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 InstantPC™ Automated on AssayMAP Technology

An automated, highly robust, high-throughput process for enzymatic deglycosylation, fluorescent labeling with InstantPC 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
- InstantDye™ labeling of N-glycans with InstantPC provides high fluorescence and MS signal
- Purified InstantPC-labeled N-glycans are eluted in 10% acetonitrile/water, ready for analysis

Product Code: GPPNG-PC

TABLE OF CONTENTS

This product is intended for in vitro research use only.

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

Required software: AssayMAP Workbench version 3.0 and VWorks 13.1.1 (or later), including the GPPNG-PC GlykoPrep with InstantPC workflow.

If you have an earlier version of the software that does not contain the GPPNG-PC workflow, please contact ProZyme or Agilent for assistance.

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

prozyme.com/products/gppng-pc

Comments

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

KIT CONTENTS

GPPNG-PC Kit GlykoPrep-plus Rapid N-Glycan Sample Preparation with InstantPC	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-PC InstantPC Labeling Module WS0338 InstantPC Dye 6 ea (30mg) WS0339 InstantPC Dye Solvent 2 ea (600 µl)	1 ea
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-PC. 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-PC 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 Product Code.

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:

www.prozyme.com/GlykoPrep

USING THE KIT

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

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

  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.

  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.

  We provide two alternative denaturation protocols: Reduction-Denaturation with TCEP, and Reduction-Denaturation-Alkylation. For more details contact ProZyme or visit www.prozyme.com/GlykoPrep-reduction-denaturation.

- 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 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).

  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-PC Kit, must be prepared prior to dispensing into the Reagent Source Plate. Please refer to the directions in the GPPNG-PC 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.

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

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.

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 & InstantPC 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.

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.

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 Prep" 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 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.

☛ 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.

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

3. InstantPC Labeling Reagent

   InstantPC Dye (supplied with the kit)
   Dye Solvent (supplied with the kit)

   Add 150 µl of Dye Solvent directly into each 30 mg InstantPC Dye vial.

   4 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.

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 desiccant in a resealable bag, and frozen (-20°C) for storage up to 3 months and 10 freeze thaw cycles; return to RT before opening for use.

4. InstantPC Solution

   InstantPC Labeling Reagent, prepared above
   100% Acetonitrile

   In a solvent-resistant, 15-ml tube, add the amounts of InstantPC 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.

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

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.

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

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

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 InstantPC icon to launch N-Glycan Sample Prep: RX digestion & InstantPC labeling.

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

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.

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.

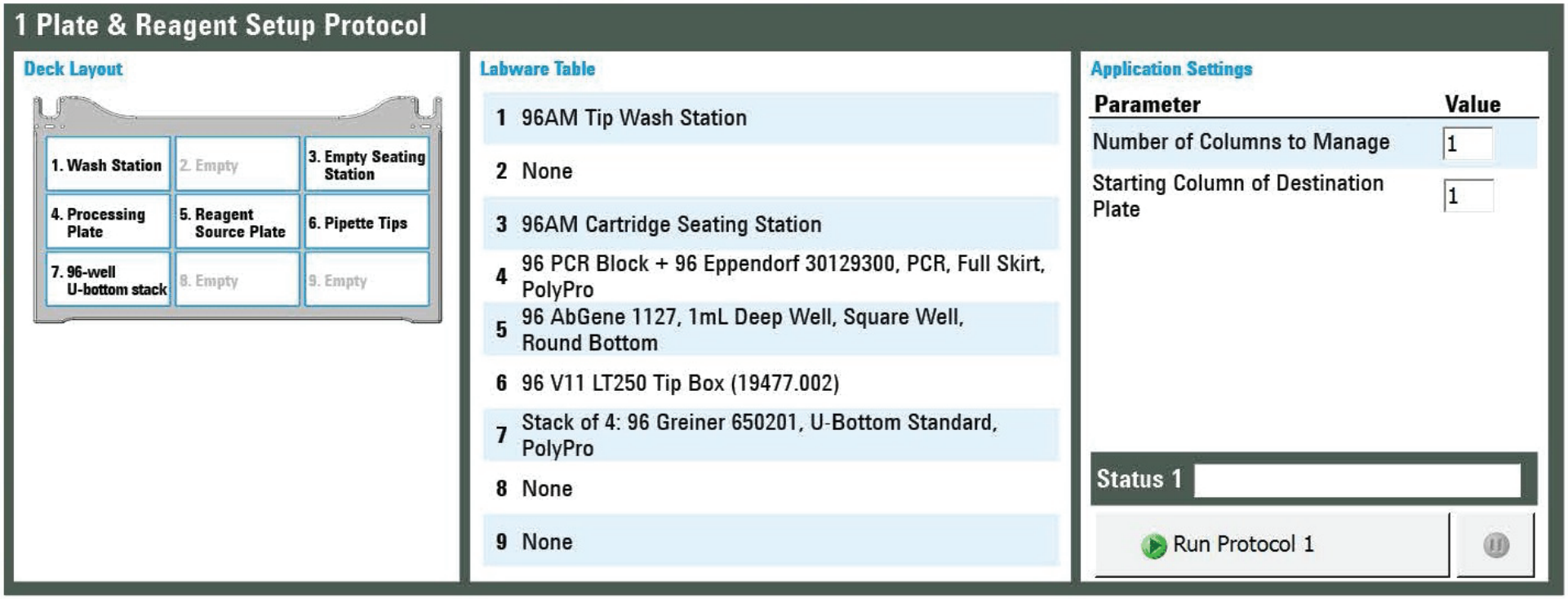
Verify that the Peltier Thermal Station with PCR Plate Adapter is installed on postion#4.

