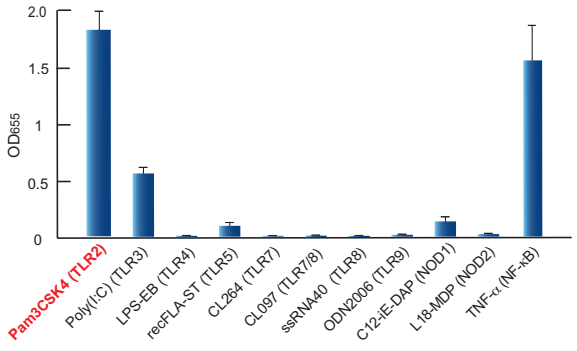
Validation sheet for HEK-Blue™ hTLR2 Cells

HEK-Blue™ hTLR2 cells are engineered HEK293 cells that stably co-express the human TLR2 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.

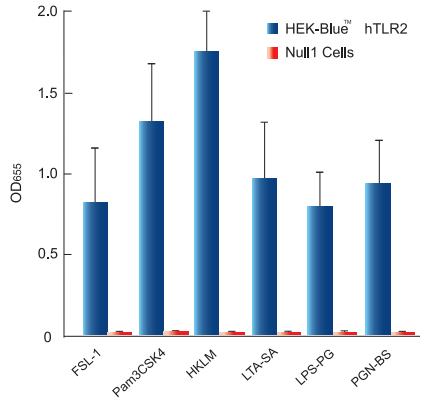
TLR/NOD INDUCTION

1- Response of HEK-Blue™ hTLR2 cells to TLR and NOD agonists



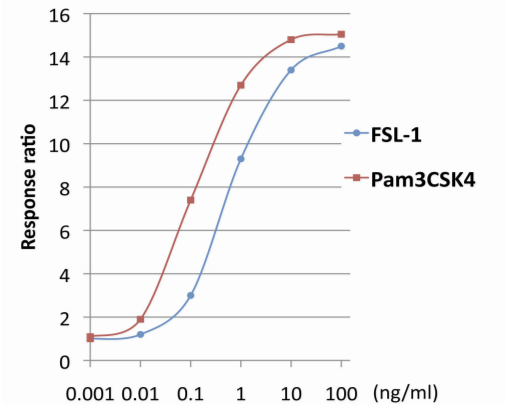
HEK-Blue™ hTLR2 cells were stimulated with various TLR and NOD agonists: Pam3CSK4 (100 ng/ml), Poly(I:C) (50 ng/ml), LPS-EB ultrapure (100 ng/ml), recombinant flagellin from *S. typhimurium* (10 ng/ml), CL264 (1 µg/ml), CL097 (1 µg/ml), ssRNA40/LyoVec™ (5 µg/ml), ODN 2006 (10 µg/ml), C12-iE-DAP (100 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 by reading the OD at 655 nm.

2- Response of HEK-Blue™ hTLR2 cells to TLR2 agonists



HEK-Blue™ hTLR2 and HEK-Blue™ Null1 (control) cells were incubated in HEK-Blue™ Detection medium and stimulated with 0.1 ng/ml FSL-1 (TLR2/6), 10 ng/ml Pam3CSK4 (TLR1/2), 10^7 cells/ml HKLM, 1 µg/ml LTA-SA, 10 ng/ml LPS-PG, or 1 µg/ml PGN-BS. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.

3- TLR2 agonists dose response



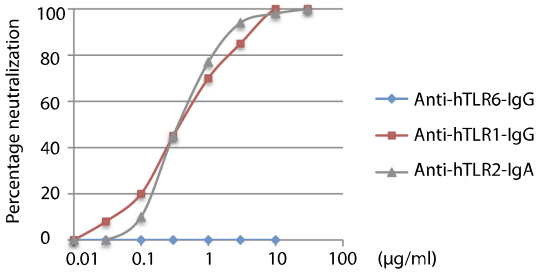
HEK-Blue™ hTLR2 cells were stimulated with increasing concentrations of TLR2 agonists. After 24h incubation, NF-κB-induced SEAP activity was assessed using HEK-Blue™ Detection by reading the OD at 655 nm. The response ratio was calculated by dividing the OD at 655 nm for the treated cells by the OD at 655 nm for the untreated cells.

Ligand	EC50	Response ratio
FSL-1	0.6 +/- 0.1 ng/ml	15
Pam3CSK4	0.1 +/- 0.1 ng/ml	14

TLR INHIBITION

1- Neutralization of TLR1/2 dose response

Anti-hTLR1 IgG IC50 400 +/-100 ng/ml
Anti-hTLR2 IgA2 IC50 350 +/-50 ng/ml



HEK-Blue™ TLR2 cells were pre-incubated for 1h with increasing concentrations of anti-hTLR2-IgA, anti-hTLR6-IgG or anti-hTLR1-IgG then stimulated with 0.5 ng/ml of Pam3CSK4 for 18h. NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ by reading the OD at 655 nm.

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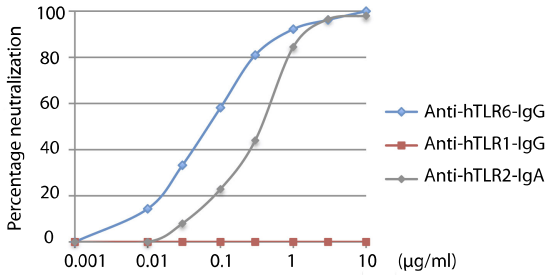
2- Neutralization of TLR2/6 dose response

Anti-hTLR2 IgA2 IC50 300 +/-100 ng/ml
Anti-hTLR6 IgG IC50 60 +/-20 ng/ml



HEK-Blue™ TLR2 cells were pre-incubated for 1h with increasing concentrations of anti-hTLR2-IgA, anti-hTLR6-IgG or anti-hTLR1-IgG then stimulated with 0.1 ng/ml of FSL-1 for 18h. NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ by reading the OD at 655 nm.