PROzyme®

GLYKO®

GLYKO Clean™

Glycan Clean-Up Station

Rapid and reproducible clean-up of fluorescently labeled glycans using GlykoClean™ miniature- and preparative-scale cartridges prior to analysis by HPLC or other methods:

- Vacuum-driven, rapid clean-up of fluorescently labeled glycans (2-AB, 2-AA and other dyes)
- Miniaturized cartridge format with minimized sample elution volumes
- Preparative-scale cartridges (selected matrices)
- Simultaneous processing of multiple samples in array format: 1 - 96 analytical samples, or 1 - 24 preparative samples
- Clean-up amenable to semi-automation and automation

Product Code: GC100

NOTICE: ProZyme was purchased by Agilent in July 2018. Documents for products and product lots manufactured before August 2019 will contain references to ProZyme. For more information about these products and support, go to: **www.agilent.com/en/contact-us.**

Agilent

TABLE OF CONTENTS

This product is intended for in vitro research use only.

CONTENTS

NOTE: Please read this entire booklet before starting the experiment to ensure successful results.

Item	Qty
Vacuum Manifold (manifold base and lid, with o-ring, lid gasket and regulator assembly)	1 ea
Trap Flask Assembly (equipped with 1/4" tubing)	1 ea
Cartridge Removal Tool	1 ea
Waste Reservoir Tray (supplied within the chamber of the Vacuum Manifold)	1 ea
Base Plate (96-well format)	2 ea
Collection Plate (96-well format), sample	1 ea
Sealing Plugs for 96-well base plate (50 x 8-ct strips)	1 bag
Base Plate (24-well format)	1 ea
Sealing Caps for 24-well base plate (1 x 25 ea)	1 bag

Additional Required Equipment

A Standard Laboratory Vacuum Pump.

SAFETY AND HANDLING

General Laboratory Procedures

All procedures should be performed using appropriate personal safety protection, including lab coat, eyeglasses, chemically resistant (e.g. nitrile) gloves and, where appropriate, in a laboratory fume hood.

Use powder-free gloves for all sample handling procedures. Ensure that all glass, plasticware or solvents are free of glycosidases and environmental carbohydrates.

Storage Conditions

The GlykoClean™ Glycan Clean-Up Station should be rinsed after use, dried and stored at room temperature.

INTRODUCTION

Analysis of fluorescently labeled glycans by HPLC and other methods typically requires removal of the excess derivatizing reagents after labeling. The GlykoClean™ Glycan Clean-Up Station (the Station) is ideal for rapid clean-up of glycans following labeling with carbohydrate-reactive fluorescent dyes, such as 2-aminobenzamide (2-AB) and 2-aminobenzoic acid (2-AA); the Station can also be used for the clean-up of glycans from proteins, salts and detergents.

The Station is a vacuum-driven, clean-up system employing a disposable cartridge array format. It is used in conjunction with GlykoClean™ cartridges developed for optimal solid-phase, extraction-based clean-up of glycans.

NOTE: GlycoClean™ S cartridges, R cartridges and H cartridges (ProZyme product codes GKI-4726, GKI-4756 and GKI-4025, respectively) are not suitable for use with the Station.

Use of the Station enables a fast and highly reproducible clean-up of multiple glycan samples, and is compatible with several cartridge sizes, allowing for clean-up on both analytical and preparative scales. A versatile array format of removable cartridges enables low, semi-high and high sample throughput. Robust, simultaneous processing of a variable number of samples (from 1 to 96 samples on analytical scale; 1 to 24 samples on semi- and preparative-scale) is facilitated by the use of different base plates capable of accommodating cartridges of various sizes.

USING THE STATION

Component Overview

The Station ships with these components:

1. Vacuum Manifold - WS0197

   The Vacuum Manifold is composed of a manifold base and a manifold lid. The base has a built-in vacuum pressure gauge, a vacuum metering valve (needle valve) and a vacuum release valve (On/Off valve), which allows the user to control and monitor vacuum levels during clean-up.

