Validation data for THP1-Dual™ MD2-CD14-TLR4 Cells

https://www.invivogen.com/thp1-dual-mc-ko-tlr4

For research use only
Version 22L01-AK

THP1-Dual™ MD2-CD14-TLR4 cells were generated from THP1-Dual™ cells through the stable expression of the human (h)*TLR4* gene as well as two co-adaptors important for lipopolysaccharide (LPS) signaling, MD-2 and CD14. Additionally, these cells 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. We observe an increased NFκB response to both smooth and rough LPS when compared to the parental THP1-Dual™ cells or the control cell line featuring a KO of the TLR4, THP1-Dual™ MD2-CD14 KO-TLR4 **(Figure 1)**. The co-expression of TLR4, CD14, and MD-2 completely restores the IRF response to rough and smooth LPS **(Figure 2)**.
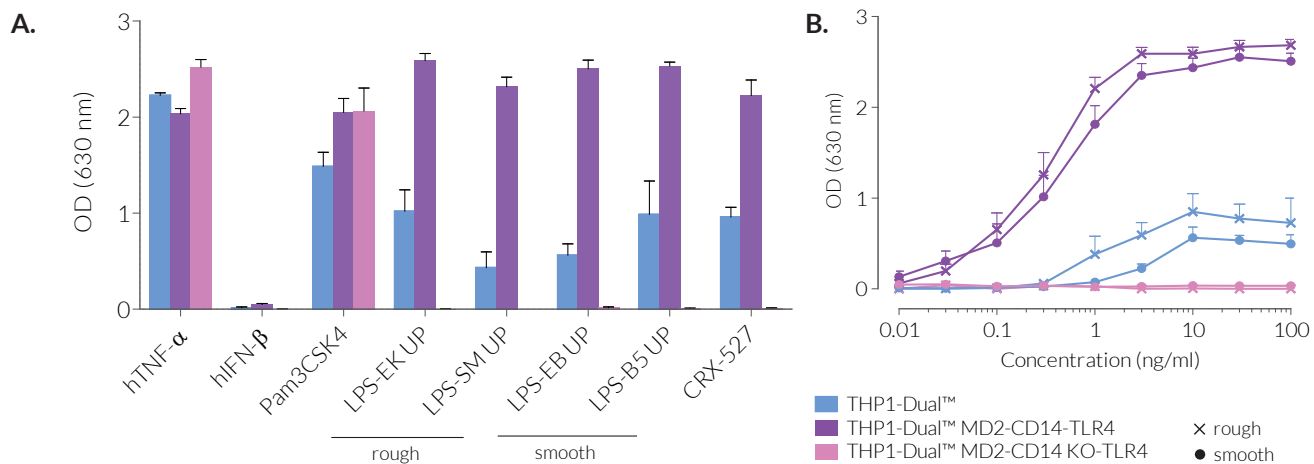
Figure 1: NF-κB responses in THP1-Dual™-derived cells. A. The cells were incubated with a variaty of rough and smooth LPS LPS-EK Ultrapure (UP) (rough; 10 ng/ml), LPS-SM UP (rough; 10 ng/ml), LPS-EB UP (smooth; 10 ng/ml), LPS-B5 UP (smooth; 1 ng/ml) and CRX-527 (synthetic; 10 ng/ml). Human (h)TNF-α (10 ng/ml) and hIFN-β (1x10³ U/ml) were used as a NF-κB-SEAP positive control and as a IRF positive control, respectively. The TLR2-TLR1 agonist Pam3CSK4 (10 ng/ml) served as a functional control. **B.** The cells were incubated with increasing concentration of rough (LPS-EK UP) and smooth (LPS-EB UP) LPS. After 24 hours, 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).
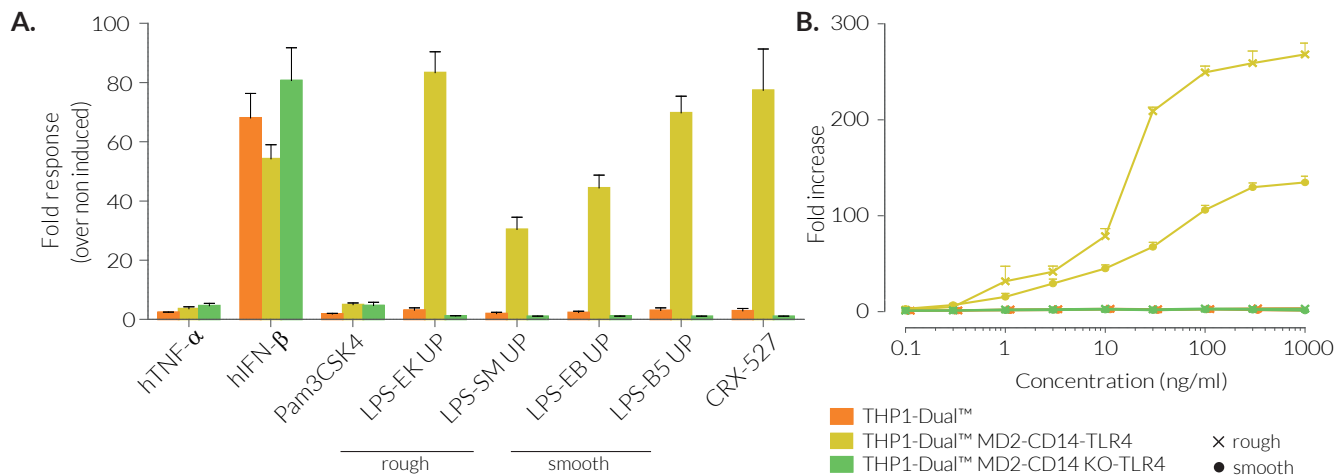
Figure 2: IRF responses in THP1-Dual™-derived cells. The cells were treated as described above *(see figure 1)*. After 24 hours, the IRF response was assessed by measuring the activity of Lucia luciferase in the supernatant using QUANTI-Luc™. Data are shown in fold response over non-induced cells (mean ± SEM).

InvivoGen
www.invivogen.com