STG-968

Conjugatable STING ligand (CL845) - VacciGrade™

Catalog Code: vac-stg968, vac-stg968-1

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

For research use only

Version 23J12-NJ

PRODUCT INFORMATION

Contents

STG-968 is provided as a lyophilized powder and is available in two pack sizes:

- 250 µg: vac-stg968
- 1 mg (4 x 250 µg): vac-stg968-1

Storage and stability

- STG-968 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-968 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-hIgG1 or Anti-TROP2-hIgG1 mAbs and subsequent activation of STING has been validated using cellular assays.

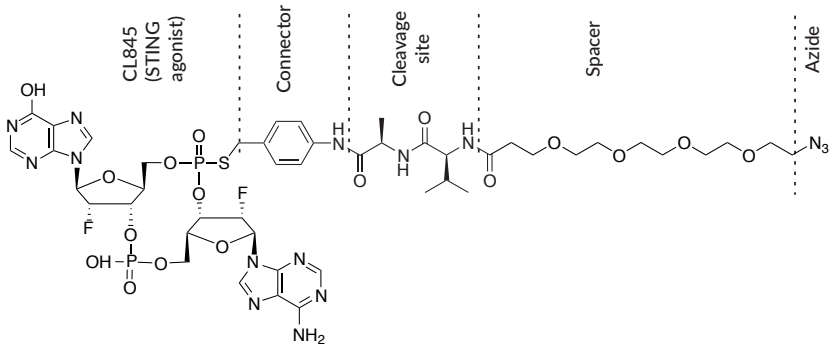
CHEMICAL PROPERTIES

Formula: $C_{46}H_{60}F_2N_{15}O_{17}P_2S \bullet Na$

Molecular weight: 1250 g/mol

Solubility: 10 mg/ml (8 mM) in H_2O

Chemical structure:



PRODUCT DESCRIPTION

STG-968 is a conjugatable STING ligand [1]. It is synthesized from the proprietary cyclic dinuleotide CL845, an analog of the clinical STING agonist $cAIM(PS)_2$ Difluor (Rp/Sp) (CL656)[2-4].

STG-968 features an azido functional group that has been coupled to the phosphorothioate group via a "built-in" linker (see below). 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 STG-968 reacts with the "commercial" linker.

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-968 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 lysososmal 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-968 is a "click-chemistry compatible" reagent, provided with one example of thioether conjugation protocol using a DBCO-maleimide linker (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 adaptative immune responses.

TECHNICAL SUPPORT

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Below is a protocol for cysteine-based thioether coupling of STG-968 to a monoclonal antibody (mAb) using a DBCO-$(Aca)_3$-maleimide linker.
Note: We do not provide the DBCO-$(Aca)_3$-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 monoclonal antibody conjugates 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-$(Aca)_3$-maleimide linker (thioether reaction). Then, the azido group of STG-968 is conjugated to the DBCO-$(Aca)_3$-maleimide linker (click-chemistry reaction): all the linker molecules that are coupled to the mAb readily and exclusively react with the STG-968 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*** *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. 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 STG-968 (10 mg/ml; 8 mM)

1. Add 25 µL sterile H_2O to 250 µg STG-968.
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-$(Aca)_3$-maleimide linker to Anti-HER2-hIgG1 mAb (step 1), and a molar excess of STG-968 to antibody-linker complex (step 2). In step 2, the click-chemistry reaction occurs for all available maleimide sites on the antibody. Therefore, it is the molar ratio of linker to antibody that determines the theoritical ratio of ligand to antibody.

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.

Step 1: Antibody and linker coupling (thioether reaction)
1. In three distinct tubes, add DBCO-$(Aca)_3$-maleimide linker (~10 mg/ml or ~14 mM) to TCEP-treated Anti-HER2-hIgG1 solution (~5 mg/ml or ~34 µM) at 12:1, 5:1, and 2:1 molar ratio.
Note: Calculated volumes for this specific example are indicated in the summary table below. MW of DBCO-$(Aca)_3$-maleimide is 709.4 g/mol. MW of Anti-HER2-hIgG1 is ~145 x 10^3 g/mol.
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 2: Antibody-linker complex coupling to STG-968 (click reaction)
Optional: Before you start step 2, measure the antibody-linker complex concentrations using a micro BCA protein assay.
1. Add STG-968 to each antibody-linker complex solutions at 12:1 molar ratio (which matches the highest linker to antibody molar ratio).
Note: This example has been used to validate the bioconjugate's potency at stimulating STING and targeting HER2 using in-house cellular assays. See STG-968 validation data sheet.
2. Vortex briefly and incubate for 180 min at room temperature using a plate or rotation shaker.
3. Desalt each ADC against **Final Buffer** using distinct spin columns.
4. Repeat Step 3.
5. Filter sterilize each ADC using distinct 0.22 µm PVDF syringe filters.
6. Store ADCs at 4°C (short-term) or -20°C (long-term).

Below is a summary table of volumes of DBCO-$(Aca)_3$-maleimide linker*, Anti-HER2-hIgG1, and STG-968 depending on desired theoritical ratio of ligand to antibody.

Theoretical ratio of ligand to mAb	Linker*/mAb Molar Ratio	Vol. of linker* per mg of mAb	Vol. of STG-968 per mg of mAb
12	12	6 µL	~ 10 µL
5	5	2.5 µL	~ 10 µL
2	2	1 µL	~ 10 µL

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.

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
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

TECHNICAL SUPPORT
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