli. RAW-Lucia™ ISG-KO-STING and RAW-Lucia™ ISG cells (parental cell line) were incubated with c-di-AMP (3 µg/ml), 2'3'-cGAMP (3 µg/ml), poly(dA:dT)/LyoVec™ (1 µg/ml), and poly(I:C) HMW/LyoVec™ (1 µg/ml). Mouse IFN-α (1x10^4 U/ml) and IFN-β (1 x 10^4 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 1 x 10^4 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 3622-3480
E-mail: info@invivogen.com