

Validation data for Poly(I:C) HMW Biotin

<https://www.invivogen.com/polyic-biotin>

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Poly(I:C) HMW Biotin is a biotin-labeled Toll-like receptor 3 (TLR3) agonist. It was chemically produced by covalent coupling of biotin with high molecular weight polyinosinic-polycytidylic acid (poly(I:C) HMW). The biological activity of Poly(I:C) HMW Biotin has been tested using InvivoGen's HEK-Blue™ hTLR3 cells. Our results demonstrate that Poly(I:C) HMW Biotin retains its ability to activate TLR3, the same as the unlabeled Poly(I:C) HMW (figure 1). The binding of Poly(I:C) HMW Biotin to TLR3 has been validated by flow cytometry using RAW-Blue™ cells (figure 2). A clear shift to the right of the fluorescence peak is noted when TLR3-expressing RAW-Blue™ cells were incubated with Poly(I:C) HMW Biotin (right) when compared to those cells incubated with unlabeled Poly(I:C) HMW (left), indicating strong and specific binding.

TLR3 activation by Poly(I:C) HMW Biotin

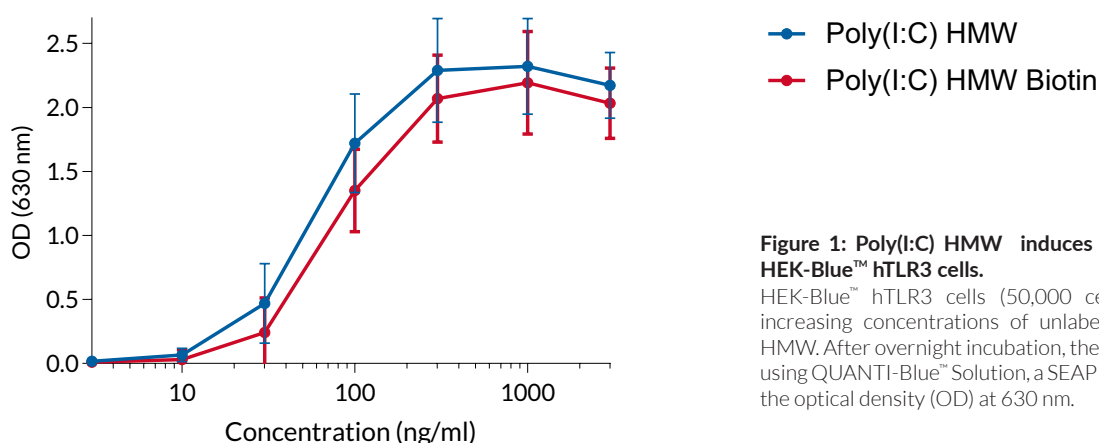


Figure 1: Poly(I:C) HMW induces a dose-dependent response in HEK-Blue™ hTLR3 cells.

HEK-Blue™ hTLR3 cells (50,000 cells/well) were stimulated with increasing concentrations of unlabeled and biotin-labeled Poly(I:C) HMW. After overnight incubation, the NF- κ B response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent, and by reading the optical density (OD) at 630 nm.

Binding of Poly(I:C) HMW Biotin to TLR3

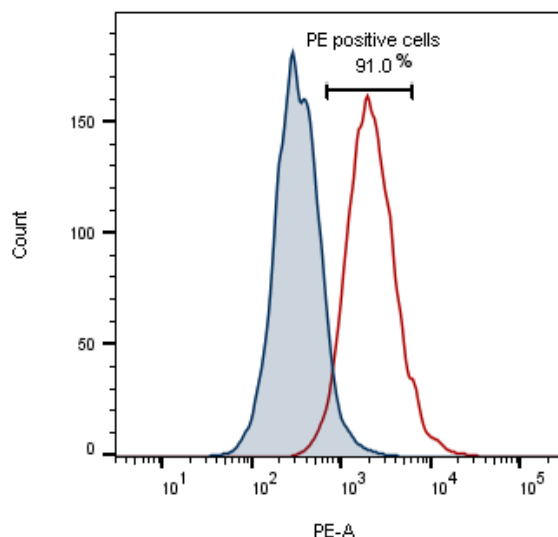


Figure 2: Binding of Poly(I:C) HMW Biotin to TLR3-expressing cells.

Poly(I:C) HMW Biotin (1 μ g), or unlabeled Poly(I:C) HMW (1 μ g), was added to 500,000 cells/ml of RAW-Blue™ cells which express TLR3 and incubated overnight at 37°C. Following this incubation, cells were permeabilized and then incubated with PE-labeled anti-biotin antibody (500 ng) for 30 minutes at 4°C. The binding affinity was then measured using flow cytometry. The unshaded red area indicates positive staining, while the shaded blue area represents the unlabeled Poly(I:C) HMW control.

TECHNICAL SUPPORT

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