Validation data for Pam3CSK4

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Version 20C31-MM

Pam3CSK4 is a synthetic triacylated lipopeptide (LP) and a TLR2/TLR1 ligand. Recognition of Pam3CSK4 is mediated by TLR2 which cooperates with TLR1 to induce a signaling cascade leading to the activation of the pro-inflammatory transcription factor NF-κB. The biological activity of Pam3CSK4 has been tested using InvivoGen's HEK-Blue™ hTLR2 cells which stably express human TLR2 and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP). Pam3CSK4 has also been tested in InvivoGen's THP1-Dual™ and THP1-Dual™ KO-TLR2 cell lines. These cells feature two reporter genes allowing the simultaneous study of the interferon regulatory factors (IRF) pathway, by monitoring the activity of an inducible secreted Lucia luciferase, and the NF-κB pathway by monitoring the activity of an inducible SEAP.
Stimulation of HEK-Blue™ hTLR2 and THP1-Dual™ cells with Pam3CSK4 results in a dose-dependent induction of the NF-κB signaling pathway. As expected, THP1-Dual™ KO TLR2 cells did not respond to Pam3CSK4. Of note, due to TLR2 not directly signaling through an IRF-dependent pathway, Pam3CSK4 did not induce a significant fold-increase in the IRF-inducible reporter Lucia luciferase in the THP1-Dual™ cells.

Evaluation of NF-κB activation with Pam3CSK4

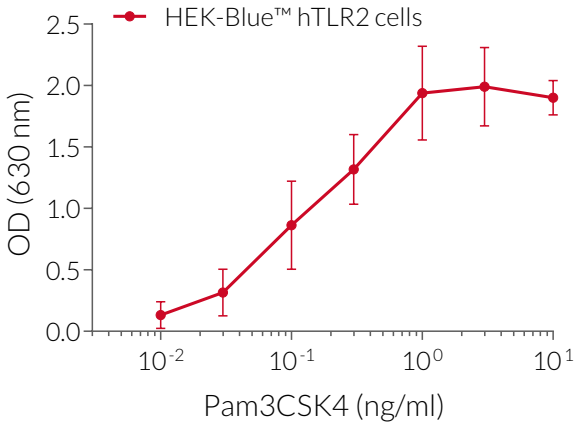

Figure 1. Pam3CSK4 induces a dose-dependent response in HEK-Blue™ hTLR2 cells. These cells were stimulated with increasing concentrations of Pam3CSK4. After overnight incubation, the NF-κB response was determined using HEK-Blue™ Detection, a SEAP detection medium, and by reading the optical density (OD) at 630 nm.

Specificity of Pam3CSK4 activation

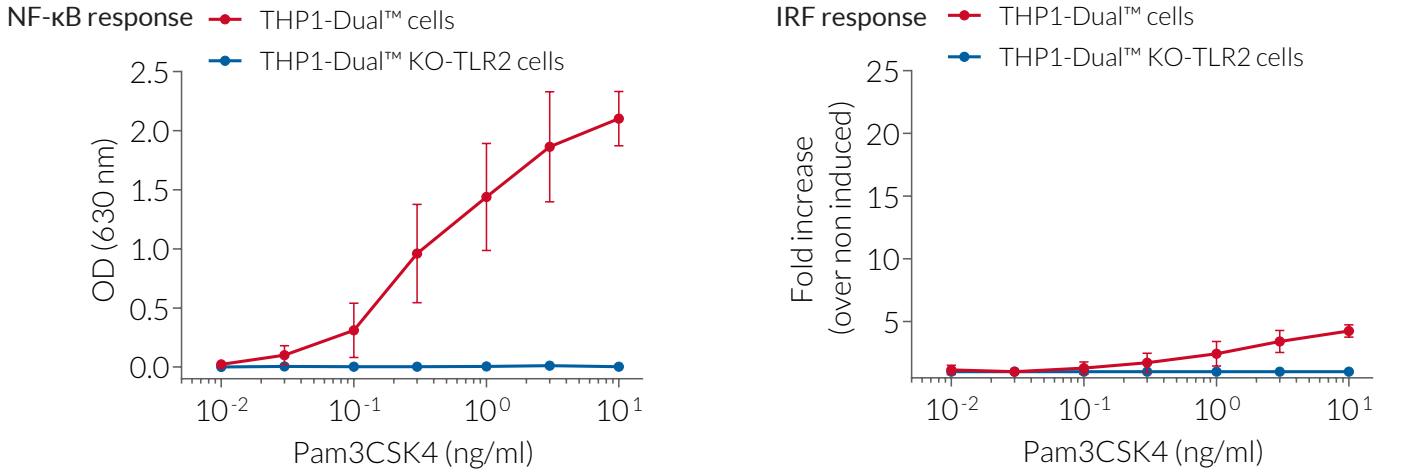

Figure 2. Specific TLR2-mediated NF-κB activation by Pam3CSK4. THP1-Dual™ and THP1-Dual™ KO-TLR2 cells were stimulated with increasing concentrations of Pam3CSK4. After overnight incubation, the NF-κB response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent, and by reading the optical density (OD) at 630 nm. The IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data for the Lucia luciferase readout are shown as a fold increase over non-induced cells.

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