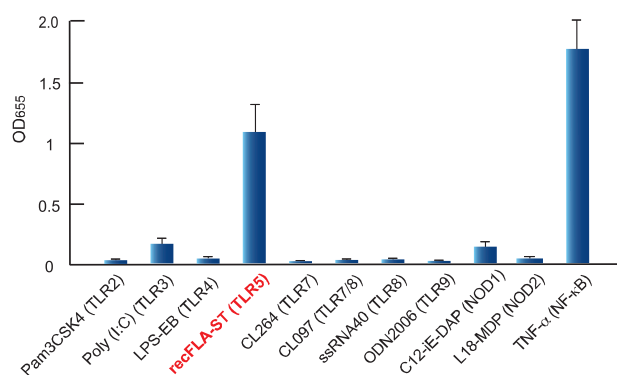


Validation sheet for HEK-Blue™ hTLR5 Cells

HEK-Blue™ hTLR5 cells are engineered HEK293 cells that stably co-express the human TLR5 gene, 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.

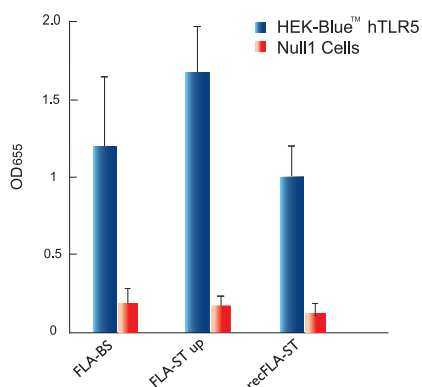
TLR/NOD INDUCTION

1- Response of HEK-Blue™ hTLR5 cells to TLR and NOD agonists



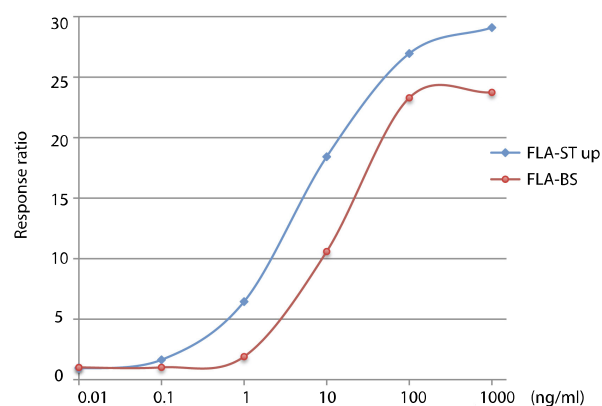
HEK-Blue™ hTLR5 cells were stimulated with TLR and NOD agonists: Pam3CSK4 (100 ng/ml), Poly(I:C) (50 ng/ml), LPS-EB ultrapure (100 ng/ml), recombinant flagellin from *S. typhimurium* (10 ng/ml), CL264 (1 μg/ml), CL097 (3 μg/ml), ssRNA40/LyoVec™ (5 μg/ml), ODN 2006 (10 μg/ml), C12-iE-DAP (100 ng/ml), 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™ by reading the OD at 655 nm.

2- Response of HEK-Blue™ hTLR5 cells to TLR5 agonists



HEK-Blue™ hTLR5 and HEK-Blue™ Null1 (control) cells were incubated in HEK-Blue™ Detection medium and stimulated with 100 ng/ml FLA-BS, 10 ng/ml FLA-ST Ultrapure, or 10 ng/ml recFLA-ST. After 24h incubation, the levels of NF-κB-induced SEAP were determined by reading the OD at 655 nm.

3- TLR5 agonists dose response



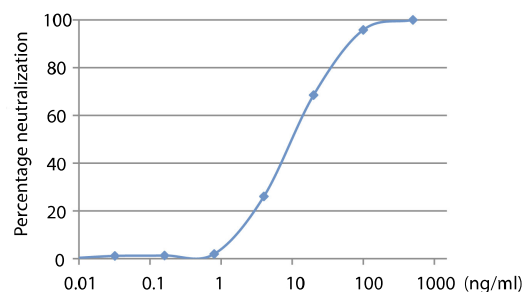
HEK-Blue™ hTLR5 cells were stimulated with increasing concentrations of TLR5 agonists. After 24h incubation, NF-κB-induced SEAP activity was assessed using HEK-Blue™ Detection by reading the OD at 655 nm. The response ratio was calculated by dividing the OD at 655 nm for the treated cells by the OD at 655 nm for the untreated cells.

Ligand	Origin	EC50	Response ratio
FLA-BS	<i>B.subtilis</i>	15 +/- 5 ng/ml	29
FLA-ST	<i>S.typhimurium</i>	6 +/- 3 ng/ml	23

TLR5 INHIBITION

1- Neutralization of TLR5 dose response

Anti-hTLR5 IgA2 IC50 10 +/-5 ng/ml



HEK-Blue™ TLR5 cells were pre-incubated for 1h with increasing concentrations of anti-hTLR5 IgA2, then stimulated with 5 ng/ml of FLA-ST Ultrapure for 18h. NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ by reading the OD at 655 nm.