PROzyme®

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GlykoPrep

Rapid N-Glycan Preparation with InstantAB™

Enzymatic deglycosylation, fluorescent labeling with InstantAB and cleanup of excess dye for analysis by LC or other methods.

- Optional purification for monoclonal antibodies and Fc proteins
- Non-selective, rapid release and recovery of intact N-Glycans from up to 96 glycoprotein samples at a time using a microplate centrifuge
- Flexible, high-throughput format: process 1 to 192 samples per run (2 kits simultaneously)
- RT InstantAB labeling with no loss of sialic acid; labeling efficiency >80%
- Purified, fluorescently labeled N-glycans are eluted in water and ready for analysis without concentrating or drying
- Compatible with high-throughput microplate liquid handling on a broad range of automation platforms

Product Code: GP96NG-LB

TABLE OF CONTENTS

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
G5524-60010 Kit AssayMAP PA50 (purchased separately)	*Optional*
G5524-60010 AssayMAP PA50 (96 Cartridges)	
WS0296 Protein A Solution Set	
WS0294 10X Wash Buffer (15 ml)	
WS0251 Eluent (30 ml)	
GP96NG-LB GlykoPrep® Rapid N-Glycan Preparation with InstantAB™	
GS96-RX GlykoPrep Digestion Module (2–8°C)	1 ea
WS0253 Digestion (RX) Cartridges (96 Cartridges)	
WS0256 Immobilization Reagent Set	
WS0226 Denaturation Reagent (30 ml)	
WS0255 Blocking Reagent (6 ml)	
WS0259 Digestion Reagent Set	
WS0278 N-Glycanase® (300 µl)	
WS0276 25x Digestion Buffer (700 µl)	
WS0229 Finishing Reagent (optional, not applicable to this kit)	
Aluminum Sealing Film (4)	
GS96-LB InstantAB Labeling Module (-20°C to Room Temp.)	1 ea
WS0224 InstantAB Dye (2 ea)	
WS0260 Dye Solvent (2 ea, N,N-Dimethylformamide)	
GS96-CU GlykoPrep Cleanup Module (2–30°C)	1 ea
WS0263 Cleanup (CU) Cartridges (96 Cartridges)	
Aluminum Sealing Film (2)	

Storage Requirements

This kit is a mixed temperature shipment (-20 to 30°C). Store components as indicated. For best results, equilibrate materials to ambient temperature prior to use. The InstantAB Dye is hygroscopic and light-sensitive; please store the GS96-LB InstantAB Labeling Module at room temperature in a dry environment protected from light.

Additional Required Reagents/Equipment

Heater and Incubation Blocks, capable of 50–100°C, available from ProZyme as product code GS150
AssayMAP Labware: Racks, Receiver Plates and Lids
Labware: Waste Plates, Cleanup Collection Plates and Gilson Diamond® D200 Pipet Tips

NOTE: Labware needed to use this kit 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 - 1000 x *g*) and deep 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 compatible with Gilson D-200 pipette tips & disposable tips (P5/P10 and P200, Gilson or equivalent)
Microplate reader (capable of reading A_{280}) for measurement of antibody concentration after Purification
Pipette basins
Microplate-compatible, centrifugal evaporator (*e.g.*, SpeedVac® or similar) for optional Finishing of released glycans (see Tips & Hints)

SAFETY AND HANDLING

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

http://www.prozyme.com

Opening the Component Ampules

Gently tap the ampule to settle the contents on the bottom. To open, hold both the body and the top of the ampule, then gently but firmly snap open at the colored break-ring. Snap away from your body.

Fluids may be pipetted into or out of the ampules with standard pipettors or syringes with slim tips or needles. Be careful to avoid sharp edges around the opening *(be sure to wear gloves and safety glasses during these operations).*

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.

All steps involving labeling reagents (InstantAB Dye and Dye Solvent) should be performed in a dry environment with dry glassware and plasticware. Procedures involving these reagents should be performed using appropriate personal protective equipment, eyeglasses, chemically resistant gloves (*e.g.*, nitrile) and, where appropriate, in a laboratory fume hood.

