

Product Information

CHS (Cholesteryl Hemisuccinate Tris Salt)

Catalog Numbers: CHS01, CHS02, CHS03

Description

CHS (cholesterol hemisuccinate Tris salt; Tris = tris(hydroxymethyl) aminomethane) is a steroid anionic surfactant additive. The molecule has a rigid cholesterol tetracyclic core as its hydrophobic backbone and is linked by succinic acid to a negatively charged polar head group. Tris acts as the counterion to achieve water solubility, mimicking the molecular conformation of natural cholesterol. CHS alone does not form micelles capable of solubilizing membrane proteins (it tends to form vesicle-like aggregates) and must be combined with a primary detergent such as LMNG. It is usually combined with main surfactants such as LMNG (Distolife catalog number: LMN01, LMN02, LMN03) for use, and is embedded inside the surfactant micelles to reconstruct the original membrane microenvironment. It can significantly enhance the thermal stability of eukaryotic membrane proteins such as G Protein-Coupled Receptors (GPCRs), inhibit protein conformational unfolding and aggregation, and retain the physiological activity of the protein.

Applications

- Extraction and purification of membrane proteins
- Protein crystallization
- Structural biology research, including cryo-EM sample preparation
- Membrane-mimicking additive for GPCR studies

Transportation and Storage

- Ship at ambient temperature.
- Store at low temperature, keep away from moisture
- Powder: -20 °C for 3 years
- In solvent: -80 °C for 1 year

Instructions for Use

Preparation of CHS Stock Solution (1% w/v)

Due to the poor water solubility of CHS, it needs to be stabilized by a primary detergent. It is recommended to prepare a low-concentration stable stock solution at 1% (10 mg/mL) for combined use with LMNG (Cat. No.: LMN01/LMN02/LMN03).

- 1) Weighing: Quickly weigh solid CHS under dry conditions to prevent moisture absorption from compromising purity.
- 2) Dissolution: Prioritize using a buffer containing 5%–10% LMNG stock solution to aid dissolution. Add deionized water or purification buffer, and perform gentle sonication or rotary shaking in a cold room until the solution becomes completely clear with no precipitate.
- 3) Filtration and aliquoting: Sterilize by filtration through a 0.22 µm membrane filter. Aliquot according to single-use dosage, and store sealed in the dark at -20 °C (stable for more than 6 months; avoid repeated freeze-thaw cycles).

Experimental Procedure

This section takes human neurotensin receptor 1 (hNTR1) and adenosine A2A receptor (A2AR) as examples to provide reference methods for membrane solubilization, extraction, and cobalt-ion affinity purification of GPCRs expressed by baculovirus using the LMNG-CHS system.

1. Materials

- 1) Detergents: Self-developed LMNG (Distolife, Cat: LMN01/LMN02/LMN03), self-developed CHS (Distolife, Cat: CHS01/CHS02/CHS03).
- 2) Auxiliary reagents: Tris-HCl, HEPES, NaCl, CaCl₂, MgCl₂, glycerol, imidazole, protease inhibitor cocktail.
- 3) Key consumables and equipment: 0.22 µm filter membrane, immobilized cobalt ion IMAC affinity chromatography resin, size exclusion SEC 250 analytical chromatography column, 4 12% SDS-PAGE gel, modular high-performance HPLC system, high-speed refrigerated centrifuge.

(Please contact us if you need detailed information.)

2. Preparation of Detergent Solution

1) 3 g of LMNG powder was dissolved in approximately 40 mL of 200 mM Tris-HCl (pH 8.0), adjusted to a final volume of 50 mL to obtain a 6% (w/v) stock solution.

2) CHS was added to a final concentration of 1.2%, followed by sonication until the solution became completely clear and colorless. The solution was brought to a final volume of 50 mL. After sterile filtration through a 0.22 μ m filter membrane, the solution was aliquoted and stored at -80 °C for subsequent use.

3. Expression of GPCR Membrane Proteins and Membrane Preparation

The Bac-to-Bac baculovirus expression system was used to construct recombinant baculoviruses encoding human GPCR family membrane proteins (including hNTR1 and A2AR). 50 mL ExpiSf9 insect cells were infected with the recombinant viruses for target protein expression. Cell pellets were collected, thawed on ice, and resuspended in low-salt buffer (20 mM HEPES pH 7.4, 100 mM NaCl, 5 mM CaCl₂, 5 mM MgCl₂, 10% glycerol, supplemented with protease inhibitor cocktail).

