Agilent

Agilent Gly-X N-Glycan Rapid Release and Labeling with InstantAB Kit (formerly ProZyme)

User Manual

Notices

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GX96-IAB
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Contents

Introduction

The Agilent Gly-X N-Glycan Rapid Release and Labeling with InstantAB kit (formerly Prozyme) uses a novel in-solution enzymatic protein deglycosylation followed by rapid labeling of released N-glycans with InstantAB dye. After a simple clean-up step, the glycan samples are ready for analysis by LC-FLR, mass spectrometry and other methods. With deglycosylation and labeling carried out in solution, the method is simple, rapid, and suitable for automation. The InstantAB dye delivers proven fluorescence and MS performance within well-established glycan analysis workflows. Other benefits include:

- Small molecule size for optimal chromatographic peak resolution
- Flexible, high-throughput format: process 1 to 96 samples
- Optimized cleanup removes excess free dye, protein, and other interfering compounds

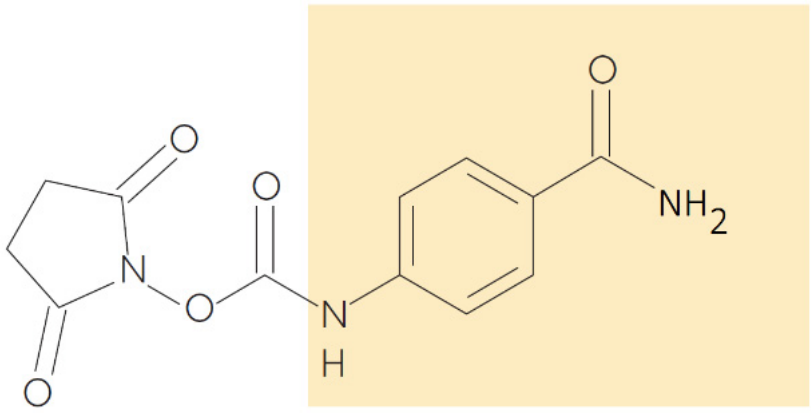

Figure 1. InstantAB dye (IAB)

Kit Components

The Gly-X with InstantAB (GX96-IAB) consists of three modules. (See **Table 1**.) Each module provides enough reagents for up to 96 samples per run.

Table 1 Kit components

Module	Component	Units	Storage
Gly-X Deglycosylation Module GX96-100	Gly-X Deglycosylation plate, 96 wells	1	RT or 4 °C
	Gly-X N-Glycanase, 1 mg/mL, 120 µL	1	4 °C
	Gly-X Digestion buffer, 240 µL	2	RT or 4 °C
	Gly-X Denaturant, 240 µL	1	RT or 4 °C
InstantAB Labeling Module GX96-501	InstantAB dye	2	-20 °C
	InstantAB dye solvent*	2	-20 °C
Gly-X InstantAB Cleanup Module GX96-502†	Gly-X Cleanup plate A	1	RT or 4 °C
	Gly-X InstantAB Eluent‡	1	RT or 4 °C
	Gly-X Waste tray	1	RT or 4 °C
	Gly-X Used-well sealing caps, black (for Cleanup plate)	96	RT or 4 °C
	Gly-X Collection plate, 96 wells	1	RT or 4 °C

* InstantAB dye solvent is N,N-Dimethylformamide (DMF).

† Gly-X Vacuum manifold spacer (GX100) is required for cleanup module, see **Figure 2**.

‡ Gly-X InstantAB Eluent is 160 mM Ammonium Formate with 10% (v/v) Acetonitrile.

Figure 2. GX100, Gly-X Vacuum manifold spacer, required

Equipment and Reagents Provided By User

A 96-well Thermocycler or two independent heat blocks, set for 90 °C and 50 °C. (for example, Corning THERM-1001, 110V; THERM-1000, 230V).

NOTE

Two GlykoPrep Heaters, WS0271, can be fitted with VWR 13259-260 Modular Heating Blocks.

- Vacuum manifold (Millipore MSVMHTS00)
- Vacuum pump (Millipore WP6211560, 110 V; WP6122050, 220V; Welch WOB-L Pump 2522)

NOTE

If you have a vacuum manifold other than the Millipore model suggested, please contact Agilent at www.agilent.com for setup instructions.

