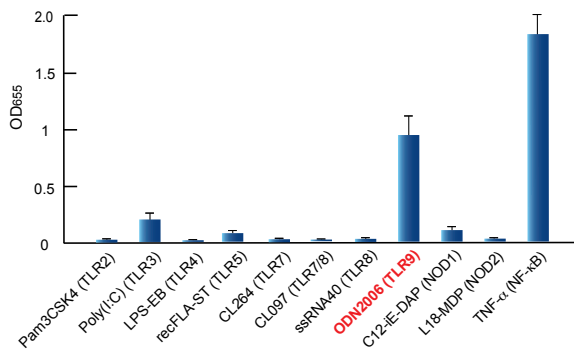


Validation sheet for HEK-Blue™ hTLR9 Cells

HEK-Blue™ hTLR9 cells are engineered HEK293 cells that stably co-express the human TLR9 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.

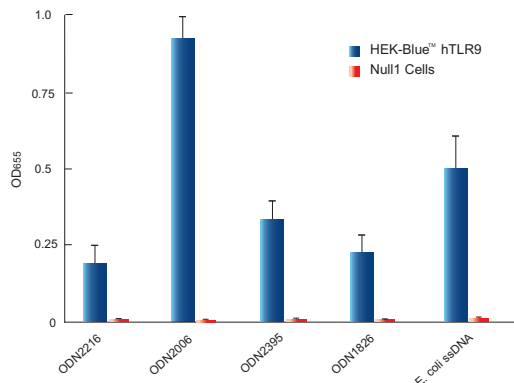
TLR/NOD INDUCTION

1- Response of HEK-Blue™ hTLR9 cells to TLR and NOD agonists



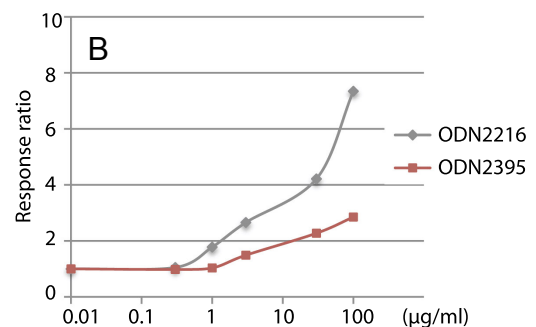
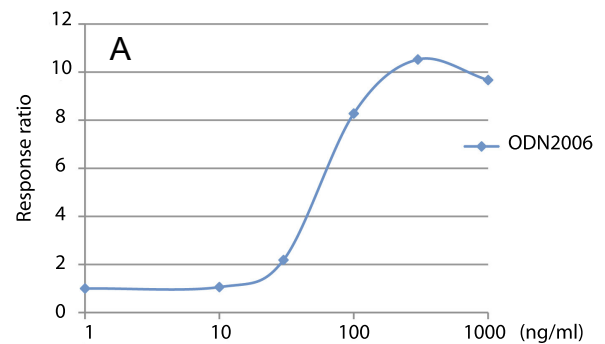
HEK-Blue™ hTLR9 cells were stimulated with various TLR and NOD agonists: Pam3CSK4 (100 ng/ml), Poly(I:C) (50 ng/ml), LPS-EB ultrapure (100 ng/ml), recombinant flagellin from *S. typhimurium* (10 ng/ml), CL264 (1 µg/ml), CL097 (1 µg/ml), ssRNA40/LyoVec™ (5 µg/ml), ODN 2006 (10 µg/ml), C12-iE-DAP (100 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™ hTLR9 cells to TLR9 agonists



HEK-Blue™ hTLR9 and HEK-Blue™ Null1 (control) cells were stimulated with 30 µg/ml ODN2216 (human specific, type A), 0.3 µg/ml ODN2006 (human specific, type B), 30 µg/ml ODN2395 (human/mouse, type C), 30 µg/ml ODN1826 (mouse specific, type B), or 5 µg/ml E.coli ssDNA/LyoVec™. After 18h incubation, NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ and reading the OD at 655 nm.

3- TLR9 agonists dose response



HEK-Blue™ hTLR9 cells were stimulated with increasing concentrations of TLR9 agonists. In graph A, the response to ODN2006 (human specific, type B) is shown. In graph B, the response to ODN2216 (human specific, type A) and ODN2395 (human/mouse, type C) is presented. After 18h incubation, NF-κB-induced SEAP activity was assessed using QUANTI-Blue™. 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.