PROzyme®

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

GlykoPrep® Microfuge Method - Rapid N-Glycan Preparation with APTS

GlykoPrep Rapid N-Glycan Preparation with APTS (product codes GP24NG-APTS and GP96NG-APTS) using a microfuge and PCR heat block

TABLE OF CONTENTS

REQUIRED REAGENTS/EQUIPMENT

Centrifuge (capable of 50–1000 x g) and rotor for 1.5/2.0-ml microcentrifuge tubes

Heater and heat block capable of 50-100°C that accommodates 0.2-ml PCR tubes

Centrifugal evaporator (e.g., SpeedVac®) for drying released N-glycans prior to labeling

Vortexer

Fume hood

Glass, graduated cylinder, 25-ml

Vials, 1-ml, polypropylene for use with organic solvents

Ultrapure, deionized water (Milli-Q® or equivalent)

Acetonitrile

Pipettors & disposable tips (P5/P10, P200 and P1000)

Nitrile gloves

Required Labware (per sample)

1 x Cartridge Adaptor A from ProZyme, Product Code AM400 (3 x 8 ea)

1 x Cartridge Adaptor B from ProZyme, Product Code AM400 (3 x 8 ea)

NOTE: Adaptors are reusable. Do not discard.

1 x 0.5-ml Microtube, screw cap, Sarstedt part number 72.730.711 or equivalent

GlykoPrep Cartridges (supplied with each Kit)

1 x 0.2-ml PCR tube, domed cap, Axygen® part number PCR-02D-C or equivalent

2 x 1.75-ml Microcentrifuge tube, flip top, E&K Scientific part number 290175 or equivalent

NOTE: Some brands of PCR tubes form a tight seal between the tube and Cartridge. Do not use these brands.

SAFETY & HANDLING

Some of the reagents in the GlykoPrep Kits are hazardous. 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, nitrile gloves for all sample handling procedures. Ensure that all glass, plasticware and solvents are free of glycosidases and environmental carbohydrates.

All procedures involving APTS Labeling Reagent or its components should be performed in a dry environment with dry glassware and plasticware, using appropriate personal safety protection, eyeglasses and nitrile gloves, and where appropriate, in a fume hood.

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. Components are available individually as a Purification Module (optional), Digestion Module and dye-specific Labeling & Cleanup Modules.

GlykoPrep is built on AssayMAP technology, performed using centrifugation to move liquid through the Cartridges (spin format). The Microfuge Method is useful for those interested in using the spin format to run only a handful of samples. Using the spin format with a 96-well microplate and microplate centrifuge, up to 192 samples can be processed simultaneously with 2 Kits. GlykoPrep-plus employs the Syringe Head on the Agilent AssayMAP Bravo Liquid Handling Workstation to move liquid through the Cartridges, for automated high-throughput.

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

USING THE KITS

Preparation of Samples

Sample Quantities

The quantitative binding for each Cartridge is:

AssayMAP PA50 Cartridge	125 µg of MAb or Fc-fusion protein
RX Cartridge	50 µg of most standard proteins
CU Cartridge	30 µg of N-glycans

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.

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

Less than the maximum quantity may be processed, for example, when the sample is available only in limited amounts. The smallest amount of sample that will give good results depends on the sensitivity requirements of the analytical methods 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. 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), as in Example 1 below. More Denaturation Reagent may be used for problematic glycoproteins, as shown in Example 2 (sample to Denaturation Reagent ratio of 9:1).

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.

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

NOTE: The final denatured Sample must be at least 50% Denaturation Reagent.

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.

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

Digestion Time, Temperature and Finishing

Time

The Digest procedure has been optimized to deliver deglycosylation of N-glycans 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 (up to 60 minutes). For glycoproteins that are comparatively easy to deglycosylate, such as monoclonal antibodies, a 15-minute incubation is generally 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 may dry out, yielding uncertain results.

Temperature

The PCR heat block is set to 45°C for the Digest procedure (deglycosylation and optional Finishing step).

NOTE: Optimal temperatures for the Microfuge Method differ from those used with a microplate centrifuge.

Finishing

Finishing Reagent converts the glycosylamine produced by N-Glycanase digestion to a free reducing end, required for labeling with APTS via Rapid-Reductive-Amination™. The incubation time for Finishing is fixed at ten minutes.

Labeling Times & Temperatures

The Rapid-Reductive-Amination with APTS Labeling incubation time is 60 minutes. The labeling procedure requires the PCR heat block to be equilibrated at 50°C.