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, Digestion and InstantPC 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

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.

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.

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. InstantPC Solution (bottom)

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

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

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.”

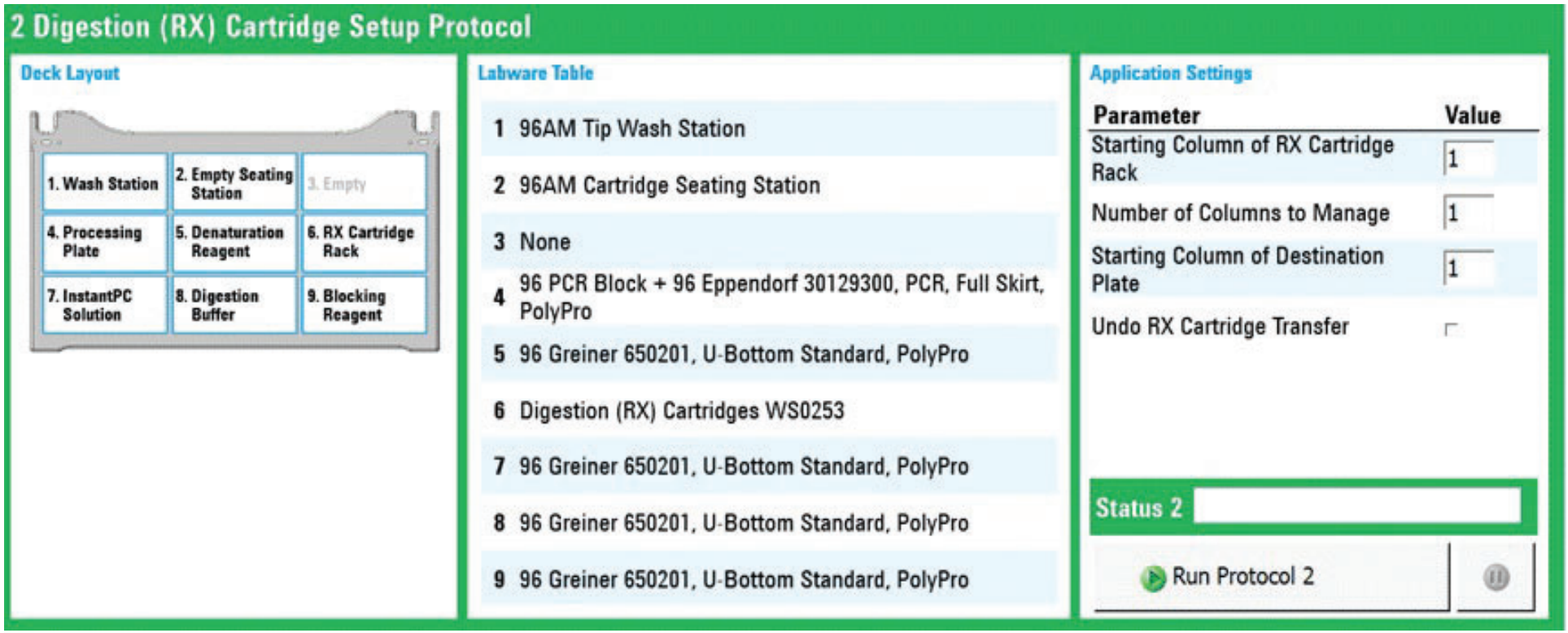
A status line will show when the protocol is complete: "Run complete. Module idle."

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.



