

# Validation data for Imiquimod (R837)

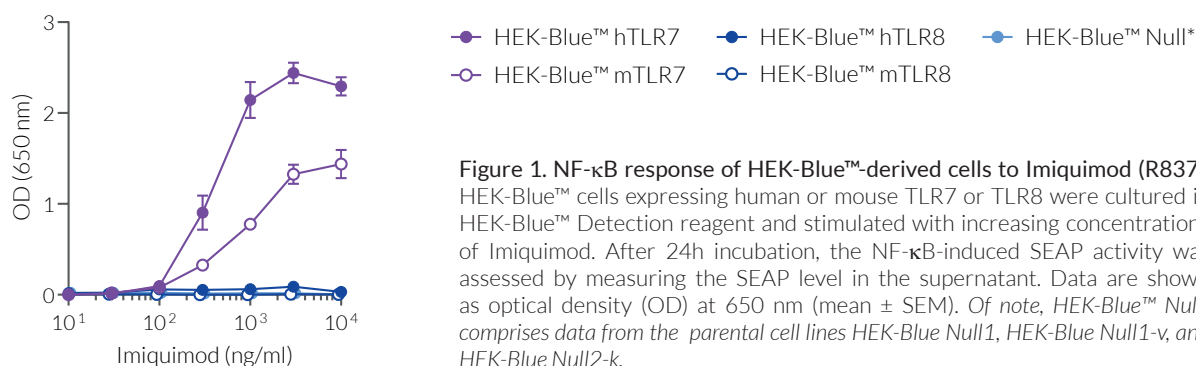
<https://www.invivogen.com/imiquimod>

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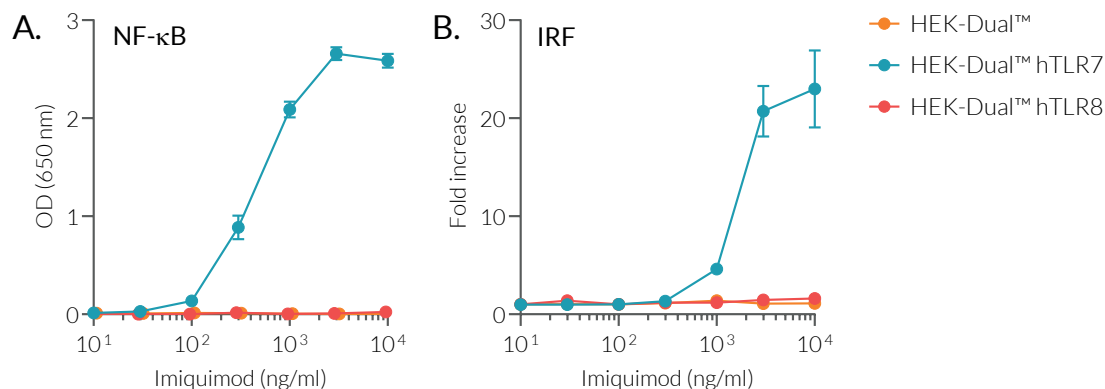
Imiquimod (R837) is an imidazoquinoline amine analog to guanosine and a specific agonist of the Toll-like receptor 7 (TLR7). The ability of Imiquimod to activate TLR7, but not TLR8 signaling was validated using a panel of InvivoGen's reporter cell lines. Imiquimod efficiently activates human (h) and 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 Imiquimod has been tested using InvivoGen's HEK-Dual™-derived 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 Imiquimod (R837). HEK-Blue™ cells expressing human or mouse TLR7 or TLR8 were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of Imiquimod. 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 HEK-Dual™-derived cells



**Figure 2.** NF- $\kappa$ B and IRF responses of HEK-Dual™-derived cells to Imiquimod (R837). HEK-Dual™, HEK-Dual™ hTLR7, and HEK-Dual™ hTLR8 cells were incubated for 24 hours with increasing concentrations of Imiquimod. 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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