USE OF ADAPTORS AND CONSUMABLES

0.5-ml Screwcap Microtube

GlykoPrep Cartridge

0.2-ml PCR Tube

1.75-ml Microcentrifuge Tube

Cartridge Adaptor A

Cartridge Adaptor B

Most Standard Operations

Basic Assembly: Nest the RX Cartridge into Cartridge Adaptor A then into a 0.5-ml screw cap Microtube.

RX Cartridge (only)

Cartridge Adaptor A

0.5-ml screw-cap Microtube

Equilibrate, Prepare, Block, Wash, Load Sample (except Cleanup).

In this configuration, flow-through remains in contact with the tip of the RX Cartridge in the Microtube ("Tips Wet").

Operations with Special Requirements

"Tips Wet" Adaptor Assembly: Insert an RX Cartridge into a 0.2-ml PCR tube. Insert into a Cartridge Adaptor B and nest into a 1.75-ml, flip-top Microcentrifuge Tube.

RX Cartridge (only)

0.2-ml PCR tube

Cartridge Adaptor B

Flip-top, 1.75-ml Microcentrifuge Tube

Incubation Assembly: Perform all incubations (including N-Glycanase, Finishing and Labeling incubations) in a 0.2-ml capped PCR tube. Note that the RX Cartridge will be nested in the uncapped PCR tube for the N-Glycanase Digestion step.

RX Cartridge (only)

0.2-ml PCR tube

OR

0.2-ml capped PCR tube

Load N-Glycanase and Elute (Digestion steps 7 & 9)

In this configuration, flow-through remains in contact with the tip of the RX Cartridge inside the 0.2-ml PCR tube ("Tips Wet").

- For N-Glycanase Digestion: when ready to Incubate, remove the RX Cartridge with the 0.2-ml PCR tube from the "Tips Wet" Assembly and place them on the PCR heat block. When the incubation is complete, return the RX Cartridge/PCR tube to the "Tips Wet" Assembly for elution of N-glycans.
- For Finishing (Rapid-Reductive-Amination with 2-AB or APTS protocols): after elution with Finishing Reagent, remove the 0.2-ml PCR tube that holds the eluted N-glycan Sample Replicates from the "Tips Wet" Adaptor Assembly, cap it and place it on the PCR heat block to incubate. The N-glycans are then dried in the same PCR tube.
- For Labeling Incubation: add Labeling Reagent directly to the 0.2-ml PCR tube with the N-glycans and perform incubation as instructed.

"Tips Free" Assembly:

CU Cartridge (only, no PCR tube)

Cartridge Adaptor B

Flip-top, 1.75-ml Microcentrifuge tube

For superior removal of free dye, the Cleanup procedure requires that the tip of the Cartridge not be in direct contact with the flow-through at the bottom of the microtube. This is accomplished by placing an adaptor on a 1.75-ml Microcentrifuge tube and then positioning the CU Cartridge on top.

Add the N-glycan Sample to the Sample Cup of the CU Cartridge in the "Tips Free" Assembly. Load the Assembly into the Microcentrifuge, balance and spin as instructed.

Keep the Cartridge in the "Tips Free" Assembly to Wash and Elute, but prior to eluting the labeled N-glycans from the CU Cartridge, transfer the Adaptor and CU Cartridge to a fresh 1.75-ml Microcentrifuge tube.

PROTOCOLS

Getting Started

Heater Setting

Turn on the heat block. Set to 45°C and allow to equilibrate for a minimum of 1 hour.

Centrifuge Settings

Convert the required RCF values (i.e., 50 x g, 100 x g, 300 x g and 1000 x g) to the microcentrifuge's RPM settings based on the radius of the rotor to be used.

Many microcentrifuges allow direct setting of RCF values.

_____ rpm = 50 x g

_____ rpm = 100 x g

_____ rpm = 300 x g

_____ rpm = 1000 x g

Preparation of Reagents

Glycoprotein Samples

This protocol begins with purified Glycoprotein Samples (see page 5 for details). Glycoprotein Samples must not contain any particulates, as they will plug the top frit, or sit on the top of the resin bed and impede the flow. Spin samples to remove particulates before processing.

Digestion Buffer

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.

Prepare 100 µl of Digestion Buffer for each sample to be processed. For example, for 10 samples add 40 µl of 25x Digestion Buffer to 960 µl of ultrapure water to make 1 ml of Digestion Buffer.

Cap tightly and vortex on high for 10 seconds to mix.

Enzyme Solution

Prepare only on the day of use. Store at RT.
Spin the N-Glycanase briefly to collect the contents in the base of the vial. Vortex the solution prior to dispensing.

N-Glycanase (supplied with the Digestion Module)

Digestion Buffer (prepared previously)

0.6-ml microcentrifuge vial

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 75 +15 = 90 µl of Digestion Buffer.

