PROzyme®

GlykoPrep

Cleanup Module

- Purified N-glycans are eluted in water; ready for analysis without concentrating or drying
- Cleanup of ≤30 µg of glycans in 5–30 µl of aqueous solution
- Particularly effective for cleanup with the InstantDye™ Labeling Modules
- Flexible, high-throughput format: process 1 to 192 samples per run (2 kits simultaneously)
- Compatible with microplate liquid handling on a broad range of automation platforms

Product Code: GS96-CU

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

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This product is intended for in vitro research use only.

KIT CONTENTS

NOTE: We want successful results for our customers, so please read this entire booklet before starting the procedure.

Item	Qty
WS0263 Cleanup (CU) Cartridges	1 rack (96 cartridges)
Aluminum Sealing Film	2 ea

Storage Requirements

This kit is shipped ambient and should be stored at room temperature upon receipt.

Additional Required Reagents/Equipment

AssayMAP Labware: Racks, Receiver Plates and Lids
Other Labware: Waste Plates/Cleanup Collection Plates, 450 µl well volume (Thermo Fisher Scientific part number 07-202-502/Corning part number 3343 or equivalent)
Gilson Diamond® D200 Pipet Tips

NOTE: Labware is available from ProZyme as a complete Starter Set (Product Code AM200), or AssayMAP labware may be purchased separately in sets of 10.

Centrifuge (capable of 50–300 x g) and microplate rotor with a height clearance of 44 mm
Ultrapure, deionized water (Milli-Q® or equivalent)
Acetonitrile (100%, HPLC-grade)
Pipettors & disposable tips (P5/P10, P200 and P1000)

Optional Reagents and Supplies

Multichannel pipettors & disposable tips (P5/P10 and P200) (Gilson or equivalent, compatible with Gilson D200 pipette tips)
Pipette basins (must be polypropylene for use with organic solvents)

SAFETY AND HANDLING

Please refer to the Safety Data Sheets (SDS) posted on ProZyme's website under the component name or Product Code.

http://www.prozyme.com

General Laboratory Procedures

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

INTRODUCTION

The GlykoPrep Sample Preparation Platform (GlykoPrep) dramatically streamlines glycoanalysis by facilitating optional protein purification, quantitative deglycosylation and separation of N-glycans, complete fluorescent labeling and efficient cleanup to reduce excess reagent peaks.

GlykoPrep is modular and can be integrated into any workflow, regardless of throughput or sample type. In order to match any standard sample preparation, Kit components are also available individually as the AssayMAP PA50 (for purification of Fc-containing antibodies only), Digestion Module and dye-specific Labeling & Cleanup Modules.

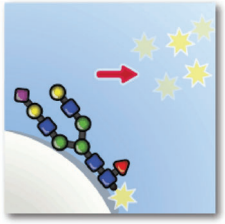
GlykoPrep is built on AssayMAP technology, microchromatography in a 96-well format, capable of automated high throughput. GlykoPrep may be performed using centrifugation to move liquid through the Cartridges (spin format), or with the Syringe Head on the Agilent AssayMAP Bravo Liquid Handling Workstation (GlykoPrep-plus). Using the spin format with a microplate centrifuge, up to 192 samples can be processed simultaneously with 2 Kits.

Important general information for achieving success with the spin format, as well as special tips particular to individual Modules, may be found in the GlykoPrep Guidebook under Using Specific Kits and Modules:

http://www.prozyme.com/documents/TNGP100.pdf

We also provide a modified Microfuge Method useful for those interested in using the spin format to run only a handful of samples with a benchtop microfuge and a PCR heater:

http://www.prozyme.com/documents/2-AB_Microfuge_Method.pdf



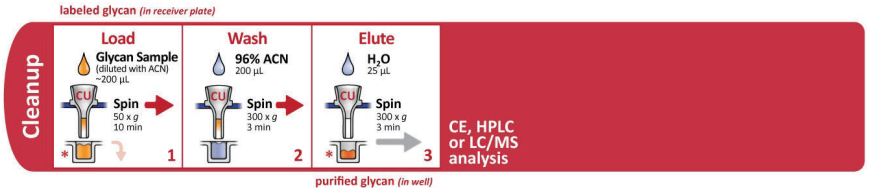
Overview

CU Cartridges allow most hydrophobic, non-glycan contaminants to be washed through; glycans are then eluted with water.

1 Load

2 Wash

3 Elute



Reagents and other Supplies

Glycan Samples (≤30 µg of glycans in 5-35 µl of aqueous solution)
CU Cartridges (supplied with the kit, 1 per sample)
Prepare two balanced CU Cartridge assemblies (Cartridges on Racks on Waste Plates with Lids)
Acetonitrile (100%, HPLC-grade), 180 µl/Sample
96% Acetonitrile Solution (prepared previously)
Ultrapure water
Cleanup Collection Plate (supplied in the AM200 Starter Labware Set, or equivalent)

PROTOCOL

Preparation of Reagents

96% Acetonitrile Solution

NOTE: May be prepared up to one week before use. Store at room temperature.

Ultrapure water
Acetonitrile (100%, HPLC-grade)

To make 25 ml (enough for a full kit) of 96% Acetonitrile Solution (v/v), add 1.0 ml of ultrapure water to a glass, graduated cylinder. Bring the volume up to 25 ml with HPLC-grade acetonitrile. Transfer to a glass or polypropylene storage vessel, cap tightly and swirl gently to mix.