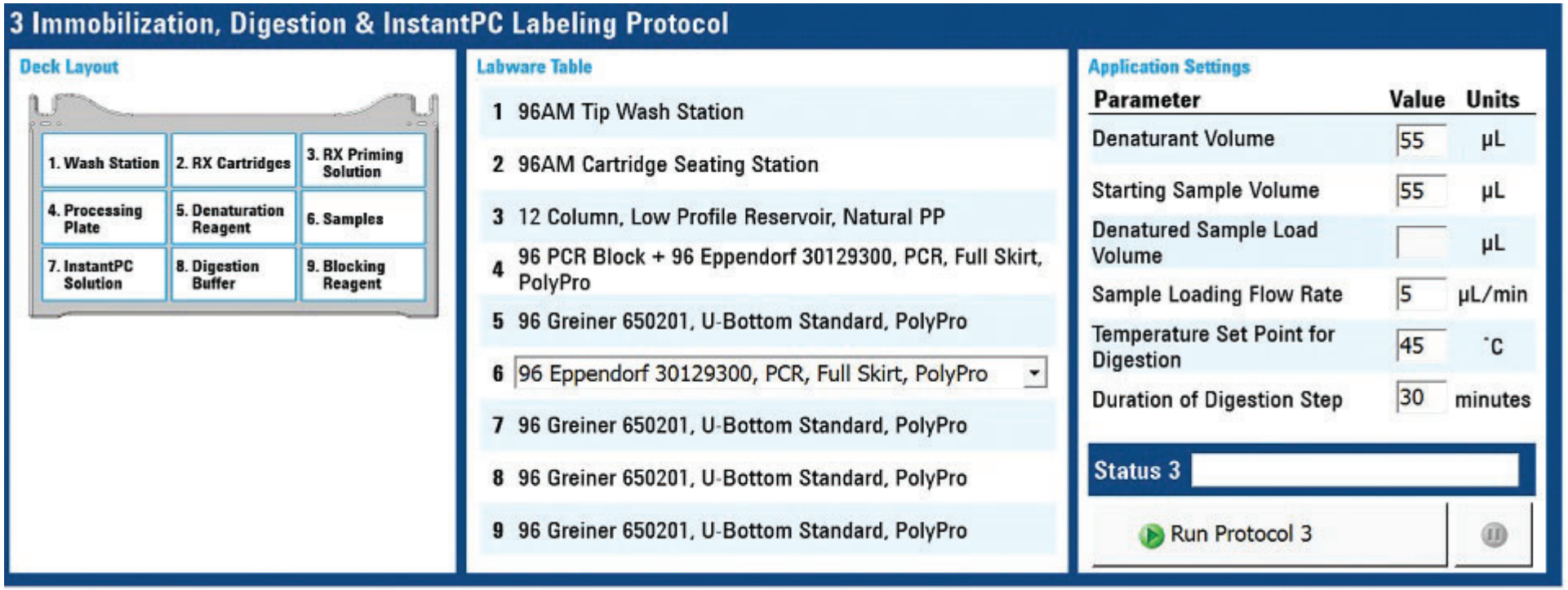
2 RX Cartridge Setup Protocol

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:	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 & InstantPC 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.

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:

Middle section of the blue graphic above.

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

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.

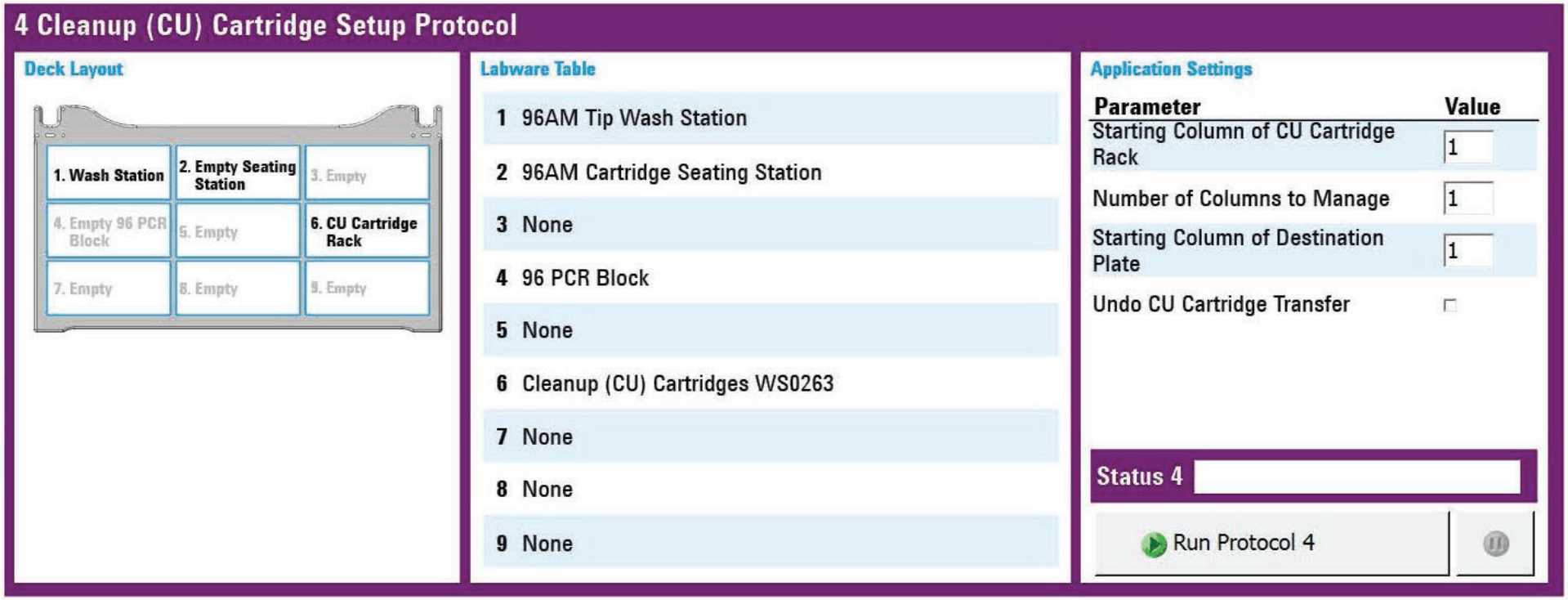
WARNING: Do not reach into the AssayMAP Bravo space while it is operating. This will break the security line; the AssayMAP Bravo will make an emergency stop, and may not be able to be restarted without intervention from a knowledgeable operator.

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

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

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 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.

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.

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."

