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

Rapid N-Glycan Preparation with APTS

Enzymatic deglcosylation, fluorescent labeling with APTS and cleanup of excess dye for analysis by Capillary Electrophoresis.

- Optional sample purification for monoclonal antibodies and Fc-fusion proteins
- Non-selective, rapid release and recovery of N-Glycans from up to 96 glycoprotein samples at a time using a microplate centrifuge
- Optimized reaction conditions help to preserve labile glycans, such as sialic acid
- Non-selective chemistry for stoichiometric labeling of glycans, independent of structure
- Complete labeling in 1 hour using Rapid-Reductive-Amination™
- Purified APTS-labeled N-glycans are eluted in water, ready for analysis
- 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: GP96NG-APTS

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-APTS GlykoPrep® Rapid N-Glycan Preparation with APTS	
GS96-RX GlykoPrep Digestion Module	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 (2 x 1 ml)	
Aluminum Sealing Film (4)	
GS96-APTS GlykoPrep Rapid-Reductive-Amination APTS Labeling Module	1 ea
WS0299 APTS Solution (130 µl)	
WS0300 Reductant Solution (325 µl)	
WS0295 APTS Catalyst (325 µl)	
GS96-C2 GlykoPrep APTS Cleanup Module	1 ea
WS0301-GP 5x APTS Sample Load Buffer (48 ml)	
WS0263 Cleanup (CU) Cartridges (96 Cartridges)	
Aluminum Sealing Film (2)	

Storage Requirements

This Kit is a mixed-temperature shipment (2–30°C). Upon arrival, store components as indicated. For best results, equilibrate materials to ambient temperature prior to use. The APTS Solution is light-sensitive and the Reductant Solution and Catalyst are hygroscopic; please store these reagents at -20°C in their original packaging.

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
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) and Gilson Diamond® D200 Pipet Tips

NOTE: Labware to use with 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)
Vortexer or plate shaker
Nitrile gloves
Vial, 1-ml, polypropylene for use with organic solvents
Fume hood (for Labeling procedure)
Miscellaneous labware for buffers and dilutions

Microplate-compatible, centrifugal evaporator (e.g., SpeedVac® or similar) for drying finished N-glycans prior to labeling. A rotor that holds two shallow-well microplates and fits into a small-capacity rotary evaporator may be purchased from Thermo Fisher Scientific (Savant Model RH2MP).
Glass graduated cylinder, 100 ml

Optional Reagents and Supplies

Multichannel pipettors & disposable tips (P5/P10 and P200) (Gilson or equivalent, compatible with Gilson Diamond® D200 pipette tips)
Microplate reader (capable of reading A_{280}) for measurement of antibody concentration after Purification
Pipette basins (must be polypropylene for use with organic solvents)

SAFETY AND HANDLING

Some of the reagents in this Kit are hazardous. 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

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 Labeling Reagents (APTS Solution, Reductant Solution and Catalyst) 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. 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/APTS_Microfuge_Method.pdf

USING THE KIT

GlykoPrep Rapid N-Glycan Preparation with APTS combines the Digestion Module, the Rapid-Reduction-Amination APTS Labeling Module and the Cleanup Module, which may be purchased individually. The Labeling and Cleanup Modules may be purchased together as GP96-APTS. 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 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.

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 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 will dry out, yielding uncertain results.

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.

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.

NOTE: If using the Microfuge Method format, PCR heat block substitutes for the GS150 Heater (with Incubation Blocks).

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

Labeling Incubation

Time

The APTS labeling incubation is fixed at 60 minutes.

Temperature

The Labeling procedure also requires the Incubation Blocks to be equilibrated at 50°C (~37°C in the Cartridge).

