



GlykoPrep[®]-plus Rapid N-Glycan Sample Preparation with InstantAB Automated on AssayMAP Technology

An automated, highly robust, high-throughput process for enzymatic deglycosylation, fluorescent labeling with InstantAB and cleanup of excess dye for analysis by LC and other methods.

- Non-selective, rapid release and recovery of N-glycans from up to 96 glycoprotein samples at a time on the Agilent AssayMAP Bravo Liquid Handling Platform (AssayMAP Bravo)
- Minimal hands-on time
- Optimized reaction conditions help to preserve labile glycans, such as sialic acid
- Non-selective chemistry for stoichiometric labeling of glycans, independent of structure
- RT InstantAB labeling with no loss of sialic acid; labeling efficiency > 80%
- Purified InstantAB-labeled N-glycans are eluted in water, ready for analysis

Product Code: GPPNG-LB

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This product is intended for *in vitro* research use only.

NOTE: The following suggestions and data are based on information we believe to be reliable. They are offered in good faith, but without guarantee, as conditions and methods of use of our products are beyond our control. We recommend that the prospective user determine the suitability of our materials and suggestions before adopting them on a commercial scale. Suggestions for use of our products or the inclusion of descriptive material from patents and the citation of specific patents in this publication should not be understood as recommending the use of our products in violation of any patent or as permission to license to use any patents of ProZyme, Inc.

EQUIPMENT

The AssayMAP Bravo and associated equipment are available from Agilent Technologies.

AssayMAP Bravo Liquid Handling Workstation (AssayMAP Bravo, p/n G5542A)

Bravo 96 AM Head (p/n G5498B#046)

Peristaltic Pump Module 2.0 (p/n G5498B#058) linked to 96AM Wash Station (p/n G5498B#057)

Peltier Thermal Station - with STC Controller (p/n G5498B#035)

Cartridge Seating Station (aka Tips Loading Station)

Orbital Shaking Station w/ Control Unit (p/n G5498B#033)

96AM Cartridge & Tip Loading Station (p/n G5409-20025)

Gripper upgrade (p/n 16545-101)

Risers, 146 mm (p/n G5498B#055)

Custom Plate Nest (p/n G5498B#017)

PCR Plate Insert (p/n G5498B#013)

Carboy to feed pump & collect waste (2 ea, G5550-17725)

Lab tape (to tape the wash station, RX/CU Cartridge Racks and Receiver Plate to the deck)

SOFTWARE AND MANUALS

For this protocol you should be running the latest version of Agilent VWorks

"GlykoPrep-*plus* Full App Installer with N-Glycan Sample Prep:

RX digestion & InstantAB labeling v1.0 or newer

The latest version of this Instruction Manual may be found here:

www.prozyme.com/~prozyme/documents/GPPNG-LB_Instruction_Manual.pdf

HOW TO USE THIS BOOKLET

We want successful results for our customers, so please read this entire booklet before starting the procedure, and follow the detailed instructions.

Once familiar with the AssayMAP Bravo and GlykoPrep-*plus* protocols, experienced users may wish to use the streamlined QuickGuide section beginning page 28.

Comments

AssayMAP Bravo must be installed, calibrated, and set up with the GlykoPrep layout and recommended software.

Print the QuickGuide separately, using Portrait Orientation.

KIT CONTENTS

GPPNG-LB Kit GlykoPrep-*plus* Rapid N-Glycan Sample Preparation with InstantAB

1 ea

GSP-RX GlykoPrep-*plus* Digestion Module

WS0253 RX Cartridges

WS0256-GP Immobilization Reagent Set

WS0226-GP Denaturation Reagent (35 ml)

WS0255-GP Blocking Reagent (14 ml)

WS0259-GP Digestion Reagent Set

WS0229-GP Finishing Reagent (10 ml)

WS0276-GP 25x Digestion Buffer (1.3 ml)

WS0278-GP N-Glycanase (700 µl)

1 ea

GSP-LB InstantAB Labeling Module

WS0224-GP InstantAB Dye (2 ea)

WS0260-GP Dye Solvent (2 ea, N,N-Dimethylformamide)

GS96-CU Cleanup Module

1 ea

Labware Set from ProZyme (product code AM96-NG when purchased alone)

WS0304 1 ea, 12-Column, Reservoir Plate (5-pack)

WS0305 4 ea, PCR Plate

WS0306 2 ea, Square, Deep-Well Plate

WS0307 1 ea, Pipette-Tip Box

WS0308 1 ea, U-Bottom Plate (10-pack)

Aluminum Sealing Film (2)

1 ea

Confirm all materials are present before beginning.

Ships with Product Code GPPNG-LB. These are the names used throughout the protocols.

WARNING: *The supplied protocols and application support a specific list of labware, which is included in the purchase of the GPPNG-LB Kit. If labware other than those listed here are to be used, the protocol and app require editing. Using undefined or incorrectly identified labware on the AssayMAP Bravo deck can cause damage to the Bravo 96AM Head.*

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.

Additional Required Reagents/Equipment

Ultrapure, deionized water (~100 ml, Milli-Q® or equivalent)
100% Acetonitrile (~250 ml, HPLC-grade)
Formic Acid (HPLC-grade)
Vortexer
Nitrile Gloves
Calibrated pipettes and disposable tips (P5/10, P200 and P1000)
Volumetric Pipettes (5-ml, 1 ea)
Glass Graduated Cylinder (50-ml, 1 ea)
Glass Bottle with Screw Cap (50-ml, 1 ea)

Optional Reagents and Supplies

Multichannel pipettors & disposable tips (P5/P10 and P200)

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

INTRODUCTION

The GlykoPrep-*plus* Sample Preparation Platform (GlykoPrep-*plus*) dramatically streamlines glycoanalysis by automating deglycosylation and separation of N-glycans, complete fluorescent labeling and efficient cleanup to reduce excess reagent peaks using the Bravo 96AM Syringe Head on the AssayMAP Bravo.

GlykoPrep-*plus* 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 may be made available individually; for more information please contact us.

GlykoPrep-*plus* is built on AssayMAP technology, microchromatography in a 96-well format using the Syringe Head on the Agilent AssayMAP Bravo to move liquid through the Cartridges. The same procedures may be performed using centrifugation (GlykoPrep, spin format). For more information about GlykoPrep, please visit our website:

<http://www.prozyme.com/GlykoPrep/>

USING THE KIT

GlykoPrep-*plus* Rapid N-Glycan Preparation with InstantAB combines the Digestion Module, the InstantAB Labeling Module and the Cleanup Module.

