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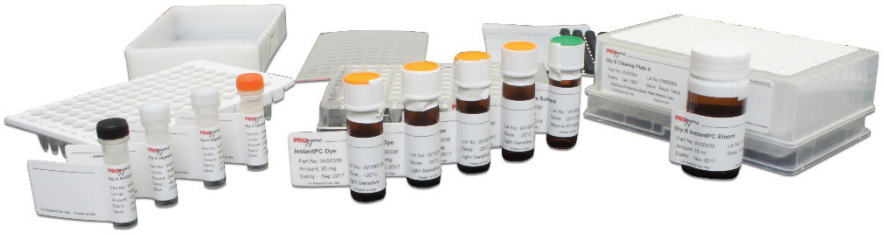
Agilent AdvanceBio Gly-X N-Glycan Prep with InstantPC Kit, 96-ct (formerly ProZyme)

User Manual



Notices

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Introduction

The Gly-X N-Glycan Rapid Release and Labeling with InstantPC kit uses a novel in-solution enzymatic protein deglycosylation followed by rapid labeling of released N-glycans with InstantPC dye. After a simple clean-up step, the glycan samples are ready for analysis by UHPLC, LC-MS, MS/MS and other methods. With deglycosylation and labeling carried out in solution, the method is simple, rapid, and suitable for automation. The InstantPC dye delivers unmatched fluorescent brightness and MS signal, which enables a single labeling method to be deployed across different glycan analysis workflows. Other benefits include:

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

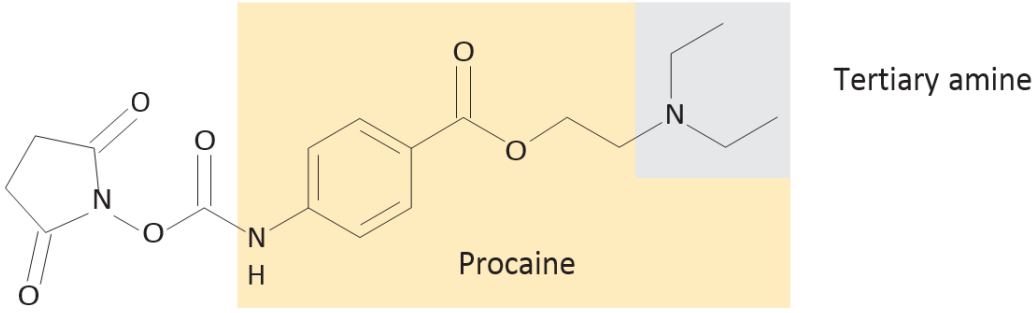


Figure 1. InstantPC dye (IPC)

Kit Components

The Gly-X with InstantPC (GX96-IPC) Dye Kit consists of three modules. 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
	Gly-X N-Glycanase, 1 mg/mL, 120 µL	1	4 °C
	Gly-X Digestion Buffer, 240 µL	2	4 °C
	Gly-X Denaturant, 240 µL	1	4 °C
Gly-X InstantPC Labeling Module GX96-101	InstantPC Dye (lyophilized)	4	-20 °C
	InstantPC Dye Solvent, 600 µL	1	-20 °C
Gly-X InstantPC Cleanup Module GX96-102	Gly-X Cleanup Plate A	1	RT
	Gly-X InstantPC Eluent	1	RT
	Gly-X Waste Tray	1	RT
	Gly-X Used-Well Sealing Caps, Black (for Cleanup Plate)	96	RT
	Gly-X Collection Plate, 96 wells	1	RT
	Sealing film (preslit)	1	RT
	Storage sealing foil	1	RT

NOTE **Gly-X Eluent is 160 mM Ammonium Formate w/10% (v/v) Acetonitrile.**

NOTE **Gly-X with InstantPC 24 ct kit (GX24-IPC) contains 30 µL Gly-X N-Glycanase, one vial Gly-X Digestion Buffer, one vial InstantPC Dye, and all other kit components listed in the table.**

