

# TL7-887

Conjugatable TLR7 ligand (CL307) - VacciGrade™

Catalog Code: vac-tl7887, vac-tl7887-1

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

For research use only

Version 22E31-NJ

## PRODUCT INFORMATION

### Contents

TL7-887 is provided as a lyophilized powder and is available in two pack sizes:

- 250 µg: vac-tl7887
- 1 mg (4 x 250 µg): vac-tl7887-1

### Storage and stability

- TL7-887 is shipped at room temperature. Upon receipt, store at -20°C. Lyophilized product is stable for 6 months when properly stored.
- Upon resuspension, prepare aliquots and store at -20°C. Resuspended product is stable for at least 6 months when properly stored. Avoid repeated freeze-thaw cycles.

### Quality control

- Purity: ≥ 95% (UHPLC)
- TL7-887 is VacciGrade™ (preclinical grade). It is prepared under strict aseptic conditions and is guaranteed sterile. Absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ hTLR2 and HEK-Blue™ hTLR4 cells, and its endotoxin level is ≤5 EU/mg (measurement by kinetic chromogenic LAL assay).
- Biological activity has been confirmed using cellular assays.
- Conjugation to an Anti-HER2-hIgG1 mAb and subsequent activation of TLR7 has been validated using cellular assays.

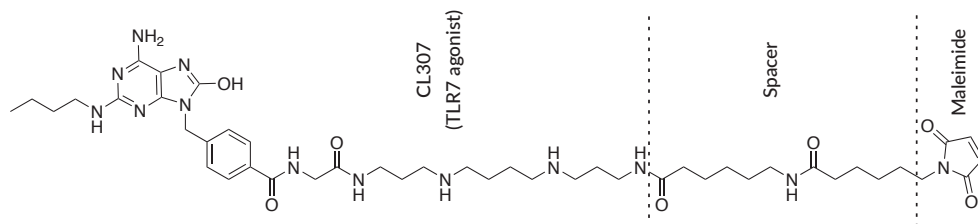
## CHEMICAL PROPERTIES

Formula: C<sub>45</sub>H<sub>69</sub>N<sub>13</sub>O<sub>7</sub>

Molecular weight: 904 g/mol

Mol. ext. coefficient  $\epsilon_{280\text{nm}}$ : 1140 M<sup>-1</sup>cm<sup>-1</sup>

Solubility: 10 mg/ml (11 mM) in H<sub>2</sub>O



## PRODUCT DESCRIPTION

TL7-887 is a conjugatable TLR7 ligand, synthesized from the base molecule CL307, a well-known specific TLR7 agonist. TL7-887 features a maleimide functional group that has been added to CL307 via a spacer. The bioconjugation to a protein of interest (POI) is driven by the maleimide reaction with free thiols on the POI, which generates a covalent thioether bond.

TL7-887 is a ready-to-use, "pre-linked" reagent, provided with one example of thioether conjugation protocol (see next page).

## APPLICATIONS

TL7-887 conjugatable TLR7 ligand has been designed to allow the generation of a bioconjugate after attachment to a biomolecule with a chemical linker. Examples of therapeutic bioconjugates include immune-stimulating antibody conjugates (ISACs) and antigen-adjuvant conjugates (AACs).

- **ISACs** allow localized TLR activation and antibody-mediated effector functions<sup>1,2</sup>.
- **AAC vaccines** allow the codelivery of antigen and TLR agonist to antigen presenting cells (APCs) and thus, better antigen processing and presentation for the induction of adaptive immune responses<sup>3</sup>.

1. Gadd A.J, *et al.* 2015. Targeted activation of toll-like receptors: conjugation of a toll-like receptor 7 agonist to a monoclonal antibody maintains antigen binding and specificity. *Bioconjug. Chem.* 26(8):1743. 2. Ackerman S.E, *et al.* 2021. Immune-stimulating antibody conjugates elicit robust myeloid activation and durable antitumor immunity. *Nat Cancer.* 2(1):18. 3. Xu Z. & Moyle P.M, 2018. Bioconjugation approaches to producing subunit vaccines composed of protein or peptide antigens and covalently attached Toll-Like receptor ligands. *Bioconjug. Chem.* 29(3):572.

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

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Below is a protocol for cysteine-based thioether coupling of TL7-887 to a protein of interest (POI).

Samples are harvested throughout the procedure for calculation of the ligand to POI ratio (DAR) using a spectrophotometer.

*Note:* for more information on conjugation methods and DAR calculation, visit our website at <https://www.invivogen.com/faq-conjugatable-ligands>.

