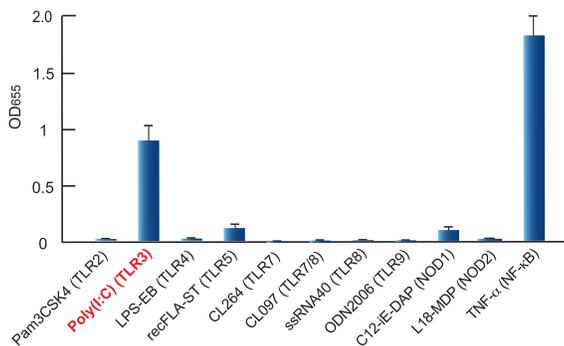


Validation sheet for HEK-Blue™ hTLR3 Cells

HEK-Blue™ hTLR3 cells are engineered HEK293 cells that stably co-express the human TLR3 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.

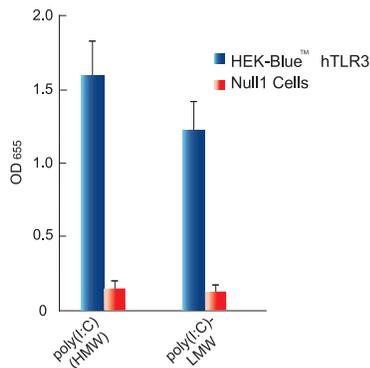
TLR/NOD INDUCTION

1- Response of HEK-Blue™ hTLR3 cells to TLR and NOD agonists



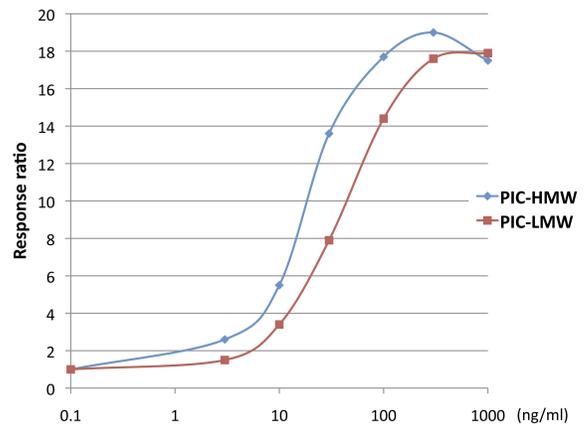
HEK-Blue™ hTLR3 cells were stimulated with various TLR and NOD agonists: Pam3CSK4 (100 ng/ml), Poly(I:C)-HMW (50 ng/ml), LPS-EB ultrapure (100 ng/ml), recombinant flagellin from *S. typhimurium* (10 ng/ml), CL264 (1 μg/ml), CL097 (1 μg/ml), ssRNA40/LyoVec™ (5 μg/ml), ODN 2006 (10 μg/ml), C12-iE-DAP (100 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™ hTLR3 cells to TLR3 agonists



HEK-Blue™ hTLR3 and HEK-Blue™ Null1 (control) cells were stimulated with poly(I:C)-HMW or poly(I:C)-LMW. All agonists were used at a concentration of 1 μg/ml. After 18h incubation, NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ by reading the OD at 655 nm.

3- TLR3 agonists dose response



HEK-Blue™ hTLR3 cells were stimulated with increasing concentrations of TLR3 agonists. After 18h incubation, NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ and 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
Poly(I:C) HMW	20 +/- 5 ng/ml	19
Poly(I:C) LMW	40 +/- 5 ng/ml	18