The mixture was centrifuged at 100,000 $\times$ g (e.g., 35,000 rpm in a Type 70 Ti rotor) for 30 min at 4 °C, and the cell membrane pellet was collected for downstream experiments.

4. Extraction and Purification of Membrane Proteins

1) Membrane resuspension and homogenization: The cell membrane pellet was resuspended in buffer (20 mM HEPES pH 7.4, 100 mM NaCl, 5 mM MgCl₂, 5 mM CaCl₂, supplemented with a protease inhibitor cocktail). The suspension was fully homogenized using a Dounce homogenizer and divided equally into two aliquots of 1 mL each.

2) Membrane protein solubilization: LMNG & CHS working solution was added to the sample. The mixture was incubated by rotation at 4 °C for 3 h to achieve solubilization of membrane proteins.

3) Clarification and affinity binding: The sample was centrifuged at 100,000 $\times$ g (e.g., 35,000 rpm in a Type 70 Ti rotor) for 30 min, and the supernatant was harvested. 60 μ L of immobilized cobalt ion IMAC affinity chromatography resin (1 CV = 30 μ L) together with a solution containing 20 mM imidazole was incubated with the supernatant overnight at 4 °C to allow binding of target proteins to the resin.

4) Washing and elution:

Washing step: Non-specifically bound impurities were removed by gradient washing with 20 column volumes (CV) of wash buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 30/50 mM imidazole, 0.05% LMNG, 0.01% CHS).

Elution step: GPCR target proteins were eluted using 5 CV of elution buffer (20 mM HEPES pH 7.4, 150 mM NaCl, 250 mM imidazole, 0.01% LMNG, 0.002% CHS).

5. Sample Analysis

1) HPLC-SEC analysis: 60 μ L protein sample was loaded onto a Sepax Nanofilm SEC-250 column. Chromatograms were recorded to evaluate protein monodispersity and aggregation status.

2) SDS-PAGE analysis: 30 μ L of eluted protein sample was mixed thoroughly with 10 μ L of 4 $\times$ protein loading buffer. A total of 30 μ L of the mixture was loaded onto a 4-12% gradient SDS-PAGE gel for electrophoresis to assess the purity of the target proteins.

Notice

1. To ensure the safety and health of operators, laboratory coats and disposable gloves must be worn throughout the entire experimental procedure.
2. Certain technical information provided with this product is intended for customer reference, academic communication, and scientific research purposes only. Our company does not warrant the absolute accuracy or completeness of the information provided.
3. This product is for research use only. It is strictly prohibited for use in clinical diagnosis, treatment, food, pharmaceuticals, or any other applications involving human use. Do not store this product in ordinary residential premises.
4. CHS solid is highly hygroscopic. Weighing should be performed quickly in a dry and well-ventilated environment. Seal the bottle cap immediately after weighing to prevent caking and deterioration caused by moisture absorption.
5. CHS does not form micelles in aqueous solution by itself. **DO NOT add CHS to buffers alone;** always co-use with glycosidic primary

detergents, including LMNG, to avoid protein precipitation.

6. The recommended ratio of CHS to main detergent is main **detergent: CHS = 10:1~5:1 (w/w)**. Excess CHS cannot be incorporated into micelles and will precipitate, leading to co-precipitation loss of membrane proteins. During chromatographic washing and elution steps, the concentrations of CHS and main detergent shall be reduced proportionally at the same time.
7. If obvious particles are observed, grind the material under dry conditions prior to dissolution to shorten the dissolution time.

Troubleshooting

Possible Problems and Solutions:

Symptoms	Solution
What are the storage and handling precautions for this product?	This product is highly hygroscopic. Store dry at -20 °C. When removing from low-temperature storage, allow the product to warm to room temperature before weighing. After weighing, flush with nitrogen and return to -20 °C storage. Avoid repeated temperature cycling to prevent caking.
What should I do if detergents (eg, CHS) remain incompletely dissolved after sonication in working buffer?	Use a small probe (probe #6) with 40% power, and sonicate for more than 30 min at room temperature. Do not place the sample on ice. Dissolution is complete when the solution changes from turbid to clear. Steroidal detergents such as CHS have low intrinsic solubility. Ice-bath conditions will further reduce solubility. Sonication with a small probe at 40% power for an extended period at room temperature is a validated, effective approach.