

# Validation data for Gardiquimod™

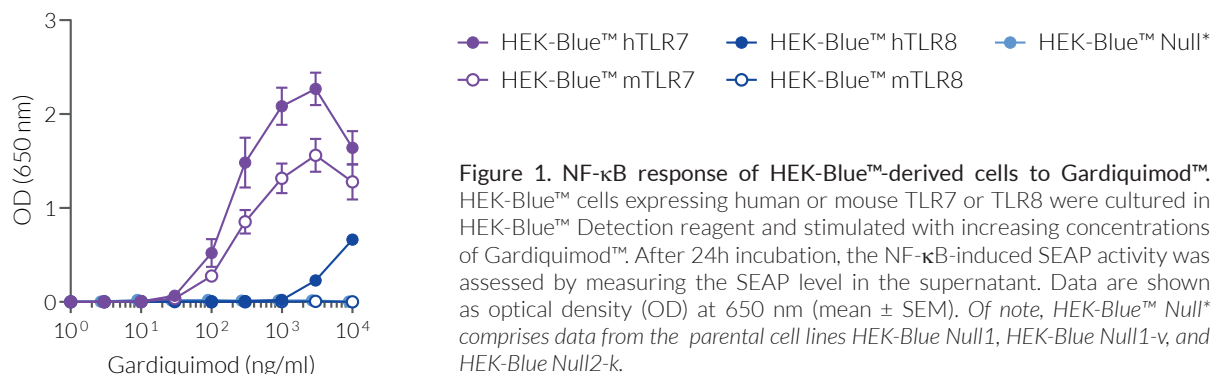
<https://www.invivogen.com/gardiquimod>

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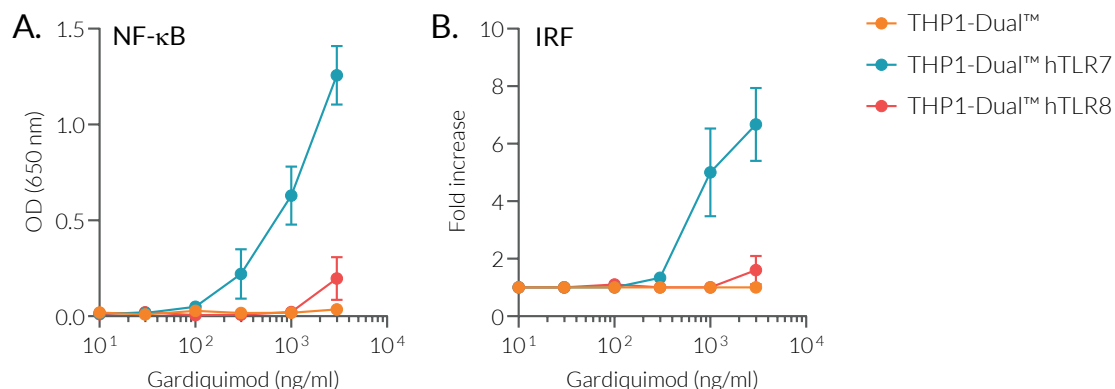
Gardiquimod™ is an imidazoquinoline and a specific agonist of the Toll-like receptor 7 (TLR7). The ability of Gardiquimod™ to activate TLR7, but not TLR8 signaling was validated using a panel of InvivoGen's reporter cell lines. Gardiquimod™ efficiently activates human (h) and mouse (m)TLR7, but not TLR8, as assessed by the expression of an NF- $\kappa$ B-inducible secreted embryonic alkaline phosphatase (SEAP) reporter in HEK-Blue™-derived cell lines (Figure 1). The induction of the NF- $\kappa$ B and IRF pathways by Gardiquimod™ has been tested using InvivoGen's THP1-Dual™-derived monocytic cell lines, which feature two reporter genes, the NF- $\kappa$ B-inducible SEAP and IRF-inducible Lucia luciferase, as well as the overexpression of TLR7 or TLR8 (Figure 2).

## Dose-dependent NF- $\kappa$ B response in HEK-Blue™-derived cells



**Figure 1. NF- $\kappa$ B response of HEK-Blue™-derived cells to Gardiquimod™.** HEK-Blue™ cells expressing human or mouse TLR7 or TLR8 were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of Gardiquimod™. After 24h incubation, the NF- $\kappa$ B-induced SEAP activity was assessed by measuring the SEAP level in the supernatant. Data are shown as optical density (OD) at 650 nm (mean  $\pm$  SEM). Of note, HEK-Blue™ Null\* comprises data from the parental cell lines HEK-Blue Null1, HEK-Blue Null1-v, and HEK-Blue Null2-k.

## Dose-dependent NF- $\kappa$ B and IRF responses in THP1-Dual™-derived cells



**Figure 2. NF- $\kappa$ B and IRF responses of THP1-Dual™-derived cells to Gardiquimod™.** THP1-Dual™, THP1-Dual™ hTLR7, and THP1-Dual™ hTLR8 cells were incubated for 24 hours with increasing concentrations of Gardiquimod™. After 24h incubation, the (A) NF- $\kappa$ B-induced SEAP activity was assessed using QUANTI-Blue™. Data are shown as optical density (OD) at 650 nm (mean  $\pm$  SEM). (B) The IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data are shown in fold response over non-induced cells (mean  $\pm$  SEM).

### TECHNICAL SUPPORT

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