This GPPNG-LB Instruction Manual provides instructions for use of the AssayMAP Bravo for N-glycan processing using GlykoPrep-*plus* Rapid N-Glycan Sample Preparation with InstantAB Kit (GPPNG-LB). It also provides additional background information and explanations to frequently-asked questions regarding:

- Glycoprotein Sample Preparation
- Using the Reagent Volume Calculator
- AssayMAP Bravo Processing Time
- GlykoPrep-*plus* Reagent Preparation

Glycoprotein Sample Preparation

This section discusses a number of considerations when preparing Glycoprotein Samples for processing on the AssayMAP Bravo.

- Optional Purification

The GPPNG-LB Kit begins with purified Glycoprotein Samples. To process cell culture or other mixtures in a single workflow, a purification module, such as Protein A or Protein G, may be employed just prior to Protocol 1 on the AssayMAP Bravo.

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.

- Sample Quantities

The quantitative binding capacity for each Cartridge is:

RX Cartridge	50 µg of any standard protein
CU Cartridge	30 µg of N-glycans

Each Cartridge is capable of binding more target, but will do so with increasing breakthrough, making the process non-quantitative. Less than the maximum quantity may be processed, for example, when the sample is available only in limited amounts. The smallest effective amount of sample will depend on the sensitivity requirements of the analytical methods used and the specific application.

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

- Sample Concentrations

For standard processing, the Glycoprotein Sample concentration should be in the range of 1–5 mg/ml, and sufficient reagents have been provided to process individual columns (8 samples) at a time. The GPPNG-LB Kit can also be used for more dilute samples (down to 0.05 mg/ml); the effective minimum Sample Concentration will depend on the denaturation requirements for the specific glycoprotein (see below).

- Sample Denaturation

Prior to the enzyme digest, the Glycoprotein Samples are denatured by pre-mixing with Denaturation Reagent to open up the protein structure and allow access to the deglycosylation enzyme. The standard protocol employs a 5-minute, relatively gentle denaturation; the Denaturation Reagent is 6 M Guanidine. Start with a 1:1 ratio of Glycoprotein Sample to Denaturation Reagent, and increase the ratio of Denaturation Reagent to 9:1 to test the effects of increasing level of Denaturation Reagent. Most glycoproteins can be adequately denatured under these conditions.

In all cases, a minimum of 50% Denaturation Reagent should be added prior to loading onto the RX Cartridge. This assures that the Glycoprotein Sample formulation matches the equilibration conditions of the RX Cartridge, and avoids precipitation of the protein, which will plug the Cartridge.

- Deglycosylation Temperature

The assay has been optimized for a setting of 45 °C. Note that this temperature is the set point, and not the actual reaction temperature, which is a few degrees lower.

The user may enter temperatures other than the recommended setting for qualification or optimization studies.

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 beginning.

NOTE: All Glycoprotein Samples will be loaded at the same volume. To achieve uniform loading across all the Cartridges, the Glycoprotein Samples must be adjusted to the same concentration.

Amount of Denaturation Reagent to be added is determined by setting the Denaturation Factor in the Reagent Volume Calculator (see Instructions page 11).

Any custom denaturation may be performed prior to processing on the AssayMAP Bravo, as long as no SDS or other detergents are used.

- Duration of the Deglycosylation Reaction

The digestion procedure has been optimized to deliver deglycosylation of N-Glycans from 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 (up to 60 minutes). For glycoproteins that are comparatively easy to deglycosylate, such as monoclonal antibodies, a 15-minute incubation is generally sufficient.

Often glycoproteins must be denatured to open the protein structure for the enzyme to gain access for cleavage. See the discussion in this section under Sample Denaturation.

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

Using the Reagent Volume Calculator

Reagents and other solutions, either provided by the user or supplied with the GlykoPrep-*plus* Kit, must be prepared prior to dispensing into the Reagent Source Plate.

Enter the sample information in the green fields at the top of the Sample Info & Reagent Prep tab. Grey fields are calculated values.

- Number of Samples: _____ (actual, green)
Enter the total number of glycoprotein Samples to be processed.
- Number of plate columns used: (calculated value, grey)
- Target Load (µg): _____ (actual, green)
Enter the desired Glycoprotein Sample Target Load.
- Sample Concentration (mg/ml): _____ (actual, green)
Enter the concentration of the Glycoprotein Samples.
- Required Starting Sample Volume (µl): (calculated value, grey)

Excel spreadsheet file that uses formulas to calculate reagent needs and recipe volumes.

NOTE: Some values incorporate a small overage required for proper operation of the probes. For example, for Required Sample Volume and Denaturation Reagent Volume, the calculator automatically adds 5 µl.

Volumes listed in the Reagent Source Plate tab and Sample Info & Reagent Prep tab are based on numbers entered into this tab.

This field is used to calculate the number of columns and volume of reagents required. Normal processing is done with complete plate columns, so the number of Samples should be 8 to 96 and divisible by 8.

This is the Number of Samples divided by 8, as only full columns are used. Used to calculate the amount of reagents to be prepared.

The maximum, quantitative Glycoprotein Sample Target Load on the RX Cartridge is 50 µg. This value is used with the Glycoprotein Sample Concentration to calculate the total Denatured Sample Load Volume.

All Glycoprotein Samples will be loaded at the same volume. To achieve uniform loading across all the Cartridges, the Glycoprotein Samples must be adjusted to the same concentration.

Calculated from the Sample Target Load and Sample Concentration. Dispense this volume accurately into the Glycoprotein Sample Plate (page 13). This cell will turn red for values greater than 250 µl as this is outside the volume limit of this parameter.

- Denaturation Reagent Factor (x:1): _____ (actual, green)

Enter the ratio of Denaturation Reagent to be added to the Glycoprotein Samples; the Denaturation Reagent Factor is the x in the relationship (X:1).

- Denaturation Reagent Volume (μl): (calculated value, grey)
- Denatured Sample Load Volume (μl): (calculated value, grey)

GlykoPrep-plus Reagent Preparation

Reagents and other solutions, either provided by the user or supplied with the GPPNG-LB Kit, must be prepared prior to dispensing into the Reagent Source Plate. Please refer to the directions in the GPPNG-LB Reagent Volume Calculator (Excel files) to determine the appropriate amount of each to prepare. The “Sample Info & Reagent Prep” tab and the “Reagent Source Plate Setup” tab of the Reagent Volume Calculator may be printed and taken to a laboratory work station to prepare and then dispense reagents and solutions as shown. The AssayMAP Bravo will dispense the reagents and solutions from the Reagent Source Plate to begin the sample preparation.

The amount of N-Glycanase provided in the kit is sufficient for the preparation of 96 samples in up to 4 separate runs. If smaller runs are desired, it is important to refer to the reagent calculator to confirm the amount of enzyme needed. Additional enzyme is needed to perform greater than 4 runs with a single kit; additional N-Glycanase (WS0278-GP) can be purchased from ProZyme.

