

# Validation data for TL7-975

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

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Version 22B23-NJ

TL7-975 is a “click-chemistry compatible” conjugatable TLR7 ligand featuring an azido group added to the N-terminus of the base molecule CL307, a well-known TLR7 agonist. TL7-975 efficiently triggers a cellular response upon recognition by either human or murine TLR7 (Figure 1). TL7-975 can be used to generate immune-stimulating antibody conjugates (ISACs) as conjugation to a Anti-HER2-hIgG1 and subsequent activation of TLR7 has been validated using cellular assays (Figure 2). The Anti-HER2/TL7-975 ISAC is more potent at inducing a TLR7-mediated response than TL7-975 only in cells expressing HER2 (Figure 2A). Of note, at high concentrations, TL7-975, Anti-HER2/TL7-975 ISAC and Anti-βGal/TL7-975 ISAC, induce a TLR7-mediated cellular response, independently of HER2 expression (Figure 2A and B). This observation could be explained by cellular uptake through endocytosis/pinocytosis.

## Biological activity of TL7-975

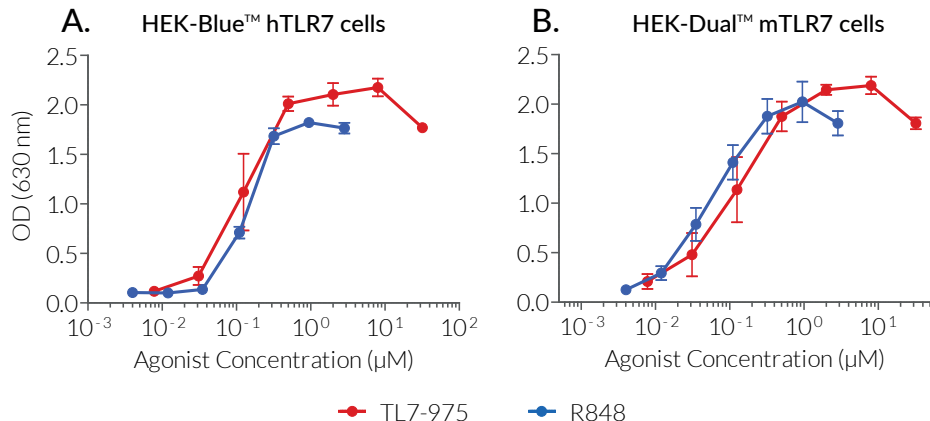


Figure 1: Dose-response of human and murine TLR7 reporter cells to TLR7 conjugatable ligand TL7-975.

~ 4x10<sup>5</sup> HEK-Blue™ hTLR7 (A) or HEK-Dual™ mTLR7 (B) cells were stimulated with increasing concentrations of TL7-975 (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 ± SEM.

## Biological activity of TL7-975 conjugated to Anti-HER2-hIgG1

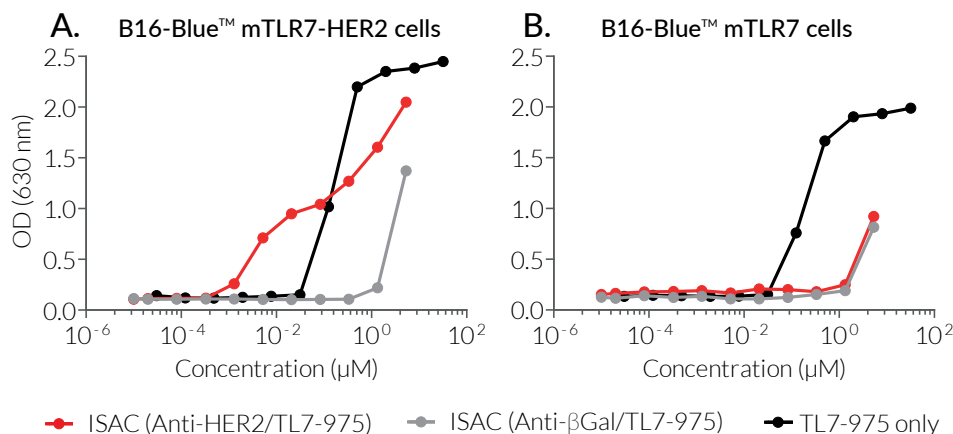


Figure 2: Dose-response of HER2-expressing and murine TLR7 reporter cells to Anti-HER2/TL7-887 ISAC.

~ 5x10<sup>5</sup> B16-Blue™ mTLR7-HER2 cells (A) or B16-Blue™ mTLR7 control cells (B) were stimulated with increasing concentrations of Anti-HER2/TL7-975 ISAC (Ratio 1:12), Anti-βGal/TL7-975 ISAC (Ratio 1:12), or TL7-975 only. After overnight incubation, the TLR7 response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent. The optical density (OD) at 630 nm is shown.

### TECHNICAL SUPPORT

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