2. Base Plates - WS0199 (96-well) & WS0202 (24-well)

   The Base Plates are used as holders for GlykoClean cartridges. The 96-well Base Plate is designed for use with mini cartridges (1-ml). The 24-well Base Plate can be used with larger cartridges (6-ml) for preparative-scale clean-up.

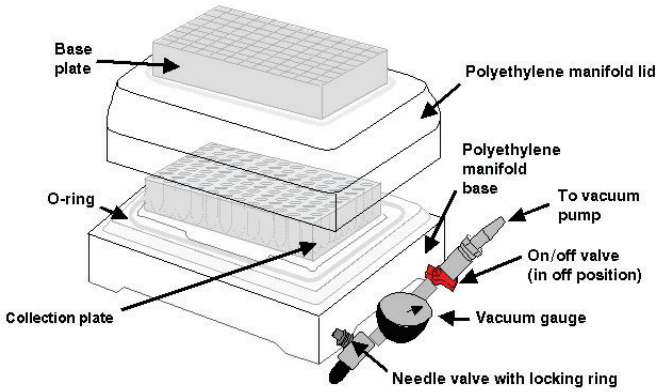

Figure 1 - Components of the Station

3. Trap Flask Assembly - WS0198

   The Trap Flask Assembly is comprised of a 1-liter polypropylene vacuum flask with a polyethylene stopper and compatible 1/4" tubing. The assembly should be connected between the Vacuum Manifold and the vacuum source (typically a vacuum pump); it protects the vacuum source from waste liquids.

4. Waste Reservoir Tray - WS0214

   The Waste Reservoir Tray is used for the collection of liquid waste during cartridge equilibration, sample application and clean-up. It ships inside the chamber of the Vacuum Manifold.

5. Sealing Plugs (for the 96-well base plate) - WS0201

   Strips of Sealing Plugs are used to adjust the number of processed samples between 1 and 96 (a full base plate), by blocking the unused wells.

6. Sealing Caps (for the 24-well base plate) - WS0203

   Sealing Caps are used to adjust the number of processed samples between 1 and 24 (a full base plate) by blocking the unused wells.

7. 96-well Collection Plate (1 ml, deep-well format) - WS0204

   A plate is provided as an example of a sample collection plate. It is also used to preset the Vacuum Manifold to a specific negative pressure. Please see Page 23 for recommended vendors of additional collection plates.

8. Cartridge Removal Tool - WS0200

   The Cartridge Removal Tool is used for removing used cartridges from the wells of the 96-well Base Plate after clean-up.

Vacuum Control - General Instructions

Become familiar with the vacuum controls on the manifold base:

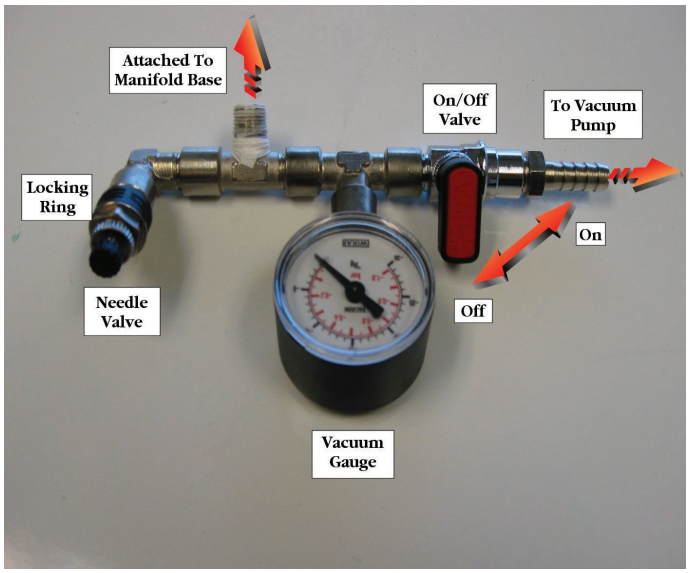

Figure 2 - Vacuum Control Apparatus

NOTE: Do not detach the Vacuum Control Apparatus from the Manifold Base. Detached Control Apparatus shown above for illustration only.