The labware provided in the kit is sufficient for a single run of up to 96 samples. If multiple runs are desired, additional labware can be purchased as a kit (AM96-NG) or in bulk from ProZyme. Please contact ProZyme for bulk purchase inquiries.

For example, recommended to start: enter 1 for a 1:1 mixture to achieve 50% Denaturation Reagent.

Calculated from previous entries. This cell will turn red for values greater than 200 μl, as this is outside the volume limit of this parameter.

Sum of the Glycoprotein Sample Volume and the Denaturation Reagent Volume, less 10 μl to ensure proper filling of the probes.

NOTE: If the sum of the Required Starting Sample Volume plus the Denaturation Reagent Volume less 10 μl is greater than 250 μl, this cell will turn yellow and the value will appear as 250 μl, which is the volume limit of the syringe.

See Preparing for the GlykoPrep-plus Protocols and GlykoPrep-plus Protocols pages 15-27 for preparation of specific reagents.

NOTE: Equilibrate all reagents to room temperature, then gently invert to mix.

AssayMAP Bravo Processing Time

The approximate time to process Glycoprotein Samples on the AssayMAP Bravo depends on the specific choices when entering values in the various protocols. The approximate times for standard processing are shown in Table 1:

Protocol	Fixed	Variable	Total (min)
1. Plate and Reagent Setup Protocol	15	15	15 - 30
2. RX Cartridge Setup Protocol	1	n/a	1
3. Immobilization, Digestion & InstantAB Labeling Protocol	75	30	75 - 105
4. CU Cartridge Setup Protocol	1	n/a	1
5. Cleanup Protocol	35	n/a	35
Total			127 - 172

The Variable times listed in Table 1 may be influenced by the following factors:

- Number of columns in use
- Digestion incubation time
- Denaturation incubation time
- Load (up to 250 µl at 5 µl/min)
- Manual pipetting efficiency

Preparing for the GlykoPrep-plus Protocols

Glycoprotein Sample Plate Preparation

Prepare the Glycoprotein Sample Plate with required volumes as described for processing on the AssayMAP Bravo.

Dispense the Glycoprotein Samples into either a PCR Plate or a U-Bottom Plate depending on the final Denatured Glycoprotein Sample Volume.

Reagent Source Plate Preparation

- Print the “Sample Info & Reagent Prep” and “Reagent Source Plate Setup” tabs of the Reagent Volume Calculator.
- Prepare the reagents and solutions needed as described below and in the **grey** section of the “Sample Info & Reagent Prep” tab.
 1. Digestion Buffer

In a separate vial, add the amounts of 25x Digestion Buffer and ultrapure water as indicated in the Reagent Volume Calculator, and then vortex to mix thoroughly.

See:

- *Sample Info & Reagent Prep tab in the Reagent Volume Calculator to compute starting volumes.*
- *“GlykoPrep Sample Preparation” above for information about Glycoprotein Sample concentration and denaturation conditions.*

Use the PCR Plate if the Total Volume is 110 μ L or less; use the U-Bottom Plate if the Total Volume is 110 to 300 μ L.

NOTE: Be sure not to introduce bubbles; spin down if necessary

NOTE: All Glycoprotein Samples will be loaded at the same volume. To achieve uniform loading across all the Cartridges, the Glycoprotein Samples must be adjusted to the same concentration.

NOTE: The color cues used here match the protocol colors in the Agilent application interface.

 *Digestion Buffer may be prepared up to one week before use. Store at 2–8 °C.*

2. Enzyme Solution

Vortex to mix the vial of N-Glycanase, and then spin down briefly to collect the contents in the base of the vial.

In a separate vial, add the amounts of Digestion Buffer (prepared above) and N-Glycanase indicated in the Reagent Volume Calculator, and then vortex to mix thoroughly.

3. InstantAB Labeling Reagent

InstantAB Dye (supplied with the kit)

Dye Solvent (supplied with the kit)

Add 1.1 ml of Dye Solvent directly into each 50 mg InstantAB Dye vial.

2 vials provide sufficient dye for 12 separate runs of 8 samples.

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

4. InstantAB Solution

InstantAB Labeling Reagent, prepared above

100% Acetonitrile

In a solvent-resistant, 15-ml tube, add the amounts of InstantAB Labeling Reagent and 100% Acetonitrile indicated in the Sample Info & Reagent Prep tab of the Reagent Volume Calculator under “Reagent Preparation for the Reagent Source Plate used in 1 Plate & Reagent Setup Protocol.”.

Cap and vortex to mix thoroughly.

5. Blocking Reagent (supplied with the kit)

 *Enzyme Solution should be prepared on the day of use; store at RT.*

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 in Acetonitrile should be prepared immediately before use; do NOT store for re-use.*

6. Denaturation Reagent (supplied with the kit)

- Pipette prepared solutions from the previous step into each well location in the Reagent Source Plate as shown in the table in the “Reagent Source Plate Setup” tab.

Preparation of the AssayMAP Bravo and Associated Equipment

Turn on the AssayMAP Bravo, computer, pump and Inheco controller.

Open the AssayMAP LaunchPad from the desktop.

Under the N-Glycan Sample Prep tab, click on the IAB icon to launch N-Glycan Sample Prep: RX digestion & IAB labeling. Verify that the Wash Station is installed on position#1.

Verify that the Wash Station is installed on position#1.

- Connect appropriate tubing from the DI water container to the pump, from the pump to the wash station, and from the wash station to the waste container.
- Fill the wash station source container with DI water (if necessary).
- Empty the wash station waste container (if necessary).
- Click on “Prime and Wash” to prime the chimneys.
- Verify that all chimneys have water flowing.

Verify that the Peltier Thermal Station with PCR Plate Adapter is installed on position#4.

Verify that the Orbital Shaking Station is installed on position#9.

NOTE: Be sure not to introduce bubbles; spin down if necessary.

NOTE: To avoid evaporation of Acetonitrile, add InstantAB in Acetonitrile last and cover if not using immediately.

Use the LaunchPad web interface to access the Reagent Volume Calculator; GlykoPrep-plus Protocols and support documents.

NOTE: Ensure that the lines are flushed if changing composition of the source.

5 L of purified, deionized water is needed.

During Prime and Wash, syringe tips are washed with 10 volumes of DI water; sufficient to eliminate carryover between steps.

GLYKOPREP-PLUS PROTOCOLS

1. Plate & Reagent Setup Protocol (**grey**)
2. RX Cartridge Setup Protocol (**green**)
3. Immobilization, Digestion and InstantAB Labeling Protocol (**blue**)
4. CU Cartridge Setup Protocol (**purple**)
5. Cleanup Protocol (**red**)

In this section, the colored tables give a Deck Layout, a Labware Table and Application Settings for each of the AssayMAP Protocols.

