Validation data for R848

https://www.invivogen.com/r848

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Version 23L21-AK

R848 (Resiquimod), 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 activates immune cells via the TLR7/TLR8 MyD88-dependent signaling pathway with the subsequent activation of the transcription factors NF-κB 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-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter **(Figure 1)**. The induction of the NF-κB and IRF pathways by TL8-506 has been tested using InvivoGen's THP1-Dual™ cells featuring two reporter genes, the NF-κB-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.

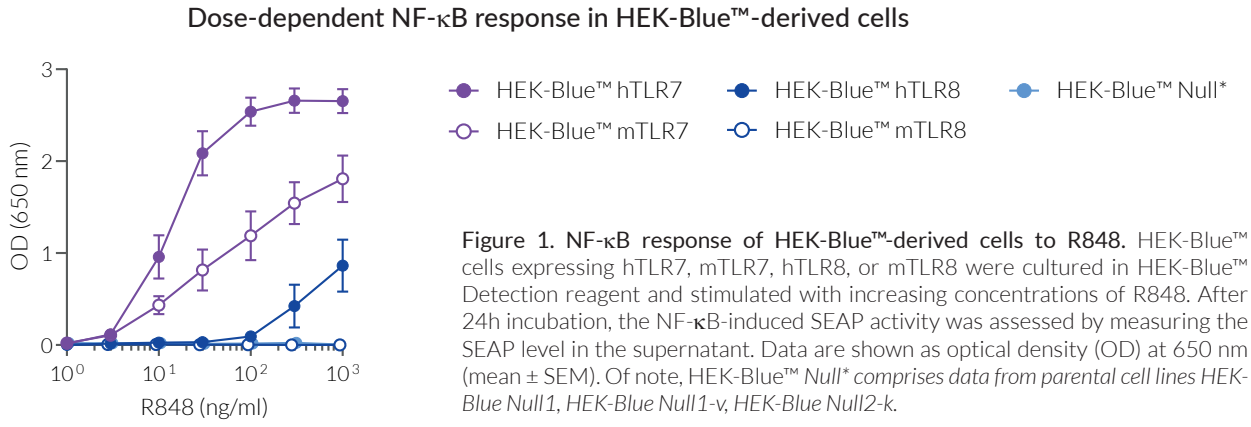

Figure 1. NF-κB response of HEK-Blue™-derived cells to R848. HEK-Blue™ cells expressing hTLR7, mTLR7, hTLR8, or mTLR8 were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of R848. After 24h incubation, the NF-κB-induced SEAP activity was assessed by measuring the SEAP level in the supernatant. Data are shown as optical density (OD) at 650 nm (mean ± SEM). Of note, HEK-Blue™ *Null* comprises data from parental cell lines HEK-Blue Null1, HEK-Blue Null1-v, HEK-Blue Null2-k.*

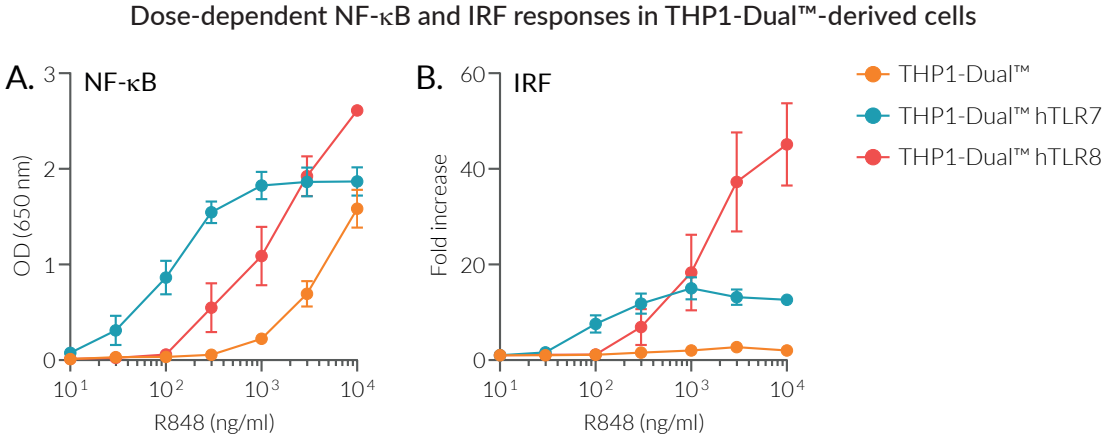

Figure 2. NF-κB and IRF responses of THP1-Dual™-derived cells to R848. THP1-Dual™, THP1-Dual™ hTLR7 cells, and THP1-Dual™ hTLR8 cells were incubated for 24 hours with increasing concentrations of R848. After 24h incubation, the **(A)** NF-κB-induced SEAP activity was assessed using QUANTI-Blue™. Data are shown as optical density (OD) at 650 nm (mean ± 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 ± SEM).

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