

Validation data for R848 VacciGrade™

<https://www.invivogen.com/r848-vaccigrade>

For research use only

Version 23L21-AK

R848 VacciGrade™, an imidazoquinoline, is a dual TLR7 and TLR8 synthetic agonist with potent antiviral activity. It induces differential TLR7 and/or TLR8 responses in human and murine immune cells. R848 (Resiquimod) activates immune cells via the TLR7/TLR8 MyD88-dependent signaling pathway with the subsequent activation of the transcription factors NF- κ B and interferon regulatory factor. The biological activity of R848 has been tested using InvivoGen's HEK-Blue™ cells expressing a human or murine TLR gene and an NF- κ B-inducible secreted embryonic alkaline phosphatase (SEAP) reporter (Figure 1). The induction of the NF- κ B and IRF pathways by TLR8-506 has been tested using InvivoGen's THP1-Dual™ cells featuring 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). Due to the low level of endogenous TLR8 in THP-1-derived cells, R848 can stimulate THP1-Dual™ cells.

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

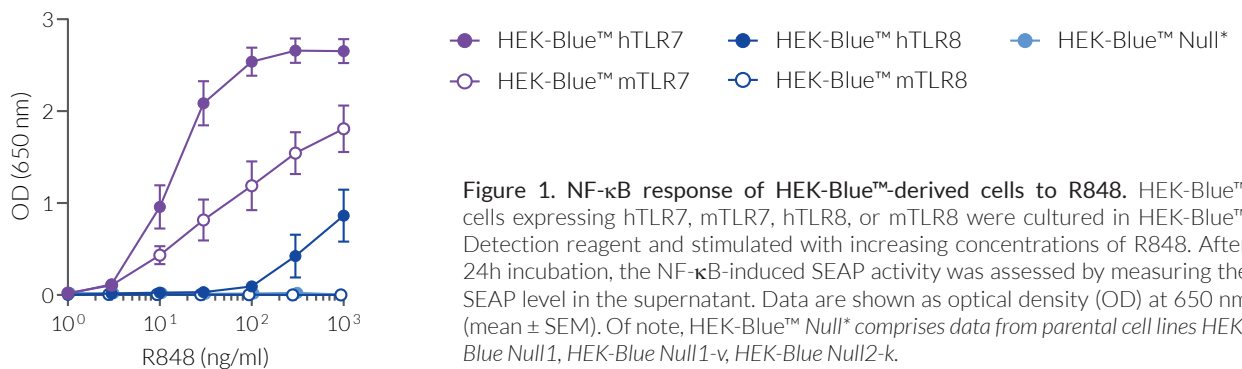


Figure 1. NF- κ B response of HEK-Blue™-derived cells to R848. HEK-Blue™ cells expressing hTLR7, mTLR7, hTLR8, or mTLR8 were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of R848. After 24h incubation, the NF- κ B-induced SEAP activity was assessed by measuring 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 parental cell lines HEK-Blue Null1, HEK-Blue Null1-v, HEK-Blue Null2-k.

Dose-dependent NF- κ B and IRF responses in THP1-Dual™-derived cells

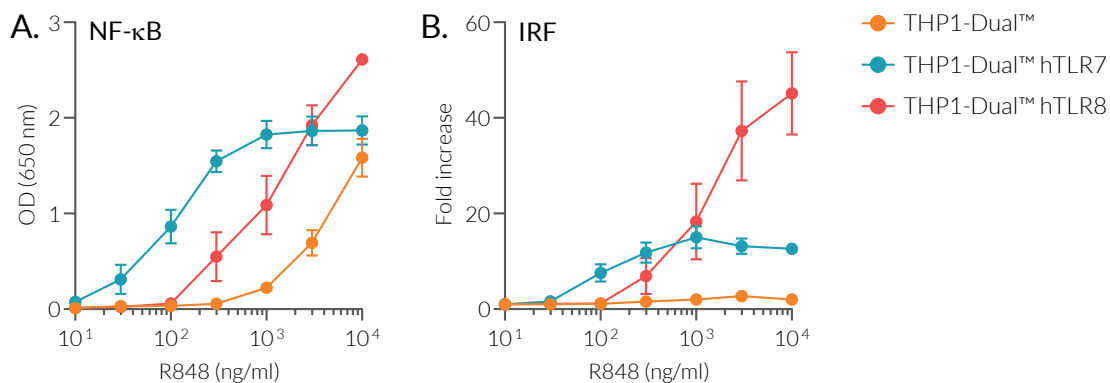


Figure 2. NF- κ B and IRF responses of THP1-Dual™-derived cells to R848. THP1-Dual™, THP1-Dual™ hTLR7 cells, and THP1-Dual™ hTLR8 cells were incubated for 24 hours with increasing concentrations of R848. After 24h incubation, the (A) NF- κ B-induced SEAP activity was assessed using QUANTI-Blue™. Data 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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