1 Plate & Reagent Setup Protocol

Deck Layout



Labware Table

1	96AM Tip Wash Station
2	None
3	96AM Cartridge Seating Station
4	96 PCR Block + 96 Eppendorf 30129300, PCR, Full Skirt, PolyPro
5	96 AbGene 1127, 1mL Deep Well, Square Well, Round Bottom
6	96 V11 LT250 Tip Box (19477.002)
7	Stack of 4: 96 Greiner 650201, U-Bottom Standard, PolyPro
8	None
9	None

Application Settings

Parameter	Value
Number of Columns to Manage	1
Starting Column of Destination Plate	1

Status 1



Run Protocol 1



1 Plate & Reagent Setup Protocol

Set the parameters in the Application Settings section:

1. Number of Columns to Manage (columns of 8 samples) = _____
2. Starting Column of Destination Plate = _____

Check the previously prepared Reagent Source Plate for bubbles; centrifuge briefly to clear bubbles if necessary.

Place the Reagent Source Plate on position#5.
Place the empty Seating Station on position#3.

Assumes full columns are processed

Third section of the **grey** graphic above.

Normally Starting Column will be set to 1 unless previously used plates are being reused. This setting will be the same for all destination plates.

From page 13, prepared using the Reagent Volume Calculator.

Label a clean PCR Plate as “Processing Plate,” and place it on the Heater Station Adaptor on position#4.

Place a Pipette-Tip Box on position#6; REMOVE THE LID.

Label and stack 4 U-Bottom Plates:

- a. Digestion Buffer (top)
- b. Blocking Reagent
- c. Denaturation Reagent
- d. InstantAB Solution (bottom)

Place the stack on position#7.

Make sure that the AssayMAP Bravo deck looks like the Deck Layout shown.

Click “Run Protocol 1.”

Proceed to Protocol 2.

If using less than a full box, the pipette tips must be on the RIGHT side of the box as you look at the AssayMAP Bravo from the front. This is counterintuitive, so please double check.

This protocol uses 40 pipette tips, which are then discarded. Make sure there are at least 40 tips in full columns in the rack of pipette tips.

Orient the plates so that the A1 wells are pointed toward deck position#1.

NOTE: At the end of Immobilization, Digestion & InstantAB Labeling, the N-glycans will be in the U-bottom Plate on position#7.

*First section of the **grey** graphic above; labware is listed in the second section.*

A status line will show when the protocol is complete: "Run complete. Module idle."

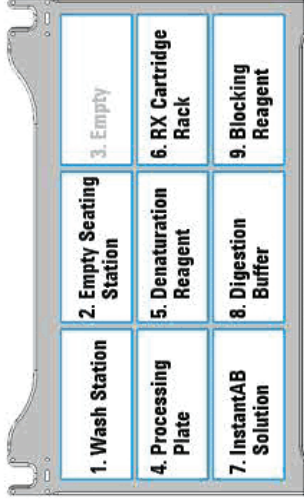
Approximate run time is 15 minutes.

WARNING: *Do not reach into the AssayMAP Bravo space while it is operating. This will break the security line; the AssayMAP Bravo will make an emergency stop, and may not be able to be restarted without intervention from a knowledgeable operator.*

May delay as much as one hour without affecting results before proceeding to the next protocol.

2 Digestion (RX) Cartridge Setup Protocol

Deck Layout



Labware Table

- 1 96AM Tip Wash Station
- 2 96AM Cartridge Seating Station
- 3 None
 - 96 PCR Block + 96 Eppendorf 30129300, PCR, Full Skirt, PolyPro
- 4 96 Greiner 650201, U-Bottom Standard, PolyPro
- 5 96 Greiner 650201, U-Bottom Standard, PolyPro
- 6 Digestion (RX) Cartridges WS0253
- 7 96 Greiner 650201, U-Bottom Standard, PolyPro
- 8 96 Greiner 650201, U-Bottom Standard, PolyPro
- 9 96 Greiner 650201, U-Bottom Standard, PolyPro

Application Settings

Parameter	Value
Starting Column of RX Cartridge Rack	1
Number of Columns to Manage	1
Starting Column of Destination Plate	1
Undo RX Cartridge Transfer	☐

Status 2

Run Protocol 2

2 RX Cartridge Setup Protocol

Rearrange the deck in preparation for processing:

1. Remove the Reagent Source Plate from position#2; discard.
2. Remove the Seating Station on position#3 and discard the used pipette tips.
3. Place the now empty Seating Station on position#2.
4. Remove the pipette tips from position#6.
5. Remove the lid from a Rack of RX Cartridges and place it with its Receiver Plate on position#6.

Assumes full columns are processed

Set the parameters in the Application Settings section:

1. Indicate the first column of RX Cartridges to be used in the RX Cartridge Rack in position#6.
2. Indicate the number of columns to transfer.
3. Indicate the first column in the Seating Station into which these columns of RX Cartridges will be placed.

Make sure that the AssayMAP Bravo deck looks like the Deck Layout shown.

Click on “Run 2 RX Cartridge Setup.”

Proceed to Protocol 3.

Third section of the **green** graphic above.

NOTE: The first column to be used must be the first available column of Cartridges as the Syringe Head cannot access a buried column of Cartridges. Remaining columns are transferred directly after the first column in the left to right direction.

Normally, the starting column of the RX Cartridge Rack will match the starting column of the Seating Station working across a plate from left to right. For example, columns 1-5 in a run using a full rack would be indicated by starting column #1 and 5 columns to be moved. The second run using that rack would start with column 6.

*First section of the **green** graphic above; labware is listed in the second section.*

NOTE: The RX Cartridge Setup module can be reversed by clicking on “Undo RX Cartridge Transfer.”

A status line will show when the protocol is complete: “Run complete. Module idle.”

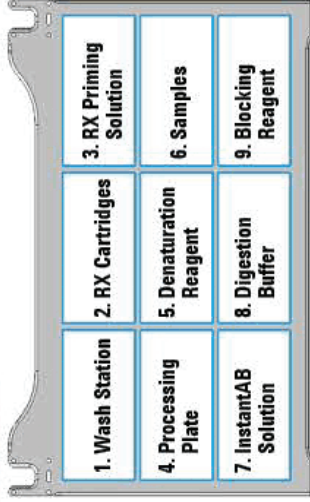
Approximate run time is 1 minute.

WARNING: *Do not reach into the AssayMAP Bravo space while it is operating. This will break the security line; the AssayMAP Bravo will make an emergency stop, and may not be able to be restarted without intervention from a knowledgeable operator.*

May delay as much as one hour before proceeding to the next protocol without affecting results.

