

S-PLUS CARTRIDGES (For clean-up of glycan samples)

Product Code:

KNBS-9730

Pack Size:

25 cartridges

Storage:

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

Introduction:

S-plus cartridges are used in conjunction with the Glycans Sample Processing Station for the rapid clean-up of glycan samples for analysis by HPLC or other methods. S-plus 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.

Application:

S-plus 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-plus 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**S-plus Cartridges Preparation:**

1. Add 1 ml of water to each cartridge.
2. Open the vacuum and the inlet valve fully to drain the cartridges.

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

3. Close the valve when the cartridges have completely drained. Release the residual vacuum.
4. Pipette 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.

Sample Processing:

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. Transfer the entire contents 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.

4. The Glycans are now ready for elution from the cartridges.
5. Elute the Glycan Samples
6. Elute each Glycan Sample with 0.5 ml of water (refer to manufacturer's instructions provided with the Station for sample elution procedures).

Sample Finishing:

Samples may be filtered or evaporated to dryness using a centrifugal evaporator and re-dissolved in a desired volume of water or other suitable solvent for further analysis.

Storage:

Samples are to be stored at -20°C, in 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:

Kornberg'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-plus Cartridge before adopting them on a commercial scale.