- Gly-X Vacuum Manifold Spacer (GX100)
- Formic acid, MS-grade (Fisher A117-50 recommended)
- Acetonitrile (ACN), MS-grade (Fisher A955-4 recommended)
- Optional - Trifluoroacetic acid (TFA)
- Optional - VWR 10 kDa Centrifugal filters (82031-348)

Sample Prep Considerations

In general, glycoprotein samples should be prepared to a maximum of 2 mg/mL in a low salt neutral buffer free of detergents and nucleophiles such as amines. Higher concentration samples should be diluted in water or 50 mM HEPES, pH 7.9.

Other sample considerations include:

- Amine buffer components (for example, Tris, arginine, glycine, histidine) should be avoided as these will react with the InstantAB glycan labeling dye. A 10 kDa molecular weight cut-off spin centrifugal filter is recommended for buffer exchange before the deglycosylation step for these samples. Water or non-interfering buffer (such as 50 mM HEPES, pH 7.9) can be used for the resuspension of the protein samples.
- When using samples prepared by protein A affinity chromatography, 0.1% formic acid should be used as an eluent rather than a glycine buffer.
- Samples in salt-containing buffers (~150 mM salt) are compatible with the kit, however, the use of PBS is not recommended (see **"FAQs"** on page 19). The preferred diluents are water or a matching buffer of 50 mM HEPES, pH 7.9.
- Samples below 2 mg/mL can be used depending on the protein sample and desired number/volumes used for injections.
- The maximum amount of protein suggested for each reaction is 40 µg (20 µL of a 2 mg/mL solution), though it may be possible to use more than 40 µg depending on the glycoprotein (see **"FAQs"** on page 19).
- Protein samples should not be below pH 5.5. Adjust the pH before starting the protocol, or add 3 µL of the Gly-X Digestion Buffer per 20 µL sample in **step 1** on **page 10**.
- For citrate-containing buffers, dilute sample with water or 50 mM HEPES, pH 7.9 to reduce citrate below 20 mM or exchange buffer with molecular weight cut-off spin centrifugal filters.

If a precipitate is observed upon incubation at 90 °C (**step 4** on **page 10**), review the sample prep and sample buffers for salts, low pH and/or possible interfering detergents. If you have questions on the compatibility of your sample buffer with the Gly-X protocol, please contact Agilent at **https://www.agilent.com/en/contact-us/page**.

Optional sample buffer exchange with 10 kDa MWCO spin columns (VWR cat# 82031-348)

- 500 µL DI water added to spin column
- Add glycoprotein (40 µg, 20 µL of 2 mg/mL)
- Centrifuge at 12,000 x g, 10 minutes
- Add additional 500 µL DI water, centrifuge for additional 10 minutes at 12,000 X g
- Bring sample up to initial starting volume with DI water (20 µL)

Protocol

Getting started

1 Prepare samples (see **"Sample Prep Considerations"** on page 8).

2 Set the thermocycler to 90 °C, or set two independent heat blocks to 90 °C and 50 °C.

3 Prepare the working solutions in **Table 2**.

Table 2 Working solution instructions. For solutions containing ACN, use tubes, basins or plates that are compatible with organic solvents (polystyrene is not compatible).

Working solution	Instructions	Notes
N-Glycanase working solution	Mix N-Glycanase and Gly-X Digestion Buffer 1:1 (v/v). Per sample, prepare 2.4 µL of working solution; for 8 wells mix 9.6 µL N-Glycanase and 9.6 µL Digestion Buffer.	2 µL required per sample, mix 20% overage of working solution.
InstantAB Dye solution	Remove InstantAB Dye and InstantAB Dye Solvent from -20 °C. Warm to room temperature, remove from desiccant pouch. Add 375 µL of InstantAB Dye Solvent to the InstantAB Dye vial, mix with pipette until dissolved.	5 µL required per sample. InstantAB Dye Solution is stable at -20 °C, one month and 10 freeze thaw cycles in desiccant pouch. Note: If transferring out of the Dye Vial, use tubes or plates that are compatible with organic solvents (polystyrene is not compatible).
Load/Wash solution* 2.5% Formic acid/97.5% Acetonitrile	Stock solution for 96 samples: Add 3 mL of Formic acid to a glass graduated cylinder. Bring the volume up to 120 mL with 100% Acetonitrile. Transfer to a glass storage vessel, cap tightly, swirl to mix.	Approximately 1.2 mL required per sample Tightly cap, store up to six months at RT

* For some proteins, a modified Load/Wash solution of 2.5% TFA/97.5% ACN may be needed. See **"Appendix A"** on page 17 for further details.