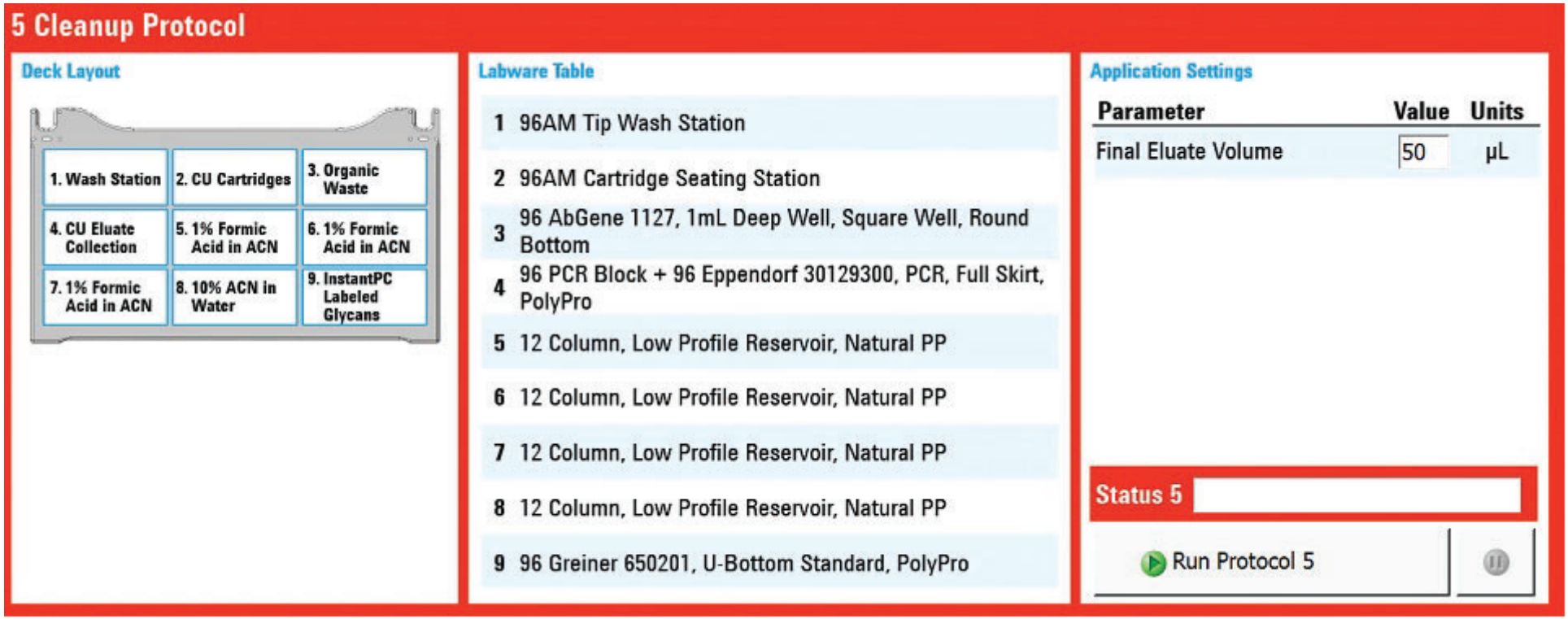
Approximate run time is 1 minute.

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.

Prepare: 1% Formic Acid in ACN Solution for Cleanup Protocol (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.



5 Cleanup Protocol

Assumes full columns are processed

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:

Third section of the red graphic above.

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

The AssayMAP Bravo elutes the Labeled N-Glycan Samples quantitatively from the CU Cartridges in 25 µl. 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 Elute Solution (6 ml each reservoir of 10% Acetonitrile in 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#4 for Eluate.

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

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

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

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 5 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 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 up to 1 month.
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

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. InstantPC Labeling Reagent

Add 150 µl of Dye Solvent directly into each 30 mg InstantPC Dye vial.

4 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. InstantPC Solution

InstantPC Labeling Reagent, prepared above
100% Acetonitrile

In a solvent-resistant, 15-ml tube, add the amounts of InstantPC 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 IPC icon to launch N-Glycan Sample Prep: RX digestion & IPC labeling.

