

STG-982

Conjugatable STING ligand (CL845) - VacciGrade™

Catalog Code: vac-stg982, vac-stg982-1

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

For research use only

Version 23J12-NJ

PRODUCT INFORMATION

Contents

STG-982 is provided as a lyophilized powder and is available in two pack sizes:

- 250 µg: vac-stg982
- 1 mg (4 x 250 µg): vac-stg982-1

Storage and stability

- STG-982 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: ≥ 85% (UHPLC)
- STG-982 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 Anti-HER2-hlgG1 or Anti-TROP2-hlgG1 mAbs and subsequent activation of STING has been validated using cellular assays.

CHEMICAL PROPERTIES

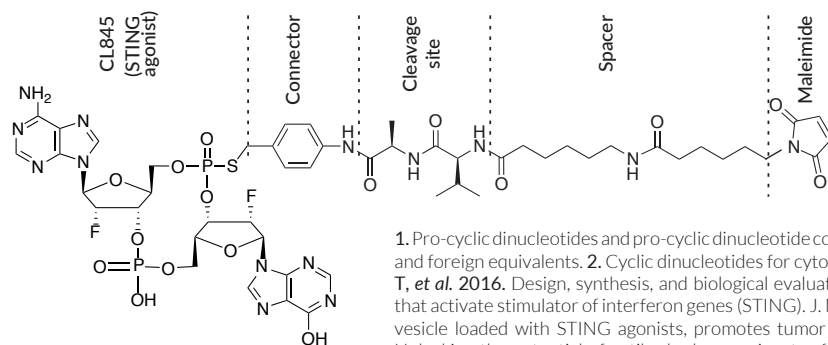
Formula: C₅₁H₆₃F₂N₁₄O₁₆P₂S • Na

Molecular weight (MW): 1283 g/mol

Mol. ext. coefficient $\epsilon_{280\text{nm}}$: 5853 M⁻¹cm⁻¹

Solubility: 10 mg/ml (7.8 mM) in DMSO

Chemical structure:



1. Pro-cyclic dinucleotides and pro-cyclic dinucleotide conjugates for cytokine induction, patent application WO 2019/129880A1 and foreign equivalents. 2. Cyclic dinucleotides for cytokine induction, patent US10011630B2 and foreign equivalents. 3. Liou T, et al. 2016. Design, synthesis, and biological evaluation of novel cyclic adenosine-inosine monophosphate (cAIMP) analogs that activate stimulator of interferon genes (STING). J. Med. Chem. 59:10253. 4. Jang S, et al. 2021. ExoSTING, an extracellular vesicle loaded with STING agonists, promotes tumor immune surveillance. Commun Biol. 4:497. 5. Drago J.Z, et al. 2021. Unlocking the potential of antibody-drug conjugates for cancer therapy. Nat Rev Clin Oncol. 18(6):327. 6. Poreba M, 2020. Protease-activated prodrugs strategies: challenges, and future directions. The FEBS Journal. 287(10):1936.

PRODUCT DESCRIPTION

STG-982 is a conjugatable STING ligand ¹. It is synthesized from the proprietary cyclic dinucleotide CL845, an analog of the clinical STING agonist cAIM(PS)₂ Difluor (Rp/Sp) (CL656)²⁻⁴.

STG-982 features a maleimide functional group that has been coupled to the phosphorothioate group via a “built-in” linker (see below). 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.

Importantly, STING signaling is triggered upon ligand binding to a small pocket that cannot accommodate large molecules. Therefore, once the bioconjugate has entered the target cell, CL845 must dissociate from the rest of the molecule.

To secure this step, STG-982 has been designed with a “cleavable linker”^{5, 6}, comprising a connector, a cleavage site, and a spacer. The spacer consists in a PEG (polyethylene glycol) sequence that minimizes steric hindrance. The cleavage site consists in a Val-Ala dipeptide, a substrate of the lysosomal protease cathepsin B⁶. A PAB (para-aminobenzoic) connector ensures accessibility of Val-Ala for the protease and subsequent release of the STING ligand—connector section⁶. Then, the PAB connector undergoes rapid and spontaneous self-immolative elimination, and the CL845 STING agonist is free⁶.

STG-982 ¹ is a ready-to-use, “pre-linked” reagent, provided with one example of thioether conjugation protocol (see next page).

APPLICATIONS

STG-982 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 STING activation and antibody-mediated effector functions.
- AAC vaccines allow the codelivery of antigen and STING agonist to antigen presenting cells (APCs) and thus, better antigen processing and presentation for the induction of adaptive immune responses.

TECHNICAL SUPPORT

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Below is a protocol for cysteine-based thioether coupling of STG-982 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 literature².

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 ($\text{Na}_2\text{B}_4\text{O}_7$)

- **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 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. **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 STG-982 (8.3 mg/ml; 6.5 mM)

1. Add 30 μl sterile DMSO to 250 μg STG-982.

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 STG-982 solution into 49 μl of **OD Buffer**. Store at 4°C until required.

Conjugation

Below is a protocol to obtain a STG-982-ADC with a DAR ~4 using a STG-982 to **Anti-HER2-hlgG1** molar ratio of 6:1.

Note: this example has been used to validate the bioconjugate's potency at stimulating STING 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 6 μl of STG-982 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 STG-982 depending on desired DAR with a standard 150 kDa mAb.

Theoretical DAR	STG-982 /mAb Molar Ratio	Volume of STG-982 per mg of mAb
~ 8	12	12 μL
~ 5 ~ 6	8	8 μL
~ 4	6	6 μL
~ 2	3	3 μL

RELATED PRODUCTS

Product	Description	Cat. Code
Anti-HER2-hlgG1	Monoclonal antibody	her2-mab1-1
Anti-TROP2-hlgG1	Monoclonal antibody	trop2-mab1-1
Anti- β -Gal-hlgG1*	Monoclonal antibody	bgal-mab1-1
2'3'-cGAMP	STING ligand	tlrl-nacga23-02
cAIM(PS) ₂ Difluor (Rp/Sp)	STING ligand	tlrl-nacairs-2
THP1-Dual™ Cells	Reporter cells	thpd-nfis
Quanti-Blue™ Solution	Detection reagent	rep-qbs
Quanti-Luc™	Detection reagent	rep-qlc-1

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