- The vacuum gauge indicates the level of negative pressure (vacuum).

- The on/off valve opens/closes the vacuum. The valve is fully open when the tap is turned parallel to the apparatus and fully shut when the tap is perpendicular to the apparatus (valve is shown closed in Figure 2). Intermediate positions of the tap will result in a partially opened valve.

- The needle valve is used to preset the manifold to a specific negative pressure to ensure reproducibility between uses. It is also used to vent the unit to release the residual vacuum. Adjusting the knurled knob on the needle valve controls the vacuum. Turn the needle valve clockwise to close. The locking ring is used to lock the position of the valve.

Residual vacuum in the chamber of the manifold should be released after each evacuation step (washing, equilibration and elution), preferably by letting it dissipate on its own (by waiting several seconds), or by either opening the needle valve (turning it counter clockwise) or by lifting the edge of one of the sealing plugs. Opening the needle valve requires re-setting the negative pressure, and lifting a plug may shock the samples. Do not release the vacuum by pulling up a corner of the plate, as it will degrade the manifold gasket.

Set-up/removal of the Cartridges (96-well Base Plate)

The wells of the Base Plate are designed to provide an airtight fit for the GlykoClean™ Cartridges. To mount a cartridge in the Base Plate, insert the cartridge into a well. Press the top of the cartridge firmly with the thumb, pushing it to the bottom of the well. For mini cartridges, a snap will indicate secure placement.

One cartridge is required for each glycan sample to be processed. Any vacant wells must be sealed using the Sealing Plugs or Caps provided, by firmly pressing them into the wells. The quality of the seal will deteriorate over time due to repeated use, creating a looser fit in the well; replace old Sealing Plugs or Caps with new ones as needed. When working with multiple cartridges, arrange them in continuous rows, rather than placing them with spaces between. While arrangement does not affect performance of the cartridges during clean-up, spaces between make cartridge removal more difficult.

The 96-well Base Plate is intended to provide long service. The thin walls of the wells are the most vulnerable parts of the Base Plate and damage to the wells (e.g. deformations or breaks in the walls) will compromise the performance due to vacuum leaks. It is essential to use proper technique when removing mini cartridges. Always use the Cartridge Removal Tool.

NOTE: Even with the Cartridge Removal Tool, improper and/or overly aggressive use may result in damage to the Base Plate.

To properly remove a mini cartridge from the Base Plate, hold the Cartridge Removal Tool in one hand and place the curved (half-moon shaped) edge of the tool to the side of the cartridge at the point where it meets the Base Plate. Keep the Cartridge Removal Tool at an angle of approximately 45° to the Base Plate (Figure 3). With the free hand, apply a moderate opposing force with a finger to the top of the cartridge on the opposing side. Using the Cartridge Removal Tool, firmly wedge the cartridge out with an upward scooping motion.

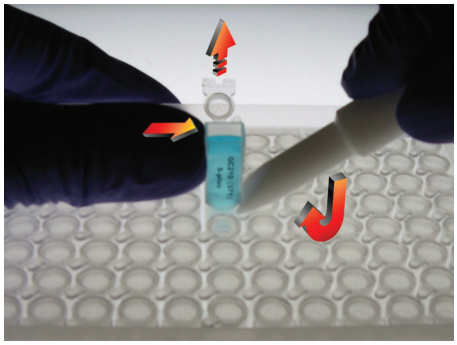

Figure 3 - Use the Cartridge Removal Tool at 45 ° initially

NOTE: The mini cartridges should never be removed from the Base Plate without the help of the Cartridge Removal Tool! For example, do not pull them from side to side or pull them out using fingers.

If several cartridges are lined up, the opposing force can be applied to the cartridge at the end of the row (Figure 4). A continuous arrangement of cartridges in row(s) is suggested, since the space between cartridges in a discontinuous arrangement may not provide adequate accessability, making removal more difficult.

