Validation data for TL8-506

https://www.invivogen.com/tl8-506

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Version 23K30-AK

TL8-506 is a benzoazepine compound, analog of the Toll-like receptor 8 (TLR8) agonist VTX-2337. The ability of TL8-506 to activate TLR8 signaling was validated using a panel of InvivoGen's reporter cell lines. TL8-506 efficiently activates human (h)TLR8, murine (m)TLR7 and mTLR8, but not hTLR7, as assessed by the expression of an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) reporter in HEK-Blue™ -derived cell lines **(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 and/or an knockout (KO) of a TLR gene **(Figure 2).** Due to the low level of endogenous TLR8 in THP-1-derived cells, high concentrations of TL8-506 can stimulate THP1-Dual™ and THP1-Dual™ hTLR7 cells.

Dose-dependent NF-κB response in HEK-Blue™-derived cells

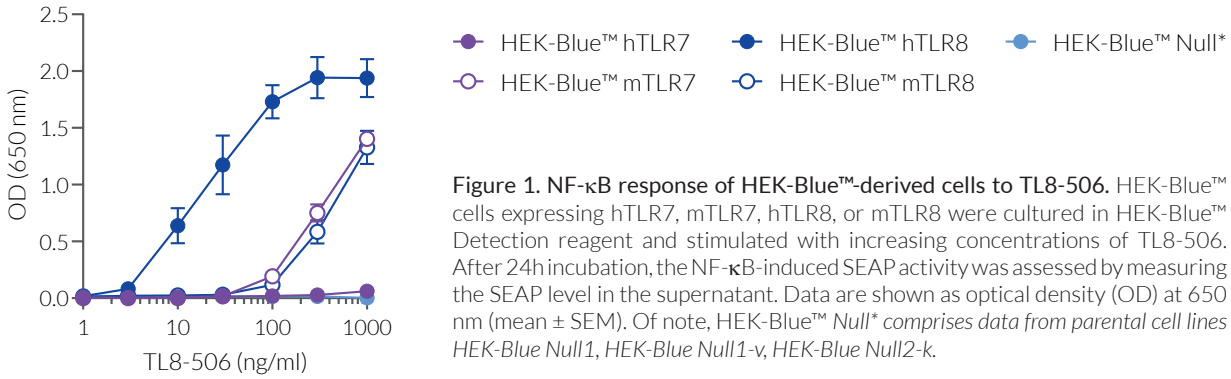

Figure 1. NF-κB response of HEK-Blue™-derived cells to TL8-506. HEK-Blue™ cells expressing hTLR7, mTLR7, hTLR8, or mTLR8 were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of TL8-506. After 24h incubation, the NF-κB-induced SEAP activity was assessed by measuring 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.*

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

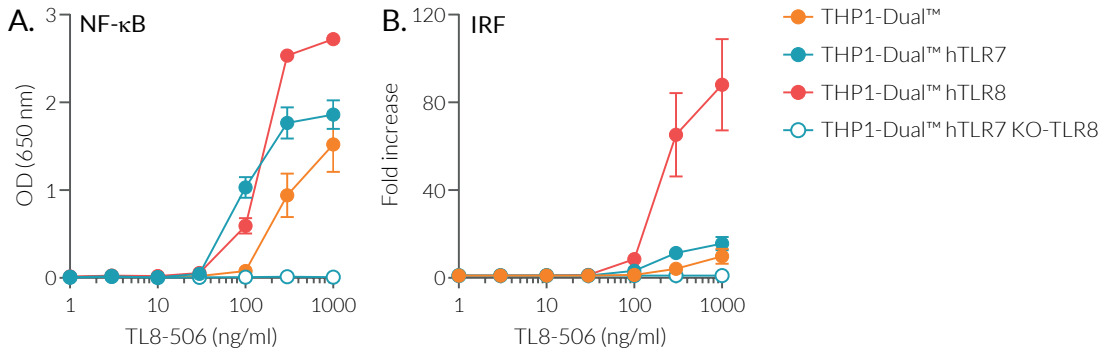

Figure 2. NF-κB and IRF responses of THP1-Dual™-derived cells to TL8-506. THP1-Dual™, THP1-Dual™ hTLR7 cells, THP1-Dual™ hTLR8 cells, and THP1-Dual™ hTLR7 KO-TLR8 cells were incubated for 24 hours with increasing concentrations of TL8-506. After 24h incubation, the **(A)** NF-κB-induced SEAP activity 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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