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 24J24-NJ

PRODUCT INFORMATION

Contents

TL7-887 is available in two pack sizes:

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

Note: TL7-887 appears as a lyophilized pellet or a light-yellow droplet.

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].

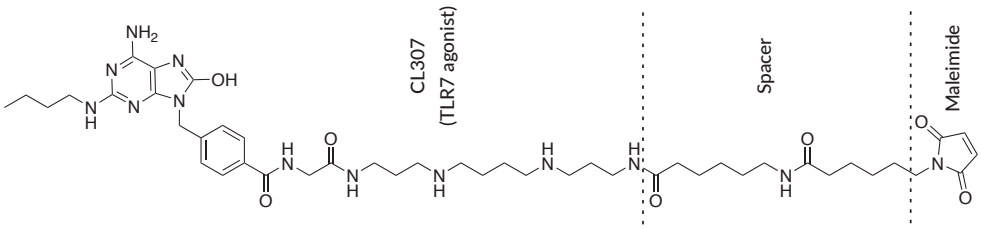
CHEMICAL PROPERTIES

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

Molecular weight: 904 g/mol

Mol. ext. coefficient ε_{280nm}: 1140 M^{-1} cm^{-1}

Solubility: 10 mg/ml (11 mM) in H_2O



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)

***Note:** Ensure you see the lyophilized pellet or light-yellow droplet before resuspension.*
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