

# Validation data for THP1-Dual™ KO-TLR2 Cells

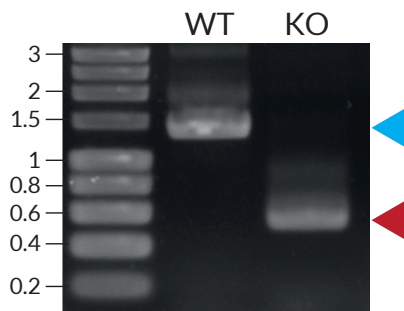
<https://www.invivogen.com/thp1-dual-ko-tlr2>

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Version 20C12-ED

THP1-Dual™ KO-TLR2 cells were generated from the THP1-Dual™ cell line through the verified stable knockout of the TLR2 gene (Figure 1). These cells feature two reporter genes allowing the simultaneous study of the NF-κB pathway by monitoring the activity of an inducible SEAP (secreted embryonic alkaline phosphatase)(Figure 2) and the IRF pathway by monitoring the activity of an inducible secreted Lucia luciferase (Figure 3). Lucia luciferase and SEAP activities are readily assessable in the cell culture supernatant using QUANTI-Luc™ and QUANTI-Blue™ Solution detection reagents, respectively.

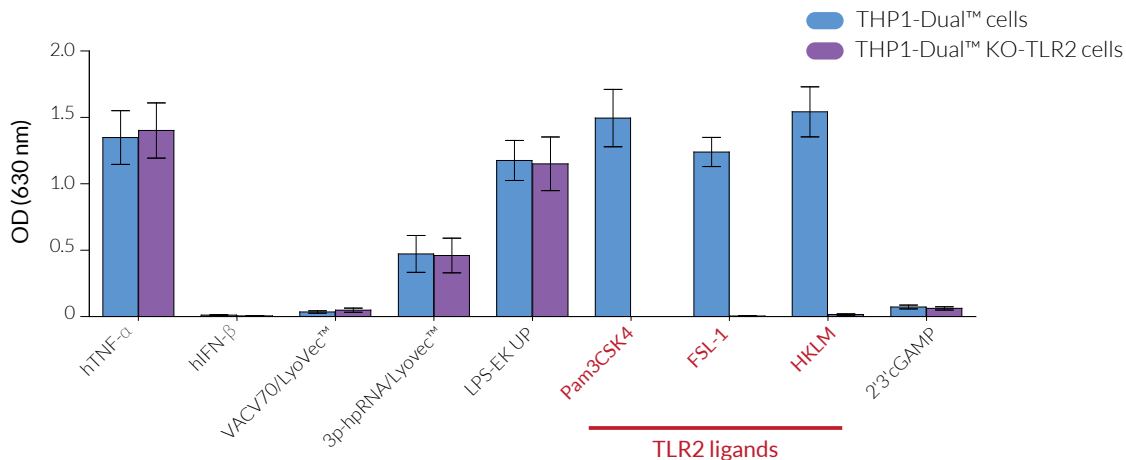
## Validation of TLR2 knockout



**Figure 1: PCR validation of TLR2 KO.** The targeted TLR2 region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-TLR2 (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-TLR2 cells feature a frameshift deletion, causing an early stop codon and inactivation of TLR2.

## Functional validation of TLR2 knockout (NF-κB response)

TLR2 forms a heterodimer on the cell surface with its co-receptor, TLR1 or TLR6, which is crucial for signaling and ligand specificity. Ultimately, TLR2 signaling leads to MyD88 and MAL/TIRAP-dependent activation of pro-inflammatory transcription factors such as NF-κB. As expected, the NF-κB response is abolished in THP1-Dual™ KO-TLR2 cells upon incubation with TLR2-specific ligands such as Pam3CSK4 (TLR1/2), FSL-1 (TLR2/6), and heat killed *Listeria monocytogenes* (HKLM; TLR2/6) when compared to the THP1-Dual™ cells (Figure 1), with no notable difference for the other ligands tested.