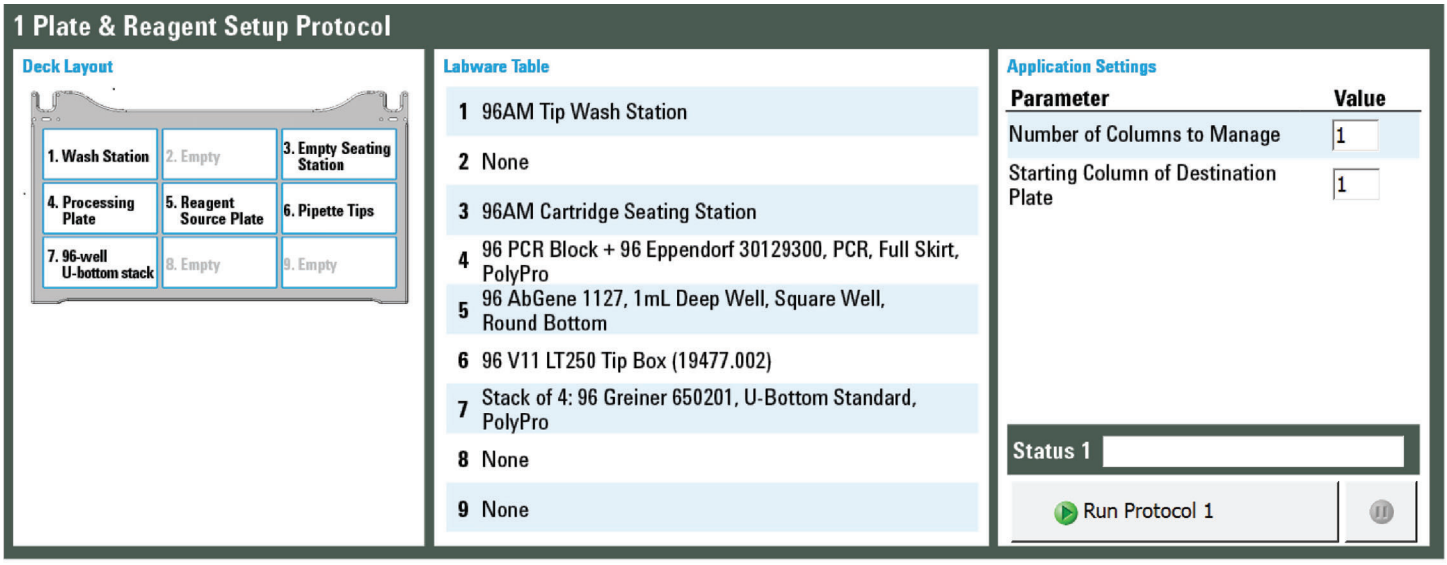
Verify that the Wash Station is installed on position#1.

- 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 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:

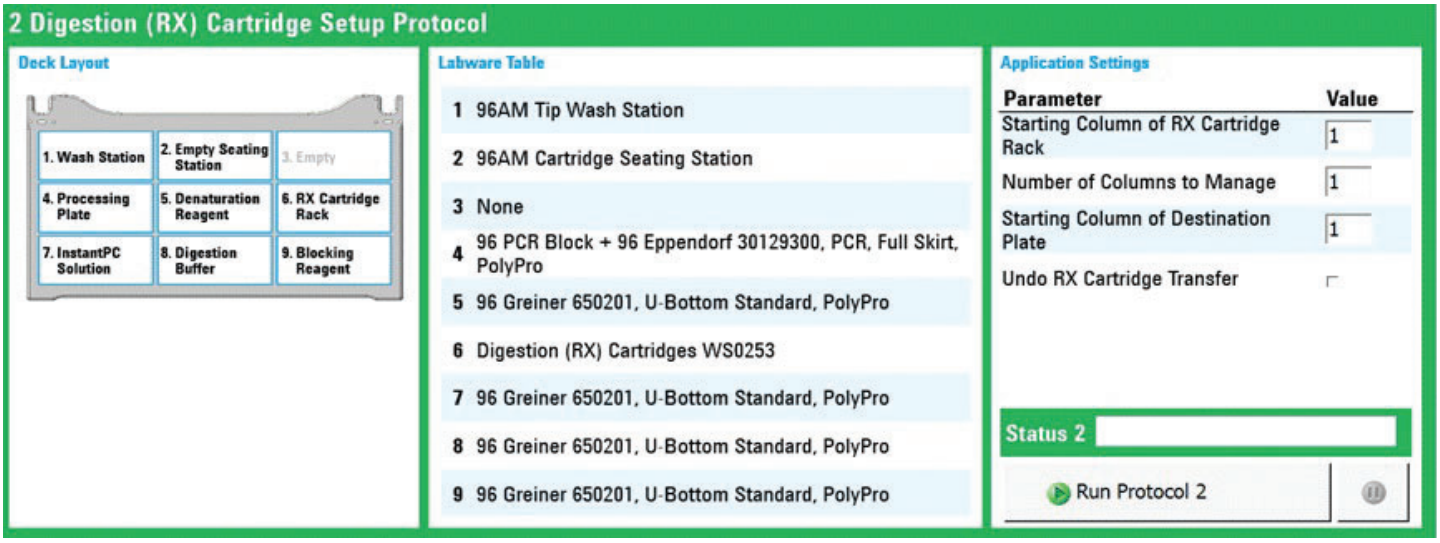
a. Digestion Buffer (top)
b. Blocking Reagent
c. Denaturation Reagent
d. InstantPC Solution (bottom)

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 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:

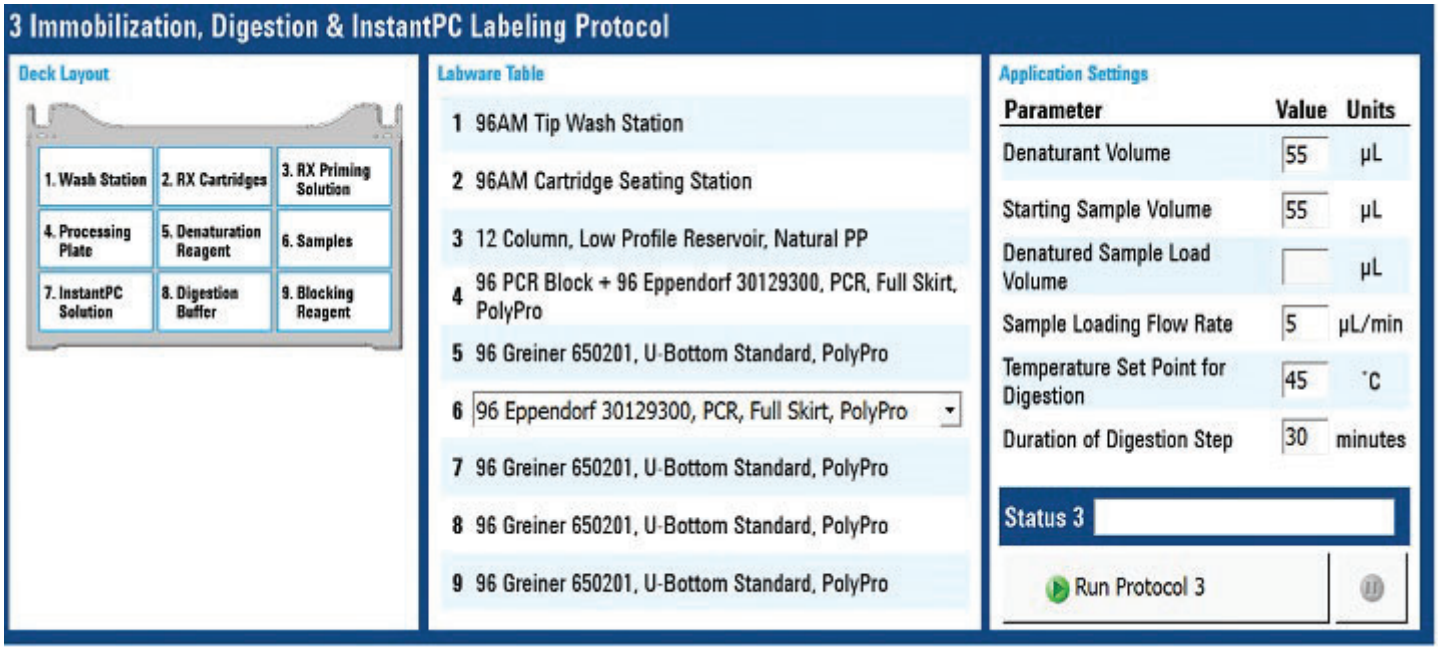
1. Indicate the first column of RX Cartridges to be used in the RX Cartridge Rack in position#6.
2. Indicate the number of columns to transfer. The columns directly follow the first column indicated above in the left to right direction.
3. Indicate the first column in the Seating Station into which these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

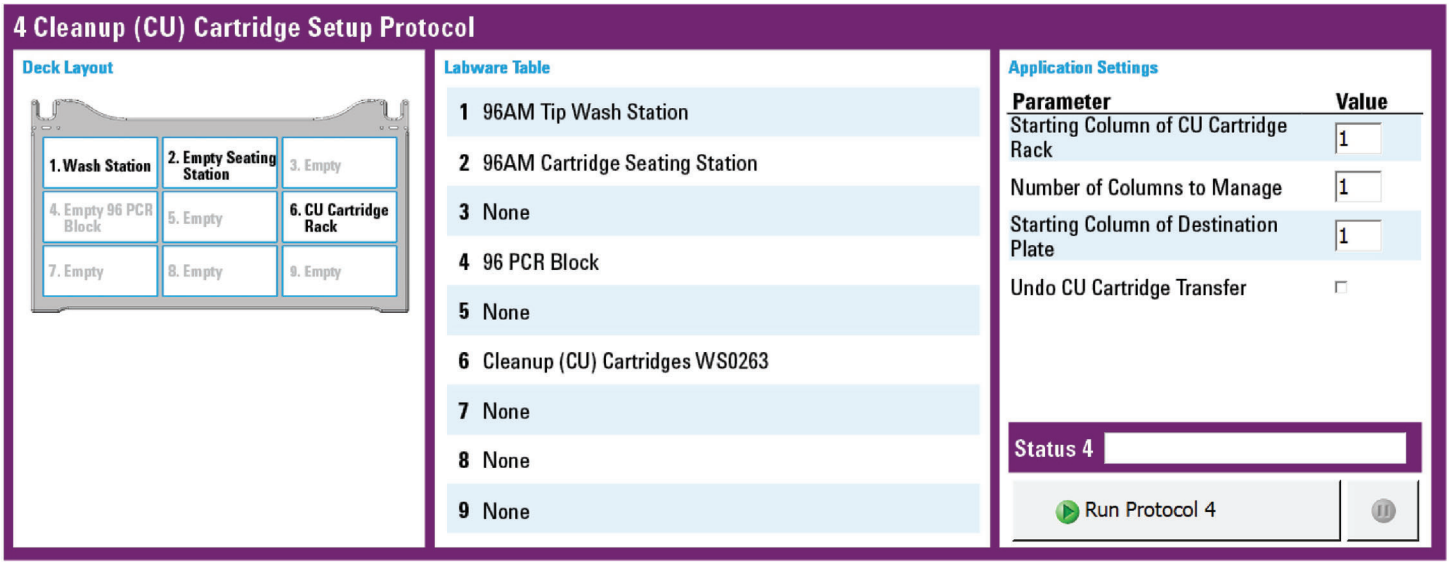
Click on “Run 3 Immobilization & Digestion.”