INTRODUCTION

The GlykoPrep Sample Preparation Platform (GlykoPrep) dramatically streamlines glycoanalysis by facilitating optional protein purification, quantitative deglycosylation and separation of 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 for glycoanalysis, Kit components are 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/InstantAB_Microfuge_Method.pdf

USING THE KIT

GlykoPrep Rapid N-Glycan Preparation with InstantAB combines the Digestion Module, the Rapid N-Glycan Preparation with InstantAB Labeling Module and the Cleanup Module, which may be purchased individually. The Labeling and Cleanup Modules may be purchased together as GP96-LB. Optional purification modules may be employed just prior to Digestion to allow glycoanalysis directly from cell culture as a single workflow (directions included for your convenience). For information on purification modules under development, please contact us.

Preparation of Samples

Sample Quantities

The quantitative binding for each Cartridge is:

PA Cartridge	125 µg of MAb or Fc-fusion protein
RX Cartridge	50 µg of most standard proteins
CU Cartridge	30 µg of N-glycans

NOTE: The binding capacity for specific glycoproteins may need to be determined.

Cartridges are capable of binding more target, but will do so with increasing breakthrough, making the process non-quantitative.

For quantitative loading, prepare an excess of 10% or more sample, and prepare replicates together. For example, for Digestion, samples should be denatured together and loaded individually.

Less than the maximum quantity may still be processed with this Kit, for example, when the sample is available only in limited amounts. The smallest amount of sample that will still give good results will depend on the sensitivity requirements of the analytical methods used and the specific application (*e.g.* screening *vs.* QC release).

Sample Denaturation

Prior to deglycosylation, the samples are denatured by pre-mixing with Denaturation Reagent. The suggested sample concentration prior to deglycosylation is 1–5 mg/ml, and sufficient reagents have been provided for the standard sample concentration range.

NOTE: If quantitation is desired, pipetting less than 10 µl is not recommended; pipetting smaller volumes introduces variability, especially when samples are highly concentrated. If necessary, dilute the sample to within the 1-5 mg/ml range with Digestion Buffer before starting.

The Kit is useful for very dilute samples without requiring further concentration, by expanding this load step to multiple spins. See the GlykoPrep Guidebook section "Loading."

When performed in a single spin, the amount loaded to each RX Cartridge should be 10–100 µl. The recommended starting ratio of Denaturation Reagent to sample is 1:1 (v/v).

NOTE: The mixture must be 50% Denaturation Reagent or more.

Example 1:
Sample concentration 1 mg/ml
Sample amount needed: 50 µg

50 µl (50 µg) Sample + 50 µl Denaturation Reagent
= 100 µl denatured sample

Example 2:
Sample concentration 5 mg/ml
Sample amount needed: 50 µg

10 µl (50 µg) Sample + 90 µl Denaturation Reagent
= 100 µl denatured sample

The current protocol employs a 5-minute, relatively gentle denaturation, but any custom denaturation may be performed and the subsequent protocol followed as described, as long as no SDS or other detergents are used. Please see the GlykoPrep Guidebook under Digestion Modules or contact us to discuss custom denaturation conditions for your glycoprotein.

Enzyme Incubation

Time

The Digest procedure has been optimized to deliver deglycosylation of most glycoproteins in 15-60 minutes. The optimal incubation time will vary depending on the specific glycoprotein; those which have proven to be resistant to deglycosylation *via* conventional enzymatic methods may require longer incubation times (*e.g.* 60 minutes). For glycoproteins that are comparatively easy to deglycosylate, such as monoclonal antibodies, a 15-minute incubation is sufficient. The selected Incubation Time will be used in the Digestion Module.

NOTE: It is critical not to exceed a 60-minute incubation, as the Cartridge resin bed will dry out, yielding uncertain results.

Temperature

The GS150 Heater and Incubation Blocks are specially designed to provide rapid heat transfer through the Receiver Plate and into the packed bed of each Cartridge.

The Incubation Blocks are sold separately (ProZyme Product Code WS0272) and can be used in any standard dry-block heater of the proper size, or pre-heated and used in an oven. Custom Incubation Blocks compatible with robotic systems are also available from ProZyme.

The GS150 Heater (with Incubation Blocks) is set to 50°C for the Digestion procedure. Please allow a minimum of 1 hour to equilibrate before beginning the procedure. The Incubation Blocks have been designed with a thermometer well in the corner. We have verified that when the thermometer there reads 50°C, the temperature is 37°C, the optimal temperature for deglycosylation. If using a different heater, confirm the block temperature.