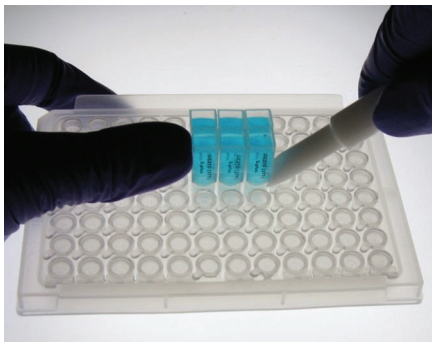

Figure 4 - Apply Opposing Force at the End of Row

Maintenance of the Glycan Clean-Up Station

After clean-up, discard the organic waste from the Waste Reservoir Tray to prevent deformation of the plastic. Rinse all components with water and allow to air dry.

PROTOCOLS

Outline of the procedure:

1. Setup of the Station
2. Equilibration of Cartridges/Sample Clean-Up
3. Elution and Collection

Setup of the Station

Before processing samples, an inspection of all of the components of the Station is recommended. Check for cracks in the Base Plate(s), tears in the lid gasket or base O-ring and any other noticeable defects. Inspect the orientation of the lid gasket and ensure that it is positioned with the rubber-side up. Test the vacuum to ensure that the preset pressure is achievable. Common sources for vacuum leaks are the Sealing Plugs and Caps. For convenience, additional Plugs and Caps are provided.

Installation of the Trap Flask Assembly:

1. Connect the On/Off valve of the Vacuum Manifold to the inlet of the Trap Flask Assembly (located on the top of the flask) using the hose barb provided.
2. Connect the outlet of the trap flask (located on the side of the flask) to the vacuum pump.

NOTE: Installation of the Trap Flask Assembly between the Vacuum Manifold and the vacuum pump prevents liquids from entering the vacuum pump.

Presetting Negative Pressure of the Vacuum Manifold:

The Vacuum Manifold should be preset to a negative pressure reading of approximately –0.5 bar.

1. Place the 96-well Collection Plate on the Vacuum Manifold onto the gasket positioned in the recess in the manifold lid.

NOTE: This prevents air from entering the Vacuum Manifold, except through the needle valve.

2. Turn on the vacuum and fully open the On/Off valve.

3. Adjust the negative pressure (indicated on the vacuum gauge) to approximately –0.5 bar, by slowly adjusting the needle valve (turn counter-clockwise to decrease the negative pressure). Lock the needle valve by tightening the locking ring (clockwise).

4. Turn off the vacuum, allow the residual vacuum to dissipate and remove the collection plate.

NOTE: Test the negative pressure periodically.

Setup of the Cartridges:

1. Remove the manifold lid and place the Waste Reservoir Tray inside the manifold base for waste collection. Replace the manifold lid.

2. Place the chosen Base Plate (96- or 24-well) on the top of the Vacuum Manifold (onto the gasket positioned in the recess of the manifold lid).

3. Insert cartridges into the Base Plate as described on Page 10. Add additional cartridges contiguously to accommodate all samples and controls.

4. Block off unused wells with Sealing Plugs or Caps provided.

NOTE: The plug strips can be arranged to seal any configuration of unused wells. If necessary the plug strips can be cut into shorter pieces.

Equilibration of Cartridges/Sample Clean-Up

NOTE: A detailed protocol for the equilibration of cartridges and clean-up of glycans is provided with each type of GlykoClean™ cartridge. Consult ProZyme's website at:

http://www.prozyme.com/glyko

Generally, GlykoClean™ S-Plus and G cartridges, when used for the clean-up of fluorescently labeled glycans, are first primed with water and then with an aqueous solution of acetonitrile (typically 96%; v/v). The glycan sample is then premixed with the acetonitrile solution and applied on the equilibrated cartridge. The glycans adsorb on the matrix of the cartridge and the excess labeling reagents are removed by a set of washes with the acetonitrile solution. Finally, the glycans are eluted with water and collected. Several elution methods are described in the following section.

Elution and Collection

Elution and collection of glycans after clean-up can be performed in three different ways, depending on the number of samples to be processed:

- Elution into individual collection tubes by centrifugation
- Elution into a collection plate by vacuum
- Elution into a collection plate by centrifugation

Elution into Individual Collection Tubes by Centrifugation (mini cartridges only)

Elution of glycans into individual collection tubes is very convenient when processing a small number of samples, and is limited only by the capacity of the microcentrifuge used (typically 18 - 24 tubes). Elution into individual tubes eliminates the possibility of cross-contamination and minimizes the volume of the eluted glycans.

