**PRODUCT INFORMATION**

**Contents**
TL7-975 is provided as a lyophilized powder and is available in two pack sizes:
- 250 µg: vac-tl7975
- 1 mg (4 x 250 µg): vac-tl7975-1

**Storage and stability**
- TL7-975 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)
- TL7-975 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_{29}H_{45}N_{13}O_{3}

**Molecular weight (MW):** 624 g/mol

**Solubility:** 10 mg/ml (16 mM) in DMSO

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**PRODUCT DESCRIPTION**

TL7-975 is a conjugatable TLR7 ligand, synthesized from the base molecule CL307, a well-known specific TLR7 agonist. TL7-975 features an azido functional group that has been added to the N-terminus of CL307. It allows a flexible choice among commercially available linkers featuring a functional group for click-chemistry conjugation (e.g. alkyne, BCN, DBCO, or TCO). The bioconjugation to a protein of interest (POI) occurs in two steps. First, cysteines or lysines on the POI react with a functional group (e.g. maleimide or carboxylic acid) on the “commercial” linker. Second, the azido group of TL7-975 reacts with the “commercial” linker.

TL7-975 is a “click-chemistry compatible” reagent, provided with one example of thioether conjugation protocol using a DBCO-maleimide linker (see next page).

**APPLICATIONS**

TL7-975 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\(^1,2\).
- **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 adaptive immune responses\(^3\).

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Below is a protocol for cysteine-based thioether coupling of TL7-975 to a monoclonal antibody (mAb) using a DBCO-(Aca)₂-maleimide linker.

**Note:** We do not provide the DBCO-(Aca)₂-maleimide linker. DBCO-maleimide linkers with or without spacers are commercially available. Spacers such as (Aca) or (PEG) sequences minimize steric hindrance.

**CYSTEINE-BASED THIOETHER & AZIDE-CLICK COUPLING**

This strategy aims at generating immune-stimulating antibody conjugates (ISACs) by targeting the cysteine residues forming the four inter-chain disulfides of the IgG. This a two-step conjugation process. First, the mAb is conjugated to the maleimide function of the DBCO-(Aca)₂-maleimide linker (thioether reaction). Second, the azido group of TL7-975 is conjugated to the DBCO-(Aca)₂-maleimide linker (click-chemistry reaction): all the linker molecules that are coupled to the mAb readily and exclusively react with the TL7-975 molecules under mild conditions.

**Materials required (not supplied):**
- DBCO-maleimide linker
- 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
- Final Buffer (pH 6.5-7.5): 20 mM Sodium Phosphate, 0.15 M NaCl
- Desalting spin columns
- PVDF Syringe filters 0.22 µm, 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. Add Reducing Agent (1 mM final) to the mAb solution.
3. Vortex briefly and incubate for 90 to 120 min at 37°C using a plate or rotation shaker.

**Preparation of TL7-975 (5 mg/ml; 8 mM)**

1. Add 50 µL sterile DMSO to 250 µg TL7-975 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.

**Conjugation**

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

Here, we provide a protocol with a variable molar ratio of the DBCO-(Aca)₂-maleimide linker to Anti-HER2-hlgG1 mAb (step 1), and a molar excess of TL7-975 to antibody-linker complex (step 2). In step 2, the click reaction occurs for all available maleimide sites on the antibody. Therefore, it is the molar ratio of linker to antibody that determines the theoretical ratio of ligand to antibody.

**Preparation of the mAb of interest**

1. Add TL7-975 to each antibody-linker complex solutions at 1:12, 1:5, and 1:2 molar ratio.
2. Vortex briefly and incubate for 120 min at 4°C using a plate or rotation shaker.
3. Desalt each antibody-linker complex against Final Buffer using distinct spin columns.
4. Optional: Filter sterilize each antibody-linker complex using a 0.22 µm PVDF syringe filter.
5. Store antibody-linker complexes at 4°C (short-term) or -20°C (long-term).

**Step 1: Antibody and linker coupling (thioether reaction)**

1. In three distinct tubes, add DBCO-(Aca)₂-maleimide linker (~10 mg/ml or ~14 mM) to TCEP-treated Anti-HER2-hlgG1 solution (~5 mg/ml or ~34 µM) at 1:12, 1:5, and 2:1 molar ratio. **Note:** Calculated volumes for this specific example are indicated in the summary table below. MW of DBCO-(Aca)₂-maleimide is 709.4 g/mol. MW of Anti-HER2-hlgG1 is ~145 x 10^5 g/mol.
2. Vortex briefly and incubate for 120 min at 4°C using a plate or rotation shaker.
3. Filter sterilize each ISAC using distinct 0.22 µm PVDF syringe filters.
4. Store antibody-linker complexes at 4°C (short-term) or -20°C (long-term).

**Step 2: Antibody-linker complex coupling to TL7-975 (click reaction)**

**Optional:** Before you start step 2, measure the antibody-linker complex concentrations using a micro BCA protein assay.

1. Add TL7-975 to each antibody-linker complex solutions at 12:1 molar ratio (which matches the highest linker to antibody molar ratio).
2. Vortex briefly and incubate for 180 min at room temperature using a plate or rotation shaker.
3. Desalt each ISAC against Final Buffer using distinct spin columns.
4. Repeat Step 3.
5. Filter sterilize each ISAC using distinct 0.22 µm PVDF syringe filters.
6. Store ISACs at 4°C (short-term) or -20°C (long-term).

**Below is a summary table of volumes of DBCO-(Aca)₂-maleimide linker*, Anti-HER2-hlgG1, and TL7-975 depending on desired theoretical ratio of ligand to antibody.**

<table>
<thead>
<tr>
<th>Theoretical ratio of ligand to mAb</th>
<th>Linker/mAb Molar Ratio</th>
<th>Vol. of linker* per mg of mAb</th>
<th>Vol. of TL7-975 per mg of mAb</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>12</td>
<td>6 µL</td>
<td>~ 10 µL</td>
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<tr>
<td>5</td>
<td>5</td>
<td>2.5 µL</td>
<td>~ 10 µL</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>1 µL</td>
<td>~ 10 µL</td>
</tr>
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</table>

Calculation of the ligand to antibody ratio (DAR) using the spectrophotometer method is not optimal when using a DBCO-based linker because of its maximal absorbance at 305 nm. For information on DAR optimization and calculation, visit our website at [https://www.invivogen.com/faq-conjugatable-ligands](https://www.invivogen.com/faq-conjugatable-ligands).

**RELATED PRODUCTS**

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<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat. Code</th>
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<td>Monoclonal antibody</td>
<td>her2tra-mab1</td>
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<tr>
<td>CL307</td>
<td>TLR7 agonist</td>
<td>tlr7-c307</td>
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<tr>
<td>HEK-Blue™ hTLR7 cells</td>
<td>Human TLR7 reporter cells</td>
<td>hkb-htlr7</td>
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<td>HEK-Blue™ Detection</td>
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<td>hb-dt2</td>
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