PROzyme®

GLYKO®

GLYCOCLEAN™ S CARTRIDGES

(For clean-up of glycan samples)

Product Code: GKI-4726

Pack Size: 12 cartridges

S Cartridges should be used only once. Maximum sample size is 10 µl and/or 20 µg.

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

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

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

Additional Required Reagents:
Water, HPLC grade
Acetonitrile, HPLC grade
Acetic acid (glacial), HPLC grade

INTRODUCTION

S cartridges contain a membrane that retains a wide range of glycans in >90% acetonitrile solutions; monosaccharides and disaccharides generally interact with the membrane too weakly for efficient retention. Most hydrophobic non-glycan contaminants either pass through the membrane or are retained weakly and may be washed off. The glycans are then eluted from the membrane with water.

The cartridge is first primed with acetonitrile and then a sample is loaded. The glycans adsorb while excess dye is removed by washing with acetonitrile. The glycans are then desorbed by washing with water.

In addition to GlycoClean S cartridges, ProZyme has a range of other products for cleaning up glycans in a variety of situations (see TechNote TNGK100 *Glycan Cleanup Strategies*).

GLYCAN CLEANUP PROTOCOL

Reagents

GlycoClean S Cartridges, one cartridge per sample

NOTE: use only HPLC-grade reagents

Water, ~3 ml per sample

Acetic Acid Solution [30% acetic acid, 70% water (v/v)], ~5 ml per sample

Acetonitrile, ~5 ml per sample

96% Acetonitrile Solution [96% acetonitrile, 4% water (v/v)], ~5 ml per sample

NOTE: A higher percentage of water in the acetonitrile solution will cause glycans (especially small molecular mass sugars) to elute from the cartridge prematurely.

Procedure

Prepare GlycoClean S Cartridges:
- wash each cartridge with 1 ml water
- wash with 5 ml Acetic Acid Solution (allow to drain completely)
- wash with 3 ml acetonitrile (allow to drain completely)
- finally, wash with an additional 1 ml acetonitrile and allow to drain completely

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

Before sample application, make sure the samples are at or below room temperature.

Spot each sample onto a freshly washed cartridge membrane, spreading the sample over the entire membrane surface (be sure that the membrane is still wet with acetonitrile).

NOTE: If the membrane has dried, it must be re-wetted by washing with 0.5 ml acetonitrile prior to loading the sample.

Leave for 15 minutes to allow the glycans to adsorb onto the membrane.

Optional: for maximum recovery, rinse each sample vial with 100 µl of acetonitrile, apply to the corresponding cartridge membrane and allow time for penetration into the membrane.

Wash each cartridge with 1 ml of acetonitrile, followed by 5 x 1 ml of 96% Acetonitrile Solution, allowing each aliquot to drain before the next is applied. Discard these into a suitable waste container.

Place each cartridge over a collection vessel suitable for drying 1.5 ml water or, if filtration is required, place the cartridge over a 5 ml syringe fitted with a PTFE filter (0.45µ).

Elute the glycans with 3 washes of 0.5 ml water, allowing each wash to drain before the next is applied.

Sample Finishing

Filter the sample (if appropriate) and evaporate to dryness using a centrifugal evaporator.

Redissolve in a desired volume of water or other suitable solvent for further analysis.

Store the remaining sample 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:

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 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. See TechNote TNGK200 *Glyko Enzyme Guide* for the use of these enzymes for glycan analysis.

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.

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-aminobenzamide 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

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