

S CARTRIDGES (For clean-up of glycan samples)

Product Code:

KNBS-9726

Pack Size:

12 cartridges

Storage:

Store at Room Temperature. Avoid moisture and place in a dry environment.

Introduction:

S cartridges comprise a membrane layer which retains a wide range of glycans in >90% acetonitrile solutions. Monosaccharides and Disaccharides 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.

Application:

S Cartridges are for single use only. Maximum sample size recommended is 10 µl and/or 20 µg. Purification of small amounts of glycan samples after a variety of procedures, include:

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

GLYCAN CLEANUP PROTOCOL

Reagents:

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.

Procedure1. Prepare **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** and allow to drain completely
 - Do a **Final 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.*

2. Before **Sample** application, make sure the samples are at or below room temperature.

3. Pipette 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.

4. 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.

5. Wash each **Cartridge** with **1 ml Acetonitrile**, followed by **5 x 1 ml 96% Acetonitrile Solution**, allowing each dispensed solution to drain before the next is applied. Discard the eluate into a suitable waste container.

6. 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 (0.45 µ)**.
7. **Elute the Glycans** with **3 Washes** of **0.5 ml Water**, allowing each wash to drain before the next is applied.

Sample Finishing:

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

LABELED GLYCAN ANALYSIS

Glycan mixtures labeled with 2-AB may be analyzed by HPLC and/or Mass spectrometry.

HPLC Analysis:

Glycan mixtures labeled with 2-AB may be separated and analyzed by HPLC with HPLC columns:

Enzymatic Analysis:

Kreative's Glycosylation 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.

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.

Precautions:

We recommend the user to determine the suitability of the S Cartridge before adopting them on a commercial scale.