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 InstantPC in Acetonitrile and instantly labeled.

Proceed to 4 CU Cartridge Setup Protocol



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.

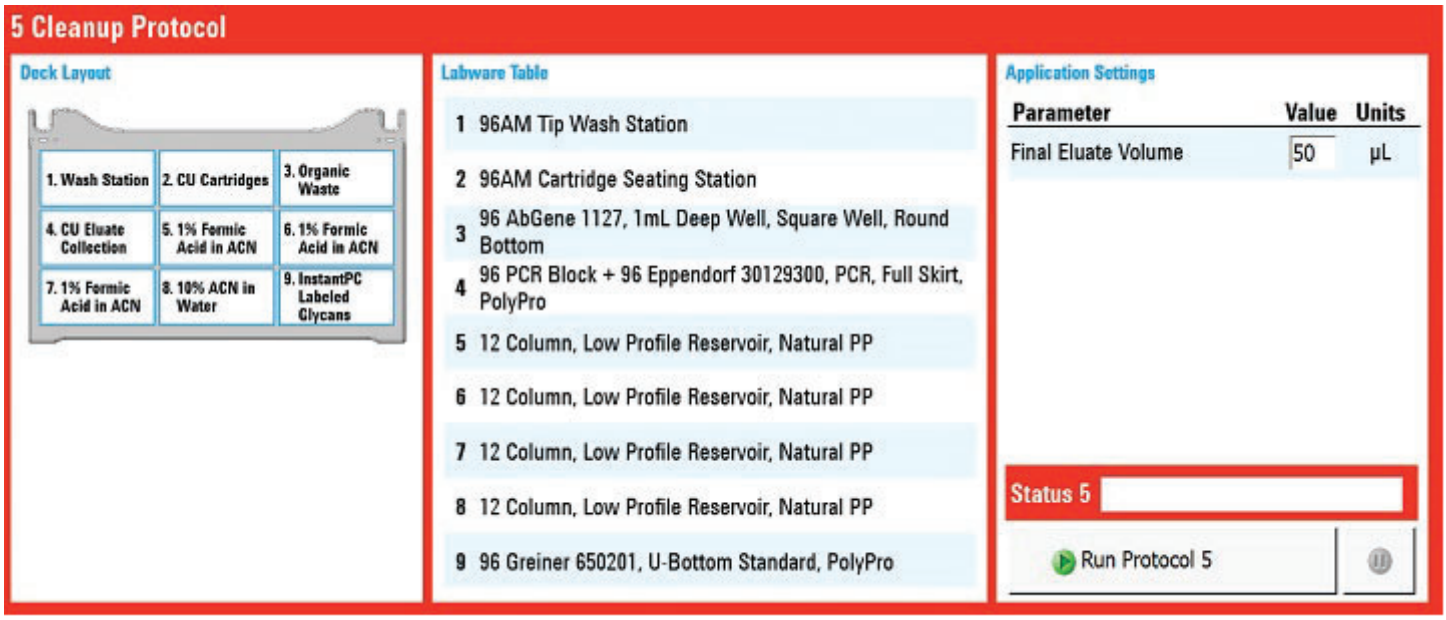
Click on "Run 4 CU Cartridge Setup."

Prepare 1% Formic Acid in Acetonitrile Solution for Cleanup Protocol (CU Priming, Sample Load and Washing Solutions):

Add of 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

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 Elute Solution (6 ml each reservoir of 10% Acetonitrile in 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 on position#4.
- Place the U-bottom Plate containing the InstantPC 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 up to 1 month.

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 GLYCANS

Standard techniques such as Hydrophilic Interaction Liquid Chromatography (HILIC) may be used to analyze labeled glycans.

Optimizing Excitation/Emission Wavelengths

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

Excitation: 285 nm
Emission: 345 nm

LC Injections

Injection of 1 µl InstantPC-glycans in InstantPC Eluent (10% (v/v) Acetonitrile) is recommended for UHPLC.

For larger injection volumes (>1 µl) of InstantPC-glycans, do not use ACN alone to dilute the sample. Use 1 part sample in eluent to 3 parts 50:50 ACN:DMF, for a final concentration of 22.5% aqueous buffer, 37.5% DMF, 40.0% ACN.

Suggested HILIC Conditions

5-Minute screening UHPLC method, Agilent AdvanceBio Glycan Mapping column:
2.1 x 100 mm, 2.7 µm. Column temperature 35 °C, excitation 285 nm, emission 345 nm.

Time (min)	Flow rate (ml/min)	% ACN	% 100 mM Ammonium Formate, pH 4.4
0.00	1.4	77	23
4.00	1.4	60	40
4.15	0.75	40	60
4.30	0.75	40	60
4.40	1.4	77	23
5.00	1.4	77	23

60-Minute high resolution UHPLC method, Agilent AdvanceBio Glycan Mapping column:
2.1 x 150 mm, 2.7 µm. Column temperature 45 °C, excitation 285 nm, emission 345 nm.

