

GlykoPrep H Cleanup Module

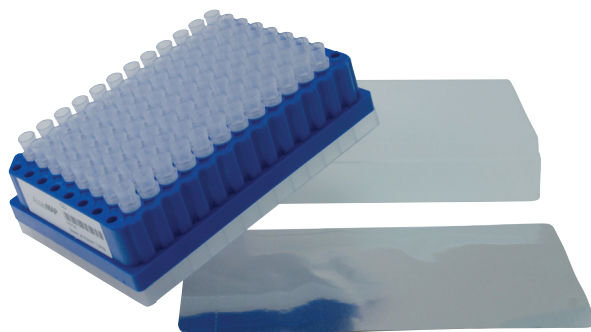
User Guide

Product Codes:

GS24-HC, 24 Samples

GS96-HC, 96 Samples

VERSION: AB



This product is intended for *in vitro* experimental research use only

NOTE: The following suggestions and data are based on information we believe to be reliable. They are offered in good faith, but without guarantee, as conditions and methods of use of our products are beyond our control. We recommend that the prospective user determine the suitability of our materials and suggestions before adopting them on a commercial scale. Suggestions for use of our products or the inclusion of descriptive material from patents and the citation of specific patents in this publication should not be understood as recommending the use of our products in violation of any patent or as permission to license to use any patents of ProZyme, Inc. See prozyme.com/patents.

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INTRODUCTION

GlykoPrep HC cartridges are solid-phase extraction cartridges, packed with ~5 mg of Hypercarb™ porous graphitic carbon (PGC).

When samples are loaded on the column, salts pass through, while glycans and protein adsorb onto the matrix. The glycans may then be selectively eluted from the column. Please note that some proteins or peptides with similar polarity (e.g. proteolytic degradation products) may also elute with the glycans, so the use of a GlykoPrep CU cartridge (product code GS24-CU or GS96-CU) is recommended prior to the H cartridge for samples to be analyzed by mass spectrometry.

HC Cartridges should be used only once.

Storage: Shipped ambient, store at room temperature in a dry environment upon arrival.

APPLICATION

GlykoPrep HC Cartridges are designed for purification of glycans from non-carbohydrate material, including salts and proteins. Applications include:

- Desalting of N-glycans released by native digestion with e.g. PNGase F
- Protein removal and desalting following exoglycosidase digestion of released glycans (e.g. sialidase digestion, fucosidase digestion prior to 2-AB labeling)
- Cleanup of glycans released by hydrazinolysis
- Desalting glycan samples including anionic species prior to mass spectrometry
- Cleanup of fluorescently-labeled glycans (e.g. glycans labeled with 2-AB, InstantAB™) from labeling reactions*

Note: *these cartridges are not suitable for removing detergent.*

**Not recommended for cleanup of InstantPC-labeled N-glycans, as these must be stored in Gly-X/InstantPC Eluent with 10% ACN. Samples applied to HC cartridges must be aqueous or low organic (<5%).*

MODULE COMPONENTS

GlykoPrep H Cleanup Module, GS24-HC & GS96-HC

The GlykoPrep H Cleanup Module consists of 96 GlykoPrep cartridges packed with ~5 mg of Hypercarb material.

Module	Component	Units	Storage
GlykoPrep H Cleanup Module GS24-HC	GlykoPrep HC Cartridges (24 count)	1	Room temp
	Aluminum Sealing Film	1	Room temp
GlykoPrep H Cleanup Module GS96-HC	GlykoPrep HC Cartridges (96 count)	1	Room temp
	Aluminum Sealing Film	1	Room temp

EQUIPMENT & REAGENTS PROVIDED BY THE USER

Reagents

- Water
- Acetonitrile
- Trifluoroacetic acid

Solvent A: [50% acetonitrile plus 0.1% trifluoroacetic acid (v/v) in water]
~100 µl per sample

Solvent B: [5% acetonitrile plus 0.1% trifluoroacetic acid (v/v) in water]
~350 µl per sample

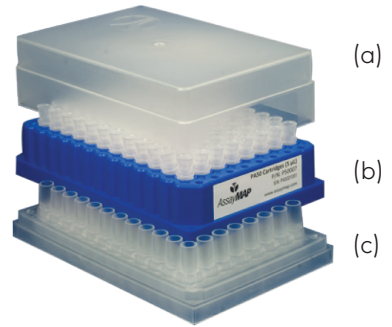
NOTE: Use only HPLC-grade reagents

Labware

The cartridges may be run in 1) plate format or 2) microtube format.