PROTOCOLS

Overview of the Procedure

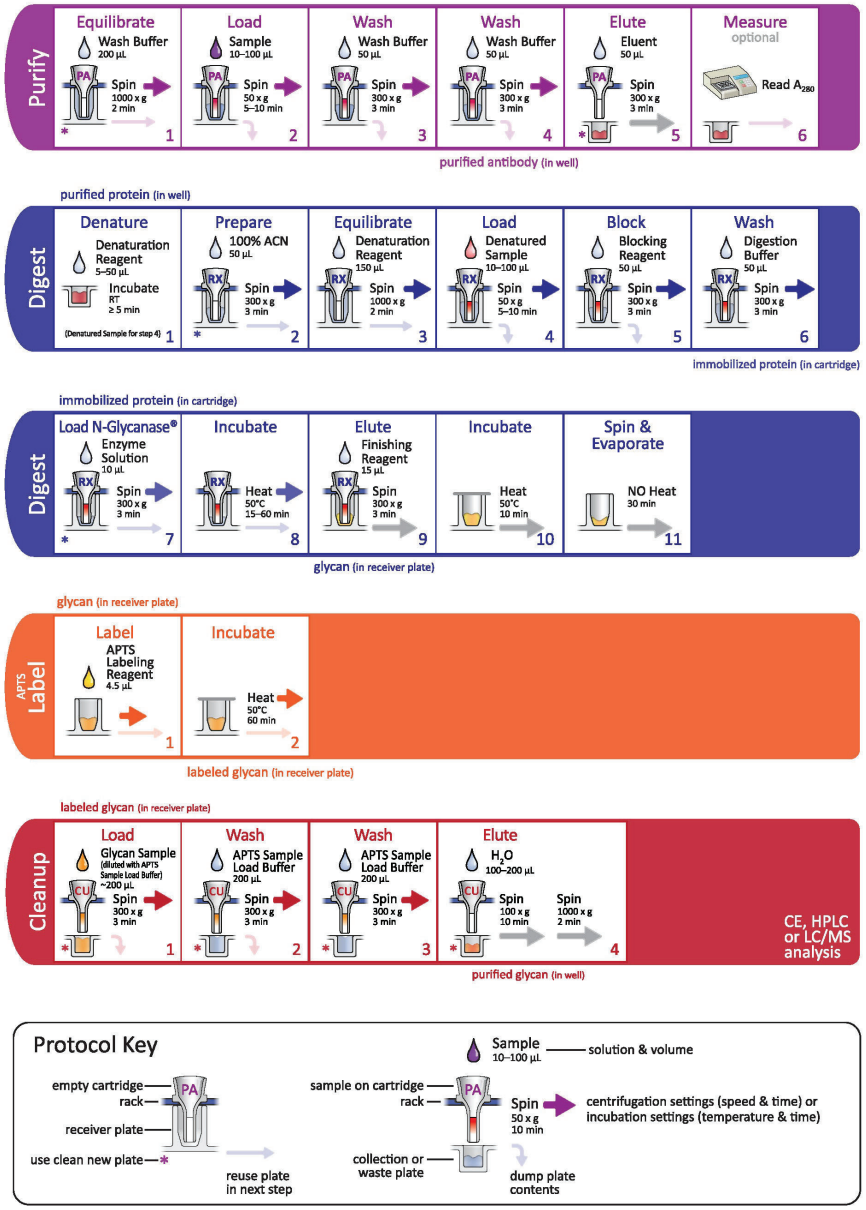
Purify (optional, purchased separately)
Antibodies or Fc-fusion proteins may be purified from crude samples using Protein A.

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

N-glycans are released by N-Glycanase® digestion and eluted, finished and dried prior to labeling.

Label
Dried N-glycans are labeled with APTS by Rapid-Reductive-Amination.

Cleanup
Buffer salts and excess labeling reagents are removed; labeled N-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

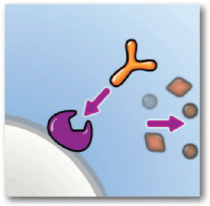
If the centrifuge does not have x g settings, determine the setting and the specific microplate rotor combination by consulting the operation manual or the manufacturer's website.

_____ rpm = 50 x g

_____ rpm = 100 x g

_____ rpm = 300 x g

_____ rpm = 1000 x g



Purify *(optional)*

Protein A purifies 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

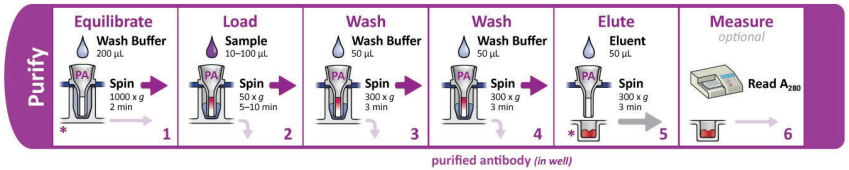
2 Load

3 Wash

4 Wash (second time)

5 Elute

6 Measure (optional)



Reagents and Other Supplies

AssayMAP PA50 (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 may interfere with the CE electropherogram.