4 Prepare cleanup station with the following items as seen in **Figure 3**:

- **a** Gly-X Cleanup plate A
- **b** Vacuum manifold connected to vacuum pump
- **c** Waste tray
- **d** Gly-X Collection plate (PCR plate)
- **e** Gly-X Manifold spacer (GX100)

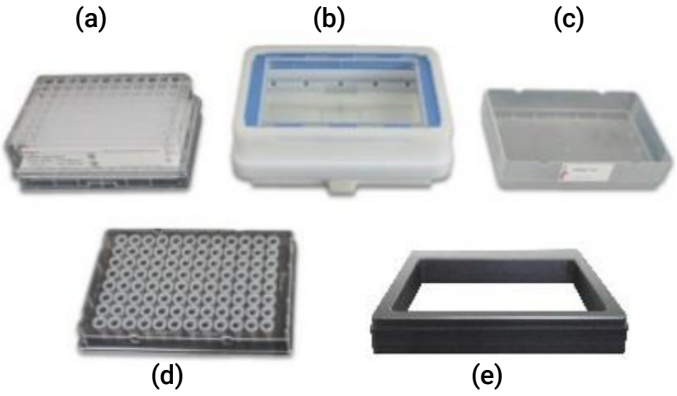

Figure 3. Cleanup station items

Gly-X Deglycosylation

1 Add 2 µL of Gly-X Denaturant (orange cap vial) to the bottom of the Gly-X Deglycosylation plate. (See **Figure 4**.)

NOTE **For samples with a pH of 5.5 or lower, also add 3 µL Gly-X Digestion Buffer (white cap vial).**

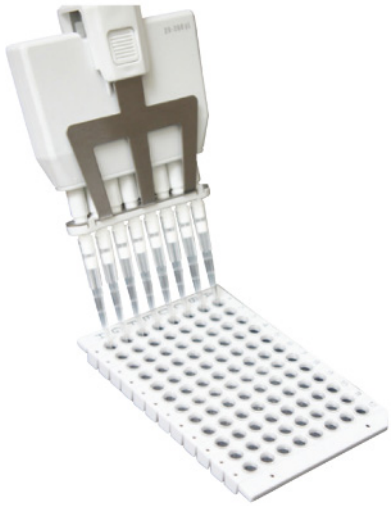

Figure 4. Gly-X Deglycosylation plate

2 Add 20 µL of each glycoprotein sample (~2 mg/mL) to the bottom of the Gly-X Deglycosylation plate. After each addition, mix thoroughly with a pipette.

3 Tap the plate on benchtop to collect samples on bottom of wells (or spin).

4 Incubate at 90 °C for three minutes.

NOTE **If a precipitate forms at this point, review the sample buffer composition.**

5 Remove plate, place at room temp for two minutes before adding N-Glycanase.

6 Add 2 µL of N-Glycanase working solution to each sample. Mix well using a pipette.

7 Tap plate on benchtop to collect samples on bottom of wells (or spin).

8 Incubate uncapped at 50 °C for five minutes.

9 Remove plate from heat and proceed directly to InstantAB labeling.

InstantAB labeling

1 Add 5 µL of InstantAB Dye solution, prepared above, per sample. After each addition, mix thoroughly with a pipette. Repeat until InstantAB Dye solution has been added to each sample.