3 Immobilization, Digestion & InstantAB Labeling Protocol

Deck Layout



Labware Table

- 1 96AM Tip Wash Station
- 2 96AM Cartridge Seating Station
- 3 12 Column, Low Profile Reservoir, Natural PP
- 4 96 PCR Block + 96 Eppendorf 30129300, PCR, Full Skirt, PolyPro
- 5 96 Greiner 650201, U-Bottom Standard, PolyPro
- 6 96 Eppendorf 30129300, PCR, Full Skirt, PolyPro
- 7 96 Greiner 650201, U-Bottom Standard, PolyPro
- 8 96 Greiner 650201, U-Bottom Standard, PolyPro
- 9 96 Greiner 650201, U-Bottom Standard, PolyPro

Application Settings

Parameter	Value	Units
Denaturant Volume	55	µL
Starting Sample Volume	55	µL
Denatured Sample Load Volume		µL
Sample Loading Flow Rate	5	µL/min
Temperature Set Point for Digestion	45	°C
Duration of Digestion Step	30	minutes

Status 3

Run Protocol 3

3 Immobilization, Digestion & InstantAB Labeling Protocol

Dispense the RX Priming Solution (100 % Acetonitrile) into a 12-Column Reservoir Plate. Fill the channels with the specified volume corresponding to the columns of RX Cartridges to be processed.

Rearrange the deck in preparation for processing:

1. Remove the RX Cartridge Rack, Receiver Plate and tape from position#6. Replace the lid to protect any remaining RX Cartridges, and store appropriately.
2. Place the 12-Column, Reservoir Plate containing the RX Priming Solution on position#3, making sure that the filled reservoirs correspond to the RX Cartridge positions.

Assumes full columns are processed

Blue section of the printed Reagent Volume Calculator "Sample Info & Reagent Prep" tab.

3. Place the Glycoprotein Sample Plate on position #6.

Set the sample plate type in the “Labware” section, line 6:

Select PCR Plate (low volume) or U-Bottom Plate (high volume).

Set the parameters in the Application Settings section:

1. Denaturant Volume: Enter the volume of Denaturation Reagent to be added to each Glycoprotein Sample.
2. Starting Sample Volume: Enter the starting volume of Glycoprotein Sample to be loaded.
3. Denatured Sample Load Volume (calculated value, grey).

4. Enter the Sample Loading Flow Rate.

5. Temperature Setpoint for Digestion: Enter the incubation temperature for the deglycosylation reaction.

The positions of the columns of Glycoprotein Samples should correspond to the positions of the columns of RX Cartridges.

NOTE: The Glycoprotein Sample Plate can be a PCR Plate or a U-Bottom Plate, depending on the Denatured Sample Load Volume. Use a PCR Plate if this value is 110 µL or less; use a U-Bottom Plate if it is between 110 and 300 µL.

*Middle section of the **blue** graphic above.*

WARNING: *The correct sample plate type **MUST** be chosen or the AssayMAP Bravo Head may be damaged.*

*Third section of the **blue** graphic above.*

Cell D16 in the Reagent Volume Calculator. The maximum allowable volume is 200 µL.

Cell D15 in the Reagent Volume Calculator. The maximum allowable volume is 250 µL.

The Denatured Sample Load Volume is calculated automatically (from cells D15 and D16 of the Reagent Volume Calculator; less 10 µL to ensure proper filling of the probes) and displayed on the form after the protocol begins.

The maximum volume that can be loaded on a Cartridge is 250 µL; if the sum of the Denaturant Volume and the Starting Sample Volume is greater than 260 µL, only 250 µL will be loaded.

The recommended flow rate is 5 µL/minute, although faster or slower rates are possible. Flow rates other than the recommended rate of 5 µL/minute may affect the maximum quantitative binding capacity.

Note that this temperature is the set point and not the actual reaction temperature, which is a few degrees lower. The assay has been optimized for a set point of 45 °C, although laboratory conditions may cause this optimum to vary slightly.

6. Duration of Digestion Step: Enter the duration for the deglycosylation reaction.

Make sure that the AssayMAP Bravo deck looks like the Deck Layout.

Click on “Run 3 Immobilization & Digestion.”

In the last step of Immobilization & Digestion, the released N-glycans are added to the InstantAB in Acetonitrile and instantly labeled.

The temperature can be monitored on the digital readout of the STC Controller.

The level of glycosylation varies from glycoprotein to glycoprotein; see “Duration of the Deglycosylation Reaction” page 9.

*First section of the **blue** graphic above; labware is listed in the second section.*

The protocol will take between 90 and 120 minutes depending on the sample volume and incubation time.

WARNING: *Do not reach into the AssayMAP Bravo space while it is operating. This will break the security line; the AssayMAP Bravo will make an emergency stop, and may not be able to be restarted without intervention from a knowledgeable operator.*

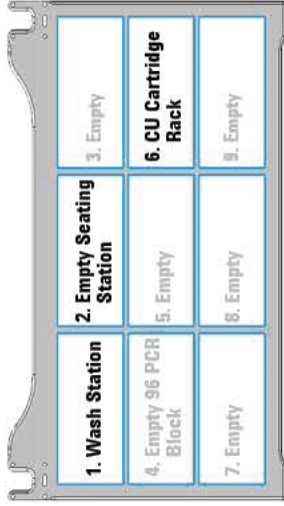
A status line will show when the protocol is complete: “Run complete. Module idle.”

The N-glycans are now in the U-bottom Plate on position #7.

NOTE: *Plates at position #3 and #7 contain Acetonitrile; discard according to waste disposal procedures.*

4 Cleanup (CU) Cartridge Setup Protocol

Deck Layout



Labware Table

1	96AM Tip Wash Station
2	96AM Cartridge Seating Station
3	None
4	96 PCR Block
5	None
6	Cleanup (CU) Cartridges WS0263
7	None
8	None
9	None

Application Settings

Parameter	Value
Starting Column of CU Cartridge Rack	1
Number of Columns to Manage	1
Starting Column of Destination Plate	1
Undo CU Cartridge Transfer	☐

Status 4

Run Protocol 4

4 CU Cartridge Setup Protocol

Rearrange the deck in preparation for processing:

- Remove all Immobilization and Digestion labware from deck.
- Discard used RX Cartridges.

Remove the lid from a Rack of CU Cartridges and place it with its Receiver Plate on position#6.

Tape the CU Cartridge Rack and Receiver Plate to the deck.

Place the empty Seating Station in position#2.

Assumes full columns are processed

May process a partial Rack.

Set the parameters in the Application Settings section:

1. Indicate the first column from which to take the CU Cartridges from Rack in position#6.
2. Indicate the number of columns to transfer.
3. Indicate the first column in the Seating Station where the CU Cartridges are to be placed.

