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

GlykoPrep®-*plus* Rapid N-Glycan Sample Preparation with 2-AB Automated on AssayMAP Technology

An automated, highly robust, high-throughput process for enzymatic deglycosylation, fluorescent labeling with 2-AB (2-aminobenzamide) 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 Workstation (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
- Complete labeling in 1 hour using Rapid-Reductive-Amination™
- Purified 2-AB-labeled N-glycans are eluted in water, ready for analysis

Product Code: GPPNG-AB

TABLE OF CONTENTS

This product is intended for in vitro research use only.

EQUIPMENT

Comments

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)

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

SOFTWARE

Agilent VWorks "GlykoPrep-plus Full App version 11.2.0.1182" or newer
Installer with N-Glycan Sample Prep: RX digestion & 2-AB labeling v1.0 or newer

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 33.

Print the QuickGuide separately, using Portrait Orientation.

KIT CONTENTS

GPPNG-AB Kit GlykoPrep-plus Rapid N-Glycan Sample Preparation with 2-AB

Confirm all materials are present before beginning.

GSP-RX GlykoPrep-plus Digestion Module — 1 ea
- 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)

GS96-AB GlykoPrep Rapid-Reductive-Amination 2-AB Labeling Module — 1 ea
- WS0302 Rapid 2-AB Solution (325 µl)
- WS0300 Reductant Solution (325 µl)

GSP-CU GlykoPrep-plus Cleanup Module — 1 ea
- WS0263 CU Cartridges (96)
- WS0303-GP 5x 2-AB Sample Load Solution (12 ml)
- Aluminum Sealing Film (2)

Labware Set from ProZyme (product code AM96-NG when purchased alone) — 1 ea
- 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)

Ships with Product Code GPPNG-AB. 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-AB 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. The 2-AB Solution and Reductant Solution are hygroscopic and the 2-AB Solution is light-sensitive; please store these reagents at -20°C in their original packaging.

Additional Required Reagents/Equipment

Ultrapure, deionized water (~100 ml, Milli-Q® or equivalent)
100% Acetonitrile (~250 ml, HPLC-grade)
Microplate-compatible, centrifugal evaporator (e.g., SpeedVac® with plate rotor or similar)
Fume hood
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) (Gilson or equivalent, compatible with Gilson Diamond® D200 pipette tips)

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 (2-AB Solution, Reductant Solution and 2-AB Labeling Reagent) 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-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 2-AB combines the Digestion Module, the Rapid-Reduction-Amination 2-AB Labeling Module and the Cleanup Module, which may be purchased individually. The Labeling and Cleanup Modules may be purchased together as GPP-ABCU.

This GPPNG-AB Instruction Manual provides instructions for use of the AssayMAP Bravo for N-glycan processing using GlykoPrep-plus Rapid N-Glycan Sample Preparation with 2-AB Kit (GPPNG-AB). 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-AB 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

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

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.

- 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-AB 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).

  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.

- 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.

  The 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.

- 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.

- 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 optional Labeling Source Plate 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).

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

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

  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.

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

  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.

GlykoPrep-*plus* Reagent Preparation

Reagents and other solutions, either provided by the user or supplied with the GPPNG-AB Kit, must be prepared prior to dispensing into the Reagent Source Plate. Please refer to the directions in the GPPNG-AB Reagent Volume Calculator (Excel files) to determine the appropriate amount of each to prepare. The "Sample Info and 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 (700 µl plus overfill) provided in the kit is sufficient for the preparation of 96 samples, in a single run or in 4 runs totaling 96 replicates. 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.

See Preparing for the GlykoPrep-plus Protocols and GlykoPrep-plus Protocols pages 13-32 for preparation of specific reagents.

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

GPPNG-AB Kit components are shipped at concentrations that assure maximum stability; some will be diluted prior to dispensing and others will be dispensed directly from the kit containers.

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 and Digestion Protocol	90	30	90 - 120
Drying N-Glycans Prior to Labeling	20	40	20 - 60
4. CU Cartridge Setup Protocol	1	n/a	1
Labeling the N-Glycans with 2-AB	n/a	5 - 15	5 - 15
5. Labeling Protocol	70	10	70 - 80
6. Cleanup Protocol	35	n/a	35
Total			237 - 342

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
- Drying time
- Load (up to 250 µl at 5 µl/min)
- Manual pipetting efficiency
- Preheating of the heater before the labeling step (saves 10 minutes)

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.

See:
1. Sample Info & Reagent Prep tab in the Reagent Volume Calculator to compute starting volumes.
2. "GlykoPrep Sample Preparation" above for information about Glycoprotein Sample concentration and denaturation conditions.

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

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

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.

Reagent Source Plate Preparation

- Print the "Sample Info & Reagent Setup" and "Reagent Source Plate Setup" tabs of the Reagent Volume Calculator.

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

- Prepare the reagents and solutions needed as described below and in the grey section of the "Sample Info & Reagent Setup" 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.

☛ 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

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

thoroughly.

3. Blocking Reagent
4. Denaturation Reagent
5. Finishing Reagent

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.

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

Optional Labeling Source Plate Preparation

No more than one hour prior to use, Labeling Reagent may be prepared in Column 1 of the Labeling Source Plate and transferred manually by multi-channel pipette to the N-glycan Samples in the Processing Plate.

For preparation of Labeling Reagent, see Manual Processing - Labeling the N-Glycans with 2-AB, page 26 and Labeling Source Plate tab of the Reagent Volume Calculator.

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.

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

Under the N-Glycan Sample Prep tab, click on the 2-AB icon to launch N-Glycan Sample Prep: RX digestion & 2-AB labeling.

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

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

- 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.

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.