NOTE **Gly-X Vacuum Manifold Spacer (GX100) is required for Cleanup Module.**

Figure 2. GX100, Gly-X Vacuum Manifold Spacer, required

Equipment and Reagents Provided by User

96-well Thermocycler or two independent heat blocks, set for 90 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 and pump other than the Millipore model suggested, please contact Agilent at https://www.agilent.com/en/contact-us/page for guidance. A Waters vacuum manifold may be used with a GX200 Spacer in place of GX100 (see ЛҒAQsˇ on page 19).**

Gly-X Vacuum Manifold Spacer (Agilent GX100)

Formic acid, MS-grade (Fisher A117-50 recommended)

Acetonitrile (ACN), MS-grade (Fisher A955-4 recommended)

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 InstantPC 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 noninterfering 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** of **Gly-X deglycosylation** 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 2** of **Gly-X deglycosylation** on page 10), review the sample prep and sample buffers for salts, low pH 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 and 50 °C.

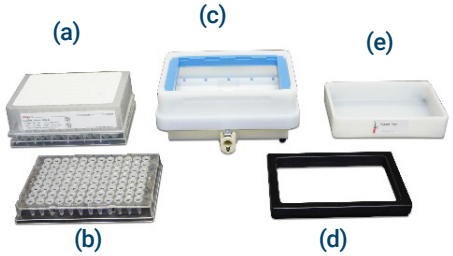
3 Prepare the working solutions shown in **Table 2**.

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 & Gly-X Digestion Buffer 1:1 (v/v). Per sample, prepare 2.4 µL of working solution; for eight wells mix 9.6 µL N-Glycanase and 9.6 µL Digestion Buffer.	2 µL required per sample, mix 20% overage of working solution.
InstantPC Dye Solution	Remove InstantPC Dye and InstantPC Dye Solvent from -20 °C. Warm to room temperature, remove from desiccant pouch. Add 150 µL of InstantPC Dye Solvent to the InstantPC Dye vial, vortex until dissolved. **Note:** retain Dye Solvent vial for use with other Dye vials.	5 µL required per sample. Reconstituted InstantPC Dye and Dye Solvent may be repackaged with desiccant in resealable bag, resealed and stored at -20 °C for up to three months and four freeze thaw cycles. Return dissolved Dye to room temperature before opening for use. **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 6 mL of Formic acid to a glass graduated cylinder. Bring the volume up to 240 mL with 100% Acetonitrile. Transfer to a glass storage vessel, cap tightly, swirl to mix.	Approximately 2.4 mL required per sample Tightly cap, store up to six months at room temperature (RT).

4 Prepare the cleanup station with these items:

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

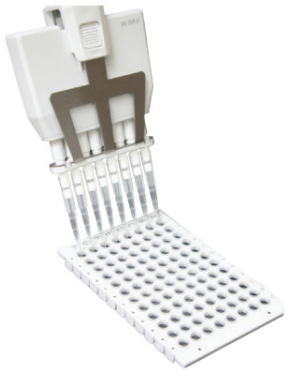


Gly-X deglycosylation

1 Add 2 µL of Gly-X Denaturant (orange cap vial) to the bottom of the Gly-X Deglycosylation Plate.

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

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 uncapped at 90 °C for three minutes.

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

5 Remove plate, place at RT 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 InstantPC labeling.

InstantPC labeling

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

NOTE **A precipitate is normal.**

2 Incubate uncapped at 50 °C for one minute.

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

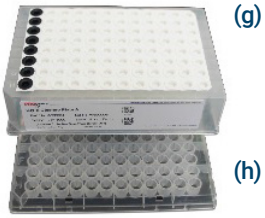
InstantPC cleanup

SET UP

1 Prepare Gly-X Cleanup Plate (f) for the required number of wells by carefully removing white caps.

NOTE **Black cap strips (g) should be placed on wells used in previous cleanup. procedures to prevent re-use of wells.**

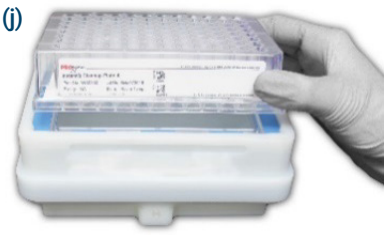
NOTE **Cleanup Plate storage plate (h) should be set aside and used to store Cleanup Plate after use. Store in foil bag provided.**



2 Place Waste Tray in vacuum or pressure manifold.

3 Assemble the complete manifold (i).

4.Install Gly-X Cleanup Plate A on top of the manifold (j).



