Validation data for HCT116-Dual™ cells

For research use only

Version # 15F29-MM

HCT116-Dual™ cells have been derived from the human HCT116 colorectal carcinoma cell line which expresses numerous pattern recognition receptors (PRRs), including the NOD-like receptors (NLRs) NOD1 and NOD2, the RIG-I-like receptor (RLR) RIG-I, and the Toll-like receptors (TLRs) TLR3 and TLR5 but not TLR2 or TLR4. HCT116-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, HCT116-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).

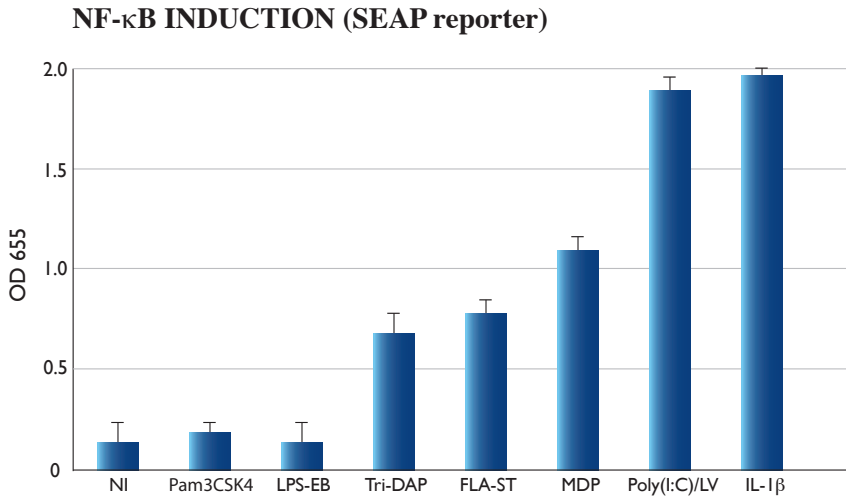

Figure 1: Stimulation of HCT116-Dual™ cells with the following PAMPs, Tri-DAP (NOD1 ligand, 10 µg/ml), FLA-ST Ultrapure (TLR5, 1 µg/ml), MDP (10 µg/ml), and Poly(I:C) (TLR3 ligand, 1 µg/ml) leads to the activation of NF-κB. IL-1β (100 ng/ml) has been included as positive controls to activate the NF-κB signaling pathway. Non-induced cells (NI), the TLR2 ligand (Pam3CSK4; 300 ng/ml), and the TLR4 ligand (LPS-EB Ultrapure; 10^4 EU/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.

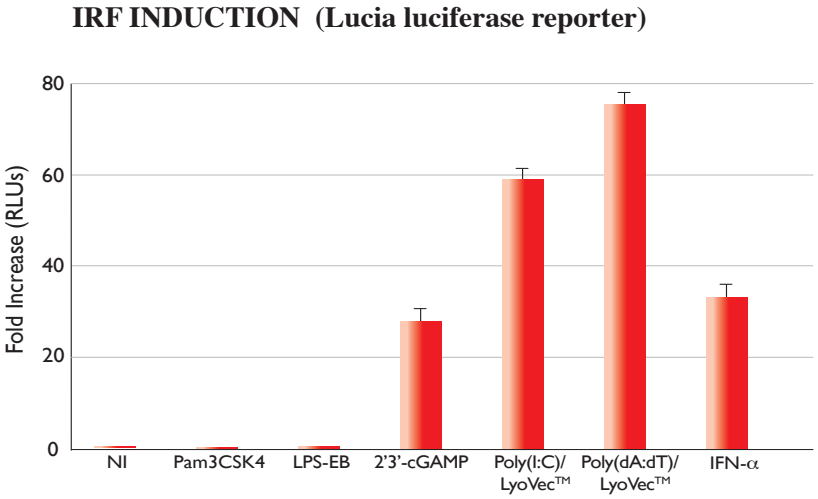

Figure 2: Stimulation of HCT116-Dual™ cells with the STING agonist 2'3'-cGAMP (30 µg/ml) or RLR ligands, such as transfected poly(I:C) (100 ng/ml) or poly(dA:dT) (100 ng/ml), triggers the IRF pathway. IFN-α ($1x10^4$ U/ml) has been included as a positive control to activate the IRF signaling pathway. Non-induced cells (NI), the TLR2 ligand (Pam3CSK4; 300 ng/ml), and the TLR4 ligand (LPS-EB Ultrapure; 10^4 EU/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.

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