Validation data for THP1-Dual™ hTLR3 cells

https://www.invivogen.com/thp1-dual-htlr3

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Version 23C24-AK

THP1-Dual™ hTLR3 cells were generated from the THP1-Dual™ cell line through the stable expression of the human Toll-like receptor 3 (hTLR3) as well as elements important for its signaling. These cells also feature two reporter genes allowing the simultaneous study of of NF-κB- and IRF-induced responses, by monitoring the SEAP (secreted embryonic alkaline phosphatase) and Lucia luciferase activities, respectively. Due to the stable expression of hTLR3, these cells show strong NF-κB and IRF responses upon incubation with synthetic double-stranded (ds)RNA analogs, such as Poly(A:U) (polyadenylic–polyuridylic acid) or Poly(I:C) (polyinosinic-polycytidylic acids), when compared to their parental cells **(Figures 1 & 2)**. Of note, as THP-1 cells express endogenous levels of various TLRs, THP1-Dual™ - derived cells respond to the cognate ligands including Pam3CSK4, LPS, or flagellin.

Functional validation of THP1-Dual™-derived cells (NF-κB responses)

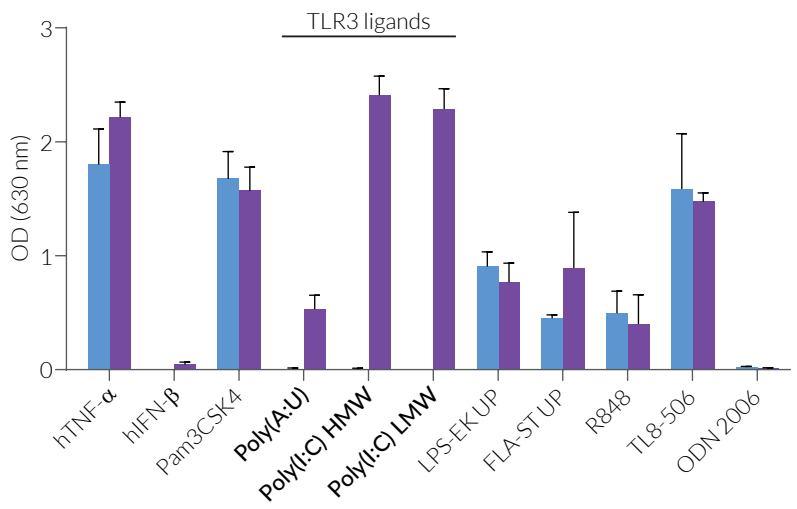

Figure 1. NF-κB responses in THP1-Dual™ -derived cells. THP1-Dual™ and THP1-Dual™ hTLR3 cells were incubated for 24 hours with various TLR agonists: Pam3CSK4 (TLR2 ligand, 10 ng/ml), Poly(A:U) (TLR3 ligand, 100 ng/ml), Poly(I:C) LMW and HMW (TLR3 ligands, 10 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 10 ng/ml), FLA-ST UP (TLR5 ligand, 10 µg/ml), R848 (TLR7/8 ligand, 3 µg/ml), TL8-506 (TLR8 ligand, 1 µg/ml), and ODN 2006 (TLR9 ligand, 10 µg/ml). Human TNF-α (10 ng/ml) was used as an NF-κB-positive control. After 24h incubation, the NF-κB-induced SEAP activity was assessed using QUANTI-Blue™. Data are shown as optical density (OD) at 630 nm (mean ± SEM).

Functional validation of THP1-Dual™-derived cells (IRF response)

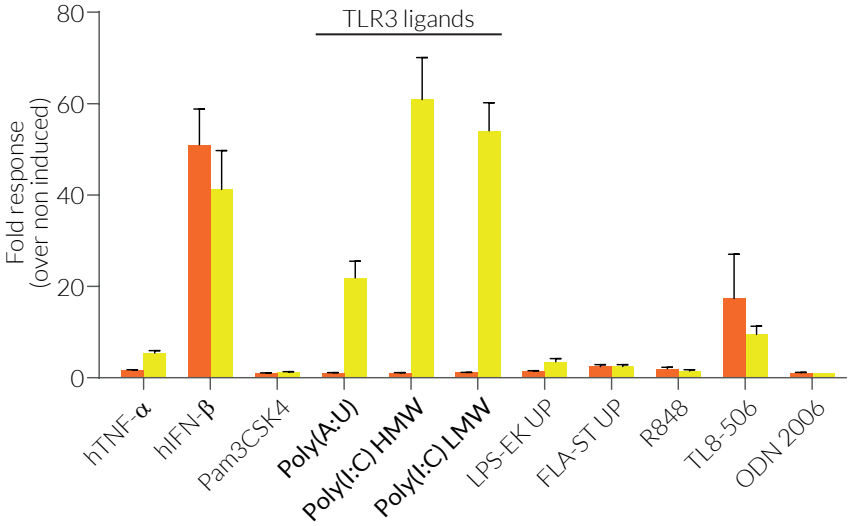

Figure 2. IRF responses in THP1-Dual™ -derived cells. THP1-Dual™ and THP1-Dual™ hTLR3 cells were incubated for 24 hours with various TLR agonists: Pam3CSK4 (TLR2 ligand, 10 ng/ml), Poly(A:U) (TLR3 ligand, 100 ng/ml), Poly(I:C) LMW and HMW (TLR3 ligands, 10 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 1 ng/ml), FLA-ST UP (TLR5 ligand, 10 µg/ml), R848 (TLR7/8 ligand, 3 µg/ml), TL8-506 (TLR8 ligand, 1 µg/ml), and ODN 2006 (TLR9 ligand, 10 µg/ml). Human IFN-β (1000 U/ml) was used as an IRF-positive control. After 24h incubation, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data are shown in fold response over non-induced cells (mean ± SEM).

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