PROTOCOLS

Overview of the Procedure

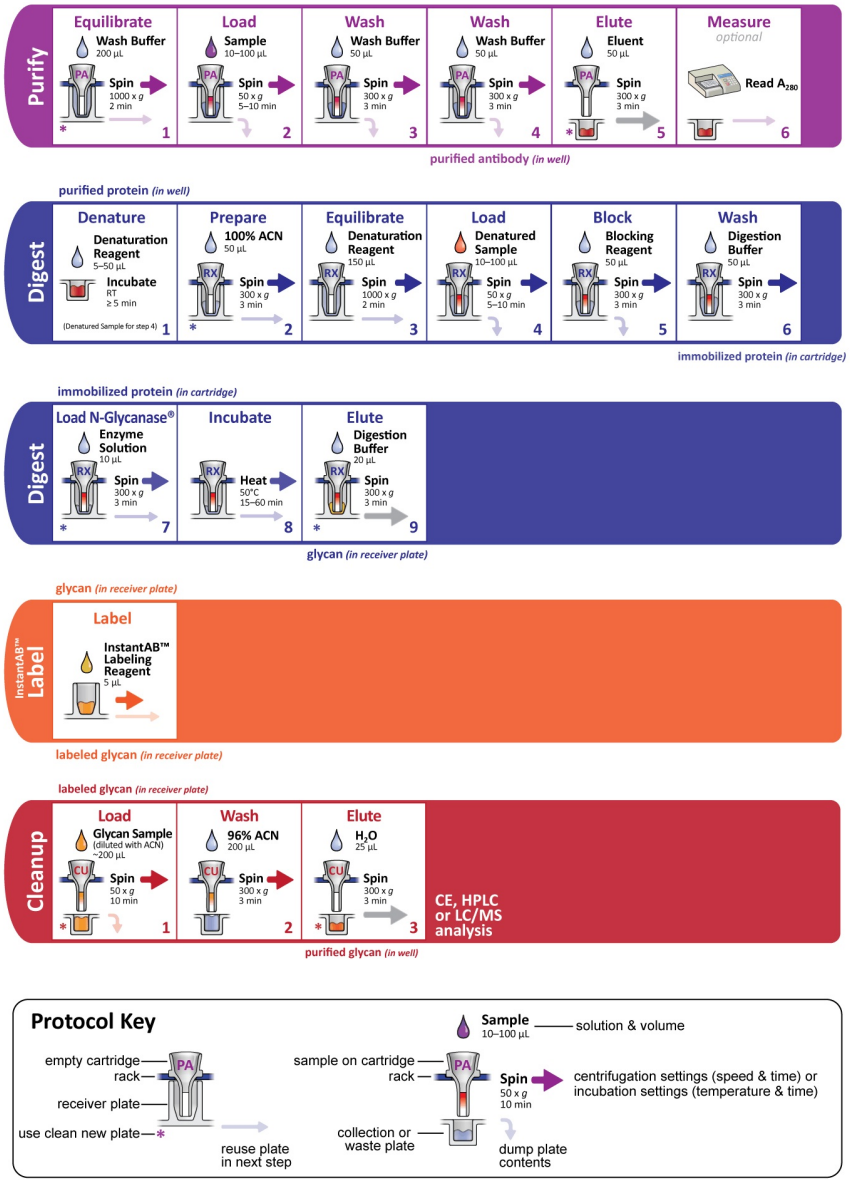
Purify *(optional, purchased separately)*
Antibodies or Fc-fusion proteins may be purified from crude samples using Protein A.

Digest
Samples (antibodies or other proteins) are denatured and immobilized.

N-Glycans are released by N-Glycanase digestion and eluted.

Label
Released glycans are labeled with InstantAB.

Cleanup
Buffer salts and excess labeling reagents are removed; labeled glycans are eluted in water, ready for analysis.



Getting Started

Heater Setting

Turn on the GS150 Heater (with 2 Incubation Blocks). Set to 50°C and allow to equilibrate for a minimum of 1 hour.

