

Validation data for HEK-Blue-Lucia™ mTLR7 (NF/IL8) cells (NF-κB-SEAP/KI-[IL-8]Lucia)

<https://www.invivogen.com/hek-blue-lucia-mtlr7>

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Version 23K28-AK

HEK-Blue-Lucia™ mTLR7 (NF/IL8) cells are designed for studying the stimulation of murine TLR7 (mTLR7) by monitoring the activation of the NF-κB/AP-1 and the IL-8 pathways. They stably express mTLR7 and 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 mTLR7 signaling without interference from other TLRs. They respond to very low concentrations of TLR7 agonists such as the imidazoquinoline compound Gardiquimod™. 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™ mTLR7 cells (SEAP reporter cells) using QUANTI-Blue™ Solution, a SEAP detection medium (see figure 1). Furthermore, HEK-Blue-Lucia™ mTLR7 (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 (see figure 2).

NF-κB (SEAP) response

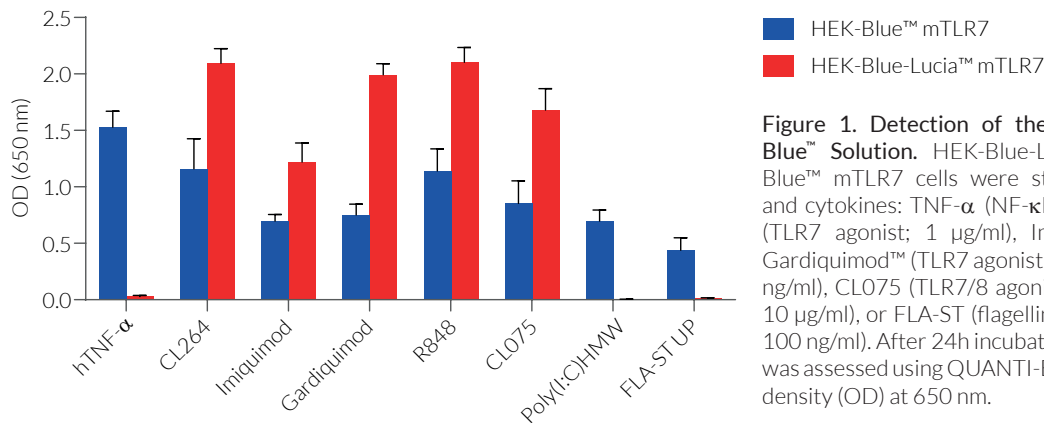


Figure 1. Detection of the NF-κB response using QUANTI-Blue™ Solution. HEK-Blue-Lucia™ mTLR7 (NF/IL8) and HEK-Blue™ mTLR7 cells were stimulated with various TLR ligands and cytokines: TNF-α (NF-κB positive control; 10 ng/ml), CL264 (TLR7 agonist; 1 μg/ml), Imiquimod (TLR7 agonist; 1 μg/ml), Gardiquimod™ (TLR7 agonist; 1 μg/ml), R848 (TLR7/8 agonist; 100 ng/ml), CLO75 (TLR7/8 agonist; 1 μg/ml), Poly(I:C) (TLR3 agonist; 10 μg/ml), or FLA-ST (flagellin from *S. typhimurium*, TLR5 agonist; 100 ng/ml). After 24h 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

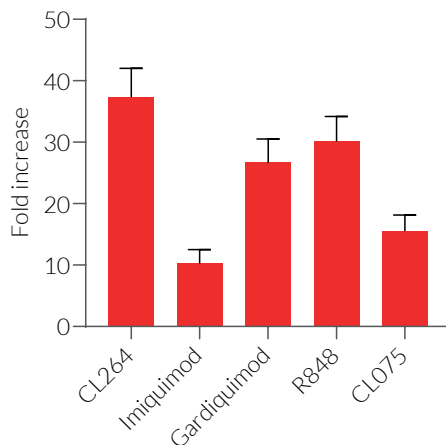


Figure 2: Detection of the IL-8 response using QUANTI-Luc™ 4 Lucia/Gaussia. HEK-Blue-Lucia™ mTLR7 (NF/IL8) cells were stimulated with TLR7/8 agonists: CL264 (TLR7 agonist; 1 μg/ml), Imiquimod (TLR7 agonist; 1 μg/ml), Gardiquimod™ (TLR7 agonist; 1 μg/ml), R848 (TLR7/8 agonist; 100 ng/ml), or CLO75 (TLR7/8 agonist; 1 μg/ml). After 24 h 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

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