Validation data for CL264

https://www.invivogen.com/cl264

For research use only

Version 23L18-AK

CL264 is a 9-benzyl-8 hydroxyadenine derivative containing a glycine on the benzyl group *(in para)* and a potent agonist of the Toll-like receptor 7 (TLR7). The ability of CL264 to activate TLR7, but not TLR8 signaling was validated using a panel of InvivoGen's reporter cell lines. CL264 efficiently activates human (h) and mouse (m)TLR7, but not h/mTLR8, 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 CL264 has been tested using InvivoGen's HEK-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)**.

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

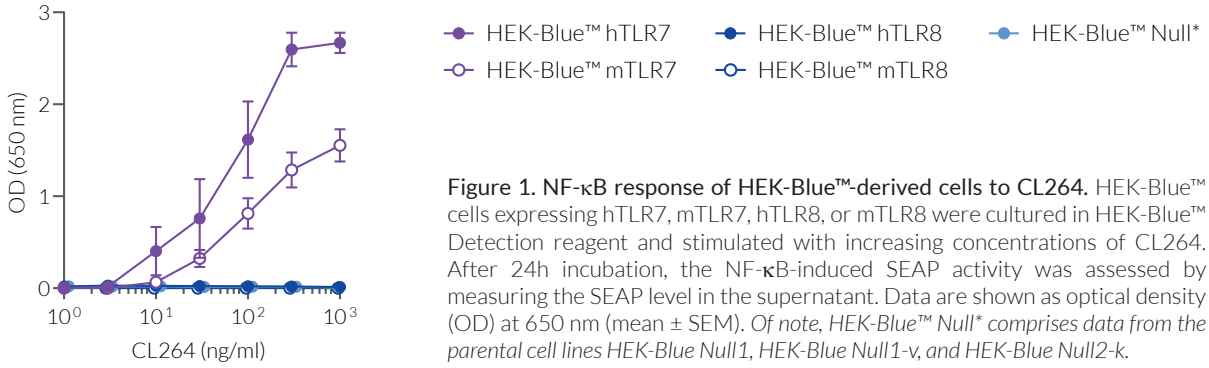

Figure 1. NF-κB response of HEK-Blue™-derived cells to CL264. HEK-Blue™ cells expressing hTLR7, mTLR7, hTLR8, or mTLR8 were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of CL264. After 24h incubation, the NF-κB-induced SEAP activity was assessed by measuring the 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 HEK-Dual™-derived cells

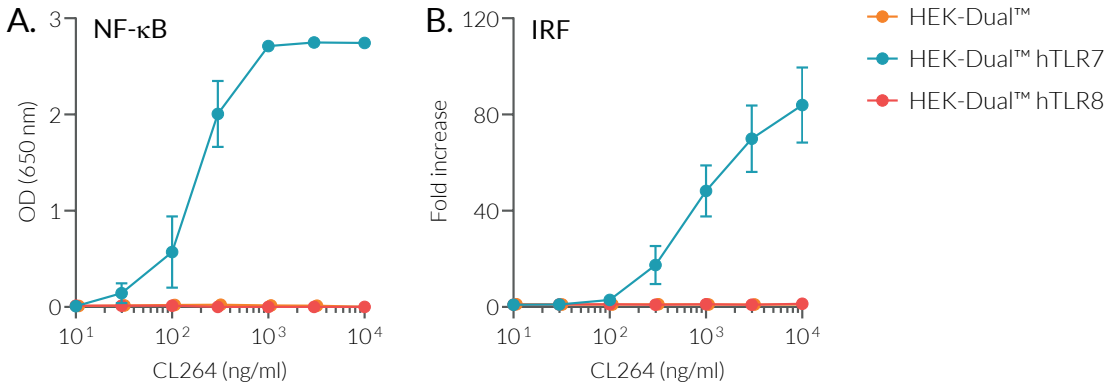

Figure 2. NF-κB and IRF responses of HEK-Dual™-derived cells to CL264. HEK-Dual™, HEK-Dual™ hTLR7, and HEK-Dual™ hTLR8 cells were incubated for 24 hours with increasing concentrations of CL264. After 24h incubation, the **(A)** NF-κB-induced SEAP activity was assessed using QUANTI-Blue™. Data are shown 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).

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

InvivoGen
www.invivogen.com