Centrifuge Settings

If the centrifuge does not have x *g* settings, determine the setting for the centrifuge and the specific microplate rotor by consulting the operation manual or the manufacturer's website:

_____ rpm = 50 x *g*

_____ rpm = 300 x *g*

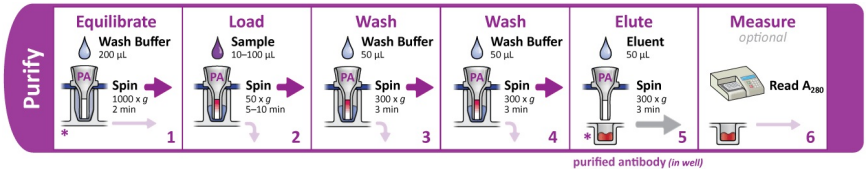
_____ rpm = 1000 x *g*

Purify *(optional)*

Protein A is used to purify antibodies or Fc-fusion proteins from cell-culture supernatants. All other samples must be purified by other methods; proceed to Digestion Module.

Overview

1	Equilibrate
2	Load
3	Wash
4	Wash (second time)
5	Elute
6	Measure *(optional)*



Reagents and other Supplies

PA Cartridges (supplied in the G5524-60010 Kit, 1 per sample)
Prepare two balanced PA Cartridge assemblies (Cartridges on Racks on Receiver Plates with Lids)

Wash Buffer (prepared below)

Eluent (supplied with the kit)

NOTE: The prepared eluent must NOT contain glycine because it leaves a signature peak on the LC chromatograph.

Purification Collection Plate (supplied in the AM200 Starter Labware Set, or equivalent)

UV-compatible, flat-bottom, half-area plate for direct protein assay *(optional)*

PCR plate *(optional)*

Crude antibody or Fc-fusion protein samples (samples may contain NO MORE than 125 µg total protein, the quantitative binding capacity of the PA Cartridge; the amount loaded onto the downstream RX Cartridge may contain NO MORE than 50 µg total protein, the quantitative binding capacity of the downstream RX Cartridge). Samples should be between pH 6.5 and 8.5 and clear of particulates.

Preparation of Reagents

Wash Buffer

NOTE: May be prepared up to one week before use. Store at 2–8° C.

10x Wash Buffer

Ultrapure water

Dilute one volume of 10x Wash Buffer stock with nine volumes of ultrapure water to obtain Wash Buffer. Specifically, add 4 ml of 10x Wash Buffer stock to 36 ml of ultrapure water to make 40 ml of Wash Buffer.

For fewer samples, prepare 400 µl of Wash Buffer for each sample to be processed.

Procedure

Equilibrate

1.a Pipet 200 µl of Wash Buffer into the sample cup of each PA Cartridge.

1.b Spin at 1000*x g* for 2 minutes; do not empty the Receiver Plates.

Load

2.a Load 10–100 µl of sample into each PA Cartridge (see Sample Loading Technique in the GlykoPrep Guidebook).

2.b Remove the Racks from the Receiver Plates. Empty the Receiver Plate and blot with a paper towel to avoid cross-contamination. Replace Racks.

2.c Spin at 50*x g* until all sample cups are empty. The estimated spin time is 5 minutes for volumes between 10 and 50 µl or 10 minutes for volumes up to 100 µl.

Wash

3.a Pipet 50 µl of Wash Buffer into the sample cup of each PA Cartridge.

3.b Empty the Receiver Plate and blot with a paper towel.

3.c Spin at 300*x g* for 3 minutes.

Wash (second time)

4.a Pipet 50 µl of Wash Buffer into the sample cup of each PA Cartridge.

4.b Empty the Receiver Plate and blot with a paper towel.

4.c Spin at 300 x *g* for 3 minutes.

Elute

5.a Remove the Racks from the Receiver Plates and place on top of a collection plate.

NOTE: For a colorimetric measurement (A_{590}/A_{450}) of protein concentration, use the Purification Collection Plate. For direct measurement (A_{280}) of concentration, use a UV-compatible, flat-bottom, half-area plate. If no protein determination will be made, a PCR plate may be used.

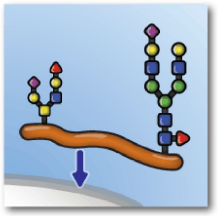
5.b Pipet 50 µl of Eluent into the sample cup of each PA Cartridge.

5.c Spin at 300 x *g* for 3 minutes.

5.d Remove the Racks and dispose of the Cartridges.

