Validation data for HEK-Blue™ hNOD2 cells

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HEK-Blue™ hNOD2 cells were engineered from the human embryonic kidney HEK 293 cell line to assess the role of the Nucleotide-binding Oligomerization Domain-containing protein 2 (NOD2). These cells stably express the human (h) *NOD2* gene and an NF-κB-inducible SEAP reporter gene. A strong SEAP activation upon stimulation of NOD2 using the specific ligands L18-MDP (Muramyl dipeptide), MDP, and Murabutide can be readily monitored by performing the assay in HEK-Blue™ Detection medium **(Figure 1)**. HEK293 cells express endogenous levels of various pattern recognition receptors (PRRs), including TLR3 and TLR5. Therefore, HEK-Blue™ hNOD2 cells also respond to their cognate ligands Poly(I:C) and flagellin, respectively. HEK-Blue™ hNOD2 cells are non-responsive to NOD1-specific ligands, as verified using Tri-DAP **(Figure 2)**.

Dose-dependent NF-κB responses of HEK-Blue™ hNOD2 to NOD2-specific ligands

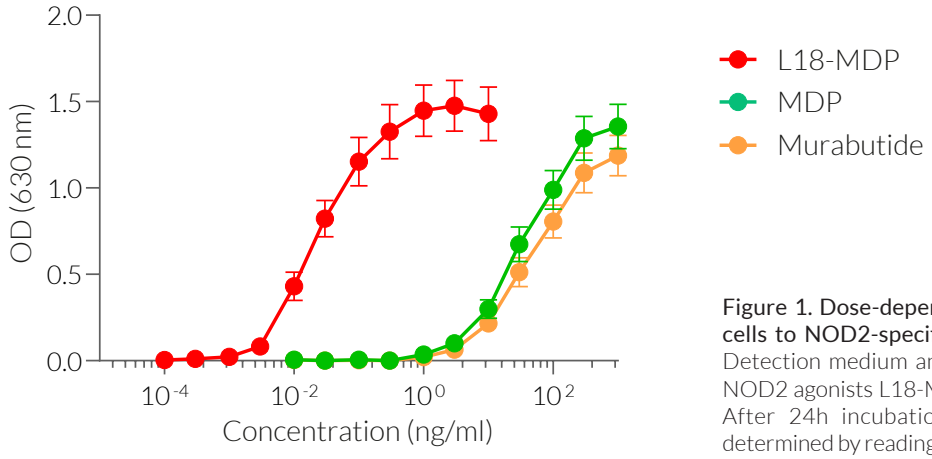

Figure 1. Dose-dependent NF-κB responses of HEK-Blue™ hNOD2 cells to NOD2-specific ligands. Cells were incubated in HEK-Blue™ Detection medium and stimulated with increasing concentrations of NOD2 agonists L18-MDP (Muramyl dipeptide), MDP, and Murabutide. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the optical density (OD) at 630 nm (mean ± SEM).

NF-κB response of HEK-Blue™ hNOD2 cells to various PRR agonists and cytokines

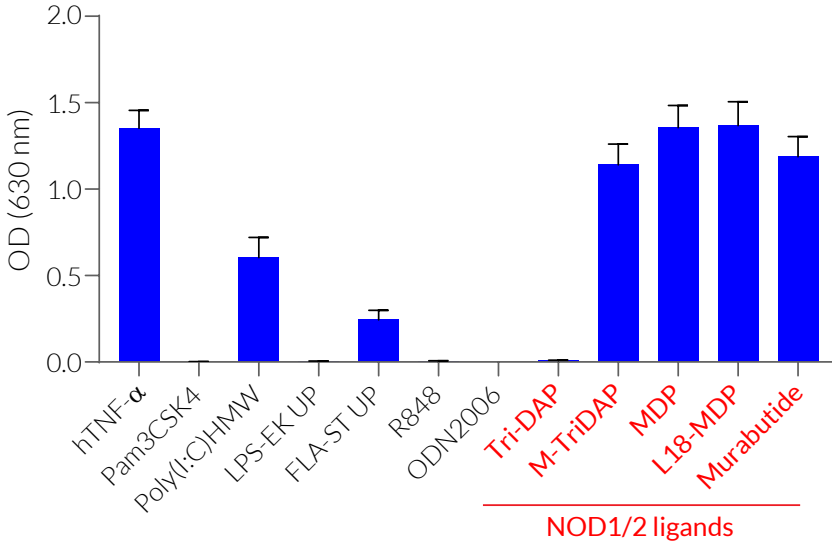

Figure 2. NF-κB responses of HEK-Blue™ hNOD2 cells to various PRR agonists and cytokines. Cells were incubated in HEK-Blue™ Detection medium and stimulated for 24h with various cytokines and PRR agonists: Human (h)TNF-α (NF-κB-positive control, 10 ng/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 1 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), R848 (TLR7/8 ligand, 10 µg/ml), ODN 2006 (TLR9 ligand, 10 µg/ml), Tri-DAP (NOD1 ligand, 10 µg/ml), M-Tri-DAP (NOD1/2 ligand, 1 µg/ml), MDP (NOD2 ligand, 1 µg/ml), L18-MDP (NOD2 ligand, 1 ng/ml), and Murabutide (NOD2 ligand, 1 µg/ml). After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the optical density (OD) at 630 nm (mean ± SEM).

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