



Cleanup Module

- Purified N-glycans are eluted in water; ready for analysis without concentrating or drying
- Cleanup of $\leq 30 \mu\text{g}$ of glycans in 5–30 μl of aqueous solution
- 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
- Particularly effective for cleanup with the InstantDye™ Labeling Modules

Product Code: GS96-CU

TABLE OF CONTENTS

	<i>page</i>
Kit Contents	2
Additional Required Reagents/Equipment	
Safety and Handling	3
Protocol	4
Preparation of Reagents	4
Getting Started	5
Analysis of Purified Glycans	10
References	10
Tips & Hints	10
Technical Assistance	11
License to Use	12
Trademarks and Tradenames	13
Product Use and Warranty	13
Other ProZyme Products & Kits	14
Ordering Information	14

Storage Conditions

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

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

Additional Required Reagents/Equipment

AssayMAP labware: Racks, Receiver Plates and Lids
Other labware: Waste Plates, Cleanup Collection Plates and
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 (*no polystyrene*)

SAFETY AND HANDLING

Please read the Material Safety Data Sheets (MSDS) included with the kit.

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.

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.

For fewer samples, prepare 250 μ l of 96% Acetonitrile Solution for each sample to be processed.

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

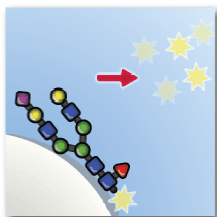
This Cleanup Module is intended for use with the GlykoPrep InstantAB™ (product code GS96-LB) and Rapid-Reductive-Amination™ with 2-AB (product code GS96-AB) Labeling Modules.

For InstantAB and 2-AB labeling, 100% Acetonitrile is used to bring the starting sample to a recommended final concentration of ~90% Acetonitrile before loading on the CU Cartridge. Be aware that the capacity of the CU Cartridge is ~200 μ l. Volumes greatly exceeding 200 μ l should be loaded onto the CU Cartridges in succession.

If continuing from GlykoPrep Rapid N-glycan Preparation with InstantAB, N-glycans will be suspended in ~25 μ l of Digestion Buffer and Labeling Reagent; add 180 μ l of 100% Acetonitrile before loading.

If continuing from GlykoPrep Rapid N-Glycan Preparation with 2-AB, add 20 μ l of water and 180 μ l of 100% Acetonitrile to the 2-AB-labeled N-glycans before loading.

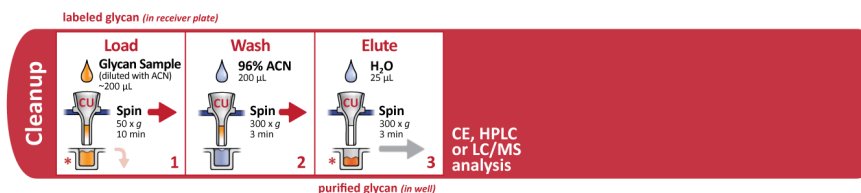
If using other fluorescent labels or cleaning up after non-GlykoPrep labeling, please contact us to discuss your particular workflow.



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

Step

- 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)

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 glass or 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. Glass vial systems designed for instrument autosamplers can also be used.

- 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.

Glycan samples are now ready to be analyzed. If not analyzed immediately, store at -20°C in the dark.

ANALYSIS OF PURIFIED GLYCANS

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

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.

TIPS & HINTS

Collection Plates for Direct HPLC Analysis

If glycan samples will be analyzed by HPLC directly following elution from the CU Cartridges, the 96-well, polypropylene plate with a pierceable lid available from MicroLiter Analytical Supplies (cat# 07-1211N) represents a convenient option for use as a Collection Plate.

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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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)

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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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