LOAD

1 With a multichannel pipette, add 400 µL Load/Wash Solution to the required number of wells in the Gly-X 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 with pipette and then transfer the entire sample (~172 µL) to corresponding wells in the Gly-X Cleanup Plate. Mix well with a pipette.

NOTE

When multiple samples are prepared, it is recommended to load the samples row-by-row or column-by-column with a multichannel pipette to minimize Load/Wash Solution loss from the plate wells.

3 Repeat until all samples have been transferred to the cleanup plate

4 Apply vacuum to <5 inHg. Let the solution pass through until the wells are empty.

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 2 inHg, 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 inHg.

NOTE

If processing more than 48 samples, empty the Waste Tray after second wash (Waste Tray holds approximately 120 mL).

3 Release the vacuum, remove the Clean-up Plate and blot on paper. Empty the Waste Tray.

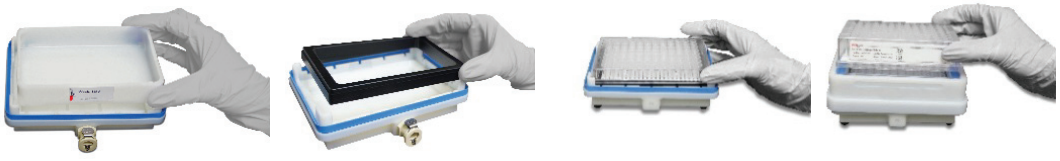
ELUTE

1 Remove the Waste Tray from manifold.

2 Place Gly-X Manifold Spacer (Agilent GX100) into manifold.

3 Place Collection Plate on top of the Manifold Spacer and reassemble vacuum manifold

4 Install the Gly-X Cleanup Plate on top of the vacuum manifold.



1 2 3 4

5 Add 100 µL of Gly-X InstantPC Eluent to each well. Using vacuum, (2 or less inHg) elute samples into the Collection Plate.

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

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 pre-slit 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 Place Cleanup plate on storage plate (i), add Black 'Used Well' Sealing Caps to used Cleanup Plate wells (h), return Cleanup Plate to foil bag and store at RT.

10 InstantPC-labeled glycan samples are ready for analysis. Samples may be stored at -20 °C for up to six months in Gly-X InstantPC eluent, or 4 °C for up to five days in either Gly-X InstantPC Eluent or after dilution with ACN/DMF (see **Analysis of Labeled Glycans** on page 14).

(h)

(i)

Analysis of Labeled Glycans

For fluorescence detection, optimal excitation and emission wavelengths for InstantPC conjugated to an N-glycan are:

Excitation: 285 nm

Emission: 345 nm

UV detection may also be used for InstantPC-labeled glycans, using a UV detector set to the excitation wavelength of 285 nm (1). The amount of sample loaded may need to be increased for optimal signal with UV detection, please contact Agilent for further details.

Injection of 1 µL InstantPC-glycans in Gly-X InstantPC Eluent (160 mM Ammonium Formate, 10% (v/v) Acetonitrile) is recommended for UHPLC with fluorescence detection. Do not inject more than 1 µL of InstantPC Eluent for UHPLC-HILIC as this may affect peak shape.

For larger injection volumes (>1 µL) of InstantPC-glycans, do not use ACN alone to dilute the sample as this may cause sialylated glycans to precipitate. Use 1 part sample in eluent to three parts 1:1 [v/v] ACN:DMF, for a final concentration of 22.5% aqueous buffer, 37.5% DMF, 40.0% to more closely match the high organic % at the start of a HILIC method.