**Figure 2: NF-κB responses in THP1-Dual™-derived cells.** THP1-Dual™ and THP1-Dual™ KO-TLR2 cells were incubated with 0.3 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1 x 10<sup>4</sup> U/ml hIFN-β (IRF-Lucia positive control), 1 μg/ml VACV70/LyoVec™ (CDS ligand), 300 ng/ml 3p-hpRNA/LyoVec™ (RIG-I agonist), 1 μg/ml LPS-EK Ultrapure (UP; TLR4), 1 ng/ml Pam3CSK4 (TLR2/1 agonist), 0.3 ng/ml FSL-1 (TLR2/6 agonist), 10<sup>7</sup> c/ml HKLM (TLR2 agonist), and 3 μg/ml 2'3'cGAMP (STING agonist). After overnight incubation, the activation of NF-κB was assessed by measuring the activity of SEAP in the supernatant using QUANTI-Blue™ Solution. Data are shown as optical density (OD) at 630 nm (mean ± SEM).

### TECHNICAL SUPPORT

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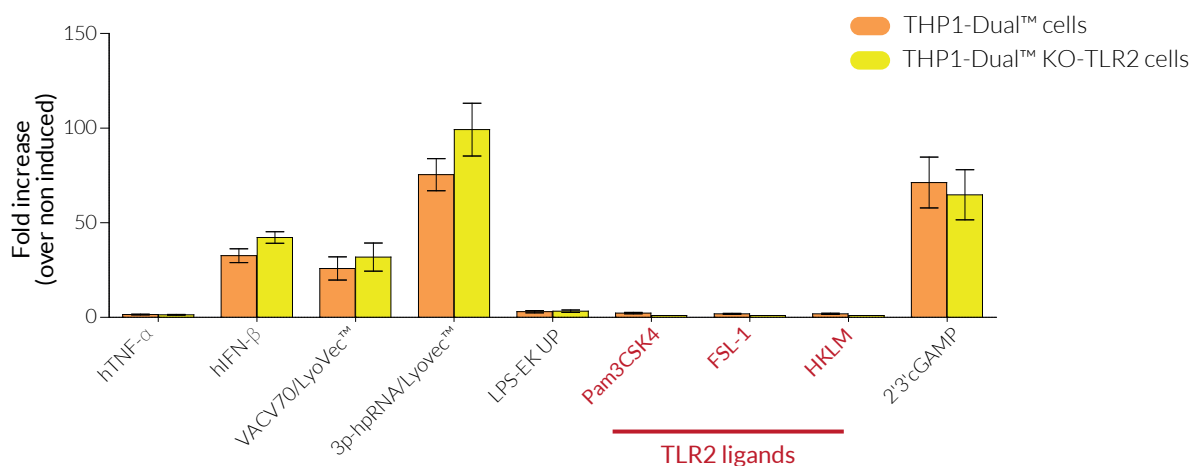
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## Functional validation of TLR2 knockout (IRF response)

As expected, due to TLR2 not directly signaling through an IRF-dependent pathway, the secretion of Lucia luciferase was unimpaired in THP1-Dual™ KO-TLR2 when tested across a range of ligands. Additionally, the response of ligands that signal in an IRF-dependent manner, such as 2'3'-cGAMP (STING agonist) and human interferon  $\beta$  (hIFN- $\beta$ ) was confirmed to be unaltered between the parental cell line, THP1-Dual™, and THP1-Dual™ KO-TLR2.



**Figure 3: IRF responses in THP1-Dual™-derived cells.** THP1-Dual™ and THP1-Dual™ KO-TLR2 cells were incubated with 0.3 ng/ml human (h) TNF- $\alpha$  (NF- $\kappa$ B-SEAP positive control),  $1 \times 10^4$  U/ml hIFN- $\beta$  (IRF-Lucia positive control), 1  $\mu$ g/ml VACV70/LyoVec™ (CDS ligand), 300 ng/ml 3p-hpRNA/LyoVec™ (RIG-I agonist), 1  $\mu$ g/ml LPS-EK Ultrapure (UP; TLR4), 1 ng/ml Pam3CSK4 (TLR2/1 agonist), 0.3 ng/ml FSL-1 (TLR2/6 agonist),  $10^7$  c/ml HKLM (TLR2 agonist), and 3  $\mu$ g/ml 2'3'-cGAMP (STING agonist). After overnight incubation, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data are shown as a fold increase over non-induced cells (Lucia luciferase readout).

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