1. Plate Format

- WS0266 Lid (a)
- WS0264 Cartridge Rack (supplied with GS96-HC)(b)
- WS0265 Receiver Plate (c)
- WS0267 Collection Plate (or equivalent e.g., Thermo Fisher Scientific part number 07-202-502/Corning #3343)



NOTE: The above components are available individually and in the AM200 Starter Labware Set.

2. Microtube format

- AM400 AssayMAP Cartridge Adapter Set, including WS0337 Cartridge Adaptor A and WS0336 Cartridge Adaptor B.
- 0.5 ml screw-cap microtubes, Sarstedt part number 72.730.711 or equivalent
- 1.75 ml flip-top microtubes, E&K part number 290175 or equivalent

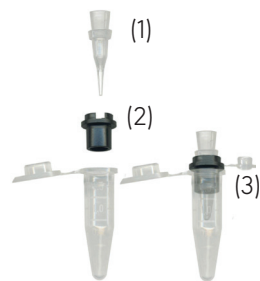
For equilibration & sample loading requiring a Receiver Plate, use the assembly below:

1. HC Cartridge
2. Cartridge Adaptor A
3. 0.5 ml screw-cap microtube



For washing and elution requiring a Collection plate, use the assembly below:

1. HC cartridge
2. Cartridge Adaptor B
3. 1.75 ml flip-top microtube



SAMPLE PREP CONSIDERATIONS

- Glycan samples - samples to be cleaned must be in an aqueous buffer or a buffer containing a low percentage (<5%) of organic solvent.
- If the sample contains organic solvent, dilute with water until the organic solvent content is less than 5% by volume. For example, dilute a 5 µl 2-AB labeling reaction with 95 µl water prior to loading.
- Samples should not contain detergents such as SDS.
- Suggested starting upper limit for the HC cartridges is ~50 µg glycans. The binding capacity for specific glycoproteins & glycans may need to be determined. Minimum glycan load will depend on the sensitivity of the analytical methods used.

PROTOCOL

The cartridge is first primed with solutions containing high, then low percentages of organic solvent. The sample is loaded onto the cartridge in an aqueous buffer or in low organic solvent. Non-carbohydrate contaminants (e.g. salts) with a low binding affinity for the cleanup matrix are eluted using a solvent with a low percentage of acetonitrile. Glycans are then selectively eluted using a solvent containing a higher percentage of acetonitrile.

1) Prime HC Cartridges

1. Add HC cartridges to a Cartridge Rack, and place over a Receiver Plate (or if using microtubes, see page 7)
2. Add 50 µl of Solvent A to each cartridge and spin at 300 x g for 3 min. Do not empty the Receiver Plate.
3. Add 150 µl of Solvent B to each cartridge and spin at 1000 x g for 3 min.

NOTE: *This prepares the surface of the resin for adsorption of the glycans. The protocol is designed to keep the cartridge tip wet during priming, sample load & wash steps. Solvent and samples will not pass through the cartridge if it dries out.*

2) Load Samples on HC Cartridges

4. Apply the 10 to 100 µl of sample to each cartridge, making sure that bubbles are not introduced into the sample cup. Spin at 50 x g for 10 min, or until sample cups are empty.

Sample volumes of >100 µl may be loaded, but will require multiple spins. When loading >100 µl, add up to 200 µl to the sample cup, spin for 10 minutes, apply additional sample, empty the Receiver Plate and spin again. The final spin should not exceed 100 µl in order to ensure complete sample loading.

