

Validation data for HEK-Blue™ hTLR3 cells

<https://www.invivogen.com/hek-blue-htlr3>

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Version 23111-AK

HEK-Blue™ hTLR3 cells are engineered HEK293 cells that stably co-express the human (h)Toll-like receptor 3 (TLR3) and an NF- κ B-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- κ B 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), when compared to their parental cells HEK-Blue™ Null1 (Figures 1 & 2). Of note, as HEK293 cells express endogenous levels of TLR5, HEK-Blue™ hTLR3 cells respond to the cognate ligand flagellin (Figure 3).

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

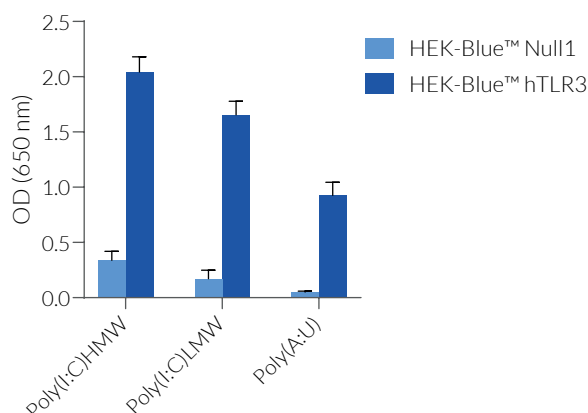


Figure 1. Response of HEK-Blue™-derived cells to TLR3 agonists. HEK-Blue™ Null1 and HEK-Blue™ hTLR3 cells were cultured in HEK-Blue™ Detection reagent and stimulated for 24 hours with 1 μ g/ml of the following TLR3 agonists: Poly(I:C) HMW, Poly(I:C) LMW, and Poly(A:U). After 24h incubation, the NF- κ B-induced SEAP activity was assessed by measuring the SEAP level in the supernatant. Data are shown as optical density (OD) at 650 nm (mean \pm SEM).

Dose-response of HEK-Blue™ hTLR3 cells to TLR3 agonists

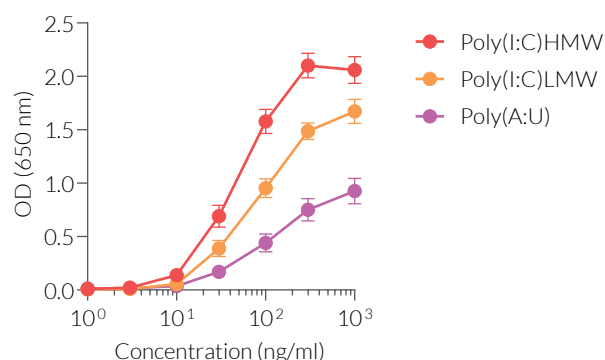


Figure 2. Dose-response of HEK-Blue™ hTLR3 cells to TLR3 agonists. Cells were cultured in HEK-Blue™ Detection reagent and stimulated with increasing concentrations of Poly(I:C) HMW, Poly(I:C) LMW, and Poly(A:U). After 24 hour incubation, the NF- κ B-induced SEAP activity was determined by reading the OD at 650 nm (mean \pm SEM).

Response of HEK-Blue™ hTLR3 cells to various PRR agonists and cytokines

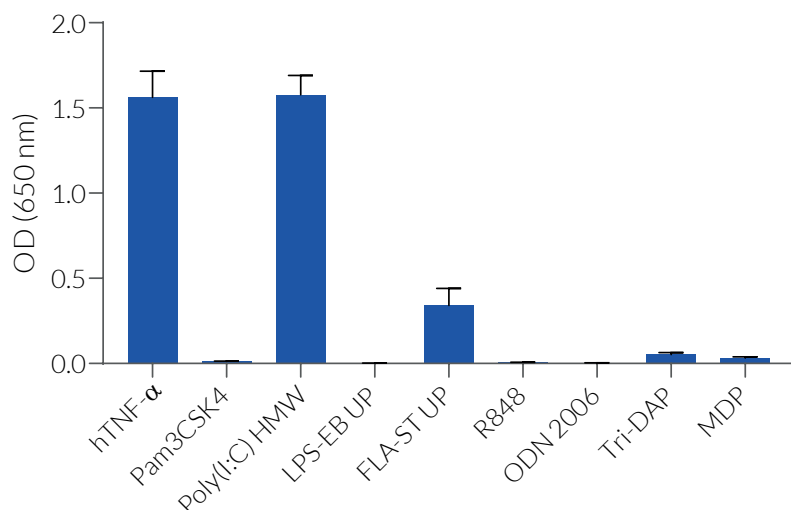


Figure 3. Response of HEK-Blue™ hTLR3 cells to various PRR agonists and cytokines. Cells were cultured in HEK-Blue™ Detection reagent and stimulated for 24 hours with cytokines and various TLR agonists: Human TNF- α (NF- κ B-positive control, 1 ng/ml), Pam3CSK4 (TLR2 ligand, 100 ng/ml), Poly(I:C) HMW (TLR3 ligand, 100 ng/ml), LPS-EK Ultrapure (UP) (TLR4 ligand, 100 ng/ml), FLA-ST UP (TLR5 ligand, 100 ng/ml), R848 (TLR7/8 ligand, 10 μ g/ml), ODN 2006 (TLR9 ligand, 10 μ g/ml), Tri-DAP (NOD1 ligand, 100 ng/ml), and MDP (NOD2 ligand, 100 ng/ml). After 24h incubation, the NF- κ B-induced SEAP activity was assessed by measuring the SEAP level in the supernatant. Data are shown as OD at 650 nm (mean \pm SEM).

TECHNICAL SUPPORT

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