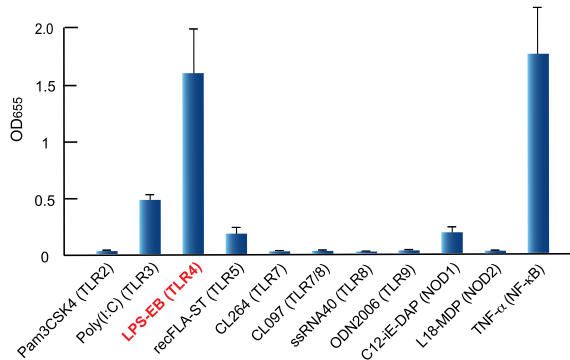


Validation sheet for HEK-Blue™ hTLR4 Cells

HEK-Blue™ hTLR4 cells are engineered HEK293 cells that stably co-express the human TLR4 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.

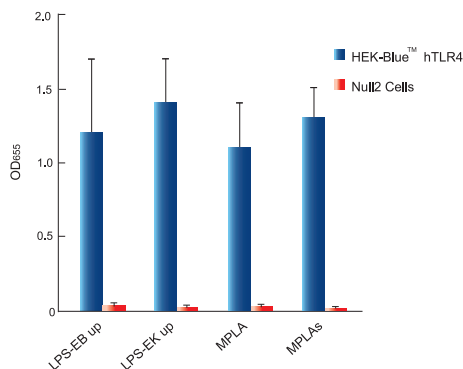
TLR/NOD INDUCTION

1- Response of HEK-Blue™ hTLR4 cells to TLR and NOD agonists



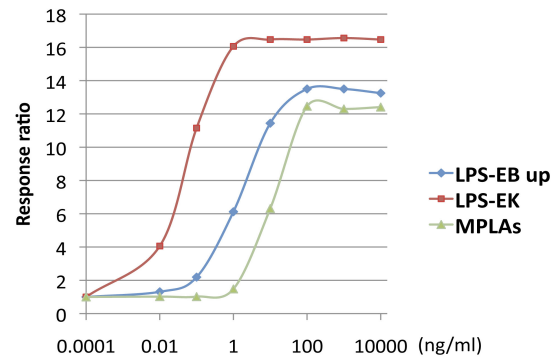
HEK-Blue™ hTLR4 cells were stimulated with various TLR and NOD agonists: Pam3CSK4 (100 ng/ml), Poly(I:C) (50 ng/ml), LPS-EB ultrapure (100 ng/ml), recombinant flagellin from *S. typhimurium* (10 ng/ml), CL264 (1 μg/ml), CL097 (1 μg/ml), ssRNA40/LyoVec™ (5 μg/ml), ODN 2006 (10 μg/ml), C12-iE-DAP (100 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™ hTLR4 cells to TLR4 agonists



HEK-Blue™ hTLR4 and HEK-Blue™ Null2 (control) cells were incubated in HEK-Blue™ Detection medium and stimulated with 10 ng/ml LPS-EB ultrapure, 10 ng/ml LPS-EK ultrapure, 100 ng/ml MPLA or 100 ng/ml MPLAs. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.

3- TLR4 agonists dose response



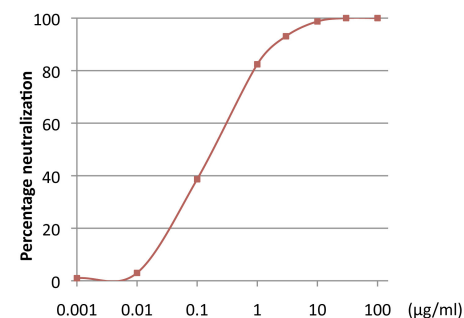
HEK-Blue™ hTLR4 cells were incubated in HEK-Blue™ Detection medium and stimulated with increasing concentrations of TLR4 agonists. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm. The response ratio was calculated by dividing the OD at 655 nm for the treated cells by the OD at 655 nm for the untreated cells.

Ligand	EC50	Response ratio
LPS-EB up	2 +/- 1 ng/ml	13
LPS-EK	0.07 +/- 0.01 ng/ml	16
MPLAs	10 +/- 3 ng/ml	12

TLR4 INHIBITION

1- Neutralization of TLR4 dose response

Anti-hTLR4 IgA2 IC50 200 +/- 60 ng/ml



HEK-Blue™ TLR4 cells were pre-incubated for 1h with increasing concentrations of anti-hTLR4 IgA2, then stimulated with 5 ng/ml of LPS-EB Ultrapure for 18h. NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ by reading the OD at 655 nm.