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 (supplied with the Kit)
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 the Sample Cup of each PA Cartridge (see Sample Loading Technique in the GlykoPrep Guidebook).

2.b Remove the Racks from the Receiver Plates. Empty the Receiver Plates 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.

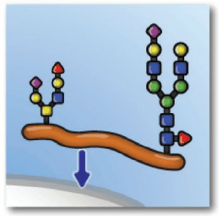
5.b Pipet 50 µl of Eluent into the Sample Cup of each PA Cartridge.

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

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

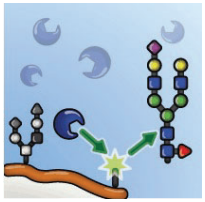
Measure (optional)

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



Digest

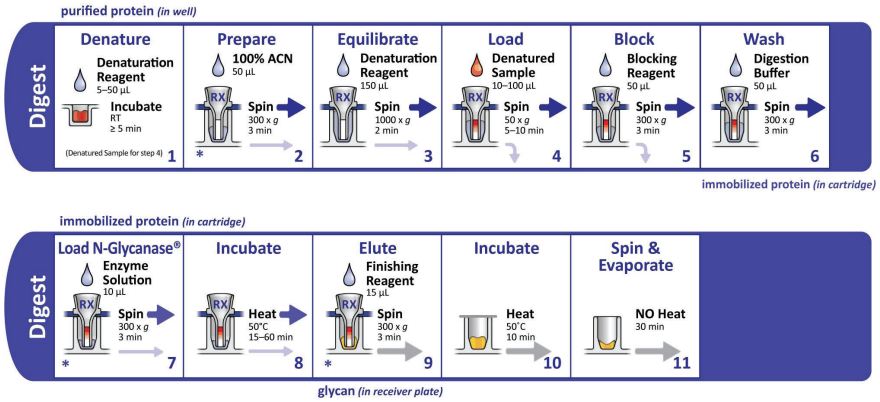
Samples (antibodies or other glycoproteins) are denatured and immobilized.



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

Overview

1	Denature
2	Prepare
3	Equilibrate
4	Load
5	Block
6	Wash
7	Load N-Glycanase
8	Incubate
9	Elute (and Finish)
10	Incubate
11	Spin & Evaporate



Reagents and other Supplies

Glycoprotein Samples

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)
Finishing Reagent (supplied with the Kit)
Aluminum Sealing Film (supplied with the Kit; film may be cut with scissors if using less than the full 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.

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

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 to collect the contents in the base of the vial, and pipet up and down several times to mix prior to use.

In a separate vial, prepare a mixture of 3 µl of N-Glycanase and 9 µl of Digestion Buffer for each sample to be processed.

To prepare 96 samples, add 288 µl of N-Glycanase to 864 µl of 1x Digestion Buffer in a pipette basin. Pipet up and down several times to mix.

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 the Sample Cup of 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 containing acetonitrile 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 (Heater setting 50°C) for the chosen Incubation Time (not to exceed 60 minutes; see Time and Temperature, page 9).

Elute (and Finish)

9.a Remove the RX Cartridge assemblies from the Incubation Blocks. Keep the heat on for Incubate step.

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

9.b Pipet 15 µl of Finishing Reagent 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 N-glycans are in the Receiver Plate; DO NOT DISCARD.

Incubate

10. Cover the Receiver Plate with Aluminum Sealing Film and incubate on the equilibrated Incubation Block (with Heater still set at 50°C) for 10 minutes.

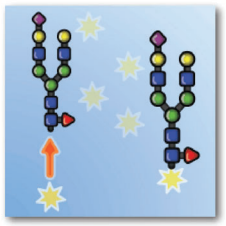
Spin & Evaporate

11. Remove the Aluminum Sealing Film and dry the N-Glycans in a centrifugal evaporator (SpeedVac, heat setting turned to the off position) for 30 minutes or until fully dry. This condenses N-glycans into a pellet small enough to be dissolved by 4.5 µl of Labeling Reagent in the next step.

