GLYOCLEAN™ 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:

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<th>Code</th>
<th>Column</th>
<th>Analyses</th>
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<tr>
<td>GKI-4721</td>
<td>GlycoSep C</td>
<td>Separation of neutral/charged glycans</td>
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<tr>
<td>GKI-4728</td>
<td>GlycoSep N</td>
<td>Profile analysis of neutral/charged glycans</td>
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<tr>
<td>GKI-4727</td>
<td>GlycoSep R</td>
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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.

**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. 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


TECHNICAL NOTES

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

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