

Validation data for HEK-Blue™ mTLR3 cells

<https://www.invivogen.com/hek-blue-mtlr3>

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Version 23128-AK

HEK-Blue™ mTLR3 cells are engineered HEK293 cells that stably co-express the murine (m)Toll-like receptor 3 (TLR3) and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. The following data were obtained using the QUANTI-Blue™ or HEK-Blue™ Detection assays. These cells show a strong NF-κB response upon incubation with synthetic double-stranded (ds)RNA analogs, such as Poly(A:U) (polyadenylic-polyuridylic acid) or Poly(I:C) (polyinosinic-polycytidylic acid) (Figures 1 & 2). Of note, as HEK293 cells express endogenous low levels of various pattern recognition receptors, including TLR3 and TLR5. Thus, HEK-Blue™-derived cells might slightly respond to the cognate ligands Poly(I:C) or flagellin (Figures 2 & 3).

Dose-response of HEK-Blue™ mTLR3 cells to TLR3 agonists

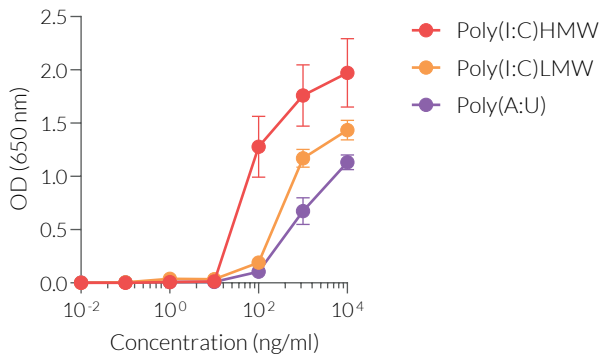


Figure 1. Dose-response of HEK-Blue™ mTLR3 cells to TLR3 agonists. Cells were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of Poly(I:C) HMW, Poly(I:C) LMW, and Poly(A:U). After 24 hour incubation, the NF-κB-induced SEAP activity was determined by reading the OD at 650 nm (mean ± SEM).

Response of HEK-Blue™-derived cells to TLR3 agonists

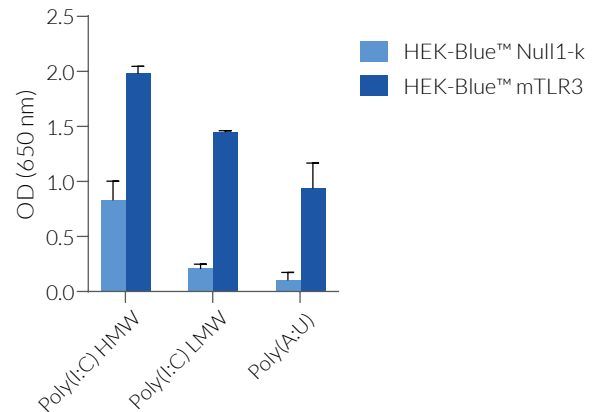


Figure 2. Response of HEK-Blue™-derived cells to TLR3 agonists. HEK-Blue™ Null1-k and HEK-Blue™ mTLR3 cells were incubated for 24 hours with 1 µg/ml of the TLR3 agonists Poly(I:C) HMW or Poly(I:C) LMW, and 10 µg/ml of Poly(A:U). After 24h incubation, the NF-κB-induced SEAP activity was assessed using QUANTI-Blue™. Data are shown as optical density (OD) at 650 nm (mean ± SEM).

Response of HEK-Blue™ mTLR3 cells to various PRR agonists and cytokines

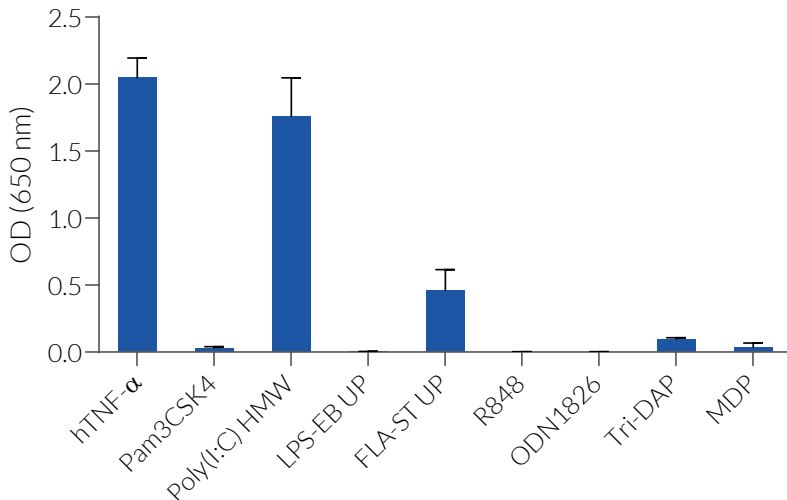


Figure 3. Response of HEK-Blue™ mTLR3 cells to various PRR agonists and cytokines. Cells were incubated for 24 hours with cytokines and various TLR agonists: Human TNF-α (NF-κB-positive control, 10 ng/ml), Pam3CSK4 (TLR2 ligand, 1 µg/ml), Poly(I:C) HMW (TLR3 ligand, 1 µg/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 1 µg/ml), FLA-ST UP (TLR5 ligand, 1 µg/ml), R848 (TLR7/8 ligand, 10 µg/ml), ODN 2006 (TLR9 ligand, 10 µg/ml), Tri-DAP (NOD1 ligand, 1 µg/ml), and MDP (NOD2 ligand, 1 µg/ml). After 24h incubation, the NF-κB-induced SEAP activity was assessed using QUANTI-Blue™. Data are shown as OD at 650 nm (mean ± SEM).

TECHNICAL SUPPORT

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