2 Incubate at 50 °C for one minute.

3 Tightly cap the InstantAB Dye Solution, place in desiccant pouch and return to -20 °C.

InstantAB cleanup

Set Up

1 Prepare Gly-X Cleanup plate (**Figure 5**) for the required number of wells by carefully removing white caps.

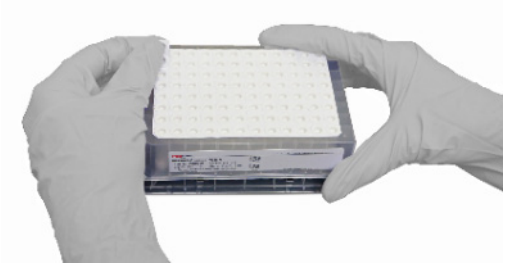

Figure 5. Gly-X Cleanup plate

2 Place the Waste tray in the Vacuum manifold and assemble the complete manifold. (See **Figure 6**.)

Figure 6. Vacuum manifold with Waste tray

3 Install Gly-X Cleanup plate A on top of the manifold. (See **Figure 7**.)

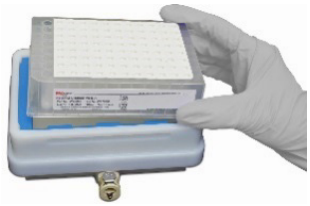

Figure 7. Gly-X Cleanup plate A with complete manifold

Prime

1 Add 400 µL of DI water to each well to be used in the Gly-X Cleanup plate, and apply vacuum at <5 in Hg until the wells are empty.

2 Wash twice with 600 µL of Load/Wash solution with vacuum <5 in Hg.

Load

1 With a multichannel pipette, add 450 µL of Load/Wash solution to one column of wells in the Cleanup plate. Do not apply vacuum.

NOTE **There will be some loss of Load/Wash solution due to pass through of the cleanup plate. Proceed to step 2 immediately or increase the Load/Wash solution volume to 500 µL to allow for more time (>three minutes).**

2 With a multichannel pipette, add 150 µL of Load/Wash solution to each sample in the deglycosylation plate, mix well, and then transfer the entire sample (~172 µL) from Deglycosylation plate into the corresponding wells of the Cleanup plate.

NOTE **When multiple samples are prepared, load the samples row-by-row or column-by-column to minimize Load/Wash solution loss from the plate wells.**

3 Repeat until all rows have been transferred to the Cleanup plate.

NOTE **When using a single channel pipette, perform steps 1-2 one sample at a time, and repeat steps for all samples.**

4 Apply <5 in Hg vacuum. Let the solution pass through.

NOTE **This step loads the sample onto the Gly-X Cleanup plate.**

Wash

1 Wash with 600 µL of Load/Wash solution, and apply vacuum to <5 in Hg, collecting wash in the Waste tray.

2 Repeat with a second and third 600 µL Load/Wash solution.

NOTE **All wells should be completely empty, if not, after two minutes, increase the vacuum to 10 in HG.**

NOTE **If processing more than 48 samples, empty the Waste tray after the first wash (Waste tray holds approximately 120 mL).**

3 Release the vacuum, remove the Cleanup plate and blot on paper. Empty the Waste tray.

4 Remove Waste tray from vacuum manifold.

Elute

1 Install the Gly-X Vacuum manifold spacer. (See **Figure 8**.)

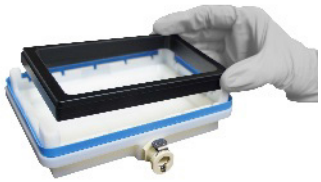

Figure 8. Gly-X Vacuum manifold spacer

2 Install the Collection plate (PCR plate). (See **Figure 9**.)

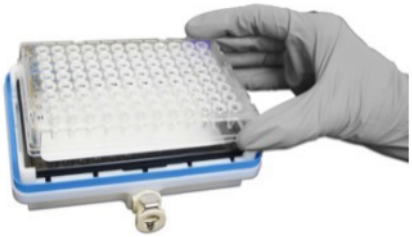

Figure 9. Collection plate (PCR plate)

3 Install the Cleanup plate on the vacuum manifold over Collection plate. (See **Figure 10**.)

Figure 10. Cleanup plate

4 Add 100 µL of Gly-X InstantAB eluent to each well. Using vacuum, (5 or less in Hg) elute samples into the Collection plate.