NOTE: The Finishing Reagent must be removed promptly by drying. Store N-glycans at -20 or -80°C in the dark if further processing will not take place immediately.

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

NOTE: If N-glycans are to be labeled, now would be a good time to bring the APTS Solution, Reductant Solution and APTS Catalyst to room temperature.



Label

APTS Labeling Reagent is optimized to label via reductive amination with high labeling efficiency in just one hour.

Overview

Label

Incubate

Reagents and other Supplies

N-Glycan Samples (≤30 µg of dried N-glycans with a free reducing end in a Receiver Plate)
Aluminum Sealing Film (supplied with the Kit; film may be cut with scissors if using less than the full Kit)
APTS Labeling Reagent (prepared just prior to use)

Preparation of Reagents

APTS Labeling Reagent

NOTE: APTS Labeling Reagent must be prepared just prior to use, after drying the N-Glycan Samples.

NOTE: The APTS Labeling Reagent components are hazardous. Please refer to the Safety Data Sheets included in the Kit or on our website.

NOTE: APTS Solution, Reductant Solution and APTS Catalyst are hygroscopic; minimize exposure to air and protect from exposure to light. These reagents may be repackaged with desiccant in a resealable bag and frozen (-20°C) for storage up to 6 months; return to RT before opening for use.

APTS Solution (supplied with the Kit)
APTS Catalyst (supplied with the Kit)
Reductant Solution (supplied with the Kit)
Vial, 1-ml, for use with organic solvents

At the time of use, allow the APTS Solution, Reductant Solution and APTS Catalyst vials to come to room temperature in the sealed desiccant bag before removing them. Before opening each vial, flick it or gently tap it on a flat surface to dislodge any liquid adhering to the underside of the cap and ensure that the contents collect at the bottom.

NOTE: Because the viscosity and high surface tension of the APTS Labeling Reagent prevent it from spreading out in a pipette basin, do not use a basin for this mixture. If using a multichannel pipette, one column of a clean, empty Receiver Plate or other 96-well plate compatible with organic solvents may be used to hold APTS Labeling Reagent, which may then be dispensed into rows of the N-Glycan sample plate.

In a separate vial, prepare a master mixture of 1.2 µl of APTS Solution, 3.0 µl of APTS Catalyst, and 1.2 µl of Reductant Solution for each sample to be processed.

Cap tightly and vortex on high for 10 seconds to mix; briefly spin down in a centrifuge.

NOTE: 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.

Tightly cap the APTS Solution, Reductant Solution and APTS Catalyst vials, return them to the desiccant-containing bag and store at -20°C.

Procedure

NOTE: Perform this procedure using appropriate personal safety protection, eyeglasses and nitrile gloves.

Label

1a. Add 4.5 µl of APTS Labeling Reagent to the side of each well containing dried N-Glycan sample. Gently tap the plate on the counter to force the droplet down to the bottom of the well.

1b. Seal the plate with Aluminum Sealing Film. Vortex the plate on high for 1 minute to mix and centrifuge (1 minute at 1000 x g) to ensure the samples are collected at the bottom of the wells.

Incubate

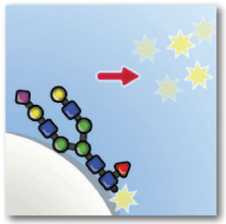
2a. Ensure that each labeling reaction is sealed using the Aluminum Sealing Film to prevent drying and incubate at 50°C on the Incubation Blocks for 1 hour.

NOTE: This is a good time to prepare the APTS Sample Load Buffer for Cleanup.

2b. Remove the Receiver Plate from the Incubation Blocks and allow to reach room temperature (5 minutes).

2c. In a fume hood, remove the Aluminum Sealing Film. It is normal for condensate to collect on the underside of the film. DO NOT centrifuge the plate to collect the condensate.

Proceed immediately to Cleanup.