Dilution examples for Gly-X InstantPC-labeled glycan samples for HILIC injections of >1 µL:

Table 3 Recommended dilutions for InstantPC-labeled glycan samples and standards

Total volume needed (µL)	IPC-labeled glycan samples in 10% organic Gly-X Eluent (µL)	IPC-labeled glycan standards (µL)*	1:1 [v/v] ACN:DMF (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		2.5	7.5	75	25
20		5	15	75	25
40		10	30	75	25

* InstantPC-labeled standards previously reconstituted in 100 mM ammonium formate, pH 4.4 to 5 or water per the instructions provided with the standards.

Recommended HILIC methods for InstantPC-labeled Glycans

Five minute screening UHPLC method for Agilent AdvanceBio Glycan Mapping column:

2.1 x 100 mm, 2.7 µm. Column temperature 35 °C, excitation 285 nm, emission 345 nm.

Table 4 5-Minute method, Agilent column

Time (min)	Flow Rate (mL/min)	% ACN	% 100 mM Ammonium Formate, pH 4.4
0.00	1.4	77.0	23.0
4.00	1.4	60.0	40.0
4.15	0.75	40.0	60.0
4.30	0.75	40.0	60.0
4.40	1.4	77.0	23.0
5.00	1.4	77.0	23.0

60-Minute high-resolution UHPLC method for Agilent AdvanceBio Glycan Mapping column:

2.1 x 150 mm, 1.8 µm. Column temperature 40 °C, excitation 285 nm, emission 345 nm.

Table 5 60-Minute method, Agilent column

Time (min)	Flow Rate (mL/min)	% ACN	% 50 mM Ammonium Formate, pH 4.4
0	0.5	80	20
2	0.5	75	25
48	0.5	62	38
49	0.5	40	60
51.5	0.5	80	20
52	0.5	80	20
60	0.5	80	20

Please refer to Agilent Application note 5991-8071EN for 15-, 30- and 37-minute HILIC methods for InstantPC N-glycans using the AdvanceBio Glycan Mapping columns.

15-Minute UHPLC methods for Waters BEH Glycan Separation Technology column:

2.1 x 100 mm, 1.7 µm column temperature 60 °C, excitation 285 nm, emission 345 nm.

Table 6 15-minute method, Waters column

Time (min)	Flow Rate (mL/min)	% ACN	% 100 mM Ammonium Formate, pH 4.4
0.0	1.0	75.0	25.0
12.0	1.0	50.0	50.0
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	75.0	25.0
15.0	1.0	75.0	25.0

60-Minute UHPLC methods for Waters BEH Glycan Separation Technology column:

2.1 x 150 mm, 1.7 µm column temperature 60 °C, excitation 285 nm, emission 345 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

Table 7 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 InstantPC-labeled Glycans

MS conditions

Agilent Jet Stream ESI source, any MS, positive mode, sheath gas 300 °C at 10.0 L/min, dry gas 150 °C at 9.0 L/min, nebulizer pressure 35 psig, VCap 2500 V, Nozzle 500 V, Fragmentor 120 V (if applicable), *m/z* range 600-3000.

Waters Xevo G2-S QTof, + 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 300-2,000 Da.

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 50-2,000 Da.

Calculating the Mass of Glycans Labeled with InstantPC

Although the glycosylamine form of the glycan is labeled by InstantPC in the protocol, the starting point for many glycan mass calculations is the free reducing end form of the unlabeled glycan. For that reason, we provide two calculations, one for the mass of the InstantPC-labeled glycan using the free reducing end mass of the glycan as a starting point, and one based on InstantPC addition to the glycosyalamine.

Mass added to glycan with a free reducing end:

Mass of Glycan (free reducing end) + $C_{14}N_3O_2H_{19}$ = Mass of InstantPC-Labeled Glycan

Mass added by $C_{14}N_3O_2H_{19}$

Monoisotopic: 261.14773 Da

Average: 261.3196 Da

Mass added to glycosylamine:

Mass of Glycan (glycosylamine) + $C_{14}N_2O_3H_{18}$ = Mass of InstantPC-Labeled Glycan

Mass added by $C_{14}N_2O_3H_{18}$

Monoisotopic: 262.13174 Da

Average: 262.3043 Da

Appendix

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 14). The ratio of reagents in the automated protocol are equivalent to the standard protocol, but minimum pipetting volume changes from 2 to 5 µL to improve reliability of automated pipetting.