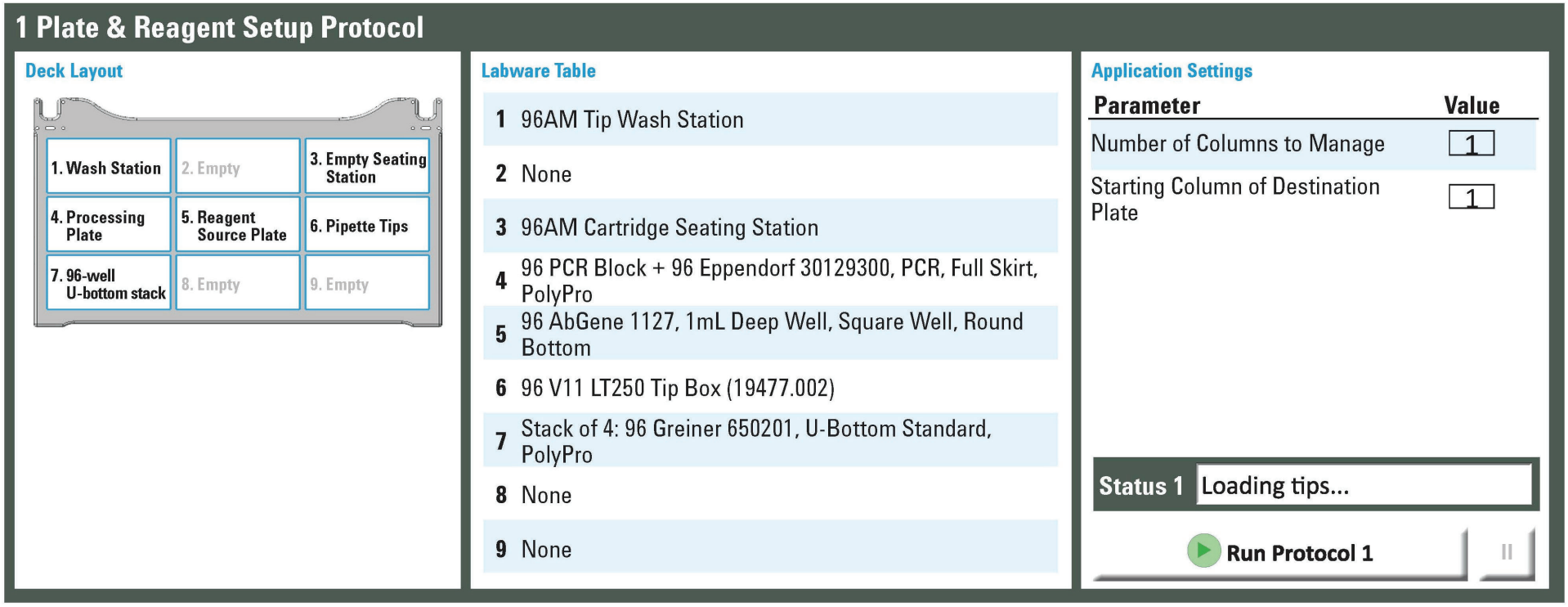
Verify that the Peltier Thermal Station with PCR Plate Adapter is installed on postion#4.

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

GLYKOPREP-*PLUS* PROTOCOLS

1. Plate & Reagent Setup Protocol (grey)
2. RX Cartridge Setup Protocol (green)
3. Immobilization and Digestion Protocol (blue)
4. CU Cartridge Setup Protocol (purple)
5. Labeling Protocol (orange)
6. 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

Set the parameters in the Application Settings section:
1. Number of Columns to Manage (columns of 8 samples) = _____
2. Starting Column of Destination Plate = _____

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.

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

Place the Reagent Source Plate on position#5.

From page 13, prepared using the Reagent Volume Calculator.

Place the empty Seating Station on position#3.

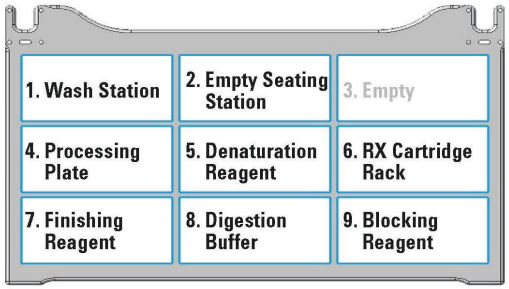
Label a clean PCR Plate as “Processing Plate,” and place it on the Heater Station Adaptor on position#4.

The Processing Plate will contain the released N-glycans after Immobilization and Digestion.

Place a Pipette-Tip Box on position#6; REMOVE THE LID.	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.
Label and stack 4 U-Bottom Plates: a. Digestion Buffer (top) b. Blocking Reagent c. Denaturation Reagent d. Finishing Reagent (bottom)	Orient the plates so that the A1 wells are pointed toward deck position#1.
Place the stack on position#7.	
Make sure that the AssayMAP Bravo deck looks like the Deck Layout shown.	First section of the grey graphic above; labware is listed in the second section.
Click “Run Protocol 1.”	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.
Proceed to Protocol 2.	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
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

Assumes full columns are processed

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 lid from a Rack of RX Cartridges and place it with its Receiver Plate on position#6.
5. Tape the Cartridge Rack and Receiver Plate to the deck.

Set the parameters in the Application Settings section:

Third section of the green graphic above.

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.

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.

3. Indicate the first column in the Seating Station into which these columns of RX Cartridges will be placed.

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.

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

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

Click on "Run 2 RX Cartridge Setup."

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

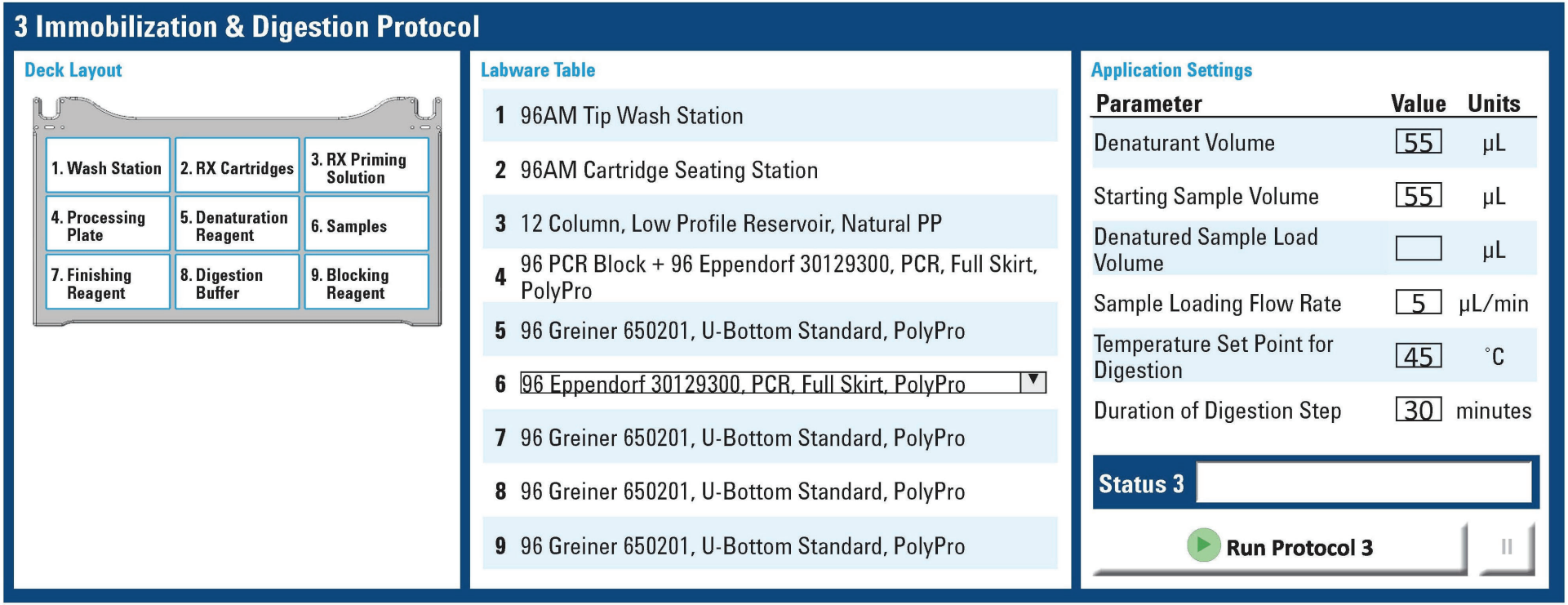
A status line will show when the protocol is complete: "Run complete. Module idle."