Make sure that the AssayMAP Bravo deck looks like the Deck Layout shown.

Click on “Run 4 CU Cartridge Setup.”

Third section of the **purple** graphic above.

NOTE: This must be the first occupied column of the CU Cartridge Rack as the Syringe Head cannot access a buried column of Cartridges.

Normally the starting column of the CU Cartridge Rack will match the starting column of the Seating Station across the plate from left to right.

First section of the **purple** graphic above; labware is listed in the second section.

NOTE: the CU Cartridge Setup module can be reversed by clicking on “Undo CU Cartridge Transfer”.

A status line will show when the protocol is complete: “Run complete. Module idle.”

Approximate run time is 1 minute.

WARNING: Do not reach into the AssayMAP Bravo space while it is operating. This will break the security line; the AssayMAP Bravo will make an emergency stop, and may not be able to be restarted without intervention from a knowledgeable operator.

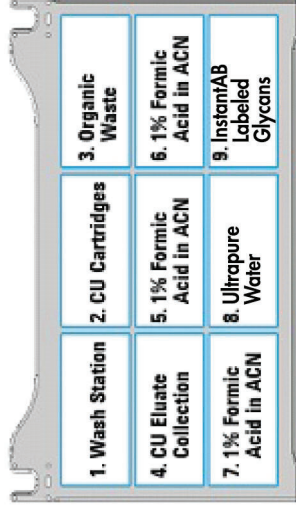
 May be prepared up to one week before use. Store sealed in a similarly sized glass container at room temperature.

Prepare: 1% Formic Acid in ACN Solution for Cleanup Protocol (CU Priming and Washing Solutions):

Add the Formic Acid to a glass, graduated cylinder. Bring the volume up to the correct volume with 100% acetonitrile. Transfer to a similarly sized glass storage vessel, cap tightly and swirl gently to mix.

5 Cleanup Protocol

Deck Layout



Labware Table

- 1 96AM Tip Wash Station
- 2 96AM Cartridge Seating Station
- 3 96 AbGene 1127, 1mL Deep Well, Square Well, Round Bottom
- 4 96 PCR Block + 96 Eppendorf 30129300, PCR, Full Skirt, PolyPro
- 5 12 Column, Low Profile Reservoir, Natural PP
- 6 12 Column, Low Profile Reservoir, Natural PP
- 7 12 Column, Low Profile Reservoir, Natural PP
- 8 12 Column, Low Profile Reservoir, Natural PP
- 9 96 Greiner 650201, U-Bottom Standard, PolyPro

Application Settings

Parameter	Value	Units
Final Eluate Volume	50	µL

Status 5

Run Protocol 5

5 Cleanup Protocol

Remove the CU Cartridge Rack, Receiver Plate and tape from position#6. Replace the lid to protect any unused Cartridges and store appropriately.

Set the parameters in the Application Settings section:

Indicate the Final Eluate Volume. (25 - 100 µL, as needed for the specific analytical method).

Assumes full columns are processed

Third section of the **red** graphic above.

The AssayMAP Bravo elutes the Labeled N-Glycan Samples quantitatively from the CU Cartridges in 25 µL of water. Any additional elution volume is added in a separate step.

The Labeled N-Glycan Samples are eluted from the CU Cartridge as continuous fractions. A 1-minute mixing step produces a homogeneous sample, ready for analysis.

Place the previously prepared 1% Formic Acid in Acetonitrile on the deck, making sure that the filled reservoirs correspond to the CU Cartridge positions:

1. Place the 12-Column, Reservoir Plate containing Load and Wash Solution (6 ml each reservoir of 1% Formic Acid in Acetonitrile) on position#5.
2. Place the 12-Column, Reservoir Plate containing Load and Wash Solution (6 ml each reservoir of 1% Formic Acid in Acetonitrile) on position#6.
3. Place a 12-Column, Reservoir Plate containing Load and Wash Solution (6 ml each reservoir of 1% Formic Acid in Acetonitrile) on position#7.
4. Place a 12-Column, Reservoir Plate containing HPLC-grade water (6 ml each reservoir) on position#8.

Place an empty Square, Deep-Well Plate on position#3 for Waste.

Label an empty PCR Plate “CU Eluate” and place it on position#4 for Eluate.

Place the U-bottom Plate containing the labeled N-glycans on position#9.

Make sure that the AssayMAP Bravo deck looks like the Deck Layout shown.

Click on “Run 5 Cleanup.”

At the end of the protocol, the purified labeled N-Glycans will be in the CU Eluate at position#4.

Each well contains Labeled N-Glycan Samples plus unreacted dye, which will be removed using HILIC chromatography.

*First section of the **red** graphic above; labware is listed in the second section.*

The protocol will take approximately 30 minutes.

Status is shown in the status bar.

WARNING: Do not reach into the AssayMAP Bravo space while it is operating. This will break the security line; the AssayMAP Bravo will make an emergency stop, and may not be able to be restarted without intervention from a knowledgeable operator.

Upon completion of the protocol:

1. Remove the CU Eluate Collection Plate containing the Labeled N-Glycan Samples from position#4.
2. Proceed directly to analysis, or seal the plate with Aluminum Seal and store at -20 °C.
3. Remove all other labware from the deck and discard any remaining solutions and waste appropriately.

Empty the Wash Station waste container.

A status line will show when the protocol is complete: "Run complete. Module idle."

The Labeled N-Glycan Samples are ready for analysis.

NOTE: Waste plate at position#3 contains Acetonitrile; discard according to waste disposal procedures.

QUICKGUIDE

Glycoprotein Sample Plate Preparation

Print the “Sample Info & Reagent Prep” and “Reagent Source Plate Setup” tabs of the Reagent Volume Calculator.

Prepare the Glycoprotein Sample Plate with required volumes using the Sample Info & Reagent Prep tab in the “Sample Info & Reagent Prep” tab to compute starting volumes.

Dispense the Glycoprotein Samples into either a PCR Plate or a U-Bottom Plate depending on the final Denatured Glycoprotein Sample Volume.

NOTE: Be sure not to introduce bubbles; spin down if necessary.

Reagent Source Plate Preparation

Prepare the reagents and solutions needed as described below and in the **grey** section of the Reagent Volume Calculator.

1. Digestion Buffer

 *Digestion Buffer may be prepared up to one week before use. Store at 2–8 °C.*

In a separate vial, add the amounts of 25x Digestion Buffer and ultrapure water as indicated in the Reagent Volume Calculator, and then vortex to mix thoroughly.

2. Enzyme Solution

 *Enzyme Solution should be prepared on the day of use; store at RT.*

Vortex the vial of N-Glycanase to mix, and then spin down briefly to collect the contents in the base of the vial.