5 Keep the vacuum for at least one minute to collect as much eluent as possible.

NOTE **All wells should be completely empty, if not, keep the vacuum at <5 in Hg for another 1 to 2 minutes. Do not increase vacuum as this may cause cross contamination.**

6 Release the vacuum, remove the Cleanup plate and rest on the Storage plate.

7 Remove the collection plate. Optional: seal the Collection plate with preslit sealing film included in the kit for use on UHPLC. For long term storage at -20 °C, use the foil sealing film included in the kit. This sealing foil can be placed on top of the preslit sealing film and removed before placing on an auto sampler.

NOTE **The sealing foil is intended for storage only, it is not compatible with UHPLC. See "FAQs" on page 19 for more options.**

8 Vortex to mix, and spin/tap to collect samples at the bottom of the wells, or mix with a pipette.

NOTE **This final, post-elution mixing step is critical for consistent results.**

9 Seal the Collection plate with Sealing film.

10 Add Black Used Well Sealing Caps to used Cleanup plate wells, return to clear bag and store at RT or 4 °C.

11 InstantAB-labeled Glycan samples are ready for analysis. Samples may be stored at -20 °C for at least six months or 4 °C for up to five days.

NOTE **Black cap strips (Figure 11) should be placed on wells used in previous cleanup procedures to prevent reuse of wells.**

NOTE **Cleanup plate storage (round bottom) plate (Figure 11) should be used to store Cleanup plate after use. Store in foil bag provided.**

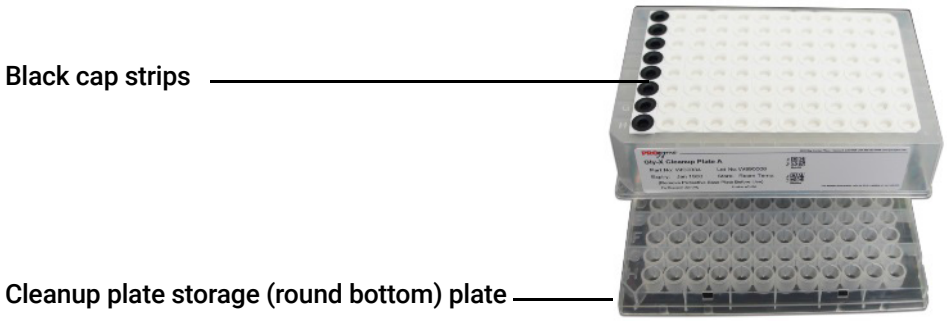

Figure 11. Cleanup plate with black cap strips

Analysis of Labeled Glycans

The optimal excitation and emission wavelengths for InstantAB Dye conjugated to an N-glycan are:

- Excitation: 278 nm
- Emission: 344 nm

Injection of 1 µL InstantAB-glycans in Gly-X InstantAB Eluent (160 mM Ammonium Formate, 10% (v/v) Acetonitrile) is recommended for UHPLC.

- For larger injection volumes (>1 µL) of InstantAB-glycans, dilute in ACN to match the high organic % at the start of your HILIC method. Dilute only what you need, as glycans should be stored in water rather than in high organic.

Dilution examples for Gly-X InstantAB-labeled glycan samples for HILIC injections of >1 µL.

Table 3 Recommended dilutions for InstantAB-labeled glycan samples and standards for UHPLC HILIC injection volumes >1 µL*

Total volume needed (µL)	InstantAB-labeled glycan samples in water (µL)	ACN (µL)	% Organic	% Aqueous
10	2.5	7.5	77.5	22.5
20	5	15	77.5	22.5
40	10	30	77.5	22.5
10		7.5	75	25
20		15	75	25
40		30	75	25

* InstantAB-labeled standards previously reconstituted in water according to the instructions provided with the standards.

Recommended HILIC methods for InstantAB-labeled Glycans

15-Minute UPLC methods for Waters BEH Glycan separation technology column

2.1 x 100 mm, 1.7 um column temperature 60 °C, excitation 278 nm, emission 344 nm.