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.

Proceed to Protocol 3.

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



3 Immobilization & Digestion 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.

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.

Set the sample plate type in the "Labware" section, line 6:
Select PCR Plate (low volume) or U-Bottom Plate (high volume).

Middle section of the blue graphic above.

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

Set the parameters in the Application Settings section:

Third section of the blue graphic above.

1. Denaturant Volume: Enter the volume of Denaturation Reagent to be added to each Glycoprotein Sample.

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

2. Starting Sample Volume: Enter the starting volume of Glycoprotein Sample to be loaded.

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

3. Denatured Sample Load Volume (calculated value, grey).

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.

4. Enter the Sample Loading Flow Rate.

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.

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

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.

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

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

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

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

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

Click on “Run 3 Immobilization & Digestion.”

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.”

Proceed to Manual Processing.

The Processing Plate (position#4) now contains the N-glycans; remove for drying. The AssayMAP Bravo may be shutdown at this point.

Manual Processing - Drying the N-Glycans prior to labeling

Rearrange the deck in preparation for processing:

- Remove the Processing Plate (PCR plate) from position#4.

The Processing Plate now contains released N-Glycans with a free reducing end, along with Finishing Reagent.

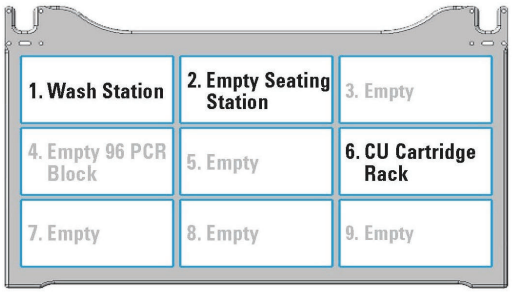
- Dry the N-Glycan Samples using a centrifugal evaporator with the heat setting turned to the off position for 20 minutes to 1 hour, or until fully dry. .

This is a good time to remove the 2-AB Solution and Reductant Solution so they can come to room temperature.

Protocol 4 may be processed simultaneously with Manual Processing.

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

Assumes full columns are processed

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.

May process a partial Rack.

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.

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.

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.

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.

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

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

Click on “Run 4 CU Cartridge Setup.”

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.

When the N-glycan Samples are dry, proceed to Manual Processing - Labeling the N-Glycans with 2-AB.

Manual Processing - Labeling the N-Glycans with 2-AB

Prepare Labeling Reagent.

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

.

☛ Prepare no more than one hour before use

Allow the 2-AB Solution and Reductant Solution vials to come to room temperature in the sealed desiccant bag before removing them. Invert each to mix gently. 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: 2-AB Labeling Reagent components are hazardous. Please refer to the Safety Data Sheets included in the Kit or on our website. Perform this procedure using appropriate personal safety protection, eyeglasses and nitrile gloves.

In a separate vial, add the amounts of 2-AB Solution and Reductant Solution as indicated in the Reagent Volume Calculator, and then vortex to mix thoroughly.

NOTE: Both the 2-AB Solution and Reductant Solution are hygroscopic; minimize exposure to air and protect from exposure to light. The individual reagents may be tightly capped, repackaged with the desiccant in the resealable bag, and frozen (-20°C) for storage up to 6 months.

Use of the Labeling Source Plate is optional.

Pipette equal volumes of 2-AB Labeling Reagent into each well of the first column of the Labeling Source Plate (PCR plate) as instructed in the Labeling Source Plate tab of the Reagent Volume Calculator.

Dried N-glycans in Processing Plate

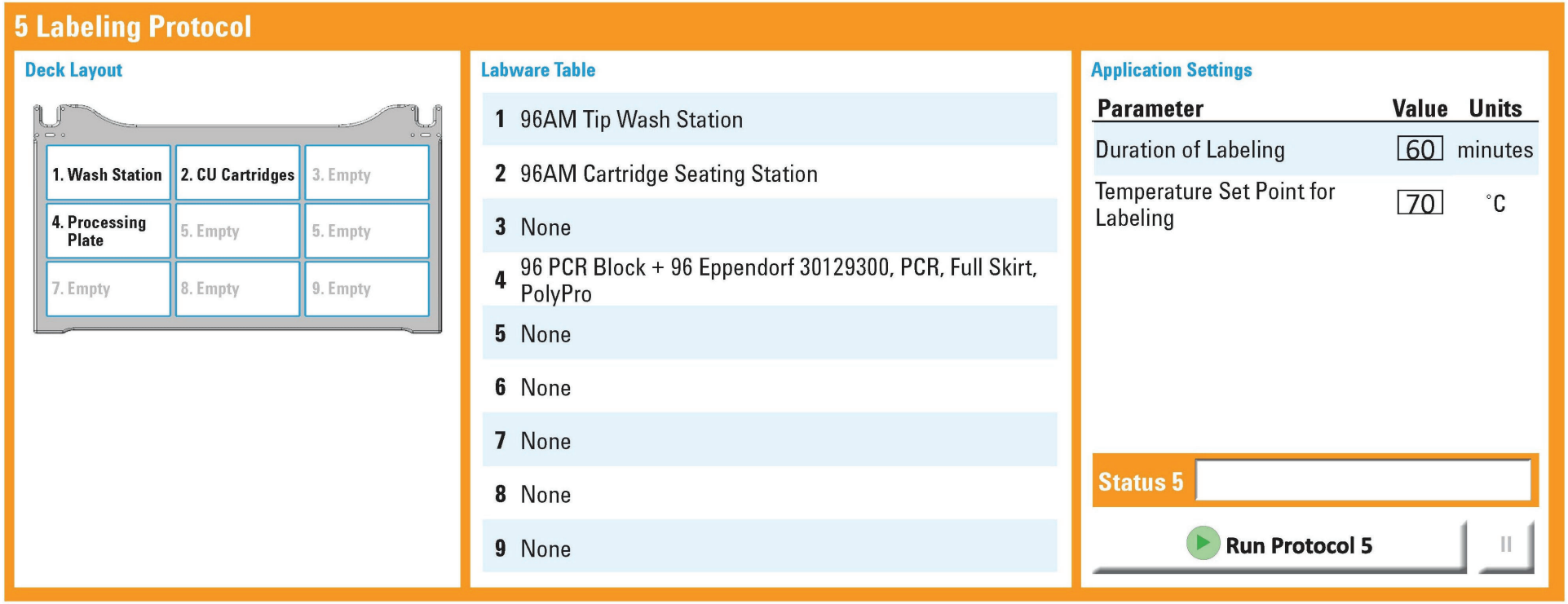
Add the Labeling Reagent:

