

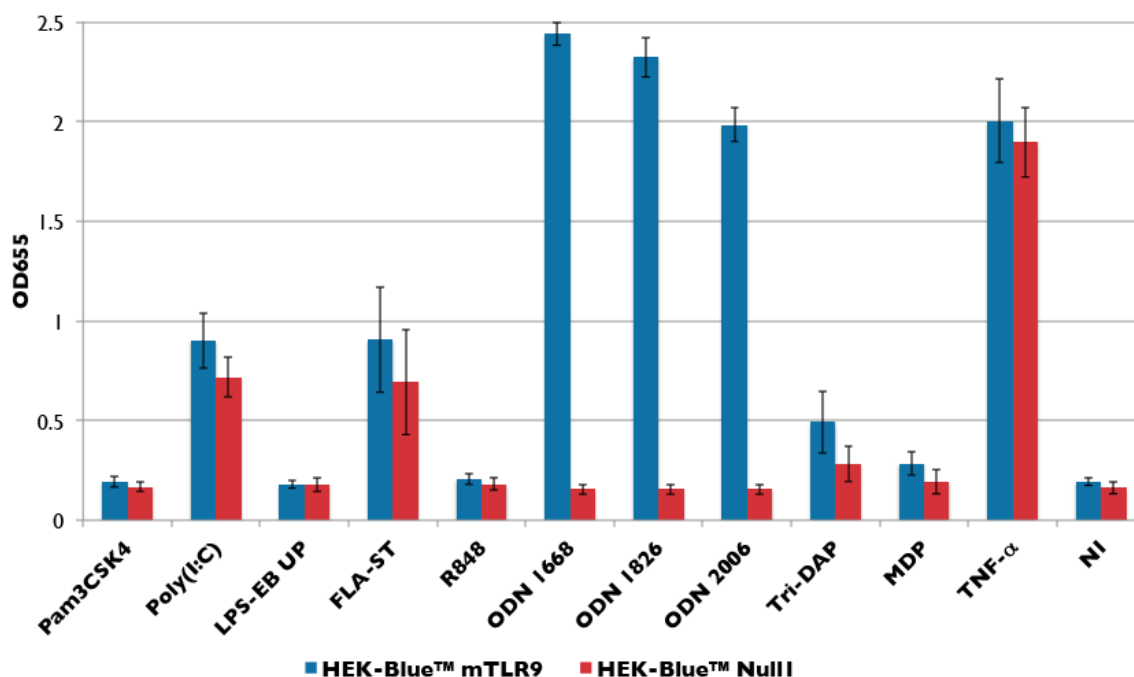
# Validation data for HEK-Blue™ mTLR9 cells

For research use only

Version # 16B26-MM

HEK-Blue™ mTLR9 are designed for studying the stimulation of mouse TLR9 (mTLR9) by monitoring the activation of NF-κB. They were obtained by co-transfection of the mTLR9 gene and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene into HEK293-derived cells. The expression of the mTLR9 gene has been confirmed by RT-PCR. The biological activity has been confirmed using HEK-Blue™ Detection, a cell culture medium that allows for real-time detection of SEAP (see figure 1).

## Response of HEK-Blue™ mTLR9 cells to PRR agonists



**Figure 1:** HEK-Blue™ mTLR9 and HEK-Blue™ Null1 cells (control cell line) were stimulated with various TLR and NOD agonists: Pam3CSK4 (TLR2 agonist; 100 ng/ml), Poly(I:C) (TLR3 agonist; 1 µg/ml), LPS-EB ultrapure (TLR4 agonist; 100 ng/ml), FLA-ST (flagellin from *S. typhimurium*, a TLR5 agonist; 100 ng/ml), R848 (TLR7/8 agonist; 1 µg/ml), ODN 1668 (class B ODN specific for mTLR9; 10 µg/ml), ODN 1826 (class B ODN specific for mTLR9; 10 µg/ml), ODN 2006 (class B ODN with a preference for human TLR9; 10 µg/ml), Tri-DAP (NOD1 agonist; 1 µg/ml), MDP (NOD2 agonist; 1 µg/ml), and TNF-α (10 ng/ml). After a 24h incubation, NF-κB-induced SEAP activity was assessed using HEK-Blue™ Detection and reading the optical density (OD) at 655 nm. TNF-α has been included as a positive control. Non-induced cells (NI) have been included as negative controls.

### TECHNICAL SUPPORT

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