HEK-Dual™ mTLR7 (NF/IL8) cells are designed for studying the stimulation of mouse 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™, a SEAP detection medium (see figure 1). Furthermore, HEK-Dual™ mTLR7 (NF/IL8) cells also enable monitoring activation of the IL-8 pathway using QUANTI-Luc™, a Lucia luciferase detection medium (see figure 2).

Table 1: EC50 values calculated for the NF-κB response using QUANTI-Blue™. HEK-Dual™ mTLR7 (NF/IL8) and HEK-Blue™ mTLR7 cells were stimulated for 24 hours with various TLR7 agonists: R848 (TLR7/8 agonist; 300 ng/ml), CL264 (TLR7 agonist; 300 ng/ml), Gardiquimod™ (TLR7 agonist; 300 ng/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™ mTLR7 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 1: Detection of the NF-κB response using QUANTI-Blue™. HEK-Dual™ mTLR7 (NF/IL8) and HEK-Blue™ mTLR7 cells were stimulated with various TLR agonists: R848 (TLR7/8 agonist; 300 ng/ml), CL264 (TLR7 agonist; 300 ng/ml), Gardiquimod™ (TLR7 agonist; 300 ng/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™ mTLR7 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™ mTLR7 (NF/IL8) cells were stimulated with TLR7 agonists: R848 (TLR7/8 agonist; 300 ng/ml), CL264 (TLR7 agonist; 300 ng/ml) and Gardiquimod™ (TLR7 agonist; 300 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™, 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.