- Add 5 µl of Labeling Reagent to the side of each N-Glycan Sample well about half way down the side of the well.
- Tap the plate sharply on the bench and centrifuge briefly to ensure that the droplet of Labeling Reagent drops to the bottom of the well.
- Cover the plate with the Aluminum Seal provided.

Visually inspect to make sure there are no bubbles at the bottom of the well.

Remove the tabs from the Aluminum Seal, as they can interfere with operation of the AssayMAP Bravo.

Proceed to Protocol 5.



5 Labeling Protocol

Place the sealed Processing Plate containing the N-Glycan Samples plus Labeling Reagent on position#4.

Set the parameters in the Application Settings section:

- Enter the desired labeling reaction duration.
- Enter the desired labeling reaction temperature.

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

Third section of the orange graphic above.

Default duration is 60 minutes.

Default temperature is 70°C; allowed temperature range is 20–70°C.

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

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

Click on “Run 5 Labeling.”

The Processing Plate containing N-Glycan Samples is moved from position#4 to position#9, where it is mixed for one minute. It will remain there until the Thermal Station temperature is within 1°C of the set point (~10 minutes to achieve 70°C). Then the plate will be moved to position#4 and the timer starts. When finished, the plate is moved to position#7 and the protocol ends. The plate movement ensures that the N-Glycan Samples are at the desired temperature for the set amount of time with no additional temperature ramp up or down.

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

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.

Prepare Cleanup Protocol Reagents during the labeling incubation.

Red section of the printed Reagent Volume Calculator “Sample Info & Reagent Prep” tab

NOTE: The maximum volume per channel is 7.0 ml.

- 96% Acetonitrile Solution (CU Priming and Washing Solutions):
  Add the ultrapure water 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.

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

- 2-AB Sample Load Solution:

☛ Prepare on the day of use. Store sealed in a similarly sized, clean, glass container until the Cleanup Protocol in order to prevent evaporation of the acetonitrile. Precise control of the acetonitrile concentration is important to ensure both retention of N-glycans and removal of excess dye during Cleanup.

Add the amount of 5x 2-AB Sample Load Solution indicated in the Reagent Volume Calculator to a glass graduated cylinder using a volumetric pipette. Bring the volume up to the final volume with HPLC-grade acetonitrile.

NOTE: Due to the viscosity and surface tension of the 5x 2-AB Sample Load Solution, we recommend using only glass cylinders. Use volumetric pipettes to assure accurate addition of the 5x 2-AB Sample Load Solution; do not use a standard pipette.

Transfer to a similarly sized glass container, cap tightly and swirl gently to mix. Before using, allow solution to come to room temperature and then gently invert to mix contents..

It is important to prevent preferential evaporation of the acetonitrile, which affects the performance of the HILIC separation.

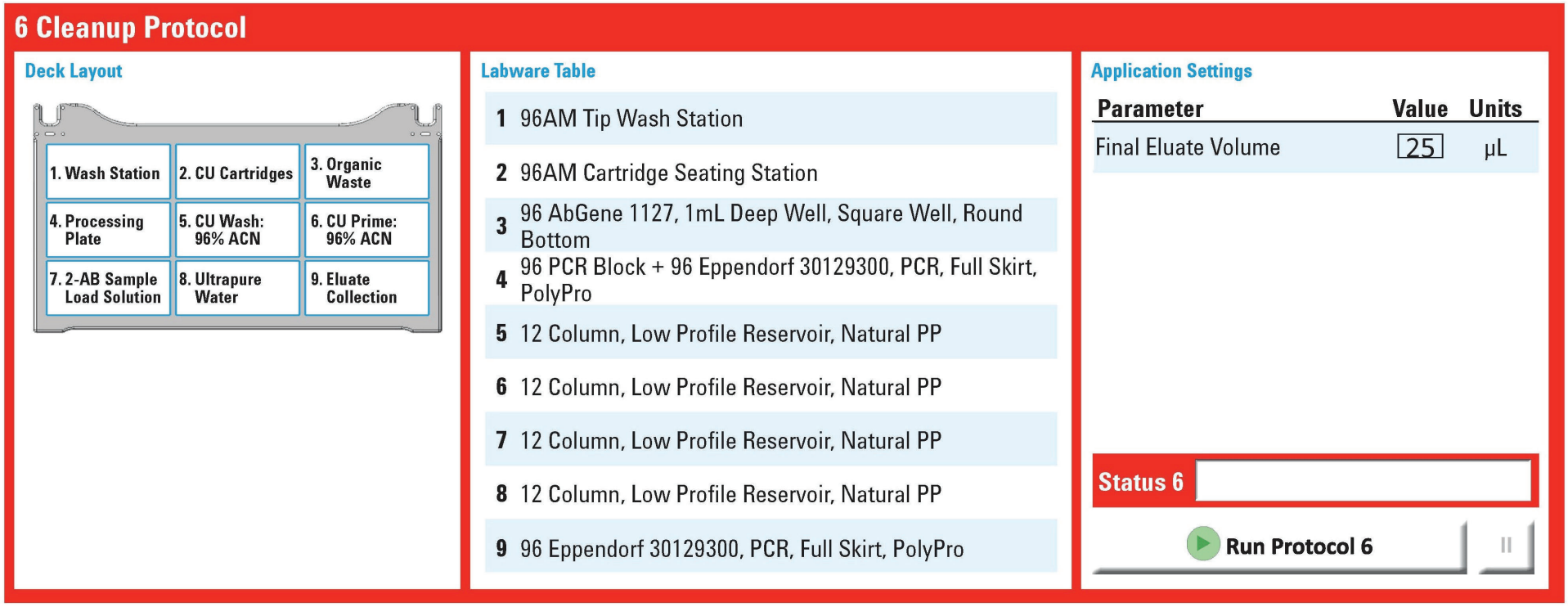
At the software prompt, prepare for resuspension of the labeling reaction:

1. Move the plate containing the labeled N-Glycan Samples from position#7 to a fume hood.
2. Allow the plate to cool for 5 minutes to room temperature.
3. Remove the Aluminum Seal.

Remove the Aluminum Seal in a fume hood to avoid inhalation of hazardous reaction products.

NOTE: Be sure to remove all excess aluminum, as it can interfere with the AssayMAP Bravo grippers and sensors

Proceed to Protocol 6.



6 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).

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 Processing Plate containing the Labeled N-Glycan Samples on position#4.

After removing the Aluminum Seal in a fume hood, each well now contains Labeled N-Glycan Samples plus unreacted dye, which will be removed using HILIC chromatography.

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

1. Place the 12-Column, Reservoir Plate containing CU Washing Solution (3 ml each reservoir of 96% Acetonitrile) on position#5.

2. Place the 12-Column, Reservoir Plate containing CU Priming Solution (6 ml each reservoir of 96% Acetonitrile) on position#6.

3. Place a 12-Column, Reservoir Plate containing 2-AB Sample Load Solution on position#7.

4 Place a 12-Column, Reservoir Plate containing HPLC-grade water 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#9.

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

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

Click on "Run 6 Cleanup."

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 Eluate Collection Plate containing the Labeled N-Glycan Samples from position#9.
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.

Glycoprotein Sample Plate Preparation

Prepare the Glycoprotein Sample Plate with required volumes using the Sample Info & Reagent Prep tab in the Reagent Volume Calculator 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. Blocking Reagent (provided with the Kit)
4. Denaturation Reagent (provided with the Kit)
5. Finishing Reagent (provided with the Kit)

Print the "Reagent Source Plate Setup" tab of the Reagent Volume Calculator.

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.

Optional Labeling Source Plate Preparation

See Labeling Source Plate tab of the Reagent Volume Calculator.

No more than one hour prior to use, Labeling Reagent may be prepared in Column 1 of the Labeling Source Plate and transferred manually by multi-channel pipette to the N-glycan Samples in the Processing Plate.

Preparation of the AssayMAP Bravo and Associated Equipment

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

Open the AssayMAP LaunchPad web interface from the desktop to access the Reagent Volume Calculator, GlykoPrep-plus Protocols and support documents.

Under the N-Glycan Sample Prep tab, click on the 2-AB icon to launch N-Glycan Sample Prep: RX digestion & 2-AB labeling.

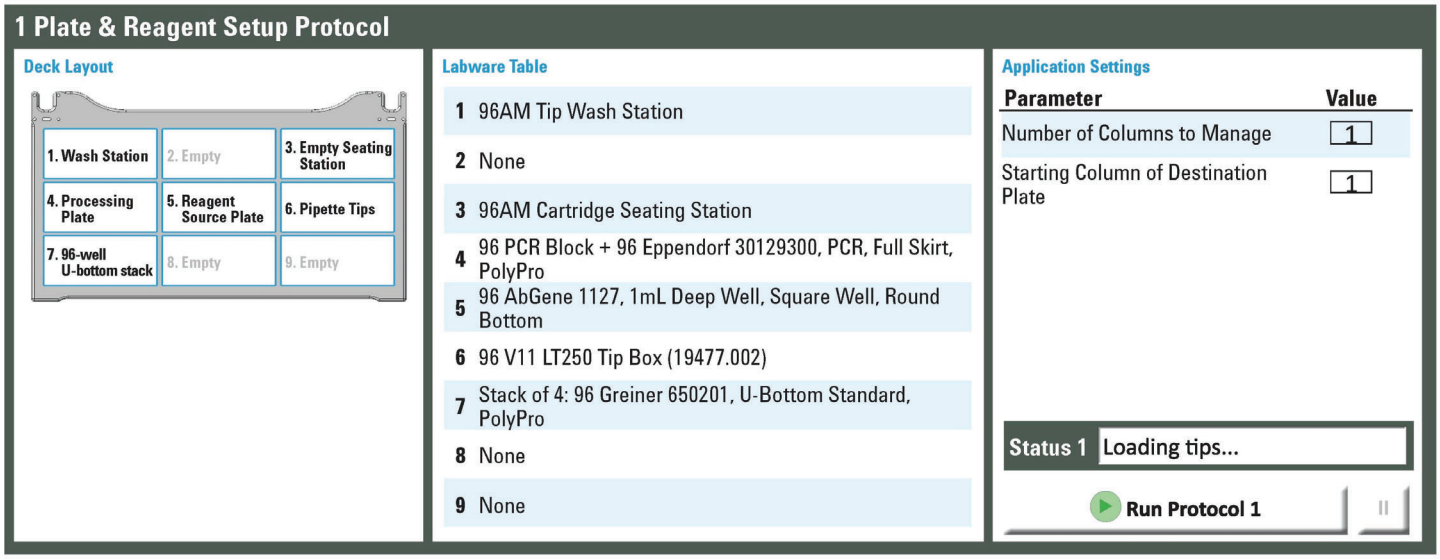
Verify that the wash station is installed on position#1.

- 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 postion#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

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.

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.