1. Complete the procedure as described in the individual cartridge technical data sheet.
2. Close the On/Off valve and allow the residual vacuum to dissipate.
3. Firmly tap the top of the base plate to release any hanging drops that may be attached to the outlet tips.

4. Remove the base plate from the vacuum manifold with the cartridge(s) still attached.

NOTE: Any drops of liquid still present on the tips of the cartridges can be removed by blotting with a piece of filter paper or tissue paper (Kimwipes®, etc.).

5. Remove the cartridge(s) from the Base Plate using the Cartridge Removal Tool (see general instructions).

6. Place each cartridge into a 2-ml polypropylene microcentrifuge tube.

NOTE: See Tips & Hints (page 23) for suggested vendors and their catalog numbers.

7. Add 500 µl of water to each cartridge.

8. Place each cartridge/collection tube assembly into a microcentrifuge and spin for two minutes at 3000 - 5000 rpm. Remove the cartridge(s) from the collection tube(s) and discard. The flow-through in the collection tube(s) contains the eluted glycans.

NOTE: The height of the cartridge/collection tube assembly may prevent the microcentrifuge rotor lid from being secured. In this case, elution is still possible if the microcentrifuge cover can close completely and the speed is set to 3000 rpm.

Elution into a 96-well Collection Plate by Vacuum

Elution of glycans into a 96-well collection plate using a vacuum is convenient when processing a larger number of samples. Elution is performed in the same Vacuum Manifold used for the clean-up.

1. Close the On/Off valve after completing the clean-up procedure. Release the residual vacuum (see general instructions).

NOTE: Tap the top of the Base Plate prior to removing to release any hanging drops that may be attached to the outlet tips.

2. Remove the Base Plate with fixed cartridges from the manifold.

NOTE: Any drops of liquid still present on the tip of the cartridge can be removed by blotting with a piece of filter paper or tissue paper (Kimwipes®, etc.).

3. Remove the manifold lid and replace the Waste Reservoir Tray with a deep-well collection plate.

NOTE: A deep-well collection plate is recommended to avoid cross contamination of samples due to entrainment. See Tips & Hints (page 23) for suggested vendors and their catalog numbers.

4. Replace the manifold lid and the Base Plate with the fixed cartridges. Ensure that the tips of the cartridges are correctly positioned and inserted into the wells of the collection plate.

5. Add 500 µl of water to each cartridge.

6. Partially open the On/Off valve to promote the flow of water (vacuum level of approximately –0.1 bar is recommended). The cartridges will begin to drain once the vacuum has been applied.

7. When all of the cartridges are completely evacuated, gradually close the On/Off valve. Release the residual vacuum.

8. Remove the Base Plate from the manifold and place it aside for further disposal of cartridges.

9. Remove the deep-well collection plate from the manifold base. The flow-through retained in the wells of the collection plate contains the glycans.

Elution into 96-well Collection Plate using Centrifugation

Elution into a 96-well collection plate using centrifugation is very convenient when processing a larger number of samples. It can be performed in a centrifuge equipped with carriers capable of accepting deep-well collection plates. Elution by centrifugation eliminates the possibility of cross-contamination and allows for the minimization of the volume of eluted sample.

1. Close the On/Off valve after completing the clean-up procedure. Release the residual vacuum (see general instructions).

NOTE: Firmly tap the top of the Base Plate prior to removing to release any hanging drops that may be attached to the outlet tips.

2. Remove the Base Plate with fixed cartridges from the manifold.

NOTE: Any drops of liquid still present on the tip of the cartridge can be removed by blotting with a piece of filter paper or tissue paper (Kimwipes®, etc.).

3. Place the Base Plate with fixed cartridges on top of a deep-well collection plate.

NOTE: A deep-well collection plate is recommended to avoid cross contamination of samples due to entrainment. See Tips & Hints (page 23) for suggested vendors and their catalog numbers.

