Validation data for THP1-Dual™ hTLR9 cells

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THP1-Dual™ hTLR9 cells were generated from the THP1-Dual™ cell line to overexpress the gene encoding human Toll-like receptor 9 (hTLR9), as verified by RT-qPCR **(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), and the IRF pathway by monitoring the activity of an inducible secreted Lucia luciferase. Human TLR9 overexpression in THP1-Dual™ cells allows potent NF-κB and IRF responses upon incubation with CpG-ODNs of class A (ODN 2216), class B (ODN 2006, ODN 1826), and class C (ODN 2395). Of note, these cells are more responsive to the class B, human-preferred, ODN 2006 **(Figures 2 and 3)**. THP1-Dual™ hTLR9 cells and their parental cells display similar NF-κB and IRF responses to other pattern recognition receptor ligands such as Tri-DAP (NOD-1 ligand) and 2'3'-cGAMP (STING ligand), respectively **(Figure 2)**.

Validation of *TLR9* overexpression

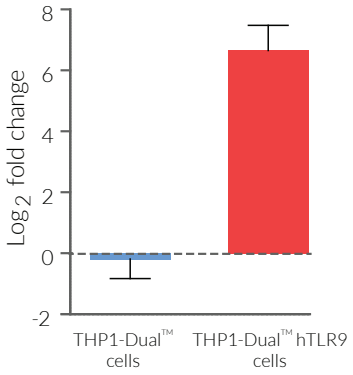

Figure 1: Human TLR9 expression in THP1-Dual™ hTLR9 cells.
Total mRNA was extracted from ~1x10^6 THP1-Dual™ (parental, blue) and THP1-Dual™ hTLR9 (red) cells. Human TLR9 (hTLR9) mRNA was amplified using quantitative RT-qPCR. Data are represented as the $\log_2$ fold change comparing hTLR9 relative expression between THP1-Dual™ and THP1-Dual™ hTLR9 cells.

Validation of the NF-κB and IRF reporter systems in THP1-Dual™ hTLR9 cells

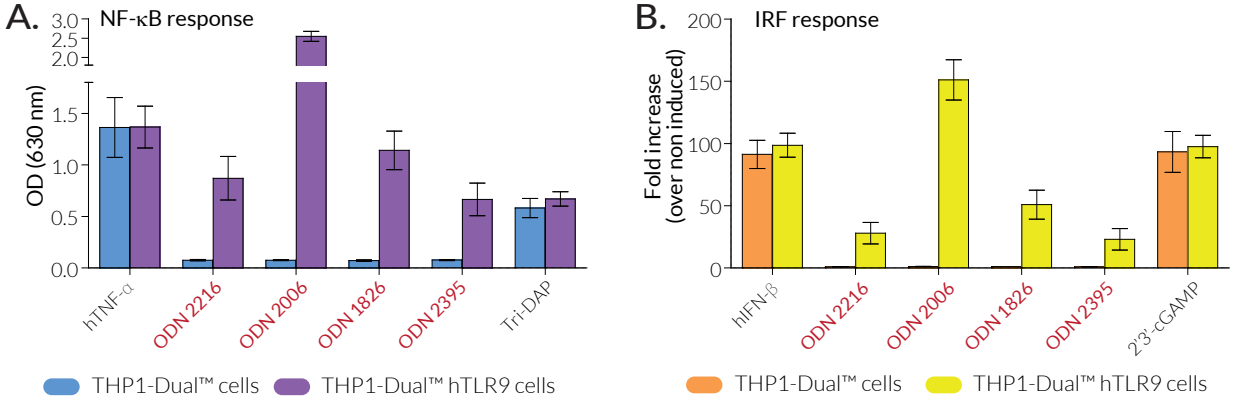

Figure 2: NF-κB and IRF responses in THP1-Dual™-derived cells.
THP1-Dual™ and THP1-Dual™ hTLR9 cells were incubated with 40 nM ODN 2216 (class A, human TLR9-preferred), ODN 2006 (class B, human TLR9-preferred), ODN 1826 (class B, mouse TLR9-preferred), or ODN 2395 (class C, human/mouse TLR9-preferred), and 1 ng/ml human TNF-α (hTNF-α), 300 ng/ml Tri-DAP, 10^4 U/ml human IFN-β (hIFN-β), or 3 µg/ml 2'3'-cGAMP as controls. After overnight incubation, the NF-κB response was assessed by measuring the SEAP activity in the supernatant using QUANTI-Blue™ Solution. Data are shown as optical density (OD) at 630 nm (mean ± SEM) **(A)**. The IRF response was assessed by measuring Lucia luciferase activity in the supernatant using QUANTI-Luc™. Data are shown as a fold increase (mean ± SEM) over non-induced cells **(B)**.

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NF-κB and IRF responses to TLR9 agonists in THP1-Dual™ hTLR9 cells

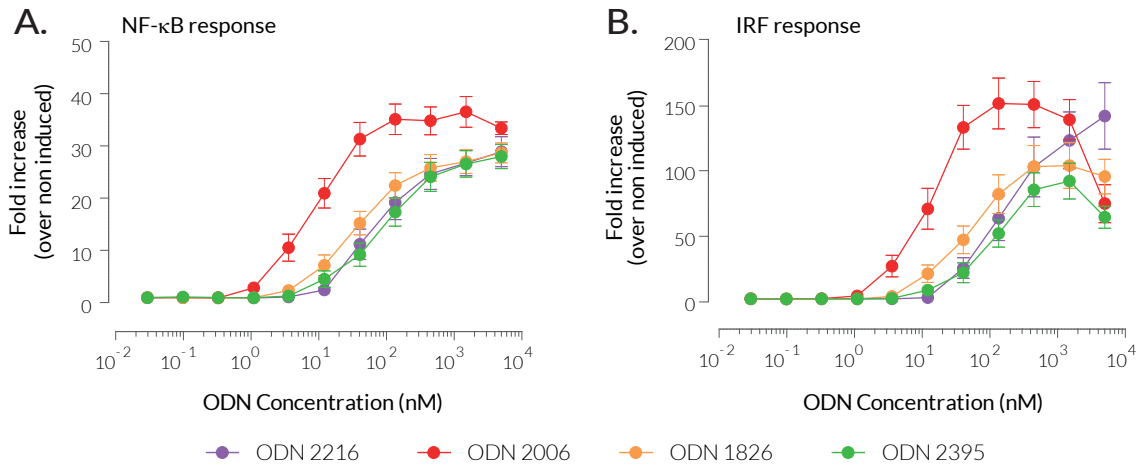

Figure 3: NF-κB and IRF responses induced by TLR9 agonist ODNs.
THP1-Dual™ hTLR9 cells were incubated with increasing concentrations of ODN 2216 (class A, human TLR9-preferred), ODN 2006 (class B, human TLR9-preferred), ODN 1826 (class B, mouse TLR9-preferred), or ODN 2395 (class C, human/mouse TLR9-preferred). After overnight incubation, the NF-κB response was assessed by measuring the SEAP and IRF activity in the supernatant using QUANTI-Blue™ Solution **(A)**, or QUANTI-Luc™ **(B)**, respectively. Data are shown as a fold increase (mean ± SEM) over non-induced cells.