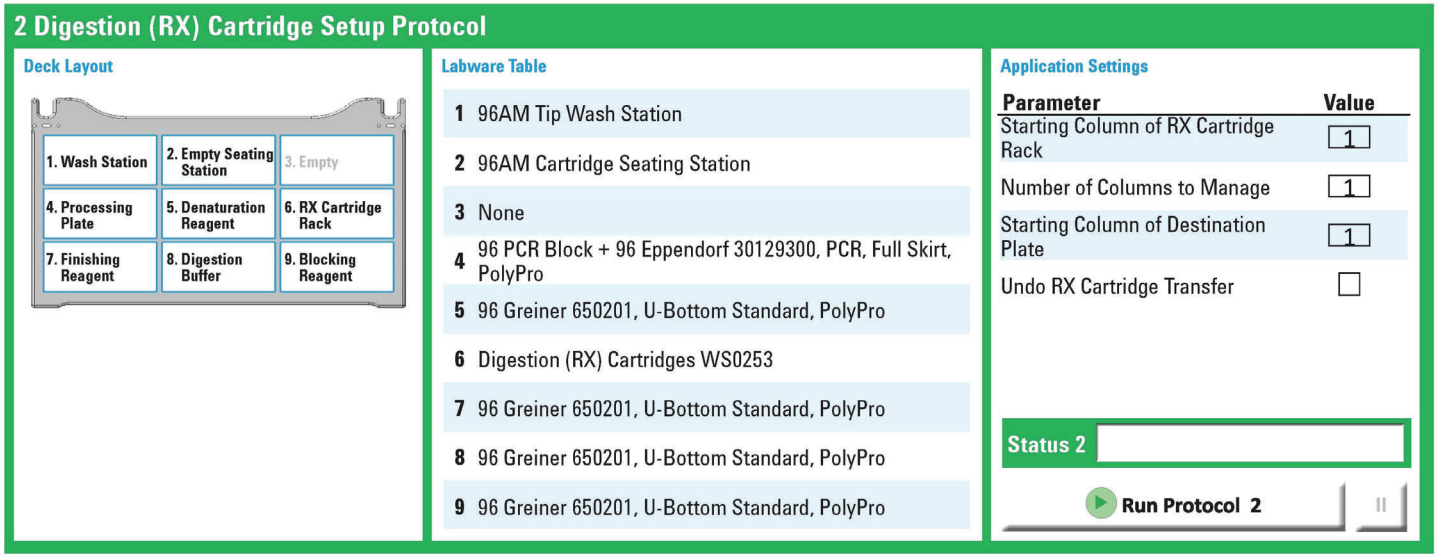
Label and stack 4 U-Bottom Plates:
a. Digestion Buffer (top)
b. Blocking Reagent
c. Denaturation Reagent
d. Finishing Reagent (bottom)

Place the stack on position#7.

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 RX Cartridge Setup Protocol

Rearrange the deck in preparation for processing:

3. Remove the Reagent Source Plate from position#2; discard.

4. Remove the Seating Station on position#3 and discard the used pipette tips.

5. Place the now empty Seating Station on position#2.

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

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

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.

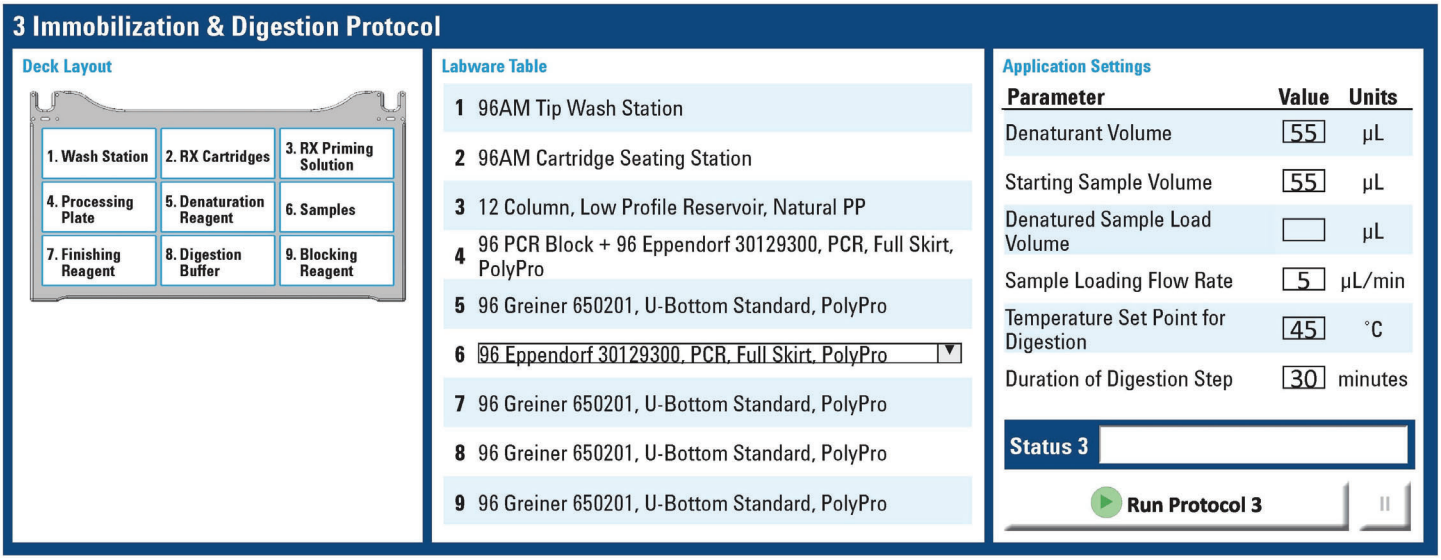
3. Indicate the first column in the Seating Station into which these columns of RX Cartridges will be placed.

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 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" 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 Processing Plate (position#4) now contains the N-glycans; remove for drying. The AssayMAP Bravo may be shutdown at this point.

