

# Validation data for RAW-Blue™ Cells

<https://www.invivogen.com/raw-blue>

For research use only

Version 23B16-AK

RAW-Blue™ cells are designed to monitor the NF- $\kappa$ B and AP-1 responses upon PRR (pattern recognition receptor) stimulation. These cells stably express an NF- $\kappa$ B/AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) reporter gene. Levels of SEAP activities are readily assessable in the supernatant using QUANTI-Blue™ Solution, a SEAP detection medium. RAW-Blue™ cells are derived from the murine RAW 264.7 macrophage cell line, which has been reported to express many pattern recognition receptors (PRRs), as verified using RT-PCR (Figure 1) and by monitoring the NF- $\kappa$ B-response upon stimulation using various PRR-ligands (Figure 2).

## Validation of PRR expression

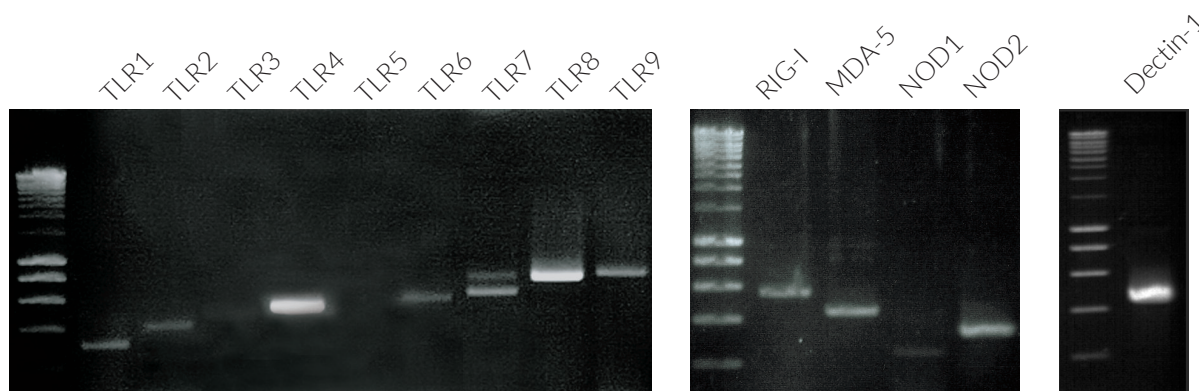


Figure 1: Expression profile of various PRRs in RAW-Blue™ cells. RT-PCR results show endogenous expression of Toll-like receptors (TLRs 1-9), RIG-I, MDA-5, NOD1, NOD2 and Dectin-1 in RAW-Blue™ cells.

## Functional validation (NF- $\kappa$ B response)

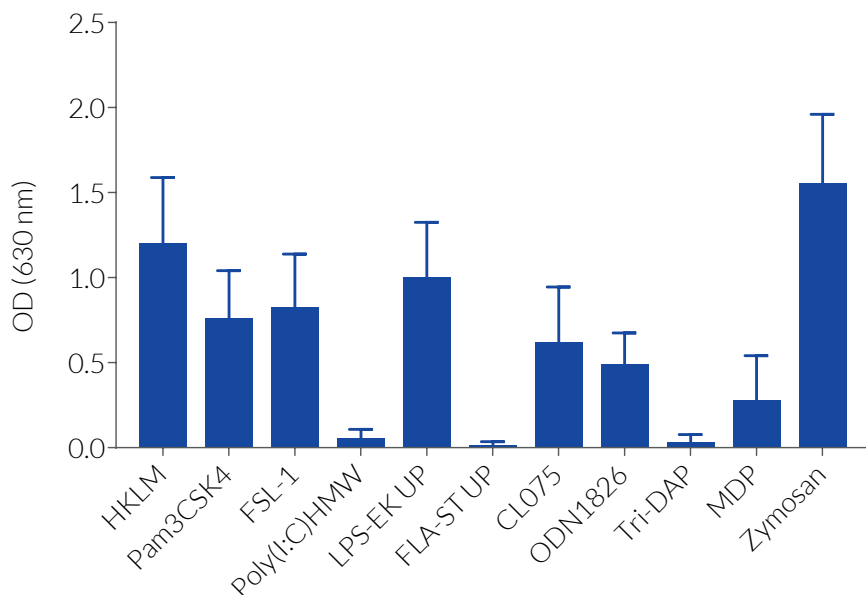


Figure 2: NF- $\kappa$ B responses in RAW-Blue™ cells to various PRR ligands. Cells were incubated with TLR, Dectin and NOD agonists: TLR2 (HKLM,  $1 \times 10^8$  cells/ml), TLR1/2 (Pam3CSK4, 1  $\mu$ g/ml), TLR2/6 (FSL-1, 1  $\mu$ g/ml), TLR3 (Poly(I:C) HMW, 100  $\mu$ g/ml), TLR4 (LPS-EK, 10  $\mu$ g/ml), TLR5 (FLA-ST Ultrapure (UP), 10  $\mu$ g/ml), TLR7 (CL075, 10  $\mu$ g/ml), TLR9 (ODN1826, 10  $\mu$ g/ml), NOD1 (Tri-DAP, 10  $\mu$ g/ml), NOD2 (MDP, 10  $\mu$ g/ml) and Dectin-1 (Zymosan, 100  $\mu$ g/ml). After 24h incubation, TLR and NOD stimulation was assessed by measuring the levels of SEAP using QUANTI-Blue™. Data are shown as optical density (OD) at 630 nm (mean  $\pm$  SEM).

### TECHNICAL SUPPORT

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