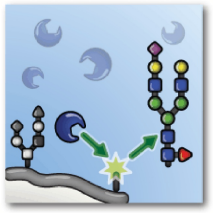
Measure (optional)

6. Measure the absorbance on a plate reader at 280 nm.



Digest

Samples (antibodies or other glycoproteins) are denatured and immobilized.



N-glycans are released by N-Glycanase and eluted.

Overview

Proceed through the Digestion, Labeling and Cleanup modules without delay.

1 Denature

2 Prepare

3 Equilibrate

4 Load

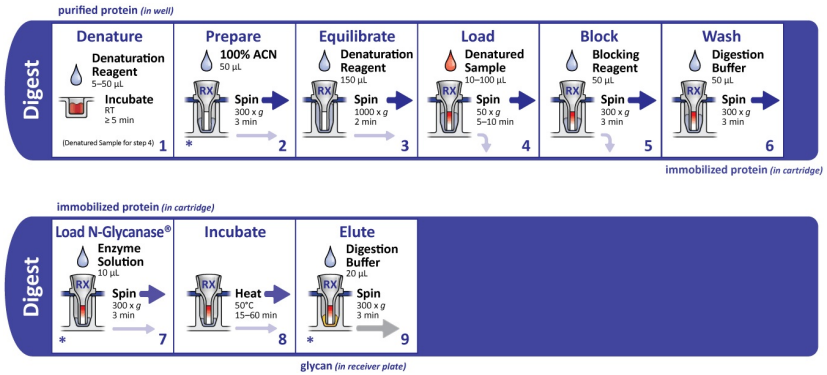
5 Block

6 Wash

7 Load N-Glycanase

8 Incubate

9 Elute



Reagents and other Supplies

Glycoprotein Samples (purified samples; may contain NO MORE than 50 µg total protein, the quantitative binding capacity of the RX Cartridge)

NOTE: The quantity of purified sample loaded to the RX Cartridge may contain NO MORE than 50 µg total protein, the quantitative binding capacity of the RX Cartridge.

RX Cartridges (supplied with the kit, 1 per sample)
Prepare two balanced RX Cartridge assemblies (Cartridges on Racks on Receiver Plates with Lids)

Denaturation Reagent (supplied with the kit)
Acetonitrile (100%, HPLC-grade), 50 µl/sample
Blocking Reagent (supplied with the kit)
Digestion Buffer (prepared below)
Enzyme Solution (prepared below)
Aluminum Sealing Film (supplied with the kit)

Preparation of Reagents

Digestion Buffer

NOTE: May be prepared up to one week before use. Store at 2–8° C.

25x Digestion Buffer (supplied with the kit)

Ultrapure water

Dilute one volume of 25x Digestion Buffer with twenty-four volumes of ultrapure water to obtain Digestion Buffer. Specifically, add 0.4 ml of 25x Digestion Buffer to 9.6 ml of ultrapure water to make 10 ml of Digestion Buffer.

For fewer samples, prepare 100 µl of Digestion Buffer for each sample to be processed.

Enzyme Solution

NOTE: Should be prepared on the day of use. Store at RT.

N-Glycanase (supplied with the kit)

Digestion Buffer (prepared above)

Spin the N-Glycanase briefly prior to use to collect the contents in the base of the vial. Mix the solution gently prior to use.

In a separate vial, prepare a mixture of 2.5 µl of N-Glycanase and 7.5 µl of Digestion Buffer for each sample to be processed, plus 20% for overage. For example, 10 samples would require 25 + 5 = 30 µl of N-Glycanase and 90 µl of Digestion Buffer.

To prepare 96 samples, add 288 µl of N-Glycanase to 864 µl of 1x Digestion Buffer in a pipette basin. Mix the solution several times by pipette action.

NOTE: The pipette basin requires a minimum of ~100 µl volume, so for fewer than 8 samples, do not use a basin.

Procedure

NOTE: An incubation at elevated temperature is required for full deglycosylation. Before beginning, be sure each Incubation Block has equilibrated to 50° C: a thermometer may be placed in the corner well of Incubation Block to monitor the temperature.

Denature

1.a Add Denaturation Reagent to each sample as described in Sample Denaturation (page 8).

1.b Pipet up and down to mix.

1.c Incubate at room temperature for at least 5 minutes.

NOTE: Proceed through the Prepare, Equilibrate and Load steps without interruption, as evaporation can lead to airlock.