Table 4 15-minute method, Waters column

Time (min)	Flow rate (ml/min)	% Acetonitrile	% 100 mM Ammonium Formate, pH 4.4
0.0	1.0	80.0	20.0
2.0	1.0	80.0	20.0
12.0	1.0	52.5	47.5
12.1	0.5	40.0	60.0
12.5	0.5	40.0	60.0
12.6	0.5	75.0	25.0
12.7	1.0	80.0	20.0
15.0	1.0	80.0	20.0

60-Minute UPLC methods for Waters BEH Glycan separation technology column

2.1 x 150 mm, 1.7 um column temperature 60 °C, excitation 278 nm, emission 344 nm.

NOTE

This method was used in our recent poster publication, Comparison of common fluorescent labels for liquid chromatography analysis of released N-linked glycans. www.agilent.com/dyecomparison

Table 5 60-minute method, Waters column

Time (min)	Flow rate (ml/min)	% ACN	% 50 mM Ammonium Formate, pH 4.4
0.0	0.4	80.0	20.0
2.0	0.4	80.0	20.0
2.5	0.4	75.0	25.0
50.0	0.4	62.0	38.0
52.0	0.4	40.0	60.0
53.5	0.4	40.0	60.0
55.0	0.4	80.0	20.0
60.0	0.4	80.0	20.0

Suggested MS conditions for InstantAB-labeled Glycans

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

Calculating the mass of Glycans labeled with InstantAB

Mass added to glycan with a free reducing end:

- Mass of Glycan (free reducing end) + $C_8N_3O_3H_7$ = Mass of InstantAB-Labeled Glycan
- Mass added by $C_8N_3O_3H_7$
  - Monoisotopic: 161.05891 Da
  - Average: 161.1 Da

Mass added to glycosylamine:

- Mass of Glycan (glycosylamine) + $C_8N_2O_4H_6$ = Mass of InstantAB-Labeled Glycan
- Mass added by $C_8N_2O_4H_6$
  - Monoisotopic: 162.0436 Da
  - Average: 162.1 Da

Appendix A

Modified cleanup protocol

In some cases, an early artifact peak may be seen in a HILIC separation of InstantAB-labeled glycans prepared with the Kit. This may depend on the protein and has been observed with cetuximab. The early peak may not affect the glycan analytical window depending on the HILIC method used.

To address this, the Load/Wash Solution of 2.5% Formic acid/97.5% Acetonitrile may be replaced by 2.5% TFA/97.5% ACN. No other alterations to the protocol are necessary.

For further information, please contact Agilent.

Appendix B

Recommended instructions for automated protocols

The following sample and reagent volume instructions have been developed to accommodate pipetting requirements for automation of Gly-X protocols (**Table 3** on page 15). The ratio of reagents in the automated protocol are equivalent to the standard protocol, but minimum pipetting volume changes from 2 µL to 5 µL to improve reliability of automated pipetting.

Table 6 Working Solution instructions for Sample, Denaturant and N-Glycanase, to accommodate a minimum pipetting volume of 5 µL. Prepare other working solutions as directed in Table 2 on page 9.

Sample and Reagents	Standard protocol	Automation protocol
Sample	20 µL at up to 2 mg/mL (40 µg max)*	15 µL at up to 2.67 mg/mL (40 µg max)*
Denaturant	Use 2 µL of neat Denaturant reagent per sample.	Dilute Denaturant with water 2:3 (v/v). Use 5 µL of diluted Denaturant reagent per sample. Always prepare diluted Denaturant reagent with 20% overage. For example, for eight samples prepare 48 µL (19.2 µL Denaturation Reagent, 28.8 µL water).
N-Glycanase Working solution	Prepare N-Glycanase Working Solution by mixing N-Glycanase and Gly-X Digestion Buffer 1:1 (v/v). Use 2 µL of N-Glycanase Working Solution per sample. Always prepare N-Glycanase Working Solution with 20% overage. For example, for eight samples prepare 20 µL.	Prepare N-Glycanase Working Solution by mixing N-Glycanase, Gly-X Digestion Buffer and water 1:1:3 (v/v/v). Use 5 µL of N-Glycanase Working Solution per sample. Always prepare N-Glycanase Working Solution with 20% overage. For example, for eight samples prepare 48 µL(9.6 µL N-Glycanase, 9.6 µL Gly-X Digestion Buffer, 28.8 µL water).

