# Validation data for HepG2-Dual™ cells

http://www.invivogen.com/hepg2-dual

For research use only

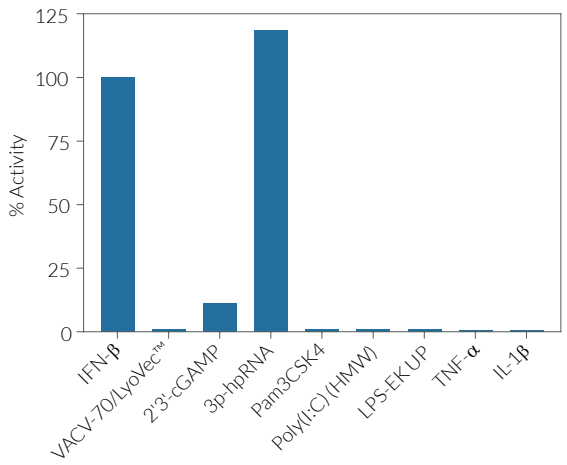
Version 18G24-MM

HepG2-Dual™ cells derive from the HepG2 adherent epithelial human hepatocellular carcinoma (HCC) cell line. This cell line expresses multiple pattern recognition receptors (PRRs), including the cytosolic RNA sensors RIG-I and MDA-5. The expression and activation profile of other PRRs such as the Toll-like receptors (TLRs) in HepG2 cells is still poorly characterized. These cells also express low levels of the cyclic dinucleotide sensor STING (stimulator of interferon genes, also known as MITA) while expression of the cyclic GMP-AMP synthase (cGAS) cytosolic DNA sensor is defective in these cells. HepG2-Dual™ cells stably express two inducible reporter constructs that enable the simultaneous study of the NF-κB pathway, through monitoring the activity of SEAP, and the interferon regulatory factor (IRF) pathway, through assessing the activity of the secreted Lucia luciferase.

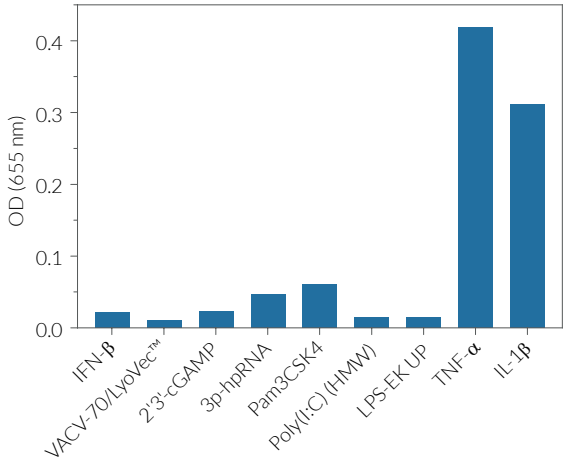
HepG2-Dual™ cells were stimulated with numerous ligands. None of the TLR ligands tested induced an NF-κB or IRF response, with the exception of the TLR2 ligand Pam3CSK4, which induced a weak NF-κB response. Consistent with the defective expression of cGAS in the parental cell line, HepG2-Dual™ cells did not respond to VACV-70/LyoVec™ stimulation. A moderate IRF response is detected in HepG2-Dual™ cells upon incubation with STING agonists such as 2'3'-cGAMP, while the 3p-hpRNA RIG-I agonist, an *in vitro* transcribed 5' triphosphate hairpin RNA, induces a strong IRF response. The cytokines IL-1β and TNF-α can be used as positive controls to activate the NF-κB pathway, while type I interferons can be used as positive controls to induce the IRF pathway.

**Evaluation of IRF- and NF-κB-induced responses**

**a. IRF induction**
(Lucia luciferase reporter)




**b. NF-κB induction**
(SEAP reporter)




HepG2-Dual™ cells were stimulated with human IFN-β (hIFN-β; 1x10⁴ U/ml), VACV/LyoVec™ (1 µg/ml), 2'3'-cGAMP (30 µg/ml), 3p-hpRNA (1 µg/ml), Pam3CSK4 (10 µg/ml), Poly(I:C) HMW (10 µg/ml), LPS-EK Ultrapure (10 µg/ml), TNF-α (100 ng/ml) and IL-1β (100 ng/ml). After overnight incubation, the IRF (panel a) and NF-κB (panel b) responses were determined. IRF induction was assessed 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 hIFN-β at 1x10⁴ U/ml (taken as 100%). NF-κB induction was assessed 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