In a separate vial, add the amounts of Digestion Buffer (prepared above) and N-Glycanase indicated in the Reagent Volume Calculator, and then vortex to mix thoroughly.

3. InstantAB Labeling Reagent

Add 1.1 ml of Dye Solvent directly into each 50 mg InstantAB Dye vial.

2 vials provide sufficient dye for 12 separate runs of 8 samples.

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

4. InstantAB Solution

InstantAB Labeling Reagent, prepared above
100% Acetonitrile

In a solvent-resistant, 15-ml tube, add the amounts of InstantAB Labeling Reagent and 100% Acetonitrile indicated in the Sample Info & Reagent Prep tab, 1 Plate & Reagent Setup section of the Reagent Volume Calculator.

Cap and vortex to mix thoroughly.

5. Blocking Reagent (supplied with the Kit)

6. Denaturation Reagent (supplied with the Kit)

Pipette prepared solutions from the previous step into each well location in the Reagent Source Plate as shown in the table in the "Reagent Source Plate Setup" tab.

Preparation of the AssayMAP Bravo and Associated Equipment

Turn on the AssayMAP Bravo, computer, pump and Inheco controller.

Open the AssayMAP LaunchPad from the desktop.

Under the N-Glycan Sample Prep tab, click on the IAB icon to launch N-Glycan Sample Prep: RX digestion & IAB labeling.

Verify that the Wash Station is installed on position#1.

- Connect appropriate tubing from DI water container to the pump, and from the pump to the wash station, and from the wash station to the waste container.
- Fill the wash station source container with DI water (if necessary).
- Empty the wash station waste container (if necessary).
- Click on "Prime and Wash" to prime the chimneys.
- Verify that all chimneys have water flowing.

Verify that the Peltier Thermal Station with PCR Plate Adapter is installed on position#4.

Verify that the Orbital Shaking Station is installed on position#9.

WARNING: *Do not ever reach into the AssayMAP Bravo space while it is operating. This will break the security line; the AssayMAP Bravo will make an emergency stop, and may not be able to be restarted without intervention from a knowledgeable operator.*

1 Plate & Reagent Setup Protocol

Deck Layout

Labware Table

1	96AM Tip Wash Station
2	None
3	96AM Cartridge Seating Station
4	96 PCR Block + 96 Eppendorf 30129300, PCR, Full Skirt, PolyPro
5	96 AbGene 1127, 1mL Deep Well, Square Well, Round Bottom
6	96 V11 LT250 Tip Box (19477.002)
7	Stack of 4: 96 Greiner 650201, U-Bottom Standard, PolyPro
8	None
9	None

Application Settings

Parameter	Value
Number of Columns to Manage	1
Starting Column of Destination Plate	1

Status 1

Run Protocol 1

1 Plate & Reagent Setup Protocol

Set the parameters in the Application Settings section:

1. Number of Columns to Manage (columns of 8 samples) = _____
2. Starting Column of Destination Plate = _____

Check the previously prepared Reagent Source Plate for bubbles; centrifuge briefly if necessary to clear bubbles.

Place the Reagent Source Plate on position#5.

Place the empty Seating Station on position#3.

Label a clean PCR Plate as "Processing Plate" and place it on the Heater Station Adaptor on position#4

Place a Pipette-Tip Box on position#6; REMOVE THE LID.

Label and stack 4 U-Bottom Plates:

- a. Digestion Buffer (top)
- b. Blocking Reagent
- c. Denaturation Reagent
- d. InstantAB Solution (bottom)

Place the stack on position#3.

Click "Run Protocol 1"

The approximate run time is 15 minutes. A Status line will show when the protocol is complete: "Run complete. Module idle."

Proceed to Protocol 2.

2 Digestion (RX) Cartridge Setup Protocol

Deck Layout

1. Wash Station	2. Empty Seating Station	3. Empty
4. Processing Plate	5. Denaturation Reagent	6. RX Cartridge Rack
7. InstantAB Solution	8. Digestion Buffer	9. Blocking Reagent

Labware Table

1	96AM Tip Wash Station
2	96AM Cartridge Seating Station
3	None
4	96 PCR Block + 96 Eppendorf 30129300, PCR, Full Skirt, PolyPro
5	96 Greiner 650201, U-Bottom Standard, PolyPro
6	Digestion (RX) Cartridges WS0253
7	96 Greiner 650201, U-Bottom Standard, PolyPro
8	96 Greiner 650201, U-Bottom Standard, PolyPro
9	96 Greiner 650201, U-Bottom Standard, PolyPro

Application Settings

Parameter	Value
Starting Column of RX Cartridge Rack	1
Number of Columns to Manage	1
Starting Column of Destination Plate	1
Undo RX Cartridge Transfer	☐

Status 2

▶ Run Protocol 2
⏏

2 RX Cartridge Setup Protocol

Rearrange the deck in preparation for processing:

1. Remove the Reagent Source Plate from position#2; discard.
2. Remove the Seating Station on position#3 and discard the used pipette tips.
3. Place the now empty Seating Station on position#2.
4. Remove the pipette tips from position#6.
5. Remove the lid from a Rack of RX Cartridges and place it with its Receiver Plate on position#6.

Set the parameters in the Application Settings section:

1. Indicate the first column of RX Cartridges to be used in the RX Cartridge Rack in position#6.
2. Indicate the number of columns to transfer. The columns directly follow the first column indicated above in the left to right direction.
3. Indicate the first column in the Seating Station into which these columns of RX Cartridges will be placed.

Make sure that the AssayMAP Bravo deck looks like the Deck Layout shown above.

Click on "Run 2 RX Cartridge Setup."

The approximate run time is 1 minute. A Status line will show when the protocol is complete: "Run complete. Module idle."

Proceed to Protocol 3.

3 Immobilization, Digestion & InstantAB Labeling Protocol

Deck Layout

Labware Table

1	96AM Tip Wash Station
2	96AM Cartridge Seating Station
3	12 Column, Low Profile Reservoir, Natural PP
4	96 PCR Block + 96 Eppendorf 30129300, PCR, Full Skirt, PolyPro
5	96 Greiner 650201, U-Bottom Standard, PolyPro
6	96 Eppendorf 30129300, PCR, Full Skirt, PolyPro
7	96 Greiner 650201, U-Bottom Standard, PolyPro
8	96 Greiner 650201, U-Bottom Standard, PolyPro
9	96 Greiner 650201, U-Bottom Standard, PolyPro

Application Settings

Parameter	Value	Units
Denaturant Volume	55	μL
Starting Sample Volume	55	μL
Denatured Sample Load Volume		μL
Sample Loading Flow Rate	5	μL/min
Temperature Set Point for Digestion	45	°C
Duration of Digestion Step	30	minutes

Status 3

Run Protocol 3

3 Immobilization, Digestion & InstantAB Labeling Protocol

Dispense the RX Priming Solution (100% Acetonitrile) into a 12-Column Reservoir Plate, corresponding to the columns of RX Cartridges to be processed.

