# Validation data for CU-T12-9

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CU-T12-9 is a synthetic, small-molecule, Toll-like receptor 2 (TLR2) agonist. CU-T12-9 triggers a TLR2-dependent NF-κB signaling cascade **(Figure 1a).** Importantly, CU-T12-9 specifically activates the TLR2-TLR1 heterodimer in a dose-dependent manner, with no response in cells expressing the other TLR2 heterodimer, TLR2-TLR6 **(Figure 1b)**.

## Specific activation of TLR2-TLR1 heterodimer by CU-T12-9

Incubation of HEK-Blue™ hTLR2 cells with CU-T12-9 results in the activation of the inducible TLR2-dependent NF-κB response in a dose-dependent manner (A). Furthermore, incubation of HEK-Blue™ hTLR2-TLR1 (*KO-TLR6*) and HEK-Blue™ hTLR2-TLR6 (*KO-TLR1*) cells with CU-T12-9 results in a dose-dependent and highly specific activation of the inducible NF-κB response in hTLR2-TLR1 cells only (B).

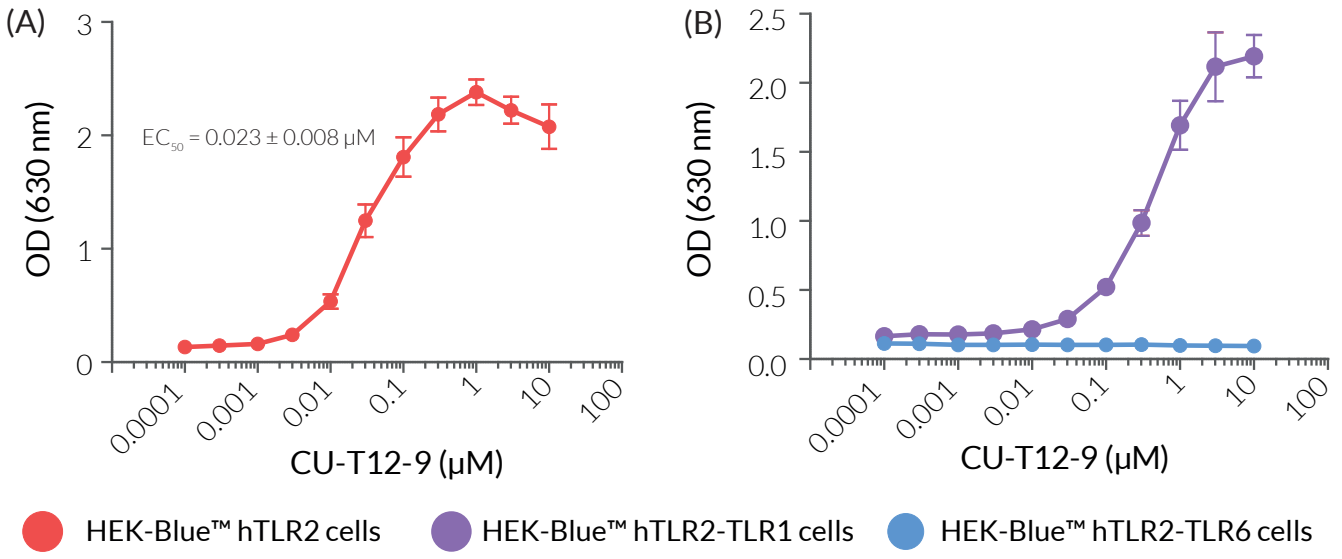


**Figure 1: Activation of hTLR2-TLR1 by CU-T12-9.** HEK-Blue™ hTLR2 cells, HEK-Blue™ hTLR2-TLR1, and HEK-Blue™ hTLR2-TLR6 cells were incubated with increasing concentrations (0-10 µM) of CU-T12-9. After overnight incubation in HEK-Blue™ detection medium, activation of (A) TLR2 or (B) TLR2-TLR1 or TLR2-TTLR6 heterodimers (NF-κB activity) was assessed by measuring SEAP activity in the supernatant. Data is shown as OD at 630 nm.


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