Prepare

2.a Pipet 50 µl of 100% Acetonitrile into the sample cup of each RX Cartridge.

2.b Spin at 300 x *g* for 3 minutes; do not empty the Receiver Plates.

Equilibrate

3.a Pipet 150 µl of Denaturation Reagent into the sample cup of each RX Cartridge.

3.b Spin at 1000 x *g* for 2 minutes into the same Receiver Plate used for Step 2.b.

NOTE: Do not empty Receiver Plate prior to loading the denatured Sample.

Load

4.a Load each Denatured Sample into an RX Cartridge (see Sample Loading Technique in the GlykoPrep Guidebook).

4.b Empty the Receiver Plate and blot with a paper towel.

NOTE: Discard waste Acetonitrile Solution according to waste disposal procedures.

4.c Spin at 50 x *g* until all sample cups are empty. The estimated spin time is 5 minutes for volumes between 10 and 50 µl or 10 minutes for volumes up to 100 µl.

Block

5.a Pipet 50 µl of Blocking Reagent into the sample cup of each RX Cartridge.

5.b Empty the Receiver Plate and blot with a paper towel.

5.c Spin at 300 x *g* for 3 minutes; do not remove the Receiver Plate.

Wash

6.a Pipet 50 µl of Digestion Buffer into the sample cup of each RX Cartridge.

6.b Spin at 300 x *g* for 3 minutes.

Load N-Glycanase

7.a Transfer RX Cartridges to fresh Receiver Plates.

7.b Pipet 10 µl of Enzyme Solution into the sample cup of each RX Cartridge.

7.c Spin at 300 x *g* for 3 minutes; do not discard flow-through.

Incubate

8. Incubate RX Cartridge assemblies on the equilibrated Incubation Blocks for the chosen Incubation Time (not to exceed 60 minutes; see Enzyme Incubation, page 8).

NOTE: If glycans are to be labeled, now would be a good time to prepare the Instant Labeling Reagent.

Elute

9.a Remove the RX Cartridge assemblies from the Incubation Blocks.

NOTE: If condensation is apparent, spin at 300 x g *for 3 minutes and tap dish gently on the bench top to release Cartridges that may be stuck to the Lid.*

9.b Pipet 20 µl of Digestion Buffer into the sample cup of each RX Cartridge; do not remove Rack from Receiver Plate.

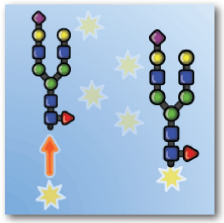
9.c Spin at 300 x *g* for 3 minutes.

9.d Remove Cartridges and Rack from the Receiver Plate. The eluted glycans are in the Receiver Plate; DO NOT DISCARD.

NOTE: Retain the RX Cartridges to recover the deglycosylated protein for further analysis (see Tips & Hints).

Proceed immediately to Labeling.

NOTE: Labeling with InstantDye requires the availability of reactive glycosylamine ends, such as those resulting from rapid digestion with N-Glycanase. Glycosylamine ends spontaneously hydrolyze over time to reducing ends which are incompatible with InstantDye chemistry. To maximize labeling efficiency, Labeling should be performed immediately following collection of the glycans from the GlykoPrep Digestion Module.



Label

InstantDye, used in excess, allows labeling at room temperature with >80% efficiency and no loss of sialic acid.

Overview

Label

Reagents and other Supplies

InstantAB Labeling Reagent (prepared blow)

N-Glycan Samples (eluted N-glycans with reactive glycosylamine ends, in a Receiver Plate)

Preparation of Reagents

Instant Labeling Reagent

NOTE: The InstantDye™ is hygroscopic; minimize exposure to air and protect from exposure to light. Should be prepared just prior to use. Reconstituted dye may be resealed, repackaged with the desiccant in the resealable bag, and frozen (-20° C) for storage up to 1 month; return to RT before opening for use.

InstantAB Dye (supplied with the kit)

Dye Solvent (supplied with the kit)

Add 375 µl of Dye Solvent directly into the InstantAB Dye vial.

Replace the cap and vortex the vial to ensure the dye is completely dissolved.

Sufficient for 48 samples; prepare both sets for a full kit.

