

# Validation data for TL7-887 VacciGrade™

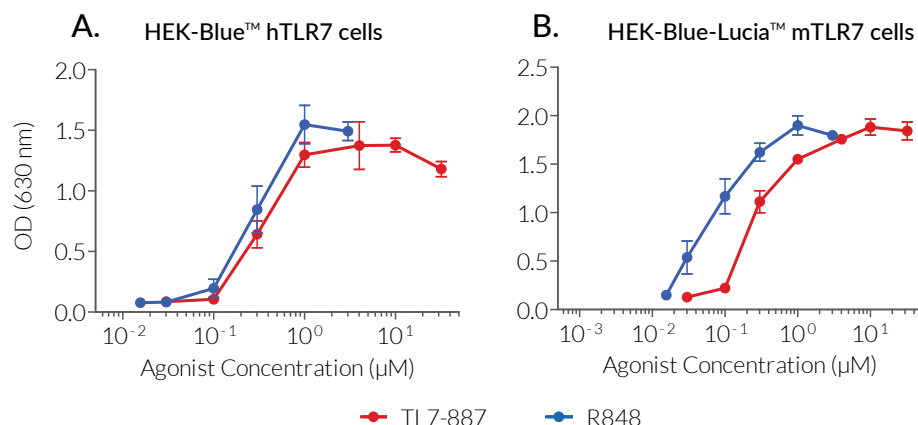
<https://www.invivogen.com/tlr7-conjugatable-ligands>

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Version 23J12-NJ

TL7-887 is a ready-to-use “pre-linked” conjugatable TLR7 ligand, synthesized from the base molecule CL307, a well-known TLR7 agonist. TL7-887 efficiently triggers a cellular response upon recognition by either human or murine TLR7 (**Figure 1**). TL7-887 can be used to generate immunostimulatory antibody-drug conjugates (ADCs) as conjugation to a Anti-TROP2-hIgG1 and subsequent activation of TLR7 has been validated using cellular assays. In a co-culture of TROP2<sup>+</sup> tumor cells (BxPC-3) and human peripheral blood monocytes (PBMCs), Anti-TROP2/TL7-887 induces a significantly higher production of IL-6 than unconjugated TL7-887 or a negative control ADC (**Figure 2**). Of note, in absence of tumor cells, PBMCs respond to higher doses of Anti- $\beta$ -gal/TL7-887 control ADC (**Figure 2B**), which could be explained by cellular uptake through endocytosis/pinocytosis.

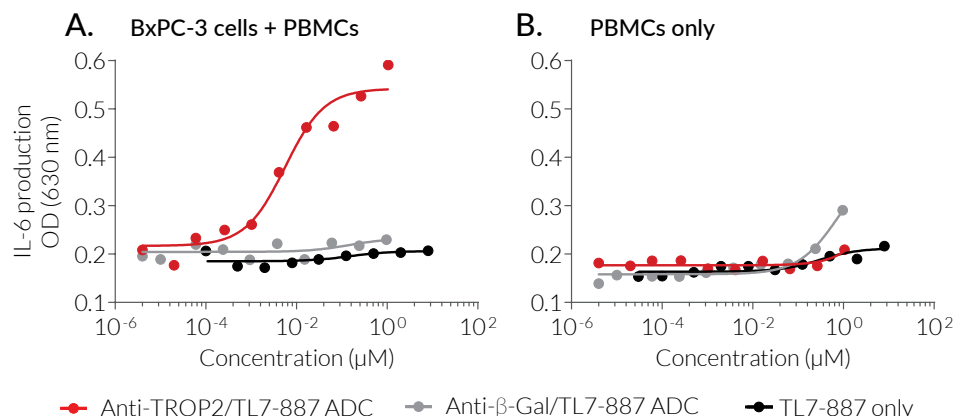
## Biological activity of TL7-887



**Figure 1:** Dose-response of human and murine TLR7 reporter cells to TLR7 conjugatable ligand TL7-887.

~  $4 \times 10^5$  HEK-Blue™ hTLR7 (A) or HEK-Blue-Lucia™ mTLR7 (B) cells were stimulated with increasing concentrations of TL7-887 (TLR7 agonist), or R848 (TLR7/8 agonist) as a control. Cells were incubated overnight in HEK-Blue™ Detection, a cell culture medium that allows real-time detection of SEAP activity in the supernatant. The optical density (OD) at 630 nm is shown as mean  $\pm$  SEM.

## Biological activity of Anti-TROP2/TL7-887 in co-cultures



**Figure 2:** Dose-response of human PBMCs co-cultured with BxPC-3 tumor cells and Anti-TROP2/TL7-887 ADC.

$1.5 \times 10^5$  human PBMCs and  $5 \times 10^4$  BxPC-3 tumor cells (A) or  $1.5 \times 10^5$  human PBMCs only (B) were incubated with increasing concentrations of Anti-TROP2/TL7-887 ADC (DAR ~6), Anti- $\beta$ -Gal/TL7-887 ADC (DAR ~6), or TL7-887 only. After overnight incubation, the TLR7-mediated response was determined using HEK-Blue™ IL-6 reporter cells. Briefly, the levels of IL-6 production in PBMC and BxPC-3 co-culture supernatants were assessed by measuring the SEAP activity of HEK-Blue™ IL-6 reporter cells, using QUANTI-Blue™, a SEAP detection reagent. The optical density (OD) at 630 nm is shown.

### TECHNICAL SUPPORT

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