* It may be possible to use up to 100 µg glycoprotein, depending on the sample type (see **"FAQs"** on page 19).

FAQs

Q: Why is PBS not recommended as a diluent for samples?

A: PBS is compatible with Gly-X InstantAB kit, however, use of PBS for sample dilution may result in artifact peaks. These peaks elute early in the separation, near the free dye peak and should not interfere with N-glycan analysis. For optimal performance, dilution of samples with water is preferred.

Q: I want to inject more than 1 µL for UHPLC-HILIC. Why do I need to dilute my sample with ACN?

A: In a HILIC separation, glycans bind to the column in a high concentration of organic solvent and are eluted from the column by increasing aqueous concentration during the gradient. We have found that aqueous injections of 1 µL are compatible with UHPLC-HILIC methods, but that aqueous injections greater than 1 µL will disrupt the high organic conditions at the start of a HILIC method, resulting in peak broadening/fronting.

Q: What are the InstantAB-glycan masses for most common N-glycans?

A: Please refer to the list of most common InstantAB-labeled glycan adducts and their masses in both positive and negative MS mode at **www.agilent.com**.

Q: Which is better for MS analysis of InstantAB-labeled glycans, positive or negative mode?

A: There are pros and cons with each. Positive mode: less noisy background, stronger signal. Negative mode: less adducts and hence MS looks less convoluted.

Q: Can I place the collection plate directly into an UHPLC autosampler? What are the plate dimensions?

A: Yes, **Figure 12** shows the settings that can be used for example on a Waters Acquity UPLC.

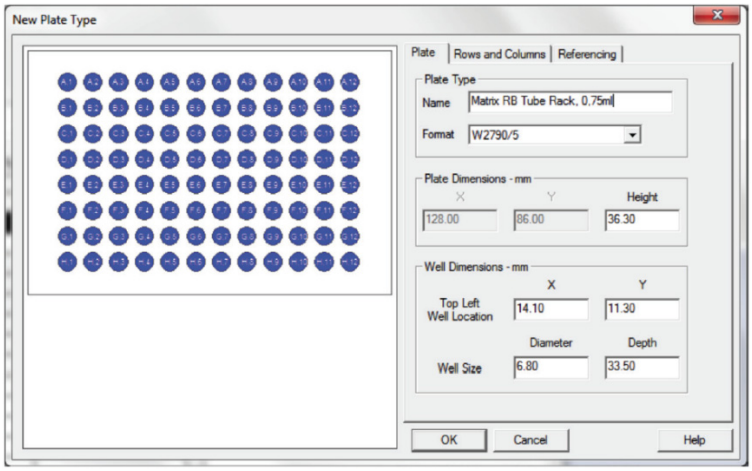

Figure 12. Collection plate settings

NOTE

When injecting from a plate, regular adhesive foils or adhesive sealing film are to be avoided as the adhesive can cause issues with the probe. Adhesive sealing foils and films need to be removed. Use the preslit sealing film included in the kit (no adhesive in the well area), or a heat sealed pierceable foil (do not use the storage sealing foil). Avoid any covers/foils that have adhesive directly above the wells; adhesive can damage the UPLC probe. The preslit film included in the kit can be covered over with the sealing foil included in the kit for storage. The included sealing foil can be removed prior to analysis leaving the preslit film in place. Additional sealing foil can be ordered from Thermo Scientific (AB-0626).

Collection Plates may be sealed with sealing film (preslit), or heat sealed with pierceable foil (for example, Thermo Easy Pierce 20 µm Foil, #AB-1720) using a microplate heat sealer (for example, Thermo ALPS 50 V Semi-automated Microplate Heat Sealer, #AB-1443).

Collection Plate dimensions are also available on the manufacturers website: Framestar 96-well skirted PCR Plates (#4ti-0960/C, E&K part number 75094). Without needle adjustment, the dead volume using this method is approximately 15 µL. The sample may also be transferred into a vial designed for greater recovery (for example, Waters Total Recovery Vial).

Q: My samples are not loading completely onto the Gly-X Cleanup Matrix (Section 4). What is happening?

A: This may be caused by the nature of your protein sample, or by matrix effects caused by the composition of your formulation buffer. This can be addressed by using less protein per reaction, or by buffer exchanging your protein prior to starting the Gly-X protocol.

