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 22I07-MM

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 an Anti-HER2-hIgG1 mAb and subsequent activation of STING has been validated using cellular assays.

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 accomodate 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 B[6]. A PAB (para-aminobenzoic) connector ensures accessibility of Val-Ala for the protease and subsequent release of the STING ligand—connector section[6]. Then, the PAB connector undergoes rapid and spontaneous self-immolative elimination, and the CL845 STING agonist is free[6].

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

CHEMICAL PROPERTIES

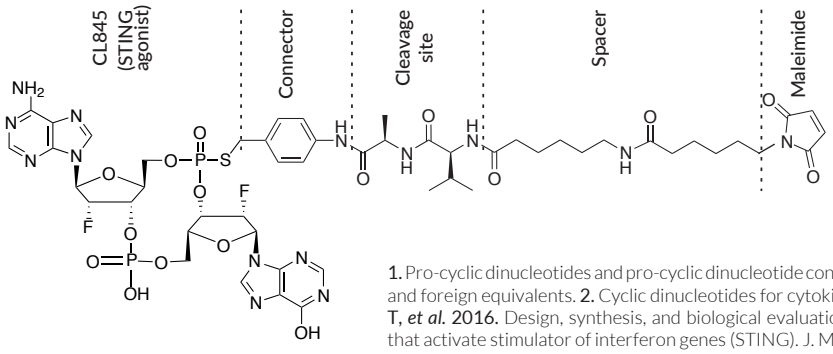
Formula: $C_{51}H_{63}F_2N_{14}O_{16}P_2S \bullet Na$

Molecular weight (MW): 1283 g/mol

Mol. ext. coefficient ε_{280nm}: 5853 $M^{-1} cm^{-1}$

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

Chemical structure:



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 immune-stimulating antibody conjugates (ISACs) and antigen-adjuvant conjugates (AACs).

- **ISACs** 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 adaptative immune responses.

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. Lioux 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.

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 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 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-ISAC with a DAR ~4 using a STG-982 to Anti-HER2-hIgG1 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 ISAC and dilute into 8 µl of the **OD Buffer**. Store at 4°C until required.
7. Filter sterilize the ISAC using a 0.22 µm 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 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-Tra-hIgG1	Monoclonal antibody	her2tra-mab1
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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