Labeling Reagent

For instructions to prepare APTS Labeling Reagent, please see Label, page 14

Digest

Reagents and other Supplies

Glycoprotein Samples

RX Cartridges (supplied with the Kit, 1 per sample)

0.5-ml Microtube, screw cap, 0.2-ml PCR tubes, Cartridge Adaptors A and B and flip-top, 1.75-ml Microcentrifuge tubes

Denaturation Reagent (supplied with the Kit)

Acetonitrile (100%, HPLC-grade), ~2 ml

Blocking Reagent (supplied with the 2- Kit)

Digestion Buffer (prepared previously)

Enzyme Solution (prepared previously)

Finishing Reagent (optional, supplied with the GlykoPrep 2-AB and APTS Kits)

GlykoPrep Digestion Module (product codes GS24-RX and GS96-RX)

Procedure

Denature

1.a Add Denaturation Reagent to each Sample as described in Sample Denaturation (page 5).

The Denaturation Reagent is viscous and needs to be mixed well.

1.b Pipet up and down to mix well.

1.c Allow to incubate at room temperature for at least 5 minutes.

Prepare

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

2.a Prepare a Basic Assembly for each sample by nesting an RX Cartridge into Cartridge Adaptor A then into a 0.5-ml screw cap Microtube.

2.b Pipet 50 µl of 100% Acetonitrile into the Sample Cup of each RX Cartridge in the Basic Assemblies.

2.c Place the Basic Assemblies in the centrifuge and spin at 300 x g for 3 minutes.

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.

Load

4.a Load 100 µl of the denatured Samples into the Sample Cup of each RX Cartridge (see Sample Denaturation, page 5).

4.b Empty the flow-through by lifting each RX Cartridge and pouring out the liquid collected in the Microtube below. Dispose of the liquid as organic waste and return each RX Cartridge to its Microtube.

4.c Spin at 50 x g until all Sample Cups are empty (~5-10 minutes).

Block

5.a Pipet 50 µl of Blocking Reagent into the Sample Cup of each RX Cartridge.

5.b Empty the flow-through (as described in 4.b).

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

Wash

6.a Pipet 50 µl of Digestion Buffer into the Sample Cup of each RX Cartridge.

6.b Empty the flow-through (as described in 4.b).

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

Load N-Glycanase

7.a Prepare a clean, 0.2-ml PCR tube for each Sample.

7.b Prepare three "Tips Wet" Adaptor Assemblies by nesting the PCR tubes into Cartridge Adaptor B and 1.75-ml Microcentrifuge tubes.

7.c Pipet 10 µl of Enzyme Solution into the Sample Cup of each RX Cartridge in the Basic Assemblies.

7.d Transfer the RX Cartridges into the corresponding "Tips Wet" Adaptor Assemblies. Dispose of Microtubes and flow-through from 6.

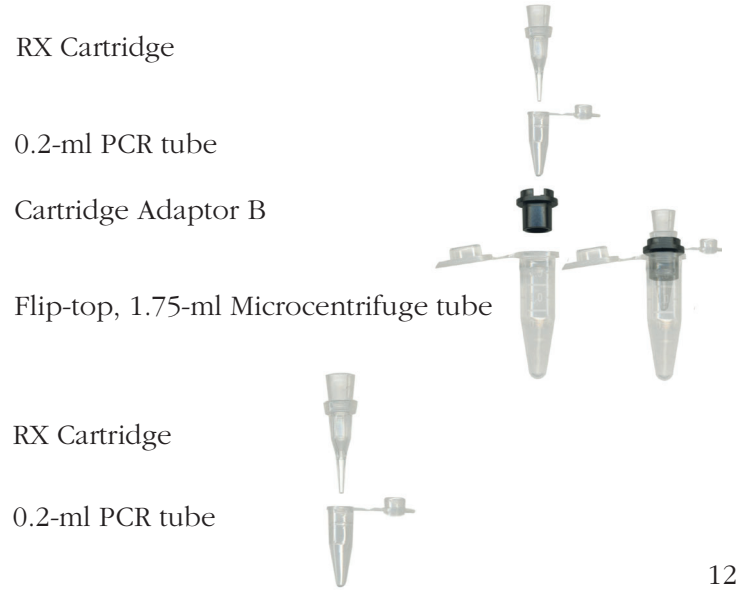
7.e Spin at 300 x g for 3 minutes; do not discard flow-through.

Incubate

8. Transfer the RX Cartridges/PCR tubes (Incubation Assembly) to the equilibrated 45°C PCR heat block and incubate for the chosen incubation time (see page 6).

