Validation data for RAW-Dual™ cells (IRF-Lucia/KI-[MIP-2]SEAP)

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Version 18L07-MM

RAW-Dual™ (IRF-Lucia/KI-[MIP-2]SEAP) cells are dual reporter cells that were designed to simultaneously study the interferon regulatory factor (IRF) pathway and the NF-κB pathway, by monitoring the activity of Lucia luciferase and of SEAP, respectively. These cells were generated from RAW 264.7 murine macrophages which express many pattern recognition receptors (PRRs) such as the Toll-like receptors (TLRs) TLR2 and TLR4, and the cyclic dinucleotide sensor STING. The IRF induction response of these cells to different ligands has been assessed (Figure 1). RAW-Dual™ cells respond to murine interferons (e.g. mIFN-α and mIFN-β) and diverse PRR ligands (e.g. the TLR4 ligand lipopolysaccharide (LPS), the TLR3 ligand Poly(I:C) and the STING ligand cyclic 2'3'-cGAMP). The NF-κB response of RAW-Dual™ cells to different PRR ligands has been assessed (Figure 2). Several TLR ligands (e.g. LPS and the TLR2 ligands HKLM, Pam3CSK4 and Zymosan) induce the NF-κB pathway in these cells.

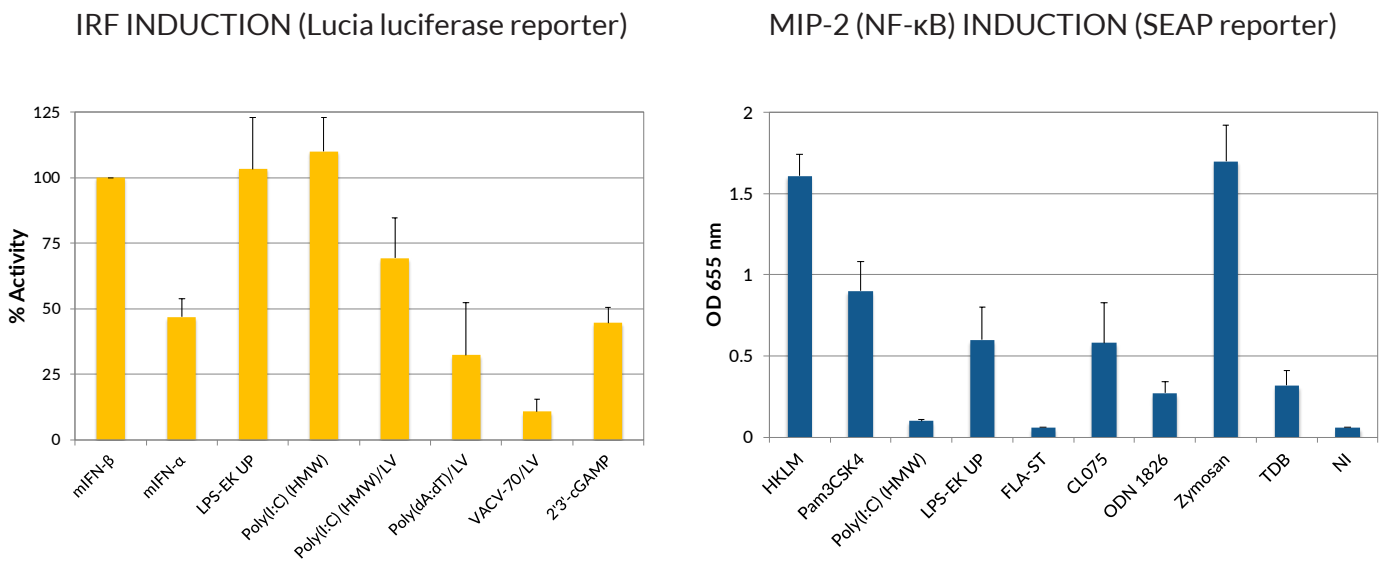

Figure 1: RAW-Dual™ cells were stimulated with murine IFN-β (mIFN-β; 1x10⁴ U/ml), mIFN-α (1x10⁴ U/ml), LPS-EK Ultrapure (100 ng/ml; TLR4 ligand), poly(I:C) HMW (1 µg/ml; TLR3 ligand), Poly(I:C) HMW/LyoVec™ (1 µg/ml; RIG-I/MDA5 ligand), poly(dA:dT)/LyoVec™ (1 µg/ml; RIG-I ligand & cytosolic DNA sensor (CDS) ligand), VACV-70/LyoVec™ (1 µg/ml; CDS ligand), or 2'3'-cGAMP (10 µg/ml; STING ligand). 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%).

Figure 2: RAW-Dual™ cells were incubated with HKLM (1x10⁸ cells/ml; heat-killed *Listeria monocytogenes*, TLR2 ligand), Pam3CSK4 (1 µg/ml; TLR2 ligand), poly(I:C) HMW (10 µg/ml; TLR3 ligand), LPS-EK Ultrapure (1 µg/ml; TLR4 ligand), FLA-ST (10 µg/ml; TLR5 ligand), CL075 (1 µg/ml; TLR 7/8 ligand), ODN 1826 (1 µg/ml; TLR9 ligand), Zymosan (10 µg/ml; TLR2 & Dectin-1 ligand), or TDB (100 µg/ml; Mincle ligand). Non-induced cells (NI) have been included as a negative control. After a 24h incubation, NF-κB activation was determined using QUANTI-Blue™, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm.

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