

## 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.*

**Procedure**1. 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.