PROzyme®

NOTICE: ProZyme was purchased by Agilent in July 2018. Documents for products and product lots manufactured before August 2019 will contain references to ProZyme. For more information about these products and support, go to: **www.agilent.com/en/contact-us.**

Agilent

GLYCOCLEAN™ H CARTRIDGES

(For clean-up of glycan samples)

Product Code: GKI-4025

Pack Size: 10 cartridges
10 5-ml syringes (non-sterile)
10 adaptors

H Cartridges should be used only once.
Maximum sample size: 100 µg (glycan)

Storage: Shipped ambient for next day delivery. Store at room temperature in a dry environment upon arrival.

Application: Designed for purification of glycans from non-carbohydrate material, including salts, proteins and detergents. Applications include the cleanup of glycans following hydrazinolysis, N-Glycanase® (PNGase F) digests and other enzyme treatments.

These cartridges are especially useful for desalting both free oligosaccharides and fluorescently labeled glycans (including anionic species) prior to mass spectrometry.

In addition to GlycoClean H cartridges, ProZyme has a range of other products for cleaning up glycans in a variety of situations.

Additional Required Materials:

- Water
- Sodium hydroxide
- Acetonitrile
- Acetic acid
- Trifluoroacetic acid

NOTE: Use only HPLC-grade reagents.

INTRODUCTION

GlycoClean H cartridges are solid-phase extraction cartridges, packed with 200 mg of Hypercarb™, developed and manufactured by Thermo Hypersil.

When samples are loaded on the column, salts pass through, while glycans, protein and detergents 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 GlycoClean S cartridge (product code GKI-4726) is recommended prior to the H cartridge for samples to be analyzed by mass spectrometry.

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.

Reagents

- GlycoClean H Cartridges, one cartridge per sample
- 5-ml syringe and adaptor
- 1 *M* sodium hydroxide, ~3 ml per sample
- Water, ~12 ml per sample
- 30% acetic acid (v/v) in water ~3 ml per sample
- Solvent A: [50% acetonitrile plus 0.1% trifluoroacetic acid (v/v) in water] ~5 ml per sample
- Solvent B: [5% acetonitrile plus 0.1% trifluoroacetic acid (v/v) in water] ~9 ml per sample
- Glycan samples - samples to be cleaned must be in an aqueous buffer or a buffer containing a low percentage (<5%) of organic solvent.

NOTE: If the sample contains organic solvent, dilute with water until the organic solvent content is less than 5% by volume.

Procedure

NOTE: If gravity is insufficient to drain the cartridges, use the adaptor and the syringe provided to push the solutions through. When changing the wash solutions, avoid pulling fluid or air back through the cartridge (i.e. *break the connection between the syringe and cartridge before withdrawing the syringe plunger).*

1. Prepare GlycoClean H Cartridges:
    i. wash with 3 ml of 1 *M* sodium hydroxide
    ii. wash with 6 ml of water
    iii. wash with 3 ml of 30% acetic acid
    iv. wash with 3 ml of water

NOTE: This removes any impurities that may have adsorbed onto the resin matrix.

NOTE: If flow is restricted, e.g. *by an air gap, apply a slight pressure to the top of the cartridge in order to resume normal flow.*

2. Prime each cartridge with 3 ml of Solvent A followed by 6 ml of Solvent B.

NOTE: This prepares the surface of the resin for adsorption of the glycans.

3. Apply the sample to the cartridge.

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

4. Wash the cartridge with 3 ml water, followed by 3 ml of Solvent B.

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

5. Place each cartridge over a collection vessel and collect the glycans by eluting with 4 x 0.5 ml of Solvent A. Allow each aliquot to drain before the next is applied.

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

Sample Finishing

1. Filter the sample (at least 0.5 µm) and evaporate to dryness using a centrifugal evaporator.
2. Redissolve in a desired volume of water or other suitable solvent for further analysis.
3. Store at -20°C in the dark.

GLYCAN ANALYSIS

Glycans purified on GlycoClean H cartridges may be studied by a variety of analytical techniques including mass spectrometry and 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 followed by analysis on high pressure liquid chromatography (HPLC) with a GlycoSep™ HPLC column[2,3].

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 range of Signal Labeling Kits available from ProZyme: GKK-402 Signal 2-AA (2-aminobenzoic acid) Labeling Kit and GKK-404 Signal 2-AB (2-aminobenzamide) Labeling Kit.

HPLC Analysis

Glycan mixtures labeled with 2-AA or 2-AB may be separated and analyzed by high pressure liquid chromatography (HPLC) with GlycoSep HPLC columns:

Code	Column	Analyses
GKI-4721	GlycoSep C	Separation of neutral/charged glycans
GKI-4728	GlycoSep N	Profile analysis of neutral/charged glycans
GKI-4727	GlycoSep R	Separation of neutral glycans

GlycoSep N is the most versatile column of the three GlycoSep columns and is routinely used to purify and analyze fluorescently labeled oligosaccharides from complex glycan mixtures.[2]

Enzymes for Glycan Analysis

ProZyme's Glyko® range of high purity, sequencing-grade enzymes is suitable for structural analysis of both N- and O-linked glycans. Please contact ProZyme's Technical Support for information on the use of these enzymes for glycan analysis.

REFERENCES

1 Bigge, J. C., Patel, T. P., Bruce, J. A., Goulding, P. N., Charles, S.M. and R. B. Parekh. Non-selective and efficient fluorescent labeling of glycans using 2-amino-benzamide and anthranilic acid. **Anal Biochem 230**: 229-238 (1995).

2 Guile, G. R., Rudd, P. M., Wing, D. R., Prime, S. B. and R. A. Dwek. A rapid and high-resolution high-performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles. **Anal Biochem 240**: 210-226 (1996).

3 Townsend, R. R., Lipniunas, P. H., Bigge, C., Ventom, A. and R. Parekh. Multimode high-performance liquid chromatography of fluorescently labeled oligosaccharides from glycoproteins. **Anal Biochem 239**: 200-207 (1996).

4 Hardy, M. R. Glycan labeling with the fluorophores 2-aminobenzamide and anthranilic acid in **Techniques in Glycobiology** (Townsend, R. R and Hotchkiss, A. T. Marcel, eds) Dekker Inc, New York (1997).

TECHNICAL NOTES

TechNotes referred to in the text may be found on ProZyme's website at:

http://www.prozyme.com/notes/tngk