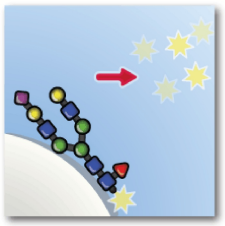
Procedure

Label

1. Pipet 5 µl of Instant Labeling Reagent into each well of N-glycan eluate in the Receiver Plate.

Glycans are now labeled and ready for cleanup.

NOTE: A precipitate may form in the labeling reaction over time. This precipitate is removed by Cleanup, though it may require longer spin times. To avoid this, proceed directly to Cleanup.



Cleanup

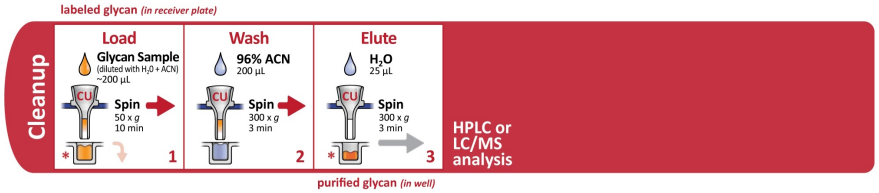
CU Cartridges allow most hydrophobic, non-glycan contaminants to be washed through; glycans are then eluted with water.

Overview

1 Load

2 Wash

3 Elute



Reagents and other Supplies

N-Glycan Samples from InstantAB Labeling

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%), 180 µl/sample

96% Acetonitrile Solution (prepared below)

Ultrapure water

Cleanup Collection Plate (supplied in the AM200 Starter Labware Set, or equivalent)

Preparation of Reagents

96% Acetonitrile Solution

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

Ultrapure water

Acetonitrile (100%, HPLC-grade)

To make 25 ml (enough for a full 96-well 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 100% acetonitrile. Transfer to a glass storage vessel, cap tightly and swirl gently to mix.

Procedure

NOTE: DO NOT use Receiver Plates in this procedure; build the stack with Waste Plates (~450 µl well volume) instead. This entire section is performed with the Cartridges "tips free."

Load

1.a Pipet 180 µl of 100% Acetonitrile into each well of glycan eluate in the Receiver Plate. Pipet up and down to mix.

1.b Transfer each N-Glycan Sample into the sample cup of a CU Cartridge. This must be done quickly because Acetonitrile has very low viscosity and may drip from the pipette tip; each sample may be pipetted in multiple rounds in order to achieve a quantitative transfer.

NOTE: Air bubbles are not a concern with this concentration of Acetonitrile..

1.c Spin at 50 x *g* for 10 minutes or until CU Cartridges are empty.

1.d Empty the Waste Plate.

NOTE: Discard waste containing acetonitrile according to waste disposal procedures.

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.

2.c Empty the Waste Plate.

Elute

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

NOTE: Because the eluate contains traces of organic solvent, polystyrene plates should NOT be used. Any glass or polypropylene ANSI/SBS 96-well microplate may 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 may 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 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 LABELED GLYCANS

Use standard techniques, such as Liquid Chromatography (LC), to analyze the aqueous eluate containing eluted, labeled glycans (see Tips & Hints, below).

REFERENCES

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

http://www.prozyme.com/glykoprep

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

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

TIPS & HINTS

Optimizing Excitation/Emission Wavelengths

The Optimal excitation/emission wavelengths for InstantAB Dye conjugated to an N-glycan are:

Excitation: 278 nm
Emission: 344 nm

Calculating the Mass of Glycans Labeled with InstantAB

The average mass of the InstantAB-labeled N-glycan is obtained using the following formula:

$$\text{Average Mass}_{\text{Glycan}} + 161.1$$

The monoisotopic mass of the InstantAB-labeled glycan is obtained using the following formula (result rounded to 4 decimal places):

$$\text{Monoisotopic Mass}_{\text{Glycan}} + 161.05891$$

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 plates (PCR plates or 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).

Recovery of the Deglycosylated Protein from the Digestion (RX) Cartridge

Often, the deglycosylated protein is analyzed to evaluate the completeness of deglycosylation using such electrophoretic methods as SDS-PAGE or microfluidic lab-on-a-chip technology. Please contact us for guidelines for eluting your glycoprotein from the RX Cartridge.

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**
FAX **(510) 638-6919**
E-MAIL **info@prozyme.com**
WEB **www.prozyme.com**

ProZyme values customer 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.

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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.

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