Use the special sample loading technique to load samples in all protocols to prevent the introduction and entrapment of air bubbles in the neck of the Sample Cup. Use a pipet to remove trapped air bubbles.

Check that Sample Cups are empty before proceeding or yield will be reduced.



Elute (and Finish)

9.a Remove the Incubation Assemblies from the heat block and reinsert into the Microcentrifuge tubes to form "Tips Wet" Adaptor Assemblies again.

9.b Pipet 15 µl of Finishing Reagent into the Sample Cup of each RX Cartridge.

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

9.d Remove RX Cartridges from the PCR tubes. The eluted N-Glycans are in the PCR tubes; DO NOT DISCARD.

Used RX Cartridges may be discarded.

10. Close the cap on each PCR tube and incubate on the equilibrated 45°C heat block for 10 minutes.

0.2-ml capped PCR tube

11. Open the PCR tubes, return them to the "Tips Wet" Adaptor Assemblies (now minus the RX Cartridges). Dry in a centrifugal evaporator (SpeedVac, heat setting turned to the off position) until fully dry (~30 minutes).

After removing the PCR tubes from the heat block, adjust the temperature setting to 50°C.

The N-glycans are condensed/spun into a pellet small enough to be dissolved by 4.5 µl of APTS Labeling Reagent in the next step.

Label

GlykoPrep Rapid-Reductive-Amination APTS Labeling Module (product codes GS24-APTS and GS96-APTS)

Reagents and other Supplies

N-Glycan Samples (dried N-glycans in 0.2-ml PCR tubes)

APTS Labeling Reagent (prepared just prior to use)

Preparation of APTS Labeling Reagent

APTS Labeling Reagent must be prepared just prior to use.

APTS Solution (supplied with the Kit)
APTS Catalyst (supplied with the Kit)

Reductant Solution (supplied with the Kit)
Aluminum Sealing Film (supplied with the Kit)

Catalyst and Reductant Solutions are hygroscopic; minimize exposure to air and protect from exposure to light.

Just prior to use, allow the APTS Solution, APTS Catalyst and Reductant Solution to come to room temperature in the sealed desiccant bag. Then invert the vials to mix.

In a separate vial, prepare a master mixture of 1.0 µl of APTS Solution, 2.5 µl of APTS Catalyst, and 1.0 µl of Reductant Solution for each sample to be processed, plus 20% for overage. For example, 10 samples would require 10 + 2 = 12 µl of APTS Solution, 25 + 5 = 30 µl of APTS Catalyst and 10 + 2 = 12 µl of Reductant Solution.

NOTE: The APTS Labeling Reagent components are hazardous. Please refer to the Safety Data Sheets on our website. Perform this procedure using appropriate personal safety protection, eyeglasses and nitrile gloves.

Return any unused APTS Solution, Reductant Solution and Catalyst to the desiccant-containing bag and store at -20°C. Do not reuse the mixed APTS Labeling Reagent.

Cap tightly and vortex on high for 10 seconds to mix.

The Labeling Reagent (APTS Solution + APTS Catalyst + Reductant) will generate bubbles over time, so spin down in a centrifuge and use immediately. If the solution begins to bubble again during use, spin down again.

Procedure

Label

1.a Add 4.5 µl of APTS Labeling Reagent to each N-Glycan Sample.

1.b Return the PCR tubes to the "Tips Wet" Adaptor Assemblies (minus the RX Cartridges) and spin at 300 x g for 1 minute to ensure the liquid is collected at the bottom of the wells.

Incubate

2.a Close the cap on each PCR tube and transfer it to the heat block.

2.b Incubate at 50°C on the equilibrated heat block for 1 hour.

2.c Remove the PCR tubes from the heat block and allow to cool to room temperature (~5 minutes).

2.d In a fume hood, open each PCR tube.

Before use, be sure each heat block has equilibrated to 50°C; a thermometer may be placed in the corner thermometer well of the heat block to test the temperature.

This is a good time to prepare the APTS Sample Load Buffer; directions may be found in the next section, Cleanup.

It is normal for condensate to collect on the underside of the lid. DO NOT centrifuge the tube to collect the condensate. Proceed immediately to Cleanup.

Cleanup

GlykoPrep APTS Cleanup Module (product code GS24-C2 and GS96-C2)

This entire section is performed with the CU Cartridges "Tips Free."

