Validation data for HEK-Dual™ hTLR2 (NF/IL8) cells

(NF-κB-SEAP/KI-[IL-8]Lucia)

http://www.invivogen.com/hek-dual-htlr2

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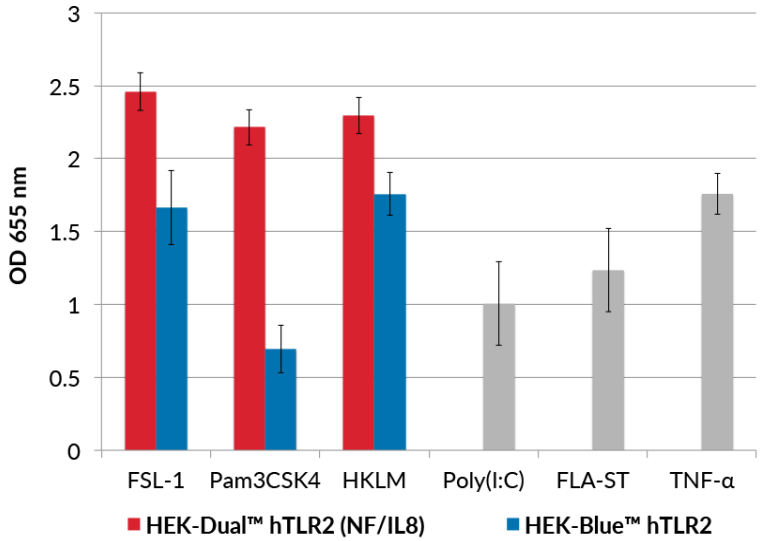
Version # 17B06-MM

HEK-Dual™ hTLR2 (NF/IL8) cells are designed for studying the stimulation of human TLR2 (hTLR2) by monitoring the activation of the NF-κB/AP-1 and the IL-8 pathways. They stably express hTLR2 together with the human CD14 co-receptor. They also express two different reporter proteins: SEAP and the secreted Lucia luciferase. In addition, these cells are triple knockout for TLR3, TLR5 and the TNF receptor, thus enabling the study of hTLR2 signaling without interference from other TLRs. They respond to very low concentrations of TLR2 agonists such as the synthetic lipopeptide Pam3CSK4. 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™ hTLR2 cells (SEAP reporter cells) using QUANTI-Blue™, a SEAP detection medium (see figure 1). Furthermore, HEK-Dual™ hTLR2 (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

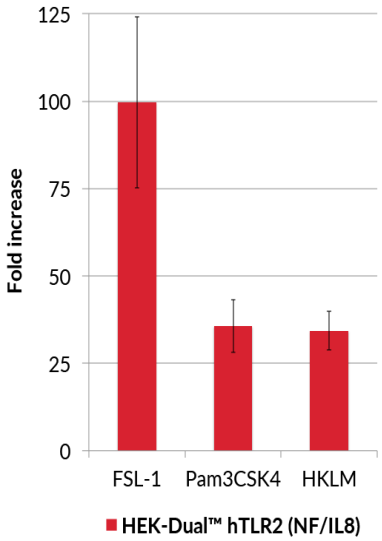


Figure 1: Detection of the NF-κB response using QUANTI-Blue™. HEK-Dual™ hTLR2 (NF/IL8) and HEK-Blue™ hTLR2 cells were stimulated with various TLR agonists: FSL-1 (TLR2 agonist; 30 pg/ml), Pam3CSK4 (TLR2 agonist; 30 pg/ml), HKLM (TLR2 agonist; $1x10^7$ cells/ml), Poly(I:C) (TLR3 agonist; 3 µg/ml), FLA-ST (flagellin from *S. typhimurium*, TLR5 agonist; 100 ng/ml), and TNF-α (10 ng/ml). The TLR3, TLR5 and TNFR activities due to the endogenous expression of these receptors in HEK-Blue™ hTLR2 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™ hTLR2 (NF/IL8) cells were stimulated with TLR2 agonists: FSL-1 (TLR2 agonist; 30 pg/ml), Pam3CSK4 (TLR2 agonist; 30 pg/ml) and HKLM ($1x10^7$ cells/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 FSL-1 (pg/ml)	EC50 for Pam3CSK4 (pg/ml)	EC50 for HKLM (cells/ml)
HEK-Dual™ hTLR2 (NF/IL8)	0.3 ± 0.1	2.5 ± 0.1	$0.5 \times 10^6 \pm 0.1 \times 10^6$
HEK-Blue™ hTLR2	2.9 ± 0.5	26.4 ± 2.9	$1.6 \times 10^6 \pm 0.5 \times 10^6$

Table 1: EC50 values calculated for the NF-κB response using QUANTI-Blue™. HEK-Dual™ hTLR2 (NF/IL8) and HEK-Blue™ hTLR2 cells were stimulated for 24 hours with various TLR2 agonists: FSL-1, Pam3CSK4 and HKLM.

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