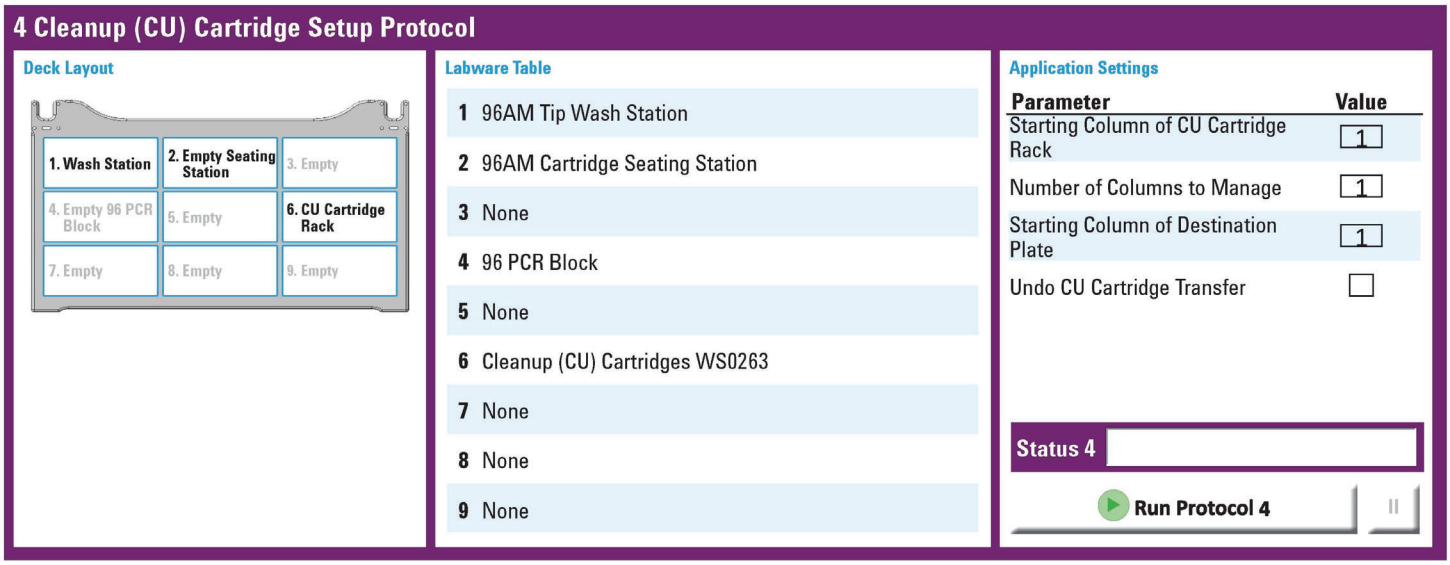
Proceed to Manual Processing.

Manual Processing - Drying the N-Glycans prior to labeling

Rearrange the deck in preparation for processing:

- Remove the Processing Plate (PCR plate) from position#4.
- Dry the N-Glycan Samples using a centrifugal evaporator with the heat setting turned off until fully dry (20 minutes to 1 hour).

Protocol 4 may be processed simultaneously with Manual Processing.



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."

The approximate run time is 1 minute. When the N-glycan Samples are dry, proceed to Manual Processing - Labeling the N-Glycans with 2-AB.

Manual Processing - Labeling the N-Glycans with 2-AB

Prepare Labeling Reagent (orange section of the printed Reagent Volume Calculator "Sample Info & Reagent Prep" tab).

☛ Prepare no more than one hour before use

Allow the 2-AB Solution and Reductant Solution vials to come to room temperature in the sealed desiccant bag before removing them. Invert each to mix gently.

NOTE: 2-AB 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.

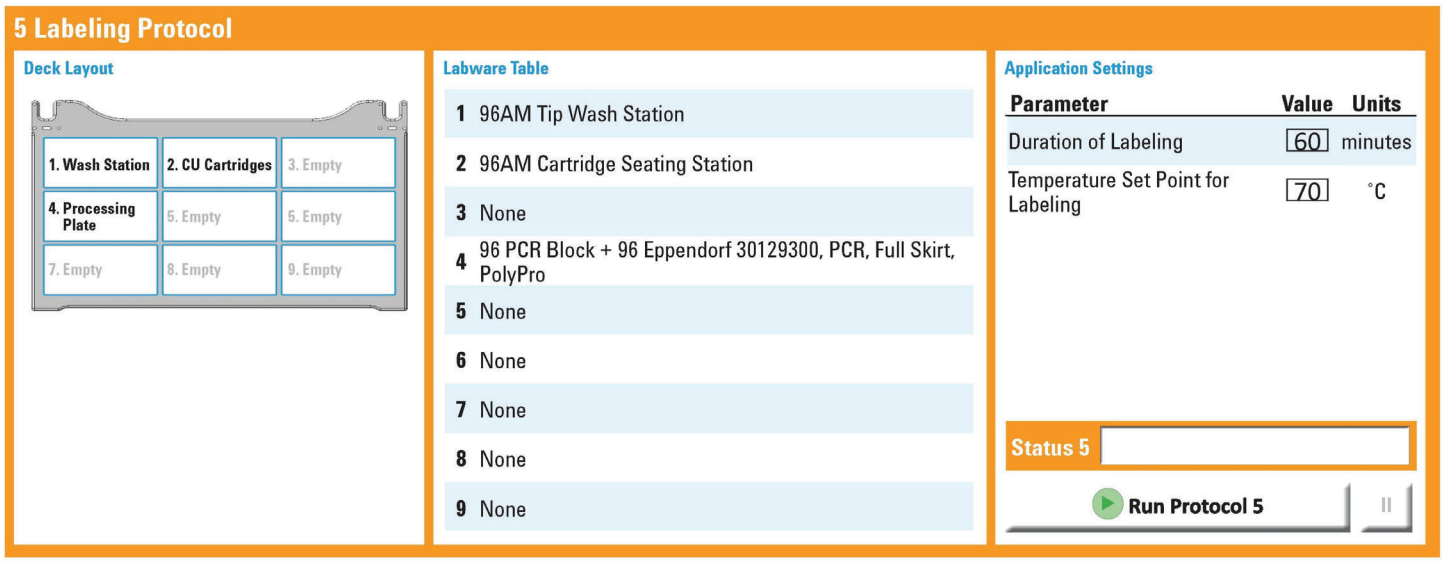
In a separate vial, add the amounts of 2-AB Solution and Reductant Solution as indicated in the Reagent Volume Calculator, and then vortex to mix thoroughly.

Optional: Pipette equal volumes of 2-AB Labeling Reagent into each well of the first column of the Labeling Source Plate (PCR plate) as instructed in the Labeling Source Plate tab of the Reagent Volume Calculator.

Add the Labeling Reagent to dired N-glycans in the Processing Plate:

- Add 5 µL of Labeling Reagent to the side of each N-Glycan Sample well about half way down the side of the well.
- Tap the plate sharply on the bench to ensure that the droplet of Labeling Reagent drops to the bottom of the well.
- Cover the plate with the Aluminum Seal provided.

Proceed to Protocol 5.



5 Labeling Protocol

Place the sealed Processing Plate containing the N-Glycan Samples plus Labeling Reagent on position#4.

Set the parameters in the Application Settings section above:

- Enter the desired labeling reaction duration. The default duration is 60 minutes.
- Enter the desired labeling reaction temperature. The default temperature is 70°C; allowed temperature range is 20 - 70°C.

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

Click on "Run 5 Labeling."