Scale as necessary (prepare 250 µl per sample).

Getting Started

Centrifuge Settings

Determine the setting for the centrifuge and the specific microplate rotor by consulting the instruction manual or the internet:

_____ rpm = 300 x g

_____ rpm = 50 x g

Sample Preparation

This Cleanup Module is intended for use with the GlykoPrep InstantAA™, InstantAB™ and Rapid-Reductive-Amination™ with 2-AB Labeling Modules (product codes GS96-LA, GS96-LB and GS96-AB, respectively) .

For these Labeling Modules, 100% Acetonitrile is used to bring the starting sample to a volume ≤200 µl before loading on the CU Cartridge. Be aware that the capacity of the CU Cartridge is ~200 µl. Volumes greatly exceeding 200 µl should be loaded onto the CU Cartridges in succession.

If continuing from GlykoPrep Rapid N-Glycan Preparation with InstantAA, N-glycans will be suspended in ~40 µl of Enzyme Solution, Digestion Buffer and Labeling Reagent; add 180 µl of 100% Acetonitrile and pipette up and down several times to mix before loading.

If continuing from GlykoPrep Rapid N-Glycan Preparation with InstantAB, N-glycans will be suspended in ~35 µl of Enzyme Solution, Digestion Buffer and Labeling Reagent; add 180 µl of 100% Acetonitrile and pipette up and down several times to mix before loading.

If continuing from GlykoPrep Rapid N-Glycan Preparation with 2-AB, add 20 µl of water and 180 µl of 100% Acetonitrile to the dry 2-AB-labeled N-glycans and pipette up and down several times to mix before loading.

If using other fluorescent labels or cleaning up after non-GlykoPrep labeling, please contact us to discuss your particular workflow.

Procedure

NOTE: DO NOT use Receiver Plates in this procedure. Waste Plates (~450 µl-well volume) can be reused throughout this protocol.

Load

1.a Prepare samples (see page 5).

1.b Transfer the entire sample into individual CU Cartridges by pipetting a portion into the CU Cartridge and then aspirating the balance and laying it onto the top surface of the first portion without concern for air bubbles.

NOTE: The CU Cartridge will accommodate the full volume.

1.c Spin at 50 x g for 10 minutes.

1.d Empty the Waste Plate.

Wash

2.a Pipet 200 µl of 96% Acetonitrile Solution into the sample cup of each CU Cartridge.

2.b Spin at 300 x g for 3 minutes.

Elute

3.a Place each racked set of CU Cartridges over a Cleanup Collection Plate.

NOTE: Because the initial eluate contains traces of organic solvent, polystyrene plates should NOT be used. Any polypropylene ANSI/SBS 96-well microplate can be used as a collection plate. To facilitate complete product recovery, we recommend plates with conical bottoms, such as PCR plates or the Cleanup Collection Plates provided in the AM200 Starter Labware Set.

3.b Pipet 25 µl of ultrapure water into the sample cup of each CU Cartridge.

NOTE: Up to 200 µl of water may be used if more dilute glycans are desired.

NOTE: To protect glycans for long-term storage, an aqueous buffer compatible with the intended analysis method may be used instead of water.

3.c Spin on Cleanup Collection Plates at 300 x g for 3 minutes.

The Cleanup Collection Plate now contains the purified glycans; DO NOT DISCARD.

Mix the eluate by pipette action or vortexing prior to analysis to ensure homogeneity. Glycan samples are now ready to be analyzed. If not analyzed immediately, store sealed at -20°C in the dark.

ANALYSIS OF PURIFIED GLYCANS

Use standard techniques, such as LC, CE and MS, to analyze the aqueous eluate containing purified N-glycans.

TIPS & HINTS

Direct LC Analysis of N-Glycans After Elution

If N-Glycan Samples will be analyzed by LC directly following elution from the CU Cartridges, the 96-well, polypropylene plate with a pierceable lid available from MicroLiter Analytical Supplies (cat# 07-1211N) may serve as a Collection Plate.

Alternatively, Cleanup Collection Plates may be heat sealed with pierceable foil (e.g., Thermo Easy Pierce 20 µm Foil, #AB-1720) using a microplate heat sealer (e.g., Thermo ALPS 50 V Semi automated Microplate Heat Sealer, #AB-1443).

REFERENCES

Visit ProZyme's website for additional information and instructional videos:

http://www.prozyme.com/glykoprep

TechNote TNGP100 GlykoPrep Guidebook - General tips, tricks and troubleshooting suggestions when using kits or modules.

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:

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

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For a complete list of ProZyme Patents, please visit our website at http://www.prozyme.com/patent.html

The AssayMAP technology is licensed from Agilent Technologies.

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Terms and conditions of sale may be found at:

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

OTHER PROZYME PRODUCTS & KITS

A wide variety of glycoanalysis products are available from ProZyme. A complete listing is accessible on our website by clicking on Glyko® Tools for Glycobiology:

http://www.prozyme.com

ORDERING INFORMATION

For North American destinations: telephone orders may be placed between 8:00 am and 5:00 pm Pacific Time. Telefax or e-mail orders may be sent or messages recorded anytime.

TOLL FREE (800) 457-9444 (US & CANADA)
PHONE (510) 638-6900
FAX (510) 638-6919
E-MAIL info@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, instructions for placing an international order may be found at:

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

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