Validation data for HEK-Dual™ hTLR5 (NF/IL8) cells

(NF-κB-SEAP/KI-[IL-8]Lucia)

http://www.invivogen.com/hek-dual-htlr5

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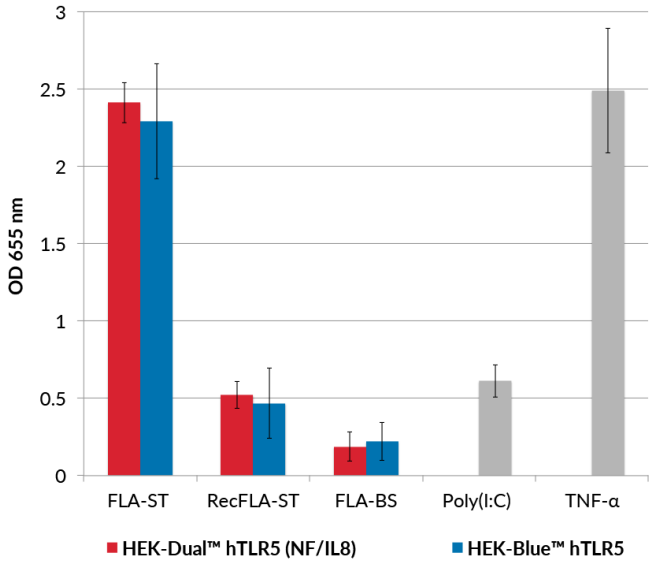
Version # 17B06-MM

HEK-Dual™ hTLR5 (NF/IL8) cells are designed for studying the stimulation of human TLR5 (hTLR5) by monitoring the activation of the NF-κB/AP-1 and the IL-8 pathways. They stably express hTLR5 and two different reporter proteins: SEAP and the secreted Lucia luciferase. In addition, these cells are double knockout for TLR3 and the TNF receptor, thus enabling the study of hTLR5 signaling without interference from other TLRs. They respond to low concentrations of the TLR5 agonist flagellin. They do not respond to other TLR agonists or to the cytokine TNF-α. The NF-κB response of these cells has been compared to that of HEK-Blue™ hTLR5 cells (SEAP reporter cells) using QUANTI-Blue™, a SEAP detection medium (see figure 1). Furthermore, HEK-Dual™ hTLR5 (NF/IL8) cells also enable monitoring activation of the IL-8 pathway using QUANTI-Luc™, a Lucia luciferase detection medium (see figure 2).

NF-κB (SEAP) response



KI-IL-8 (Lucia) response

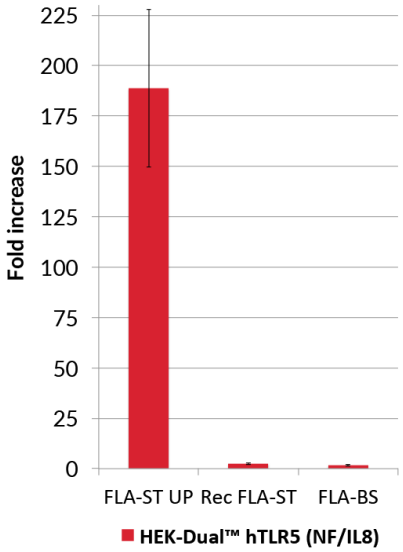


Figure 1: Detection of the NF-κB response using QUANTI-Blue™. HEK-Dual™ hTLR5 (NF/IL8) and HEK-Blue™ hTLR5 cells were stimulated with various TLR agonists: FLA-ST (flagellin from *S. typhimurium,* TLR5 agonist; 100 ng/ml), RecFLA-ST (recombinant flagellin from *S. typhimurium,* TLR5 agonist; 100 ng/ml), FLA-BS (flagellin from *B. subtilis,* TLR5 agonist; 100 ng/ml), Poly(I:C) HMW (TLR3 agonist; 1 µg/ml) and TNF-α (10 ng/ml). The TLR3 and TNFR activites due to the endogenous expression of these receptors in HEK-Blue™ hTLR5 cells are shown in gray. After 24 hour incubation, NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ and reading the optical density (OD) at 655 nm.

Figure 2: Detection of the IL-8 response using QUANTI-Luc™. HEK-Dual™ hTLR5 (NF/IL8) cells were stimulated with TLR5 agonists: FLA-ST (100 ng/ml), FLA-BS (100 ng/ml), and RecFLA-ST (1 µg/ml). After 24 hour incubation, activation of the IL-8 promoter was determined by measuring the relative light units (RLUs) in a luminometer using QUANTI-Luc™, a Lucia luciferase detection reagent. The activation of the IL-8 promoter is expressed as fold increase relative to untreated cells which was calculated by dividing the RLUs for the treated cells by the RLUs for the untreated cells.

Cell line	EC50 for FLA-ST(ng/ml)	EC50 for recFLA-ST (ng/ml)	EC50 for FLA-BS (ng/ml)
HEK-Dual™ hTLR5 (NF/IL8)	1.4± 0.7	200 ± 37.1	568.9± 195.5
HEK-Blue™ hTLR5	2.2 ± 1.7	219.4 ± 95.8	410.5 ± 65.6

Table 1: EC50 values calculated for the NF-κB response using QUANTI-Blue™. HEK-Dual™ hTLR5 (NF/IL8) and HEK-Blue™ hTLR5 cells were stimulated for 24 hours with various TLR5 agonists: FLA-ST, recFLA-ST and FLA-BS.

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