Time (min)	Flow rate (ml/min)	% ACN	% 50 mM Ammonium Formate, pH 4.4
0.0	0.4	80	20
43.5	0.4	54	46
45.0	0.4	0	100
50.0	0.4	0	100
52.0	0.4	80	20
60.0	0.4	80	20

For examples of HILIC separations of Instant-PC-labeled N-glycans using Agilent AdvanceBio Glycan Mapping columns, please see reference [1].

15-Minute UPLC method, Waters BEH GST column:
2.1 x 100 mm, 1.7 µm. Column temperature 60 °C, excitation 285 nm, emission 345 nm.

Time (min)	Flow rate (ml/min)	% ACN	% 100 mM Ammonium Formate, pH 4.4
0.0	1.0	75	25
12.0	1.0	50	50
12.1	0.5	40	60
12.5	0.5	40	60
12.6	0.5	75	25
13.0	1.0	75	25
15.0	1.0	75	25

60-Minute high resolution UPLC method, Waters BEH GST column:
2.1 x 150 mm, 1.7 µm. Column temperature 45 °C, max pressure 15,000 psi, excitation 285 nm, emission 345 nm.

Time (min)	Flow rate (ml/min)	% ACN	% 50 mM Ammonium Formate, pH 4.4
0.0	0.4	80	20
43.5	0.4	54	46
45.0	0.4	0	100
50.0	0.4	0	100
52.0	0.4	80	20
60.0	0.4	80	20

MS Analysis of InstantPC-labeled N-glycans

Suggested MS Conditions:
Waters Xevo G2-S QTof, + mode, capillary voltage 2.8 kV, cone voltage 30 V, source temperature 120 °C, desolvation temperature 350 °C, 0.8 second scan time, m/z range 300–2000 Da.

Suggested MS/MS Conditions:
Collision energy ramp of 40–60 V for +1; 15–30 V for +2; 15-25 V for +3; 1.0 second scan time, m/z range 50–2000 Da.
InstantPC is suitable for Collision Induced Dissociation (CID) MS/MS. As with other positively charged tags such as Procainamide, the CID profile contains mostly glycosidic cleavages with some cross-ring fragmentation. For an example, please see reference [1].

Adducts in MS analysis of InstantPC-labeled glycans:

In positive mode MS, most biantennary InstantPC- N-glycans will give $[M+2H]^{+2}$, larger sialylated will be majority $[M+3H]^{+3}$. InstantPC-glycan masses for some major N-glycan species present on biotherapeutics:

Glycan ID	IPC-Glycan Monoisotopic Mass	$[M+2H]^{+2}$	$[M+3H]^{+3}$
Man5	1495.5811	748.7978	499.5343
G0	1577.6343	789.8244	526.8854
G0F	1723.6922	862.8534	575.5713
G1	1739.6871	870.8508	580.9030
G1F	1885.7450	943.8798	629.5889
G2F	2047.7978	1024.9062	683.6065
A1	2192.8353	1097.4249	731.9524
A1F	2338.8932	1170.4539	780.6383
A2	2483.9307	1242.9726	828.9842
A2F	2629.9886	1316.0016	877.6701

Calculating the Mass of Glycans Labeled with InstantPC

Mass added to glycan with a free reducing end:

$Mass_{Glycan\ (free\ reducing\ end)} + C_{14}N_3O_2H_{19} = Mass_{InstantPC\text{-}Labeled\ Glycan}$

Mass added by $C_{14}N_3O_2H_{19}$ (Da):

Monoisotopic: 261.14773
Average: 261.3

Mass added to glycosylamine:

$Mass_{Glycan\ (glycosylamine)} + C_{14}N_2O_3H_{18} = Mass_{InstantPC\text{-}Labeled\ Glycan}$

Mass added by $C_{14}N_2O_3H_{18}$ (Da):

Monoisotopic: 262.13174
Average: 262.3

FAQs

Q. Why do you suggest 100 mM ammonium formate (pH 4.4) for 5- and 15-minute HILIC separations, 50 mM ammonium formate for the 60-minute separations?

A. For faster HILIC separations we use 100 mM ammonium formate. For MS in conjunction with longer separations we use 50 mM ammonium formate.

Q. Is there a method to recover the deglycosylated protein from the Digestion (RX) cartridge for analysis?

A. Please contact us for guidelines for eluting your glycoprotein from the RX Cartridge.

Q. Can inject glycans onto LC directly from the Cleanup Collection Plate?

A. PCR 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). The dimensions of the cleanup plates can be used to program positioning of the LC sample probe.
We advise against direct LC injection after sealing plates with the aluminium sealing film included with this kit. Using sealing film with adhesive may cause system problems.

REFERENCES

1. Kimzey et al. Development of an Instant Glycan Labeling Dye for High Throughput Analysis by Mass Spectrometry. Poster presented at ASMS 2015, St. Louis MO.

prozyme.com/posters/instantpc

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

www.prozyme.com

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

www.prozyme.com/documents/TNGP100.pdf

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TOLL FREE **(800) 457-9444** (US & CANADA)
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FAX **(510) 638-6919**
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Agilent AssayMAP

FAX (510) 638-6919

3832 Bay Center Place
Hayward, CA 94545-3619

TOLL FREE (800) 457-9444
PHONE (510) 638-6900

E-MAIL info@prozyme.com