Q: Can I use more than the recommended upper limit of 40 µg protein per reaction?

A: Maybe, although it will depend on the protein. You would have to test to ensure that >40 µg of protein can be successfully loaded onto the Gly-X Cleanup Matrix without blockage.

Resources and References

Visit the Agilent website for additional information, downloadable posters, publications and tech notes:

www.agilent.com

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Technical Assistance

Agilent is committed to developing rapid, automatable methods for glycan analysis. Call us to discuss products in development.

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Agilent values customer opinions and encourage you to contact us. We welcome your suggestions about product performance or new applications and techniques.

Ordering Information

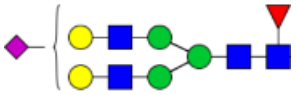
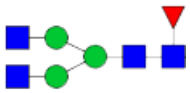
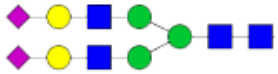
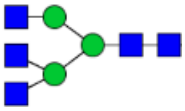
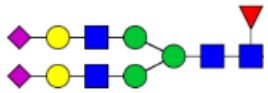
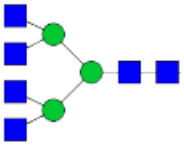
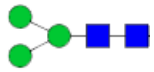
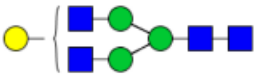
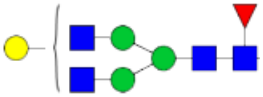
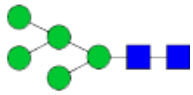
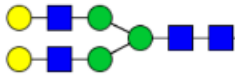
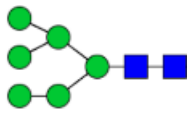
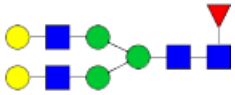
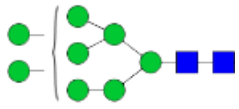
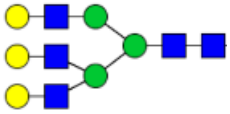
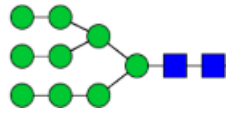
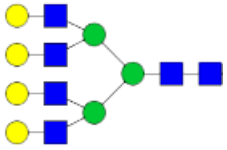
Kits and Modules

Product Code	Description
GX96-IAB	Gly-X with InstantAB Kit (96-ct)
GX96-202IAB	Gly-X Deglycosylation and Labeling Module Set (96-ct)
GX96-501	InstantAB Labeling Module (96-ct)
GX96-502	Gly-X Cleanup Module (96-ct)
GX100	Gly-X Vacuum Manifold Spacer (2 pack)
G5524-60010 KIT	AssayMAP PA50 protein A affinity purification kit (96-ct)

InstantAB labeled Glycan libraries

Product Code	Description
GKIB-005	Human IgG N-Linked Glycan Library
GKIB-025	Human IgG + RNase B N-Linked Glycan Library
GKIB-009	RNase B N-Linked Glycan Library
GKIB-520	Biantennary and High Mannose Partitioned Library
GKIB-232	α(2-3) Sialylated Biantennary Library
GKIB-233	α(2-3) Sialylated Triantennary Library
GKIB-234	α(2-3) Sialylated Tetraantennary Library
GKIB-001	Human α1-acid glycoprotein N-Linked Glycan Library
GKIB-002	Bovine Fetuin N-linked Glycan Library
GKIB-009	RNase B N-Linked Glycan Library

InstantAB labeled individual Glycan standards

Product code	Description		Product code	Description	
GKIB-301	G0		GKIB-315	A1F	
GKIB-302	G0F		GKIB-312	A2	
GKIB-307	NGA3		GKIB-313	A2F	
GKIB-309	NGA4		GKIB-101	Man3	
GKIB-317	G1		GKIB-102	Man3F	
GKIB-316	G1F		GKIB-103	Man5	
GKIB-304	G2		GKIB-104	Man6	
GKIB-305	G2F		GKIB-106	Man8	
GKIB-308	G3		GKIB-107	Man9	
GKIB-310	G4				

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