During the labeling incubation, prepare the Cleanup Protocol reagents as indicated in the Red section of the printed Reagent Volume Calculator "Sample Info & Reagent Prep" tab:

- 96% Acetonitrile Solution (CU Priming and Washing Solutions):

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

Add the ultrapure water 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.

- 2-AB Sample Load Solution:

☛ Prepare on the day of use. Store sealed in a similarly sized, clean, glass container until the Cleanup protocol.

Add the amount of 5x 2-AB Sample Load Solution indicated in the Reagent Volume Calculator to a glass graduated cylinder using a volumetric pipette.

Bring the volume up to the final volume with HPLC-grade acetonitrile.

Transfer to a similarly sized glass container, cap tightly and swirl gently to mix.

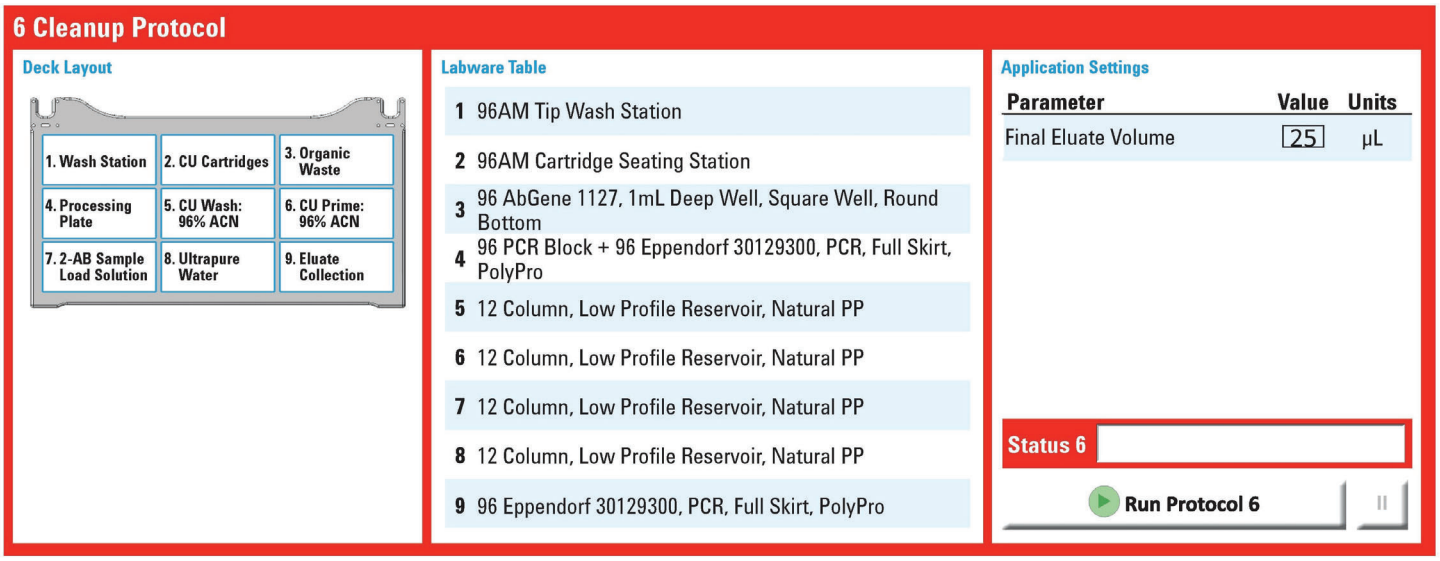
Allow solution to come to room temperature and then gently invert to mix contents before using.

At the software prompt, prepare for resuspension of the labeling reaction:

1. Move the plate containing the labeled N-Glycan Samples from position#7 to a fume hood.
2. Allow the plate to cool for 5 minutes to room temperature.
3. Remove the Aluminum Seal.

NOTE: Remove the Aluminum Seal in a fume hood to avoid inhalation of hazardous reaction products.

Proceed to Protocol 6.



6 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).

After removing the Aluminum Seal in a fume hood, place the Processing Plate containing the Labeled N-Glycan Samples on position#4.

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 CU Washing Solution (3 ml each reservoir of 96% Acetonitrile) on position#5.
- Place the 12-Column, Reservoir Plate containing CU Priming Solution (6 ml each reservoir of 96% Acetonitrile) on position#6.
- Place a 12-Column, Reservoir Plate containing 2-AB Sample Load Solution on position#7.
- Place a 12-Column, Reservoir Plate containing HPLC-grade water on position#8.

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

Place an empty PCR Plate on position#9 for Eluate.

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

Click on "Run 6 Cleanup."

Upon completion of the protocol:

a. Remove the Eluate Collection Plate containing the Labeled N-Glycan Samples from position#9. 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.

Empty the Wash Station waste container.

If the next processing is NOT a GlykoPrep workflow, remove all appliances from the AssayMAP Bravo deck.

NOTE: The presence of the Wash Station, the Thermal Station and the Orbital Shaker can cause damage to the AssayMAP Bravo Head if the next workflow has not been defined to include them.

ANALYSIS OF LABELED N-GLYCANS

Use standard techniques, such as Liquid Chromatography (LC), Mass Spectrometry (MS), or a combination of the two, to analyze the aqueous eluate containing eluted, labeled N-glycans (see Tips and Hints, below).

TIPS & HINTS

Optimizing Excitation/Emission Wavelengths

Optimal excitation/emission wavelengths for 2-AB Dye conjugated to an N-glycan may vary depending upon the optical configuration of the instrument used. Excitation/emission pairs that have been used together include 250/428 nm (Melmer et al., 2010), 330/420 nm (Bigge et al., 1995) and 360/428 nm (used by ProZyme with the Waters® Acquity® UPLC®; Haxo et al., 2012).

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 2-AB

The reductive amination reaction results in the loss of an oxygen atom. The mass of the 2-AB-labeled N-glycan is obtained using the following formula:

$$Mass_{Glycan} + Mass_{2\text{-}AB} = Mass_{2\text{-}AB\text{-}Labeled\ Glycan} - Mass_{Oxygen}$$

Mass Added to Glycan	
Monoisotopic	120.06875
Average	120.2

Direct LC Analysis of N-Glycans After Elution

If N-Glycan Samples will be analyzed by LC directly following elution from the CU Cartridges, collection plates may be heat sealed with pierceable foil (e.g. Thermo Easy Pierce 20um Foil, #AB-1720) using a microplate heat sealer (e.g. Thermo ALPS 50 V Semi automated Microplate Heat Sealer, #AB-1443).

REFERENCES

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