Validation data for CL307

https://www.invivogen.com/cl307

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Version 23L18-AK

CL307 was generated by covalently linking spermine to the adenine analog CL264. CL307 is a potent and specific agonist of Toll-like receptor 7 (TLR7). The ability of CL307 to activate TLR7, but not TLR8 signaling was validated using a panel of InvivoGen's reporter cell lines. CL307 efficiently activates human (h) and mouse (m)TLR7, but not hTLR8, 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 CL307 has been tested using InvivoGen's THP1-Dual™-derived monocytic cell lines, that features two reporter genes, the NF-κB-inducible SEAP and IRF-inducible Lucia luciferase, as well as the overexpression of TLR7 or TLR8 **(Figure 2)**.

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

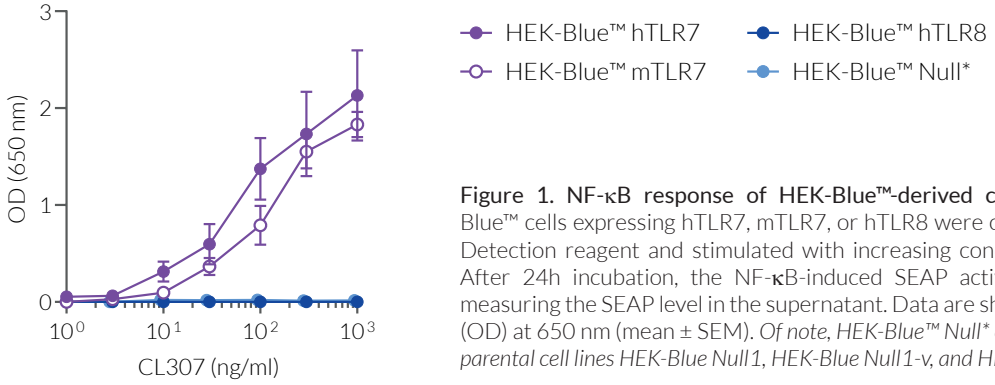

Figure 1. NF-κB response of HEK-Blue™-derived cells to CL307. HEK-Blue™ cells expressing hTLR7, mTLR7, or hTLR8 were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of CL307. After 24h incubation, the NF-κB-induced SEAP activity was assessed by measuring the SEAP level in the supernatant. Data are shown as optical density (OD) at 650 nm (mean ± SEM). *Of note, HEK-Blue™ Null* comprises data from the parental cell lines HEK-Blue Null1, HEK-Blue Null1-v, and HEK-Blue Null2-k.*

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

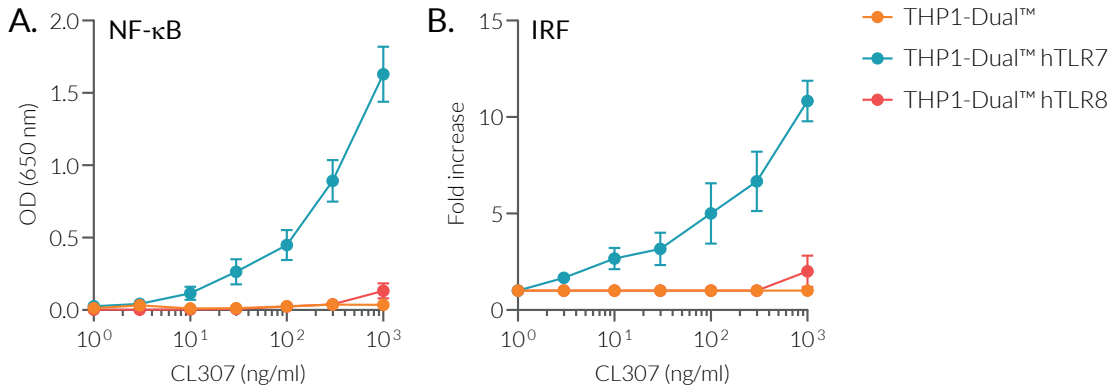

Figure 2. NF-κB and IRF responses of THP1-Dual™-derived cells to CL307. THP1-Dual™, THP1-Dual™ hTLR7, and THP1-Dual™ hTLR8 cells were incubated for 24 hours with increasing concentrations of CL307. After 24h incubation, the **(A)** NF-κB-induced SEAP activity was assessed using QUANTI-Blue™. Data are 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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