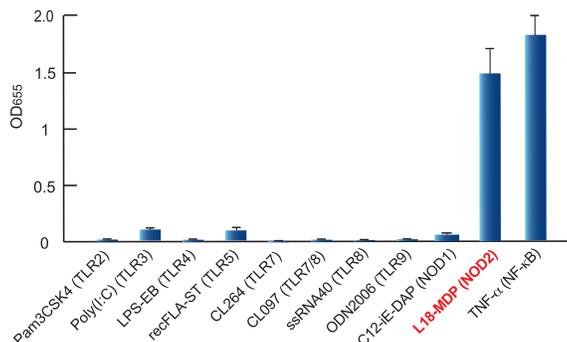


# Validation sheet for HEK-Blue™ hNOD2 Cells

HEK-Blue™ hNOD2 cells are engineered HEK293 cells that stably co-express the human NOD2 and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. These cells were thoroughly tested and validated by InvivoGen. The following data were obtained using the QUANTI-Blue™ or HEK-Blue™ Detection assays. These assays allow the detection of SEAP production following TLR/NOD activation by reading the optical density (OD) at 655 nm. Performance of these assays was validated under optimized conditions in a 96-well plate.

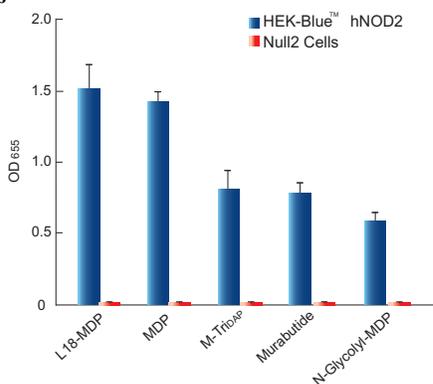
## TLR/NOD INDUCTION

### 1- Response of HEK-Blue™ hNOD2 cells to TLR and NOD agonists



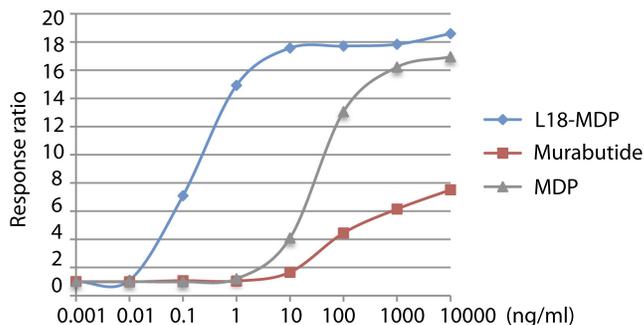
HEK-Blue™ hNOD2 cells were stimulated with various TLR and NOD agonists: Pam3CSK4 (100 ng/ml), Poly(I:C) (50 ng/ml), LPS-EB ultrapure (100 ng/ml), recombinant flagellin from *S. typhimurium* (10 ng/ml), CL264 (1 μg/ml), CL097 (1 μg/ml), ssRNA40/LyoVec™ (5 μg/ml), ODN 2006 (10 μg/ml), C12-iE-DAP (100 ng/ml), L18-MDP (100 ng/ml), and TNF-α (100 ng/ml). After 18h incubation (24h incubation for CL264, C12-iE-DAP and L18-MDP ligands), NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ and reading the OD at 655 nm.

### 2- Response of HEK-Blue™ hNOD2 cells to NOD agonists



HEK-Blue™ hNOD2 and HEK-Blue™ Null2 (control) cells were incubated in HEK-Blue™ Detection medium and stimulated with 10 ng/ml L18-MDP (NOD2), 100 ng/ml MDP (NOD2), 100 ng/ml M-TriDAP (NOD1/2), 100 ng/ml murabutide (NOD2) or 100 ng/ml N-Glycolyl-MDP (NOD2). After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.

### 3- NOD2 agonists dose response



HEK-Blue™ hNOD2 cells were incubated in HEK-Blue™ Detection medium and stimulated with increasing concentrations of NOD2 agonists. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm. The response ratio was calculated by dividing the OD at 655 nm for the treated cells by the OD at 655 nm for the untreated cells.

Ligand	EC50	Response ratio
L18-MDP	0.15+/- 0.1 ng/ml	19
MDP	35+/- 5 ng/ml	17
Murabutide	100+/- 20 ng/ml	8