Validation data for A549-Dual™ cells

For research use only

Version # 15F11-MM

A549-Dual™ cells have been derived from the human A549 lung carcinoma cell line which expresses numerous pattern recognition receptors (PRRs), including the RIG-I-like receptor (RLR) RIG-I, and the Toll-like receptors (TLRs) TLR2, TLR3 and TLR5 but not TLR4. A549-Dual™ cells have been transfected with two inducible reporter constructs: an IRF-inducible Lucia luciferase and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase). As a result, A549-Dual™ cells allow to simultaneously study the NF-κB pathway, by assessing the activity of SEAP (see figure 1), and the interferon regulatory factor (IRF) pathway, by monitoring the activity of Lucia luciferase (see figure 2).

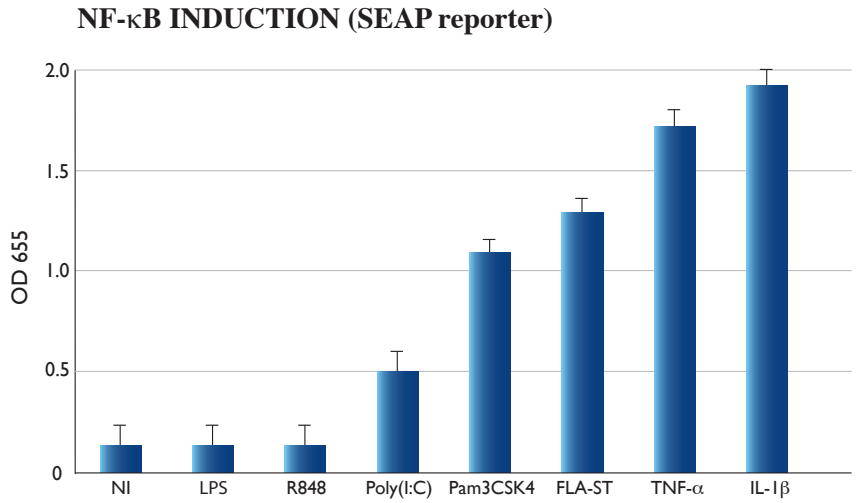

Figure 1: Stimulation of A549-Dual™ cells with the following PAMPs, Pam3CSK4 (TLR2 ligand, 300 ng/ml) Poly(I:C) (TLR3 ligand, 3 µg/ml), FLA-ST Ultrapure (TLR5, 300 ng/ml), leads to the activation of NF-κB. TNF-α (1 ng/ml) and IL-1β (1 ng/ml) have been included as positive controls to activate the NF-κB signaling pathway. Non-induced cells (NI), the TLR4 ligand (LPS-EB Ultrapure; 10^3 EU/ml), and the TLR7/8 ligand (R848; 10 µg/ml) have been included as negative controls. 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.

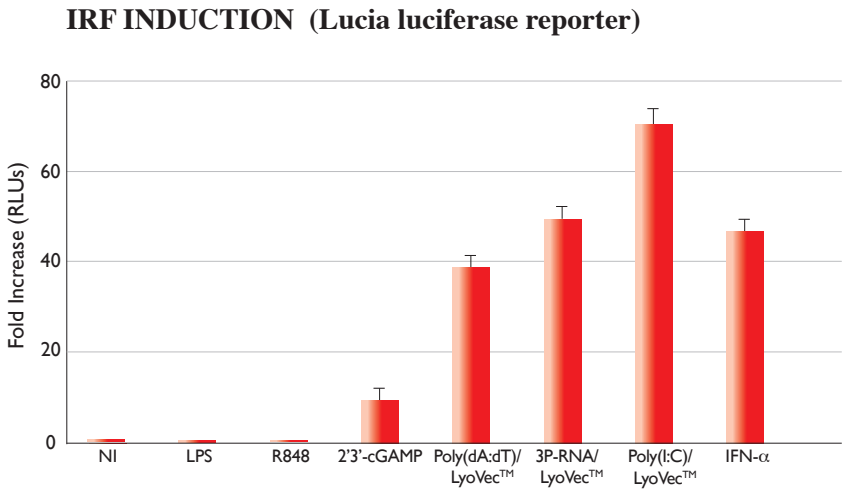

Figure 2: Stimulation of A549-Dual™ cells with RLR ligands, such as transfected poly(I:C) (100 ng/ml), 5'ppp-dsRNA (1 µg/ml) or poly(dA:dT) (100 ng/ml) triggers the IRF pathway. The STING agonist 2'3'-cGAMP (30 µg/ml) leads to low-level IRF induction in A549-Dual™ cells. IFN-α ($1x10^4$ U/ml) has been included as a positive control to activate the IRF signaling pathway. Non-induced cells (NI), the TLR4 ligand (LPS-EB Ultrapure; 10^3 EU/ml), and the TLR7/8 ligand (R848; 10 µg/ml) have been included as negative 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.

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

InvivoGen
www.invivogen.com