4. Add 500 microliters of water to each cartridge.

5. Place the Base Plate and collection plate assembly into a centrifuge and spin at 500 - 2500g for 2-3 minutes. The flow-through retained in the wells of the collection plate contains the glycans.

SPECIFICATIONS

Name

GlykoClean™ Glycan Clean-Up Station

Materials of Construction

Vacuum Manifold:	Polypropylene
Gasket:	EDPM (ethylene propylene)
O-ring:	Silicone
Maximum Operating Vacuum:	71.12 cm Hg (28 in Hg)

NOTE: The Vacuum Manifold can be used with any multi-well filter plates that meet the specifications set forth by the Society for Biomolecular Sciences (SBS).

TIPS & HINTS

Suggested Products (Vendor / Part Number)

Collection tubes (2-ml) for sample elution from individual cartridges:

Sarstedt AG & Co / Part No. 72.694.005

Deep-well collection plates for sample elution:

BD Biosciences / Part No. 353964 (1-ml wells)

BD Biosciences / Part No. 353966 (2-ml wells)

TROUBLESHOOTING

Solvents/samples flow slowly, flow unevenly and/or fail to flow through the cartridges.

Uneven flow of the solvents may be observed, especially when equlibrating cartridges with water. Typically, the flow becomes more uniform after switching to acetonitrile solution (due to its lower viscosity).

Some of the cartridges may have a lower flow rate, even after switching to acetonitrile solution. Continue evacuation of the cartridges until all the solvent has drained. Running cartridges “dry” (fully drained of solvent) for up to two minutes does not affect the recovery of glycans. Longer times have not been tested.

If cartridges drain too slowly or do not drain, look for a vacuum leak. Ensure that the manifold base and lid are securely fitted together, that the gasket in the lid is oriented rubber-side up, and that the Base Plate is properly positioned on the gasket. All unused wells must be blocked.

Inspect the wells of the Base Plate(s) for deformation or any cracks. The Sealing Plugs and/or Sealing Caps may not sufficiently seal wells when misshapen. Replace old Plugs and Caps with new ones.

Cartridges are difficult to remove from the 96-well Base Plate.

The cartridges can usually be removed with a moderate effort as described in the General Instructions.

Cartridges that are difficult to remove require greater caution when using the Cartridge Removal Tool. The Base Plate can be damaged when carelessly removing tighter cartridges.

It is often more convenient to start removal of the cartridges on the side of the array with fewer cartridges, as more direct force can be applied by hand opposite the Cartridge Removal Tool. For example, for two adjacent rows of twelve cartridges each, removal should be performed on the side of the array that is two cartridges deep, as force can be applied more directly on each side.

TECHNICAL ASSISTANCE

ProZyme is committed to developing rapid, automatable methods for glycan analysis. Call us to discuss products in development.

If you have any questions or experience difficulties regarding any aspect of our products, please contact us:

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ProZyme values customers opinions and considers customers an important source for information regarding advanced or specialized uses of our products. We encourage you to contact us. We welcome your suggestions about product performance or new applications and techniques.

OTHER PROZYME PRODUCTS & KITS

A wide variety of glycobiology products are available from ProZyme. A complete listing is accessible on our website:

http://www.prozyme.com/glyko

PRODUCT USE AND WARRANTY

Terms and conditions of sale as well as product warranties may be found at:

http://www.prozyme.com/terms.html

TRADEMARKS AND TRADENAMES

ORDERING INFORMATION

For North American destinations: telephone orders may be placed between 8:00 am and 5:00 pm Pacific Time. Fax orders, e-mail orders and voicemail messages can be received at any time.

TOLL FREE (800) 457-9444 (US & CANADA)
PHONE (510) 638-6900
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E-MAIL orders@prozyme.com
WEB www.prozyme.com

Outside North America:

A list of ProZyme's distributors, with contact information, may be found at:

http://www.prozyme.com/distributors.html

If there is no distributor in your area, place an international order using the directions at:

http://www.prozyme.com/ordering.html#outside_america

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