NOTE: *Glycans should bind to the matrix while salts and non-hydrophobic, non-glycan contaminants should pass through.*

3) Wash

5. Add 200 μ l of water to each cartridge and empty the Receiver Plate. Spin at 300 x g for 3 min.

NOTE: *This washes residual salts and non-hydrophobic, non-glycan material off the column.*

4) Elute

6. Place each cartridge over a collection plate. Add 25 to 50 μ l of Solvent A to each cartridge. Spin at 300 x g for 3 min to elute glycans.

NOTE: *Glycans should be eluted into the collection plate, while hydrophobic material, such as certain peptides, and/or proteins, remain bound to the solid phase matrix.*

5) Sample Finishing

7. Evaporate to dryness using a centrifugal evaporator.
8. Redissolve in a desired volume of water or other suitable solvent for further analysis.
9. Store at -20°C in the dark.

NOTE: *The sample finishing step is to remove TFA for storage of glycans.*

GLYCAN ANALYSIS

Glycans purified on GlykoPrep HC cartridges may be studied by a variety of analytical techniques including mass spectrometry and liquid chromatography. Detailed, high sensitivity structural analyses of complex glycan mixtures may be performed by tagging the sugars with a fluorescent dye using a Glyko® Signal™ Labeling Kit, and separation of labeled glycans with techniques such as hydrophilic interaction liquid chromatography (HILIC).^{1,2}

Fluorescent Labeling of Released Glycans

Glycans with free reducing ends (e.g. those released from glycoproteins by hydrazinolysis or N-Glycanase) may be fluorescently labeled using one of the Labeling Kits available from ProZyme, including GKK-804 Signal 2-AB (2-aminobenzamide) Labeling Kit.

FAQS

Q: Do you have a protocol for cleanup of glycans released by hydrazinolysis using the HC cartridges?

A: No, but Hypercarb material has been used to clean up glycans released using our Glycan Hydrazinolysis Kit.³

Q: Is it possible to preferentially elute neutral N-glycans vs. sialylated N-glycans from the HC resin?

A: Developmental work at ProZyme indicates that neutral glycans can be eluted alone using an eluent of 25% acetonitrile. Then, sialylated glycans may be eluted with 25% acetonitrile with 0.1% TFA. Using this two-step elution, neutral and sialylated glycans may be fractionated. Please contact ProZyme for further details.

Q. Do you have a larger version of the HC cartridge?

A. The GlycoClean H Cartridge from ProZyme (product code GKI-4025) is a larger gravity flow cartridge packed with 200 mg of Hypercarb.

REFERENCES AND RESOURCES

1. Rudd PM, Colominas C, Royle L, Murphy N, Hart E, Merry AH, Hebestreit HF and Dwek RA. A high-performance liquid chromatography based strategy for rapid, sensitive sequencing of N-linked oligosaccharide modifications to proteins in sodium dodecyl sulphate polyacrylamide electrophoresis gel bands. *Proteomics* 2001 Feb;1(2):285-94.
2. Royle L, Campbell MP, Radcliffe CM, White DM, Harvey DJ, Abrahams JL, Kim YG, Henry GW, Shadick NA, Weinblatt ME, Lee DM, Rudd PM and Dwek RA. HPLC-based analysis of serum N-glycans on a 96-well plate platform with dedicated database software. *Anal Biochem.* 2008 May 1;376(1):1-12.
3. Turyan I, Hronowski S, Lyubarskaya Y. Comparison of two approaches for quantitative O-linked glycan analysis used in characterization of recombinant proteins. *Anal Biochem.* 2014 Feb 1;446:2836
4. TechNotes referred to in the text may be found on ProZyme's website at: <http://prozyme.com/pages/tech-notes>

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Safety and Handling

Please refer to the Safety Data Sheets (SDS) posted on ProZyme's website under the Product Code.

<http://www.prozyme.com/HCCartridge>

Technical Assistance

ProZyme is committed to developing rapid, automatable methods for glycan analysis. Call us to discuss products in development.

If you have any questions or experience difficulties regarding any aspect of our products, please contact us:

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Part # GS24/96-HC Rev AB