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Table 8 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 @ up to 2 mg/mL (40 µg max)*	15 µL @ 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 & 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 InstantPC 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 or 50mM HEPES, pH 7.9 is preferred.

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

A: It may depend on the protein. Typically, protein quantities up to 100 µg are compatible with this sample prep, but users should test to ensure that >40 µg protein can be used without a) loss of linear response in glycan relative % area values, or b) precipitation after addition of Denaturant.

Q: My samples are not loading completely onto the Gly-X Cleanup Matrix (Load Step 9). 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: I want to inject more than 1 µL for UHPLC-HILIC. Why do I need to dilute my sample with DMF?

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. For this reason we suggest diluting one part sample in eluent to three parts 1:1 [v:/v] ACN:DMF for injection volumes of >1 µL.

Q: Why can I not use ACN to dilute the samples prior to HILIC injection? And can I use something other than DMF for the dilution?

A: If the InstantPC-labeled glycans in Gly-X Eluent are diluted with ACN alone, this may cause the sialylated glycans to precipitate. We have not tested alternatives to DMF for sample dilution.

Q. I am seeing low fluorescence signal of labeled glycans by LC analysis. How do I address this?

A. There are a few options for increasing signal, but first check the FLD ex/em wavelengths used (285/345 nm, **page 14**).

Options include, in order of preference:

1. Inject more labeled glycans onto LC after dilution with ACN:DMF (**page 14**).
2. During the Cleanup protocol at Elute **step 5** on **page 13**, elute labeled glycans with 50 µL of InstantPC Eluent, rather than 100 µL. This should work for most sample types. Please contact Agilent with any questions.
3. Dry down the glycans using for example, SpeedVac (no heat) and resuspend in a smaller volume, this should work for many sample types. InstantPC Eluent is 160 mM ammonium formate, 10% [v/v] acetonitrile. Ammonium formate is a volatile buffer so may be dried by rotary evaporation in a SpeedVac, with heat setting turned off to protect the sample. Samples may be resuspended in 100 mM ammonium formate, pH 4.7 ±0.3. InstantPC Eluent may also be used as an alternative for resuspension. Do not use water to resuspend

dried InstantPC labeled glycans as this may affect sample stability.

Depending on the SpeedVac, an ammonium formate film may appear on the walls of the tube during the drying process. If this is a concern, resuspend the sample and dry down again.

If you have already mixed the samples in InstantPC Eluent with 1:1 DMF:ACN for LC injections of >1 µL, we do not recommend drying down the sample. It is difficult to dry samples containing DMF by rotary evaporation without using heat (which may adversely affect the glycans) or very low pressure.

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

A: Yes.

For an Agilent LC, operated with OpenLab 2, in the Control Panel close the connection to the instrument and select **Configure Instrument**. Right-click to open the autosampler module, at the bottom of that window click **Define Sample Containers** to see the list of options. Add a new option with the dimensions shown in **Figure 3**.

