# 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 23J12-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 and Anti-TROP2-hIgG1 mAbs and subsequent activation of TLR7 has been validated using cellular assays.

## 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 immunostimulatory antibody-drug conjugates (ADCs) and antigen-adjuvant conjugates (AACs).

- **ADCs** allow localized TLR activation and antibody-mediated effector functions [1,2].
- **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 adaptative immune responses[3].

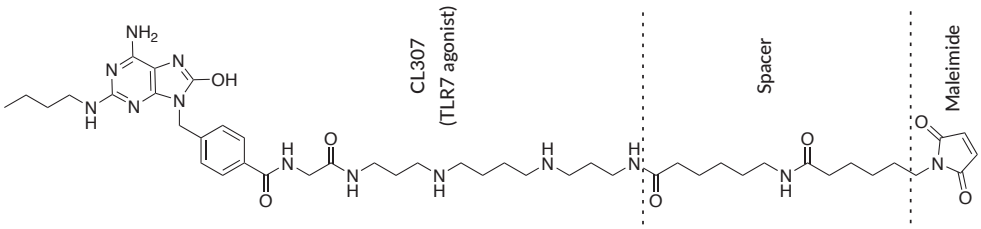
## CHEMICAL PROPERTIES

**Formula:** $C_{45}H_{69}N_{13}O_7$

**Molecular weight:** 904 g/mol

**Mol. ext. coefficient $\varepsilon_{280nm}$:** 1140 $M^{-1}$ $cm^{-1}$

**Solubility:** 10 mg/ml (11 mM) in $H_20$




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.

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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[2].*

## 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 ($Na_2HPO_4$), 0.1 M NaCl, 50 mM Sodium Tetraborax ($Na_2B_4O_7$)*
- ***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 µm, 4 mm or13 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 µl of mAb solution into 9 µl 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 µL sterile water to 250 µg 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 µl of TL7-887 solution into 49 µl of **OD Buffer**. Store at 4°C until required.

### Conjugation

Below is a protocol to obtain a TL7-887-ADC with a DAR ~8 using a TL7-887 to Anti-HER2-hIgG1 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 µl of TL7-887 to 200 µl 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 µl of the ADC and dilute into 8 µl of the **OD Buffer**. Store at 4°C until required.
7. Filter sterilize the ADC using a 0.22 µm PVDF syringe filter.
8. Store ADC 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 µL |
| ~ 5 | 9 | 9 µL |
| ~ 4 | 6 | 6 µL |
| ~ 2 | 4 | 4 µl |

## RELATED PRODUCTS

| Product | Description | Cat. Code |
|---|---|---|
| Anti-HER2-hIgG1 | Monoclonal antibody | her2-mab1-1 |
| Anti-TROP2-hIgG1 | Monoclonal antibody | trop2-mab1-1 |
| Anti-β-Gal-hIgG1* | Monoclonal antibody | bgal-mab1-1 |
| CL307 | TLR7 agonist | tlrl-c307 |
| HEK-Blue™ hTLR7 cells | Human TLR7 reporter cells | hkb-htlr7 |
| HEK-Blue™ Detection | Detection reagent | hb-dt2 |