

Product Information

GDN (Glyco-Diosgenin)

Catalog Numbers: GDN01, GDN02, GDN03, GDN04

Description

GDN (glyco-diosgenin) is a synthetic non-ionic detergent composed of a rigid sterol-based (diosgenin) hydrophobic tail and a di-maltose hydrophilic head group. It is a synthetic drop-in substitute for Digitonin. Unlike natural Digitonin extracted from *Digitalis purpurea*, GDN is produced synthetically, free of toxic byproducts such as digitoxin and digoxin, with no batch-to-batch variation, a lower critical micelle concentration (CMC), and superior water solubility.

Applications

This product meets a variety of membrane protein research needs, including but not limited to:

- Solubilization, extraction, and purification of membrane proteins (direct substitute for Digitonin)
- Single-particle cryo-EM sample preparation (GPCRs, ion channels, transporters, etc.)
- Lipidic cubic phase (in meso / LCP) crystallization
- Maintenance of membrane protein solution stability and functional studies
In Vitro Assays: Used for in vitro bioluminescence detection and quantitative determination of luciferase expression

Transportation and Storage

- Store protected from light and moisture; recommended storage temperature: -20°C.
- Ship at ambient temperature..
- Stable as a powder; aliquot stock solutions and store at -80 °C. Avoid repeated freeze-thaw cycles.

Instructions for Use

GDN Stock Solution Preparation (10% w/v)

A high-concentration stock solution of 10% (100 mg/mL) is recommended for storage.

- 1) Weighing: Quickly weigh the LMNG solid under well-ventilated, dry conditions.
- 2) Dissolution: Add deionized water (or a purified buffer) and gently sonicate or rotate and shake in a cold room until the solution becomes completely clear.
- 3) Filtration and Aliquoting: Filter through a 0.22 µm membrane filter, aliquot as needed, and store at -80°C for future use. Avoid repeated freeze-thaw cycles.

Experimental Procedure

GDN Detergent System for GPCR Extraction (Using the A2A Receptor as an Example)

1. Materials

Detergents: GDN, LMNG, and CHS (Product Codes: GND, LMN, and CHS, respectively)

Additional reagents: Tris-HCl, HEPES, NaCl, MgCl₂, glycerol, imidazole, and protease inhibitors

Target protein (example) and expression system: A2A receptor (ExpiSf9 insect cells)

Key consumables and equipment: 0.22 µm membrane filter, TALON IMAC resin, and high-speed refrigerated centrifuge

2. Detergent Solution Preparation

Weigh 0.5 g of GDN powder and add it to 9 mL of 200 mM Tris-HCl (pH 8.14) to prepare a 5% (w/v) stock solution. Add 0.1 g of CHS to each tube to obtain a final concentration of 1% (w/v). Sonicate until the solution becomes clear and colorless, then bring the final volume to 10 mL.

Filter through a 0.22 µm membrane filter and store at -80°C until use.

3. GPCR Expression and Membrane Preparation

1. Construct recombinant baculovirus encoding the A2A receptor using the Bac-to-Bac system and infect 50 mL of ExpiSf9 cells.
2. Collect the cell pellet and allow it to thaw at room temperature. Resuspend the pellet in low-salt buffer containing 10 mM HEPES (pH 7.4), 10 mM MgCl₂, 20 mM KCl, and protease inhibitors. Centrifuge at 35,000 rpm for 30 min at 4°C and collect the membrane pellet.
3. Resuspend the membrane pellet in high-salt buffer (low-salt buffer supplemented with 1 M NaCl). Centrifuge again at 35,000 rpm for 30 min at 4°C and collect the membrane pellet.

4. Membrane Protein Extraction and Purification

4.1 Membrane Resuspension and Disruption

Resuspend the membrane pellet in buffer containing 20 mM HEPES (pH 7.4), 100 mM NaCl, 5 mM MgCl₂, 5 mM CaCl₂, and protease inhibitors. Homogenize thoroughly using a Dounce homogenizer, then divide the suspension into two tubes, each containing 1 mL.

4.2 Membrane Solubilization

Add the LMNG/GDN/CHS working solution and incubate with rotation at 4°C for 2.5 h to solubilize the membrane proteins.

4.3 Clarification and Binding

Centrifuge at 15,000 rpm for 30 min and collect the supernatant. Add 40 µL of TALON IMAC resin (1 CV = 20 µL) and imidazole-containing solution to a final concentration of 20 mM. Incubate overnight at 4°C to allow the target protein to bind to the resin.

4.4 Washing and Elution

Washing: Wash the resin using 20 CV of buffer containing 20 mM HEPES (pH 7.4), 150 mM NaCl, 30 or 40 mM imidazole, 0.05% LMNG, and 0.01% CHS. Apply a stepwise or gradient wash using the specified imidazole concentrations.

Elution: Elute the target protein using 10 CV of buffer containing 20 mM HEPES (pH 7.4), 150 mM NaCl, 125 mM imidazole, 0.01% LMNG, and 0.002% CHS.

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 authority 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. Maintain the Detergent Concentration Above the CMC: Ensure that the final GDN concentration in the system remains above its critical micelle concentration (CMC; approximately 0.0021% w/v, or 18 µM). During purification, washing, elution, and subsequent sample storage, a GDN concentration of 0.005%–0.02% (w/v) is recommended. If the concentration falls below the CMC, micelle dissociation may cause membrane protein denaturation, aggregation, or precipitation.
5. Be Aware of Detergent Enrichment During Concentration: GDN forms relatively large micelles, with an approximate micellar molecular weight of 100–150 kDa. When using 50 kDa or 100 kDa MWCO centrifugal concentrators, GDN may be concentrated along with the target protein. Therefore, the final GDN concentration in the elution and sample preparation buffers should be controlled within 0.005%–0.02% (w/v), with concentrations as low as 0.005% used when necessary. Avoid excessive concentration. Excessive detergent enrichment may interfere with cryo-EM sample preparation, resulting in high background from free micelles, poor particle orientation, and abnormal ice thickness, as well as adversely affecting crystallization experiments.
6. Low-Temperature Turbidity Is Normal Physical Precipitation: GDN solubility is characterized at room temperature, and lower temperatures may reduce its solubility. At 4°C or under ice-bath conditions, highly concentrated GDN stock solutions may develop slight turbidity or a small amount of precipitate. This is considered normal physical precipitation. Before use, allow the stock solution to return to room temperature or warm it in a 25–30°C water bath. Gently sonicate or mix until the solution becomes completely clear before diluting it into the experimental system.