Validation data for THP1-Dual™ KO-DNase2 Cells

https://www.invivogen.com/thpd-kodnase2

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Version 22F15-AK

THP1-Dual™ KO-DNase2 cells were generated from the THP1-Dual™ cell line through the stable biallelic knockout of the *DNASE2* gene, as verified by PCR **(Figure 1)**. Additionally, these cells feature two reporter genes allowing the simultaneous study of NF-κB- and IRF-induced responses by monitoring the SEAP (secreted embryonic alkaline phosphatase) and Lucia luciferase activities, respectively. There are no notable differences in the NF-κB- and IRF-mediated responses in THP1-Dual™ KO-DNase2 cells when compared to their parental cell line **(Figures 2 and 3)**.

Validation of DNASE2 knockout

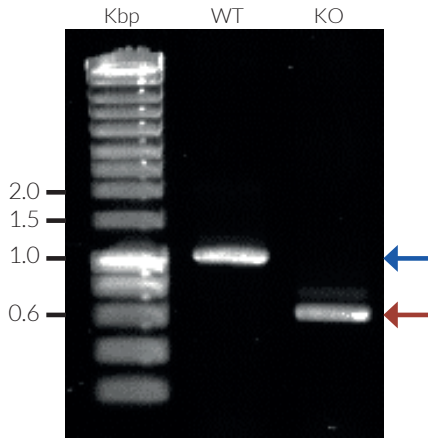

Figure 1: Validation of DNase2 KO. The targeted DNase2 region in THP1-Dual™ (WT; blue arrow) parental cells and THP1-Dual™ KO-DNase2 (KO; red arrow) cells was amplified by PCR. THP1-Dual™ KO-DNase2 cells were generated by a biallelic deletion of 443 bp in exon 2 and parts of exon 1 and 3, causing the inactivation of DNase2. The WT PCR product is 1133 bp, whereas the truncated KO band measures only 690 bp.

Functional validation of DNASE2 knockout (NF-κB response)

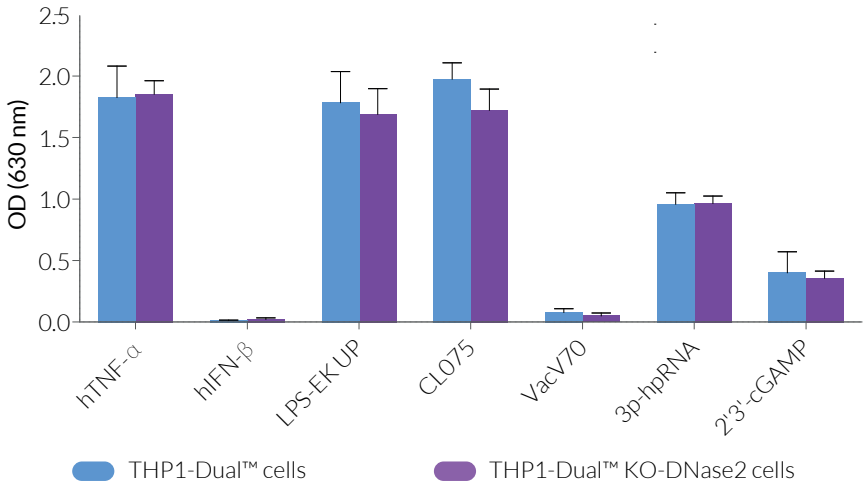

Figure 2: NF-κB responses in THP1-Dual™ -derived cells. THP1-Dual™ and THP1-Dual™ KO-DNase2 cells were incubated with 1 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 1000 U/ml hIFN-β (IRF-Lucia positive control), 10 ng/ml LPS-EK Ultrapure (UP; TLR4 agonist), 10 µg/ml CL075 (TLR7/8 agonist), 1 µg/ml VacV70 complexed with LyoVec™ (CDS agonist), 1 µg/ml 3p-hpRNA complexed with LyoVec™ (RIG-I agonist) and 30 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).

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Functional validation of DNASE2 knockout (IRF response)

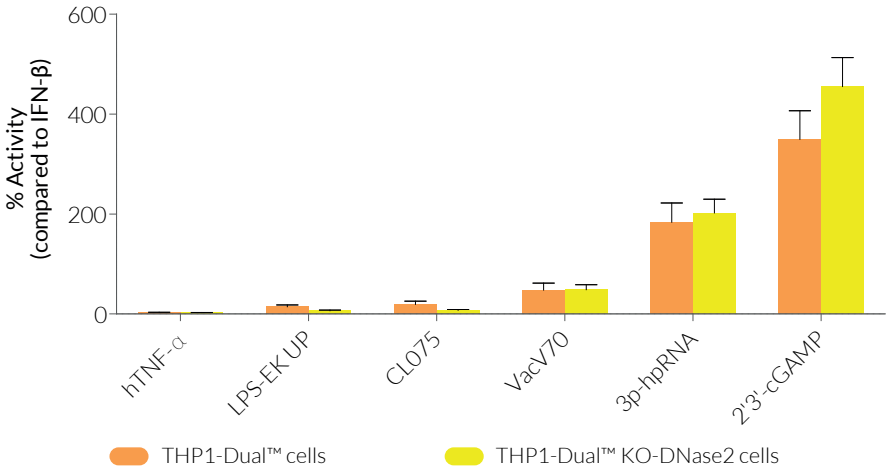

Figure 3: IRF responses in THP1-Dual™ -derived cells. THP1-Dual™ and THP1-Dual™ KO-DNase2 cells were incubated with 1 ng/ml human (h)TNF-α (NF-κB-SEAP positive control), 100 ng/ml LPS-EK Ultrapure (TLR4 agonist), 10 µg/ml CL075, 10 µg/ml R848 (both TLR7/8 agonists), 1 µg/ml VacV70 complexed with LyoVec™ (CDS agonist), 1 µg/ml 3p-hpRNA complexed with LyoVec™ (RIG-I agonist) and 30 µg/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™. The IRF induction of each ligand is expressed relative to that of hIFN-β at $1x10^3$ U/ml (mean ± SEM).