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

Glyko® GLYCOCLEAN™ R CARTRIDGES

(For clean-up of glycan samples)

Product Code: GKI-4756

Pack Size: 12 cartridges
12 5-ml syringes
(polypropylene, lubricant-free)

Maximum sample size: up to ~9 mg protein

NOTE: This amount depends on the sample formulation - the presence of co-retained compounds such as detergents will reduce the protein-binding capacity.

GlycoClean R Cartridges and syringes should be used only once.

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

Application: primarily used for the clean-up of glycans released from glycoproteins by digestion with N-Glycanase® (PNGase F) or other endoglycosidases. Also used for re-purification of free glycans after additional exoglycosidase treatment(s).

Optional Application: Proteins and/or peptides may be purified for further analysis.

Additional Required Reagents:
NOTE: use only HPLC-grade reagents

Water
Methanol
Acetic acid

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

INTRODUCTION

The GlycoClean R Cartridge has been designed for purification of glycans with free reducing ends (or glycans in the alditol form) from hydrophobic, non-carbohydrate material, such as detergents, proteins and peptides. It is a solid-phase extraction cartridge containing a reverse-phase matrix. Free glycans pass through the matrix while proteins, peptides and detergents are retained. The retained proteins and/or peptides may be recovered by elution with aqueous buffer containing an organic solvent for mass spectrometry or other analysis.

Clean-up After N-Glycanase Digestion

N-Glycanase digestion reactions may contain salt, detergents, protein (including the enzyme) and released glycans in the reducing form (*i.e.* with nothing attached to the reducing terminus GlcNAc). The standard GlycoClean R protocol given here will remove most of the protein, peptides and detergent. The glycans and salt are eluted together.

Further treatment of the glycan fraction depends on the type of analysis required and the nature of the digest buffer. Volatile salts may be removed by lyophilization, centrifugal evaporation or rotary evaporation.

NOTE: N-Glycanase digests contain complex mixtures of salts; this is especially true when using the Denaturing Protocol. The most robust glycan purification method first uses a GlycoClean R Cartridge to remove hydrophobic material, followed by a GlycoClean H Cartridge to desalt the glycan.

PROTOCOL

The cartridge is first primed with methanol and then equilibrated in 5% acetic acid. The sample is loaded onto the cartridge in an aqueous buffer. Carbohydrates and non-hydrophobic compounds (*e.g.* most salts) with a low affinity for the matrix are eluted using an aqueous wash. If desired, adsorbed hydrophobic components (such as proteins and/or peptides) may be eluted from the cartridge using an aqueous solvent containing an appropriate percentage of methanol.

Reagents

GlycoClean R Cartridges (one per sample)

Syringes (one per sample, supplied with the cartridge)

Glycan samples - must be in an aqueous buffer or a buffer containing a low percentage (<5%) of organic solvent.

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

Methanol, ~2 ml per sample (more for protein/peptide recovery)

Solvent A [5% (v/v) acetic acid in water], ~9 ml per sample

(Optional) For Protein/Peptide Recovery

Solvent B [10% (v/v) methanol in Solvent A], ~3 ml per sample

NOTE: Also have on hand a stock solution of Solvent A to mix with methanol in different proportions.

Procedure

NOTE: During priming and subsequent steps do not allow the cartridge bed to run dry.

Prime each GlycoClean R Cartridge:

- wash with 2 ml methanol
- follow with 6 ml Solvent A

*NOTE: Use the syringe 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).*

Pass the sample through the cartridge and save the eluate. Hydrophobic contaminants such as proteins, peptides and detergents should stick to the matrix while free glycans and salts should pass through.

Maximize the glycan recovery by washing the cartridge with 3 x 1 ml of Solvent A and pooling the washes with the eluate.

(Optional) For Protein/Peptide Recovery

To elute hydrophobic components, wash with 3 ml of Solvent B, followed by additional wash solutions containing progressively increasing amounts of methanol in Solvent A [15 - 100% methanol (v/v)]

NOTE: The final methanol concentration to use depends on the hydrophobicity of the components bound to the cartridge matrix; the more hydrophobic the component, the higher the methanol concentration must be to elute it.

Sample Finishing

Filter the glycan-containing sample (pore size ≤0.5 µm). If appropriate, evaporate to dryness and redissolve in water or solvent for further analysis.

(Optional) Filter the protein/peptide-containing sample (pore size ≤0.5 µm) and concentrate before further analysis.

Store samples at -20°C in the dark.

GLYCAN ANALYSIS

Glycans purified on GlycoClean R 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 Signal™ Labeling Kit followed by analysis on high pressure liquid chromatography (HPLC) with a GlycoSep™ HPLC column[2-4].

Fluorescent Labeling of Released Glycans

Glycans with free reducing ends (such as those released from glycoproteins by N-Glycanase or other endoglycosidases, or by hydrazinolysis) 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® line of high purity, sequencing-grade enzymes is suitable for structural analysis of both N- and O-linked glycans.

REFERENCES

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2 Guile GR, Rudd PM, Wing DR, Prime SB and Dwek RA. A rapid and high-resolution high-performance liquid chromatographic method for separating glycan mixtures and analyzing oligosaccharide profiles**.** Anal Biochem 1996 Sep 5;240(2):210-226.

3 Townsend RR, Lipniunas PH, Bigge C, Ventom A and Parekh R. Multimode high-performance liquid chromatography of fluorescently labeled oligosaccharides from glycoproteins. Anal Biochem 1996 Aug 1;239(2):200-207.

4 Hardy MR. Glycan labeling with the fluorophores 2-aminobenzamide and anthranilic acid. In: Townsend RR, Hotchkiss AT, editors. Techniques in Glycobiology, New York: Marcel Dekker Inc., 1997. p. 359-376

PROzyme®

3832 Bay Center Place
Hayward, CA 94545-3619

TOLL FREE (800) 457-9444
PHONE (510) 638-6900
FAX (510) 638-6919

E-MAIL info@prozyme.com
WEB www.prozyme.com