Cleanup

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

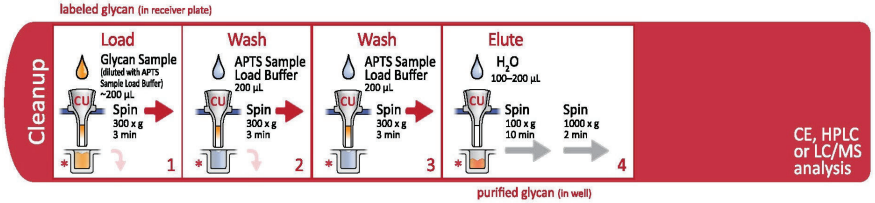
Overview

1 Load

2 Wash

3 Wash (second time)

4 Elute



Reagents and other Supplies

N-Glycan Samples from APTS Labeling (≤30 µg of N-glycans in 4.5 µl APTS Labeling Reagent)
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)
APTS Sample Load Buffer (1 ml for each sample, prepared below)
Ultrapure water
Volumetric pipettes
Glass graduated cylinder, 100 ml
Capped glass storage vessel, 100 ml

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

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

Preparation of Reagents

APTS Sample Load Buffer

NOTE: Prepare the APTS Sample Load Buffer on the day of use. Store sealed in a clean glass container until cleanup step to prevent evaporation of acetonitrile. Precise control of the acetonitrile concentration is important to ensure both retention of N-glycans and removal of excess dye during Cleanup.

Ultrapure water
Acetonitrile (100%, HPLC-grade)
5x APTS Sample Load Buffer
Volumetric pipette
Glass graduated cylinder, 100 ml
Capped glass storage vessel, 100 ml

To make 100 ml of APTS Sample Load Buffer, add 20 ml of 5x APTS Sample Load Buffer to a glass graduated cylinder. Bring the volume up to 100 ml with HPLC-grade acetonitrile using a volumetric pipette.

NOTE: DO NOT use standard air-displacement pipettes to measure acetonitrile.

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

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 CU Cartridges "tips free."

Load

1.a Add 200 µl of APTS Sample Load Buffer to each N-Glycan Sample. Pipet up and down several times 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 300 x g for 3 minutes or until the Sample Cup of each CU Cartridge is empty.

1.d Empty the Waste Plate.

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

NOTE: For best results, rotate the Waste Plate to use clean wells for the next step, or use a new Waste Plate.

Wash

2.a Pipet 200 µl of APTS Sample Load Buffer into the Sample Cup of each CU Cartridge.

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

2.c Empty Waste Plate.

NOTE: For best results, rotate the Waste Plate to use clean wells for the next step, or use a new Waste Plate.

Wash (second time)

3.a Pipet 200 µl of APTS Sample Load Buffer into the Sample Cup of each CU Cartridge.

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

Elute

4.a Place each racked set of CU Cartridges over a clean Cleanup Collection Plate. Refer to Tips and Hints for collection in PCR tubes in the rack for use with Beckman Coulter® CE instruments.

NOTE: Because the eluate contains traces of organic solvent, polystyrene plates should NOT be used. Any 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.

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 N-glycans are desired, or if the CE system requires a larger volume.

NOTE: To protect N-glycans for long-term storage, an aqueous solution compatible with the intended analysis method (e.g., 0.05% Sodium Azide in water) may be used instead of water.

4.c Spin on Cleanup Collection Plates at 100 x g for 10 minutes.

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

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

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

Mix the eluate by pipette action or vortexing prior to analysis to ensure homogeneity. 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

Use standard techniques, such as Capillary Electrophoresis (CE), to analyze the aqueous eluate containing eluted, labeled N-glycans (see Tips and Hints, below).

TIPS & HINTS

Collection Plates for Direct CE Analysis

AssayMAP Racks will hold 200 µl PCR collection tubes for use directly in the Beckman Coulter PA 800 plus Pharmaceutical Analysis System. To collect in PCR tubes, load one tube in the Rack corresponding to the location of each Cartridge in the elution plate. Stack the elution plate on top of the Rack with PCR tubes and follow elution from step 4b. For best results, elute samples in 100 µl minimum of DI water to avoid further dilution prior to CE analysis. Ensure that the eluted samples are sufficiently mixed prior to CE.

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.

Calculating the Mass of Glycans Labeled with APTS

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

$$Mass_{Glycan} + Mass_{APTS} = Mass_{APTS\text{-}Labeled\ Glycan}$$

Mass Added to Glycan	
Monoisotopic	440.96468
Average	441.5

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. 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.

Other Analytical Methods

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

Excitation λ: 473 nm
Emission λ: 520 nm

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

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

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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