*Note:* lysine-based thioether coupling protocols are available in the literature<sup>2</sup>.

## CYSTEINE-BASED THIOETHER COUPLING

This strategy is widely used to generate monoclonal antibody (mAb) conjugates. It aims at targeting the cysteine residues forming the four inter-chain disulfides of the IgG.

*Materials required (not supplied):*

- **Conjugation Buffer** (pH 7.5): 0.1 M Sodium Phosphate, 0.1 M NaCl, 1 mM EDTA

- **Reducing Agent:** 0.2 M TCEP (tris(2-carboxyethyl) phosphine), freshly prepared

- **Stopping Buffer** (pH 8.0): 0.1 M Disodium Phosphate ( $\text{Na}_2\text{HPO}_4$ ), 0.1 M NaCl, 50 mM Sodium Tetraborax ( $\text{Na}_2\text{B}_4\text{O}_7$ )

- **Final Buffer** (pH 6.5-7.5): 20 mM Sodium Phosphate, 0.15 M NaCl

- **OD Buffer:** Final Buffer + 0.2 M Cysteine

- **Desalting spin columns** 0.5 ml (refer to manufacturer's instructions)

- **PVDF Syringe filters** 0.22  $\mu\text{m}$ , 4 mm or 13 mm

### Preparation of the mAb of interest

1. Prepare a solution of antibody in **Conjugation Buffer** at ~5 mg/ml.

**IMPORTANT:** the mAb solution must contain no tris, cysteine, glycine, nor any other amine or thiol function.

2. **Sample 1:** Harvest and dilute 1  $\mu\text{l}$  of mAb solution into 9  $\mu\text{l}$  of **OD Buffer**. Store at 4°C until required.

3. Add **Reducing Agent** (1 mM final) to the mAb solution.

4. Vortex briefly and incubate for 90 to 120 min at 37°C using a plate or rotation shaker.

### Preparation of TL7-887 (6.25 mg/ml; 6.9 mM)

1. Add 40  $\mu\text{l}$  sterile water to 250  $\mu\text{g}$  TL7-887 vial.

2. Mix gently by pipetting until the product is completely dissolved.

3. Do a quick spin down to collect all the material from the tube wall.

4. **Sample 2:** Harvest and dilute 1  $\mu\text{l}$  of TL7-887 solution into 49  $\mu\text{l}$  of **OD Buffer**. Store at 4°C until required.

## Conjugation

Below is a protocol to obtain a TL7-887-ISAC with a DAR ~8 using a TL7-887 to **Anti-HER2-hlgG1** molar ratio of 12:1.

*Note:* this example has been used to validate the bioconjugate's potency at stimulating TLR7 and targeting HER2 using in-house cellular assays.

**IMPORTANT:** the user should assess different molar ratios to optimize the DAR depending on the immunoglobulin of interest and the following applications (in vitro or in vivo).

1. Add 12  $\mu\text{l}$  of TL7-887 to 200  $\mu\text{l}$  of TCEP-treated mAb solution.
2. Vortex briefly and incubate for 4 hours at 4°C using a plate or rotation shaker.
3. Desalt the conjugate against **Stopping Buffer** using a spin column.
4. Incubate at room temperature for ~30 min.
5. Desalt the conjugate against **Final Buffer** using a spin column.
6. **Sample 3:** Harvest and dilute 2  $\mu\text{l}$  of the ISAC and dilute into 8  $\mu\text{l}$  of the **OD Buffer**. Store at 4°C until required.
7. Filter sterilize the ISAC using a 0.22  $\mu\text{m}$  PVDF syringe filter.
8. Store ISAC at 4°C (short-term) or -20°C (long-term).

Below is a summary table of calculated volumes of TL7-887 depending on desired DAR with a standard 150 kDa mAb.

Theoretical DAR	TL7-887/mAb Molar Ratio	Volume of TL7-887 per mg of mAb
~ 8	12	12 $\mu\text{L}$
~ 5	9	9 $\mu\text{L}$
~ 4	6	6 $\mu\text{L}$
~ 2	4	4 $\mu\text{l}$

## RELATED PRODUCTS

Product	Description	Cat. Code
Anti-HER2-Tra-hlgG1	Monoclonal antibody	her2tra-mab1
CL307	TLR7 agonist	tlr1-c307
HEK-Blue™ hTLR7 cells	Human TLR7 reporter cells	hkb-htlr7
HEK-Blue™ Detection	Detection reagent	hb-dt2

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