

# Validation data for HEK-Blue™ mNOD1 cells

<https://www.invivogen.com/hek-blue-mnod1>

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Version 23G24-AK

HEK-Blue™ mNOD1 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 (NOD1). These cells stably express the murine (m) *NOD1* gene and an NF- $\kappa$ B-inducible SEAP reporter gene. A strong SEAP activation upon stimulation of NOD1 using the specific ligands C12-iE-DAP, Tri-DAP, and iE-DAP 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 NOD1, TLR3, and TLR5. Therefore, HEK-Blue™ mNOD1 cells also respond to their cognate ligands Tri-DAP, Poly(I:C), and flagellin, respectively (Figure 2).

## Dose-dependent NF- $\kappa$ B responses of HEK-Blue™ mNOD1 to NOD1-specific ligands

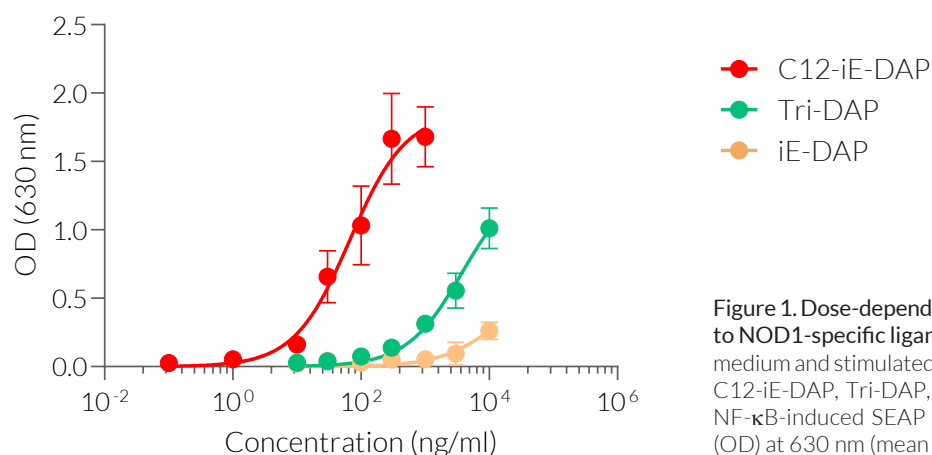


Figure 1. Dose-dependent NF- $\kappa$ B responses of HEK-Blue™ mNOD1 cells to NOD1-specific ligands. Cells were incubated in HEK-Blue™ Detection medium and stimulated with increasing concentrations of NOD1 agonists C12-iE-DAP, Tri-DAP, and iE-DAP. After 24h incubation, the levels of NF- $\kappa$ B-induced SEAP were determined by reading the optical density (OD) at 630 nm (mean  $\pm$  SEM).

## NF- $\kappa$ B response of HEK-Blue™ mNOD1 cells to various PRR agonists and cytokines

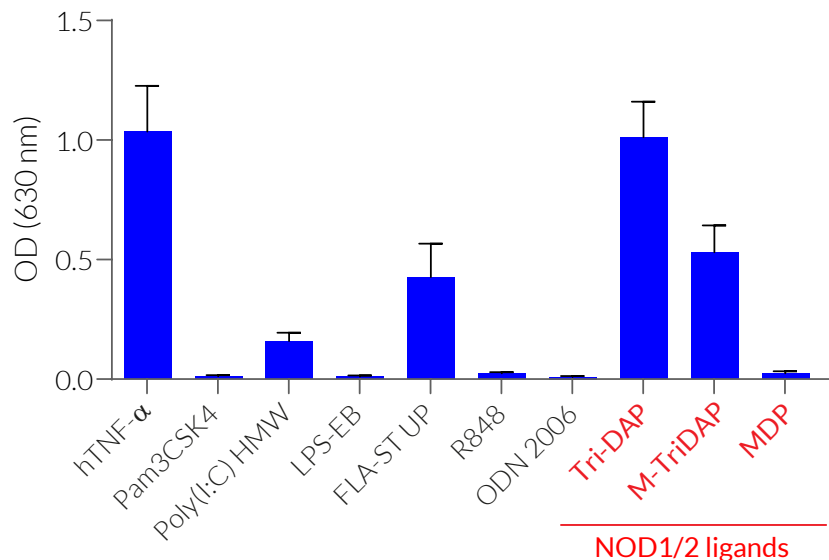


Figure 2. NF- $\kappa$ B responses of HEK-Blue™ mNOD1 cells to various PRR agonists and cytokines. Cells were incubated in HEK-Blue™ Detection medium and stimulated for 24h with various cytokines and PRR agonists: Human (h)TNF- $\alpha$  (NF- $\kappa$ B-positive control, 1 ng/ml), Pam3CSK4 (TLR2 ligand, 1  $\mu$ g/ml), Poly(I:C) HMW (TLR3 ligand, 10  $\mu$ g/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 10  $\mu$ g/ml), FLA-ST UP (TLR5 ligand, 1  $\mu$ g/ml), R848 (TLR7/8 ligand, 10  $\mu$ g/ml) ODN 2006 (TLR9 ligand, 10  $\mu$ g/ml), Tri-DAP (NOD1 ligand, 10  $\mu$ g/ml), M-Tri-DAP (NOD1/2 ligand, 10  $\mu$ g/ml), and MDP (NOD2 ligand, 10  $\mu$ g/ml). After 24h incubation, the levels of NF- $\kappa$ B-induced SEAP were determined by reading the optical density (OD) at 630 nm (mean  $\pm$  SEM).

### TECHNICAL SUPPORT

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