Reagents and other Supplies

N-Glycan Samples from APTS Labeling (in PCR tubes)

CU Cartridges (supplied with the APTS GlykoPrep Kit, 1 per N-glycan sample)

AssayMAP Cartridge Adaptors and flip-top, 1.75-ml Microcentrifuge tubes, 2 per sample

Acetonitrile (100%)

5x APTS Sample Load Buffer (supplied with the APTS GlykoPrep Kit)

Ultrapure water

Volumetric pipettes, 5-ml

Glass graduated cylinder, 10-ml

Screw cap, glass storage vessel, 10-ml

0.2-ml capped PCR tube

DO NOT use standard air-displacement pipettes to measure Acetonitrile.

Due to the viscosity and surface tension of the 5x APTS Sample Load Buffer, we recommend only using glass graduated cylinders.

Preparation of APTS Sample Load Buffer

5x APTS Sample Load Buffer

Acetonitrile

Prepare 1 ml of APTS Sample Load Buffer for each sample to be processed by diluting one volume of 5x APTS Sample Load Buffer with 4 volumes of 100% acetonitrile.

For example, to make 10 ml of APTS Sample Load Buffer, add 2 ml of 5x APTS Sample Load Buffer to a glass graduated cylinder. Bring the volume up to 10 ml with HPLC-grade acetonitrile using a volumetric pipette. Transfer to a glass storage vessel, cap tightly and swirl thoroughly to ensure complete dissolution.

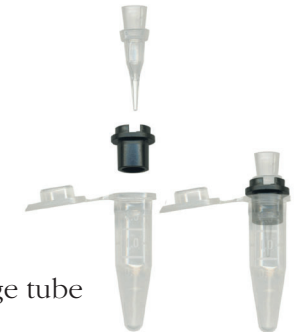
It is important to prevent evaporation of the Acetonitrile which would affect performance of the HILIC separation.

Procedure

Prepare one “Tips Free” Assembly per Sample Replicate by nesting a CU Cartridge into Cartridge Adaptor B and a 1.75-ml Microcentrifuge tube.

CU Cartridge

Cartridge Adaptor B



Flip-top, 1.75-ml Microcentrifuge tube

Load

1.a Add 200 µl of APTS Sample Load Buffer to each N-glycan Sample in the PCR tubes. Pipet up and down to mix.

Transfer each N-glycan Sample into the Sample Cup of a CU Cartridge in a “Tips Free” Assembly.

1.b Spin at 300 x g for 3 minutes or until the Sample Cup of each CU Cartridge is empty.

Transfer the mixture as quickly as possible because Acetonitrile solution 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.

Wash

2.a Pipet 200 µl of APTS Sample Load Buffer into the Sample Cup of each CU Cartridge in the “Tips Free” Assembly.

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

Wash (second time)

3.a Pipet 200 µl of APTS Sample Load Buffer into the Sample Cup of each CU Cartridge in the “Tips Free” Assembly.

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

NOTE: Discard waste containing acetonitrile and APTS Labeling Reagents according to waste disposal procedures.

Elute

4.a Transfer each CU Cartridge with its Adaptor to the new 1.75-ml Microcentrifuge Microcentrifuge tube.

4.b Pipet 100 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.

4.c Spin at 100 x g for 10 minutes.

4.d Spin at 1000 x g for 2 minutes.

Used CU Cartridges may be discarded. Adaptors are reusable, DO NOT DISCARD.

NOTE: Initial, slow spin maximizes removal of N-glycans; second, fast spin elutes all liquid from the CU Cartridge.

The 1.75-ml Microcentrifuge tubes now contain the APTS-labeled N-Glycans with free dye and buffer salts removed; DO NOT DISCARD.

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

ANALYSIS OF LABELED N-GLYCANS

CE Analysis

The APTS label is optimized for analysis using Capillary Electrophoresis (CE). We recommend using PVA coated capillaries, and CE separations using an applied electric field of 600 V/cm.

CE-MS

Mass spectrometry and various types of spectroscopic methods may also be used to analyze glycans labeled with APTS (see Tips and Hints, below). The label is stable under 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 APTS-labeled glycans to strongly acidic or alkaline conditions.

The MW of the APTS-labeled glycan is:

MW_{Glycan} + 440.9647

TIPS & HINTS

Optimizing Excitation/Emission Wavelengths

APTS-labeled glycans may also be analyzed by spectroscopic methods other than CE (i.e., HPLC) using these wavelengths:

Excitation λ: 473 nm

Emission λ: 520 nm

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.

TRADEMARKS AND TRADENAMES

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 GlykoPrep® Rapid Sample Preparation Platform:

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

3832 Bay Center Place
Hayward, CA 94545-3619
USA

TOLL FREE (800) 457-9444
PHONE (510) 638-6900
FAX (510) 638-6919
E-MAIL info@prozyme.com
WEB www.prozyme.com
Rev. AB