

Validation data for Imiquimod VacciGrade™

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

For research use only

Version 23L21-AK

Imiquimod VacciGrade™ 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- κ B-inducible secreted embryonic alkaline phosphatase (SEAP) reporter in HEK-Blue™-derived cell lines (**Figure 1**). The induction of the NF- κ B and IRF pathways by Imiquimod has been tested using InvivoGen's HEK-Dual™-derived cell lines, which feature two reporter genes, the NF- κ B-inducible SEAP and IRF-inducible Lucia luciferase, as well as the overexpression of TLR7 or TLR8 (**Figure 2**).

Dose-dependent NF- κ B response in HEK-Blue™-derived cells

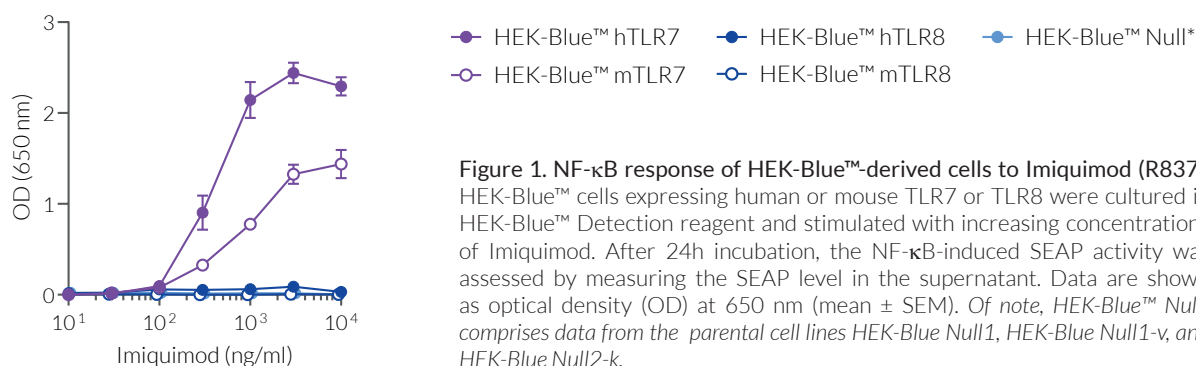


Figure 1. NF- κ B response of HEK-Blue™-derived cells to Imiquimod (R837). HEK-Blue™ cells expressing human or mouse TLR7 or TLR8 were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of Imiquimod. After 24h incubation, the NF- κ B-induced SEAP activity was assessed 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- κ B and IRF responses in HEK-Dual™-derived cells

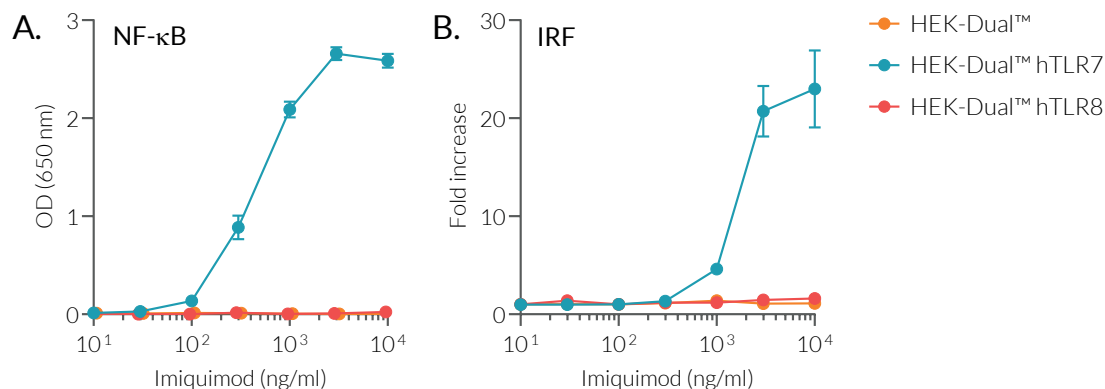


Figure 2. NF- κ B and IRF responses of HEK-Dual™-derived cells to Imiquimod (R837). HEK-Dual™, HEK-Dual™ hTLR7, and HEK-Dual™ hTLR8 cells were incubated for 24 hours with increasing concentrations of Imiquimod. After 24h incubation, the (A) NF- κ B-induced SEAP activity was assessed using QUANTI-Blue™. 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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