Validation data for TL2-C29

https://www.invivogen.com/tlr2-in-c29

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Version 22D11-NJ

TL2-C29 is a small-molecule inhibitor of Toll-like receptor 2 (TLR2). The ability of TL2-C29 to inhibit human TLR2 signaling was validated using InvivoGen's HEK-Blue™ hTLR2 reporter cells **(Figure 1)**. These cells endogenously express human TLR1 and TLR6 and have been stably transfected with human TLR2 and CD14 genes, together with an NF-κB/AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. The specific inhibition of TLR2 signaling by TL2-C29 has been verified **(Figure 2)**. The ability of TL2-C29 to inhibit human both TLR2/1 and TLR2/6 signaling pathways was also validated using InvivoGen's HEK-Blue™ hTLR2-TLR1 and HEK-Blue™ hTLR2-TLR6 reporter cells, which are knockout for TLR6 and TLR1, respectively **(Figure 3)**.

Dose-dependent inhibition of TLR2 signaling

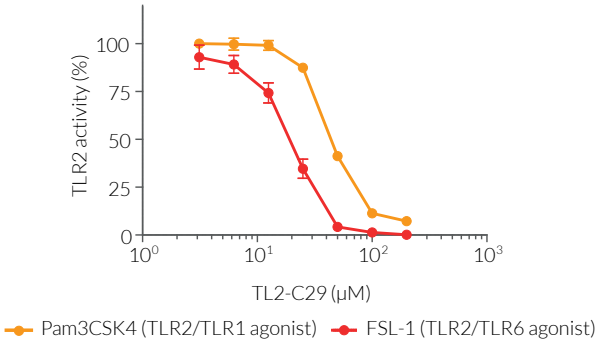

Figure 1: TL2-C29 is a potent inhibitor of human TLR2 signaling pathways. HEK-Blue™ hTLR2 cells were cultured in the presence of increasing concentrations of TL2-C29 for 3 hours at 37°C. Cells were then incubated overnight with 10 ng/ml Pam3CSK4 (TLR2/TLR1 agonist) or 0.1 ng/ml FSL-1 (TLR2/TLR6 agonist). The neutralizing activity of TL2-C29 was determined by measuring the reduction of SEAP production in the supernatant using the QUANTI-Blue™ Solution detection reagent. Data are shown as a percentage (%) of maximal TLR2 activation with each agonist.

Specific inhibition of TLR2 signaling

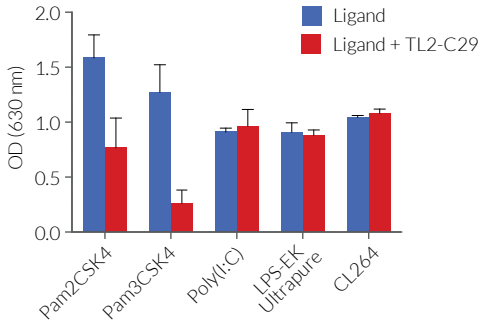

Figure 2: TL2-C29 specifically inhibits human TLR2 signaling pathways. HEK-Blue™ hTLR2, HEK-Blue™ hTLR3, HEK-Blue™ hTLR4, or HEK-Blue™ hTLR7 cells were cultured with specific agonists either alone (blue bars) or in the presence of 50 μM TL2-C29 (red bars): 1 ng/ml Pam2CSK4 (TLR2/TLR6 agonist), 10 ng/ml Pam3CSK4 (TLR2/TLR1 agonist), 1 μg/ml Poly(I:C) (TLR3 agonist), 1 ng/ml LPS-EK Ultrapure (TLR4 agonist), or CL264 (TLR7 agonist). After overnight incubation, the neutralizing activity of TL2-C29 was determined by measuring the reduction of SEAP production in the supernatant using the QUANTI-Blue™ Solution detection reagent. The optical density (OD) at 630 nm is shown as mean ± SEM.

Dose-dependent inhibition of TLR2/1 and TLR2/6 signaling

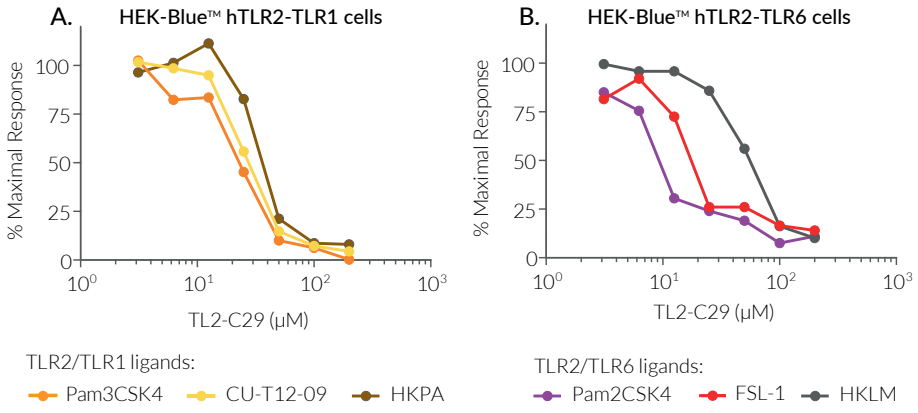

Figure 3: TL2-C29 specifically inhibits human TLR2/1 and TLR2/6 signaling pathways. HEK-Blue™ hTLR2-TLR1 **(A)** or HEK-Blue™ hTLR2-TLR6 cells **(B)** were cultured in the presence of increasing concentrations of TL2-C29 for 3 hours at 37°C. Cells were then incubated overnight with 10 ng/ml Pam3CSK4, 10 μM CU-T12-9, 10^7 CFU/ml heat-killed *pseudomonas aeruginosa* (HKPA), 1 ng/ml Pam2CSK4, 10 ng/ml FSL-1, or 10^7 CFU/ml heat-killed *listeria monocytogenes* (HKLM)). The neutralizing activity of TL2-C29 was determined by measuring the reduction of SEAP production in the supernatant using the QUANTI-Blue™ Solution detection reagent. Data are shown as a percentage (%) of maximal response with each ligand.

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