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

GLYKOCLEAN™ G CARTRIDGES

(For clean-up of glycan samples)

Product Code: GC250,
GC250-6

Pack Size: 25 cartridges,
5 cartridges

G Cartridges should be used only once. Maximum sample size for GC250 is 2.5 mg and for GC250-6 is 25 mg.

Storage: Shipped ambient for next day delivery. Store at room temperature.

NOTE: The sealed package controls cartridge moisture to facilitate equilibration. Store ambient after opening.

Application: Purification of small amounts of glycan samples after a variety of procedures, including:

- reductive amination (Signal™ fluorescent labeling) with 2-AB (2-aminobenzamide) and 2-AA (2-aminobenzoic acid)
- enzyme digestions

Additional Required Reagents:

Water, HPLC grade
Acetonitrile, HPLC grade

INTRODUCTION

G Cartridges are used in conjunction with the GlykoClean Sample Processing Station (ProZyme product code GC100) for the rapid clean-up of glycan samples for analysis by HPLC or other methods.

G Cartridges contain a proprietary matrix that retains a wide range of glycans in >90% acetonitrile solutions. Most hydrophobic non-glycan contaminants either pass through the cartridge or are retained weakly and may be washed off. The glycans are then eluted from the cartridge with water.

The cartridge is first primed with water and then with a 96% solution of acetonitrile. Then a sample (premixed with acetonitrile solution) is loaded. The glycans adsorb while excess dye is removed by washing with acetonitrile solution. The glycans are then desorbed and eluted by washing with water. Clean-up steps are vacuum-driven and performed with the help of the GlykoClean Sample Processing Station.

NOTE: G Cartridges cannot be equilibrated, washed nor eluted by gravity.

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

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

GLYCAN CLEANUP PROTOCOL

Reagents

Water

96% Acetonitrile Solution [96% acetonitrile (v/v), 4% water (v/v)], from 3 - 4.5 ml per sample.

GlykoClean G Cartridges, one cartridge per sample.NOTE: A higher percentage of water in the acetonitrile solution will cause glycans (especially smaller molecular weight glycans) to elute from the cartridge prematurely.

NOTE: Use only HPLC-grade reagents.

Procedure

Prepare the GlykoClean Sample Processing Station (refer to the instructions provided with the station).

Prepare GlykoClean G Cartridges

1. Add 1 ml of water to each cartridge.

2. Turn on the vacuum and open the valve fully to drain the cartridges.

NOTE: Water will drain slowly (<2 minutes) from the cartridges. Some differences will be observed between individual cartridges.

3. Close the valve when all of the cartridges have completely drained. Release the residual vacuum.

4. Add 1 ml of 96% Acetonitrile Solution to each cartridge.

5. Open the valve fully to drain the cartridges.

NOTE: Acetonitrile Solution will drain faster and more uniformly than water.

The cartridge is now equilibrated and ready for glycan sample clean-up.

Process the Samples

1. Dilute each sample (typically 5 to 10 µl of 2-AB/2-AA labeling reaction) with 200 µl of 96% Acetonitrile Solution. Mix by pipetting three times up and down. Transfer the entire sample to a cartridge.

2. Apply vacuum sparingly by partially opening the valve and slowly drain the cartridges.

3. Wash each cartridge three times with 0.75 ml of 96% Acetonitrile Solution. Apply vacuum sparingly by partially opening the valve to slowly drain the cartridge after each wash.

NOTE: A wash volume of 0.75 ml is suggested for the clean-up of most samples. Washes with as little as 0.5 ml of 96% Acetonitrile Solution each may result in acceptable clean-up while minimizing solvent use. If necessary, washes with up to 1 ml of 96% Acetonitrile Solution each may be used without affecting glycan recovery.

Glycans are now ready for elution from the cartridge.

Elute the Glycan Samples

Elute each sample with 0.5 ml of water (refer to instructions provided with the Station for sample elution procedures).

Finishing the Samples

Samples may be filtered (if appropriate), evaporated to dryness using a centrifugal evaporator and redissolved in a desired volume of water or other suitable solvent for further analysis.

Store the samples at -20°C in the dark.

LABELED GLYCAN ANALYSIS

Glycan mixtures labeled with 2-AB may be studied by a number of analytical methods including HPLC and mass spectrometry.

HPLC Analysis

Glycan mixtures labeled with 2-AB may be separated and analyzed by HPLC with GlycoSep™ HPLC columns (see table). GlycoSep N is the most versatile column of the three GlycoSep columns and is routinely used to purify and analyze 2-AB-labeled oligosaccharides from complex glycan mixtures.[2]

Code	Column	Analyses
GKI-4721	GlycoSep C	Separation of neutral/charged glycans
GKI-4728	GlycoSep N	Profile analysis of neutral/charged glycans
GKI-4730	GlycoSep N Plus	Profile analysis of neutral/charged glycans with higher resolution
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 2-AB-labeled oligosaccharides from complex glycan mixtures.[2]

Enzymatic Analysis

ProZyme's Glyko® range of high purity, sequencing-grade enzymes is suitable for structural analysis of both N- and O-linked glycans labeled with 2-AB.

Mass Spectrometry

Mass spectrometry may also be used to analyze glycans labeled with 2-AB. The 2-AB label is stable under extremes of acidic and alkaline conditions and does not interfere with the action of exoglycosidases.[1-4] Note, however, that glycan structures may not be stable under extremes of pH. For this reason, users are advised not to subject 2-AB-labeled glycans to strongly acidic or alkaline conditions.

TECHNICAL ASSISTANCE

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

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

REFERENCES

1 Bigge JC, Patel T, Bruce JA, Goulding PN, Charles SM, Parekh RB. Nonselective and efficient fluorescent labeling of glycans using 2-amino benzamide and anthranilic acid. Anal Biochem 1995 Sep 20;230(2):229-238.

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