Fill the channels with the volume specified in the Blue section of the printed Reagent Volume Calculator “Sample Info & reagent Prep” tab.

Rearrange the deck in preparation for processing:

1. Remove the RX Cartridge Rack, Receiver Plate and tape from position#6. Replace the lid to protect any remaining RX Cartridges and store appropriately.
2. Place the 12-Column, Reservoir Plate containing the RX Priming Solution on position#3, making sure that the filled reservoirs correspond to the RX Cartridge positions.
3. Place the Glycoprotein Sample Plate on position#6.

Set the sample plate type in the “Labware Table” section, line 6 above:

Select PCR Plate (low volume) or U-Bottom Plate (high volume).

Set the parameters in the Application Settings section (Third section of the **blue** graphic above.):

1. Enter the Denaturant Volume (cell D16 in the Reagent Volume Calculator).
2. Enter the Starting Sample Volume (cell D15 in the Reagent Volume Calculator).
3. Denatured Sample Volume (calculated value, grey).
4. Enter the Sample Loading Flow Rate.
5. Enter the Temperature Set Point for Digestion.
6. Enter the Duration of the Digestion step.

Make sure that the AssayMAP Bravo deck looks like the Deck Layout above.

Click on "Run 3 Immobilization & Digestion."

The protocol will take between 90 and 120 minutes depending on the sample volume and incubation time. The status line will show when the protocol is complete: "Run complete. Module idle."

The U-bottom plate at position#7 now contains the N-glycans.

In last step the released N-glycans are added to the InstantAB in Acetonitrile and instantly labeled.

Proceed to 4 CU Cartridge Setup Protocol

4 Cleanup (CU) Cartridge Setup Protocol

Deck Layout

Labware Table

1	96AM Tip Wash Station
2	96AM Cartridge Seating Station
3	None
4	96 PCR Block
5	None
6	Cleanup (CU) Cartridges WS0263
7	None
8	None
9	None

Application Settings

Parameter	Value
Starting Column of CU Cartridge Rack	1
Number of Columns to Manage	1
Starting Column of Destination Plate	1
Undo CU Cartridge Transfer	☐

Status 4

4 CU Cartridge Setup Protocol

Rearrange the deck in preparation for processing:

- Remove all Immobilization and Digestion labware from deck.
- Discard used RX Cartridges.

Remove the lid from a Rack of CU Cartridges and place it with its Receiver Plate on position#6.

Tape the CU Cartridge Rack and Receiver Plate to the deck.

Place the empty Seating Station in position#2.

Set the parameters in the Application Settings section:

1. Indicate the first column from which to take the CU Cartridges from Rack in position#6.
2. Indicate the number of columns to transfer.
3. Indicate the first column in the Seating Station where the CU Cartridges are to be placed.

Make sure that the AssayMAP Bravo deck looks like the Deck Layout shown above.

Click on "Run 4 CU Cartridge Setup."

Prepare 1% Formic Acid in ACN Solution for Cleanup Protocol (CU Priming and Washing Solutions):

Add the Formic Acid to a glass, graduated cylinder. Bring the volume up to the correct volume with 100% acetonitrile. Transfer to a similarly sized glass storage vessel, cap tightly and swirl gently to mix.

1% Formic Acid in ACN may be prepared up to one week before use. Store sealed in a similarly sized glass container at room temperature.

Proceed to Protocol 5.

5 Cleanup Protocol

Deck Layout

Labware Table

1	96AM Tip Wash Station
2	96AM Cartridge Seating Station
3	96 AbGene 1127, 1mL Deep Well, Square Well, Round Bottom
4	96 PCR Block + 96 Eppendorf 30129300, PCR, Full Skirt, PolyPro
5	12 Column, Low Profile Reservoir, Natural PP
6	12 Column, Low Profile Reservoir, Natural PP
7	12 Column, Low Profile Reservoir, Natural PP
8	12 Column, Low Profile Reservoir, Natural PP
9	96 Greiner 650201, U-Bottom Standard, PolyPro

Application Settings

Parameter	Value	Units
Final Eluate Volume	50	µL

Status 5

5 Cleanup Protocol

Remove the CU Cartridge Rack, Receiver Plate and tape from position#6. Replace the lid and store appropriately.

Set the parameters in the Application Settings section above:

Indicate the Final Eluate Volume (25-100 µl).

Place the previously prepared Cleanup Solutions on the deck, making sure that the filled reservoirs correspond to the CU Cartridge positions:

- Place the 12-Column, Reservoir Plate containing Load and Wash Solution (6 ml each reservoir of 1% Formic Acid in Acetonitrile) on position#5.
- Place the 12-Column, Reservoir Plate containing Load and Wash Solution (6 ml each reservoir of 1% Formic Acid in Acetonitrile) on position#6.
- Place a 12-Column, Reservoir Plate containing Load and Wash Solution (6 ml each reservoir of 1% Formic Acid in Acetonitrile) on position#7.
- Place a 12-Column, Reservoir Plate containing HPLC-grade water (6 ml each reservoir) on position#8.
- Place an empty, Square, Deep-Well Plate on position#3 for Waste.
- Label an empty PCR Plate “CU Eluate” and on position#4.
- Place the U-bottom Plate containing the InstantAB labeled N-glycans on position#9.

Make sure that the AssayMAP Bravo deck looks like the Deck Layout shown above.

Click on "Run 5 Cleanup."

Upon completion of the protocol:

- a. Remove the Eluate Collection Plate containing the Labeled N-Glycan Samples from position#4. The Labeled N-Glycan Samples are ready for analysis.
- b. Proceed directly to analysis, or seal the plate with Aluminum Seal and store at -20°C.
- c. Remove all other labware from the deck and discard any remaining solutions and waste appropriately.

NOTE: Waste plate at position#3 contains Acetonitrile; discard according to waste disposal procedures.

ANALYSIS OF LABELED N-GLYCANS

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

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

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 MW of N-Glycans Labeled with InstantAB

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

$$\text{Mass}_{\text{Glycan}} + \text{Mass}_{\text{InstantAB}} = \text{Mass}_{\text{InstantAB-Labeled Glycan}}$$

Mass Added to Glycan

Monoisotopic	161.05891
Average	161.2

Direct LC Analysis of N-Glycans After Elution

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

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

REFERENCES

Visit ProZyme's website for additional information, 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>

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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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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