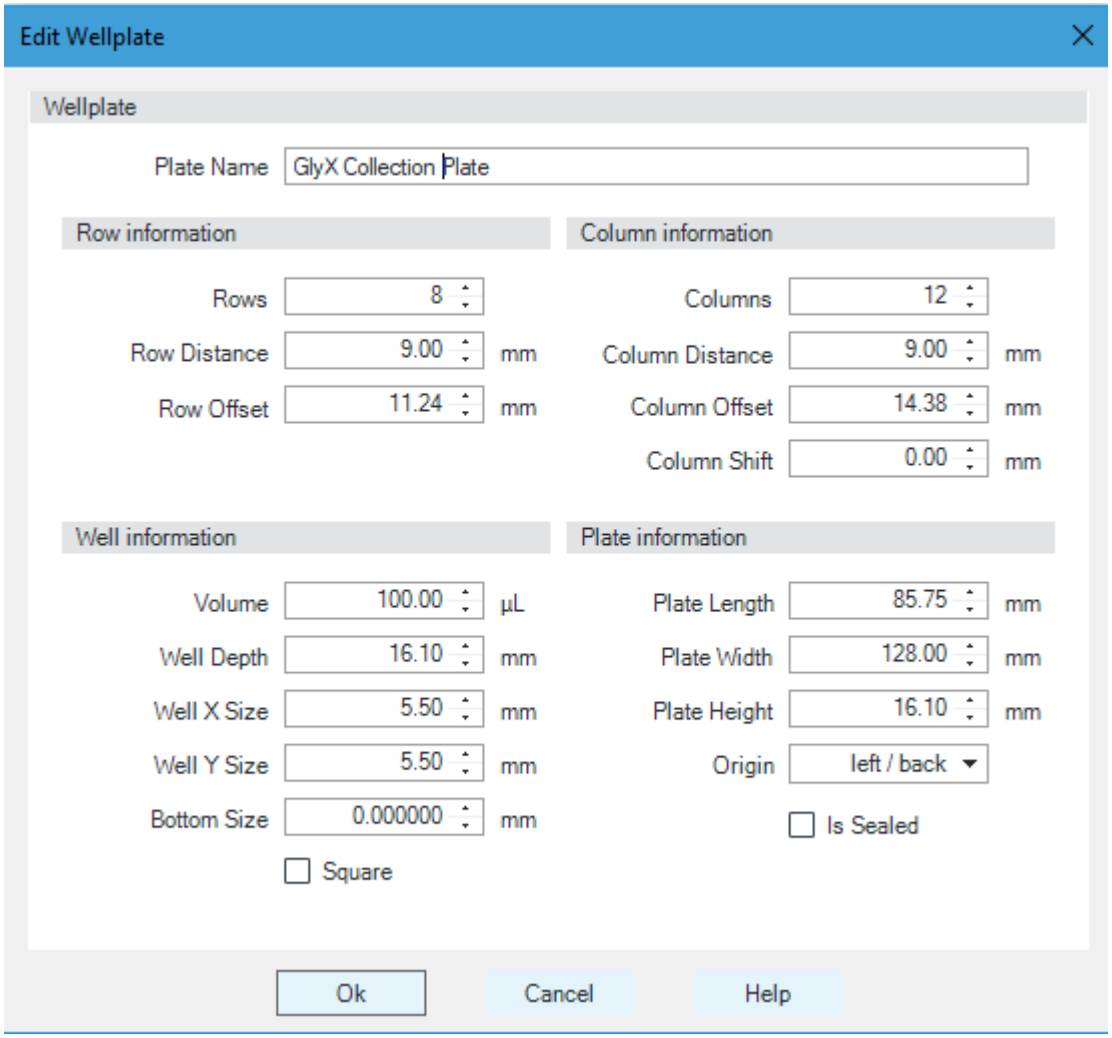


Figure 3. Edit Wellplate options

For Agilent LCs operated using MassHunter, the collection plate can be configured in a similar way using the Instrument Configuration tool.

Figure 4 shows settings that can be used for example on a Waters Acquity UHPLC.

