

Validation data for HEK-Blue-Lucia™ hTLR3 (NF/IL8) cells (NF-κB-SEAP/KI-[IL-8]Lucia)

<https://www.invivogen.com/hek-blue-lucia-htlr3>

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Version 23111-AK

HEK-Blue-Lucia™ hTLR3 (NF/IL8) cells are designed for studying the stimulation of human TLR3 (hTLR3) by monitoring the activation of the NF-κB/AP-1 and the IL-8 pathways. They stably express hTLR3 and two different reporter proteins: secreted embryonic alkaline phosphatase (SEAP) and secreted Lucia luciferase. In addition, these cells are double knockout for TLR5 and the TNF receptor, thus enabling the study of hTLR3 signaling without interference from other TLRs. They respond to very low concentrations of TLR3 agonists such as poly(I:C) a synthetic analog of double-stranded RNA. 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™ hTLR3 cells (SEAP reporter cells) using QUANTI-Blue™ Solution, a SEAP detection medium (Figure 1). Furthermore, HEK-Blue-Lucia™ hTLR3 (NF/IL8) cells also enable monitoring activation of the IL-8 pathway using QUANTI-Luc™ 4 Lucia/Gaussia, a Lucia and Gaussia luciferase detection reagent (Figure 2).

NF-κB (SEAP) response

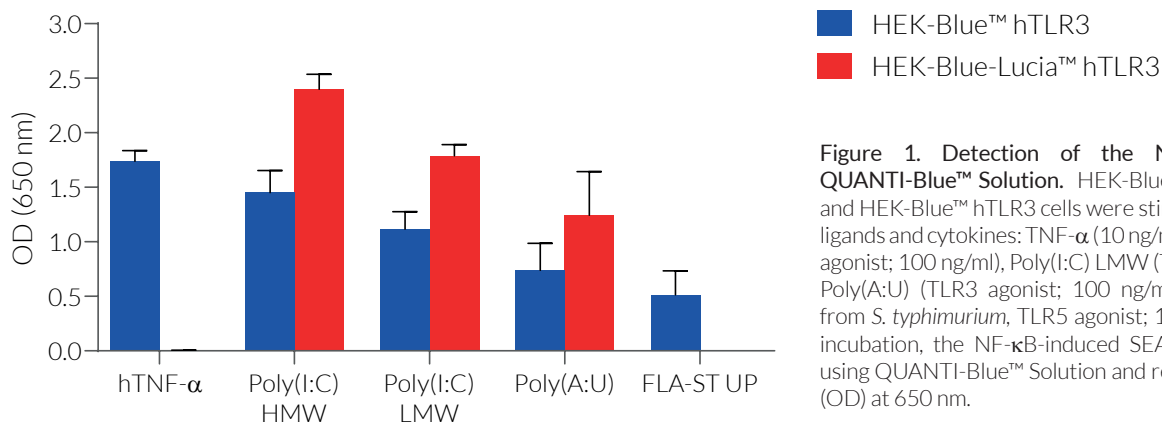


Figure 1. Detection of the NF-κB response using QUANTI-Blue™ Solution. HEK-Blue-Lucia™ hTLR3 (NF/IL8) and HEK-Blue™ hTLR3 cells were stimulated with various TLR ligands and cytokines: TNF-α (10 ng/ml), Poly(I:C) HMW (TLR3 agonist; 100 ng/ml), Poly(I:C) LMW (TLR3 agonist; 100 ng/ml), Poly(A:U) (TLR3 agonist; 100 ng/ml), and FLA-ST (flagellin from *S. typhimurium*, TLR5 agonist; 100 ng/ml). After 24 hour incubation, the NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ Solution and reading the optical density (OD) at 650 nm.

KI-IL-8 (Lucia) response

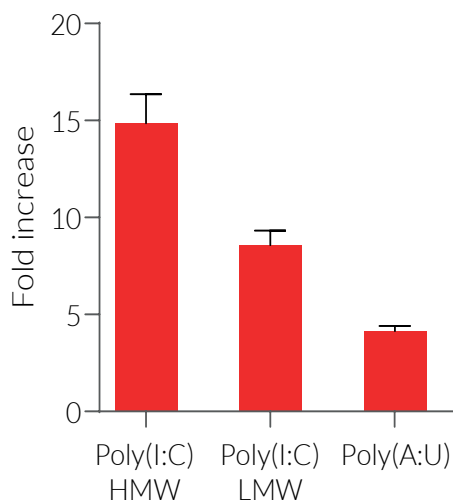


Figure 2. Detection of the IL-8 response using QUANTI-Luc™ 4 Lucia/Gaussia. HEK-Blue-Lucia™ hTLR3 (NF/IL8) cells were stimulated with TLR3 agonists: Poly(I:C) HMW (100 ng/ml), Poly(I:C) LMW (100 ng/ml) and Poly(A:U) (100 ng/ml). After 24 hour incubation, activation of the IL-8 promoter was determined by measuring the relative light units (RLUs) in a luminometer using QUANTI-Luc™ 4 Lucia/Gaussia, a Lucia and Gaussia luciferase detection reagent. Data are shown in fold response over non-induced cells (mean ± SEM).

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 Asia: +852 3-622-34-80
E-mail: info@invivogen.com