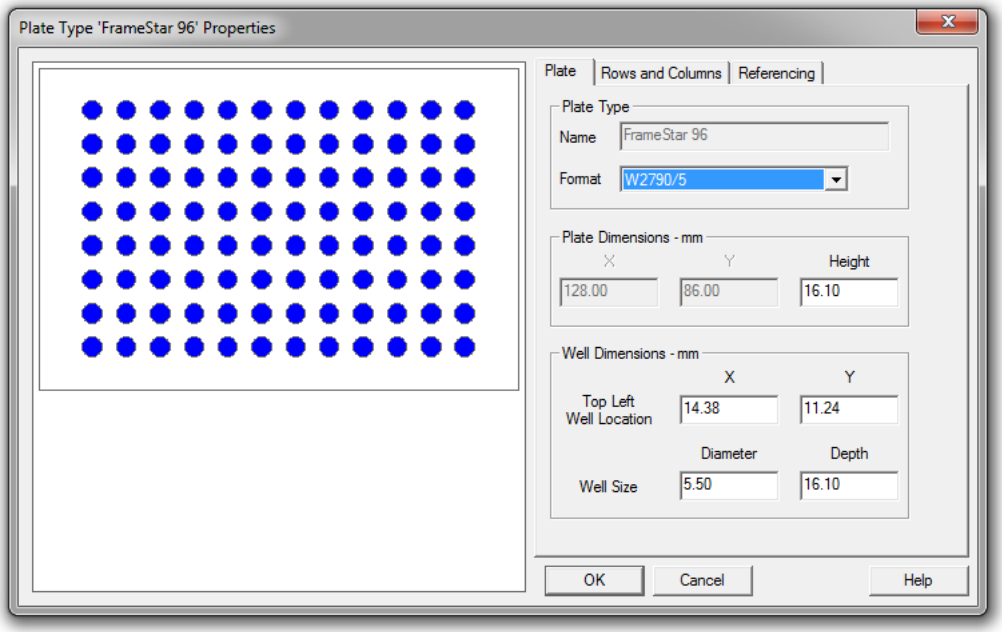


Figure 4. Waters Acquity UHPLC setting example

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 UHPLC 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 pre-slit film in place. Additional sealing foil can be ordered from Thermo Scientific (AB-0626).

Collection Plates may be sealed with sealing film (pre-slit), 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). The collection plate used for revisions AA and AB of the GX96-IPC manual was Fisher #50-823-843 (4721).

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. You suggest 100 mM ammonium formate for the 5-minute HILIC separation, 50 mM ammonium formate for the 60-minute separations. Is this correct?

A. For fast separations we use 100 mM ammonium formate, for MS in conjunction with longer separations we use 50 mM ammonium formate.

Q. What is the most common adduct seen in MS analysis of InstantPC-labeled glycans?

A. In positive mode MS, most biantennary InstantPC- N-glycans will give [M+2H]+2, larger sialylated will be majority [M+3H]+3. Please refer to the list of most common InstantPC-labeled glycan adducts and their masses in positive mode MS at www.agilent.com/glycanmass.

Q: Can I use the GX96-IPC kit with a Waters vacuum manifold?

A: Yes, if GX200 Gly-X Vacuum Manifold Spacer (Waters Manifold) is used in place of GX100 during the Elute step (**page 12**). GX200 provides the correct distance between the Cleanup Place and Collection Plate when using a Waters vacuum manifold (#186001831). This is critical to avoid crosstalk when eluting labeled N-glycans into the Collection Plate. Please contact Agilent at **https://www.agilent.com/en/contact-us/page** for further details.

Resources and References

Visit Agilent's website for additional information, publications and technotes:

www.agilent.com

Product Use, Warranty and License to Use

Terms and conditions of sale may be found at: **www.agilent.com**

Virtual Patent Marking

Visit **www.agilent.com** for a list of Agilent products and patents.

References

1 Nwosu C, *et al*. Evaluation of UV Detection as a Novel Method for Antibody N-glycan Analysis. ASMS Poster, **2017**.

Technical Assistance

Agilent is committed to developing rapid, automatable methods for glycan analysis. Call us to discuss products in development. We value customer opinions and encourage you to contact us. We welcome your suggestions about product performance or new applications and techniques.

If you have questions or comments, please contact us at **https://www.agilent.com/en/contact-us/page.**

Ordering Information

Table 9 Kits and modules

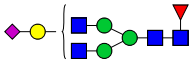
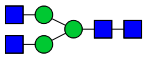
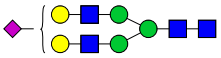
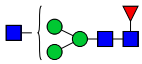
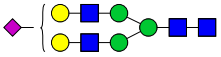
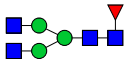
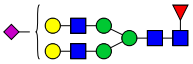
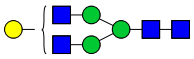
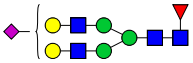
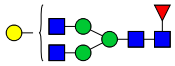
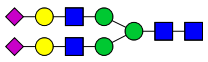
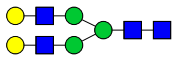
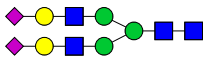
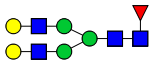
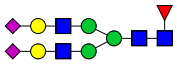
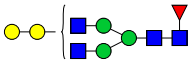
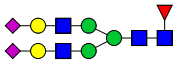
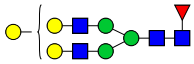
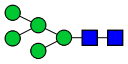
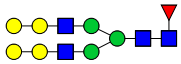
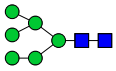
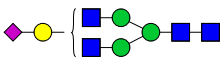
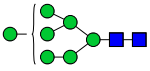
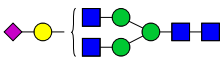
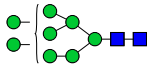
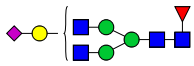
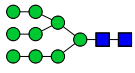
Product Code	Description
GX96-IPC	Gly-X with InstantPC Kit (96-ct)
GX24-IPC*	Gly-X with InstantPC Kit (24-ct)
GX96-201PC	Gly-X with InstantPC Deglycosylation and Labeling Module Set (96-ct)
GX24-201PC	Gly-X with InstantPC Deglycosylation and Labeling Module Set (24-ct)
GX96-101	Gly-X InstantPC Labeling Module (96-ct)
GX24-101	Gly-X InstantPC Labeling Module (24-ct)
GX96-102	Gly-X Cleanup Module for InstantPC (96-ct)
GX100	Gly-X Vacuum Manifold Spacer (2 pack)
GX200	Gly-X Vacuum Manifold Spacer (Waters Manifold)
G5524-60010 KIT	AssayMAP PA50 protein A affinity purification kit (96 ct)

* 24-ct kit (GX24-IPC) contains a 96-well cleanup plate. Store the cleanup plate at room temp, and order 24-ct refills of Gly-X InstantPC Deglycosylation and Labeling Modules (GX24-201PC).

Table 10 InstantPC Labeled Glycan Libraries

Product Code	Description
GKPC-005	Human IgG N-Linked Glycan Library
GKPC-503	Glucose Homopolymer
GKPC-020	CHO mAb N-Linked Glycan Library
GKPC-020-P	CHO mAb N-Linked Glycan Library + CHO mAb Glycoprotein
GKPC-233	Ω(2-3) Sialylated Triantennary Library
GKPC-263	Ω(2-6) Sialylated Triantennary Library
GKPC-234	Ω(2-3) Sialylated Triantennary Library
GKPC-264	Ω(2-6) Sialylated Tetraantennary Library

Table 11 InstantPC Labeled Individual Glycan Standards

Product code	Description		Product code	Description	
GKPC-401	G0-N		GKPC-320	G1FS1 (Ω2,6)	
GKPC-301	G0		GKPC-321	A1 (Ω2,3)	
GKPC-402	G0F-N		GKPC-311	A1 (Ω2,6)	
GKPC-302	G0F		GKPC-325	A1F (Ω2,3)	
GKPC-317	G1		GKPC-315	A1F (Ω2,6)	
GKPC-316	G1F		GKPC-322	A2 (Ω2,3)	
GKPC-304	G2		GKPC-312	A2 (Ω2,6)	
GKPC-305	G2F		GKPC-323	A2F (Ω2,3)	
GKPC-403	G1F + 1aGal		GKPC-313	A2F (Ω2,6)	
GKPC-405	G2F + 1aGal		GKPC-103	Man5	
GKPC-318	G2F + 2aGal		GKPC-104	Man6	
GKPC-329	G1S1 (Ω2,3)		GKPC-105	Man7	
GKPC-319	G1S1 (Ω2,6)		GKPC-106	Man8	
GKPC-330	G1FS1 (Ω2,3)		GKPC-107	Man9	

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