Gly-*X*™ N-Glycan Rapid Release and Labeling with InstantQ™ Dye Kit

User Manual

Product Code: GX96-IQ

Version: AE

PROzyme Advancing Glycosciences

This product is intended for in vitro experimental research use only

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INTRODUCTION

The Gly-*X* N-Glycan Rapid Release and Labeling with InstantQ kit utilizes a 5-minute in-solution enzymatic protein deglycosylation followed by rapid labeling of released N-glycans with InstantQ dye. After a simple clean-up step, the glycan samples are ready for analysis using the Gly-Q™ instrument. With deglycosylation and labeling carried out in solution phase, the method is simple, rapid and suitable for automation.

InstantQ dye is specifically designed for glycan separation and detection using the Gly-Q instrument and a Gly-Q InstantQ Cartridge. Other benefits include:

- Flexible, high-throughput format: process 1 to 96 samples
- 2-minute CE separations using the Gly-Q system
- High sensitivity detection
- Rapid N-glycan analysis using Gly-Q Manager software

KIT COMPONENTS

The Gly-*X* with InstantQ (GX96-IQ) consists of five modules. Each module provides enough reagents for up to 96 samples per run.

Module	Component	Units	Storage
Gly-*X* Deglycosylation Module for InstantQ Dye GX96-300	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* Blocker, 300 µl	1	4°C
Gly-*X* InstantQ Labeling Module GX96-301	InstantQ Dye (lyophilized)	4	-20°C
	InstantQ Dye Solvent, 1 ml	2	-20°C
	InstantQ Activation Reagent 1 ml	2	-20°C
Gly-*X* InstantQ Cleanup Module GX96-302	InstantQ Cleanup Plate A	1	RT
	Gly-*X* Collection Plate, 96 wells	1	RT
	Waste Tray for vacuum manifold	1	RT
	Gly-X Used-Well Sealing Caps, Black (for cleanup plate)	1	RT
Gly-Q Cartridge Module GQ103	Gly-Q Separation Buffer	1	RT
	Gly-Q Mineral Oil	1	RT
	Gly-Q Reagent Tray	1	RT
	200 µl tubes (clear) for optional standards	2	RT
	Gly-Q Cartridge	1	4°C
Gly-Q Alignment Standards Set GKSQ-505	Gly-Q GU Ladder (GKSQ-503), 100 µl	1	-20°C
	200 µl tubes (blue) for Migration Standards and (yellow) for GU Ladder	4 each	RT
	Gly-Q Migration Standards GKSQ-500, 100 µl	1	-20°C

Note: *Allow for Gly-Q cartridge to equilibrate to room temperature before first use (~1hr). The cartridge can be stored at RT after the first use.*

Note: *Gly-X InstantQ Eluent is ultrapure water (provided by user)*

Note: *Gly-X with InstantQ 24ct kit (GX24-IQ) contains 30 µl Gly-X N-Glycanase, 1 vial Gly-X Digestion Buffer, 2 vials InstantQ Dye, 1 vial InstantQ Dye Solvent, 1 vial InstantQ Activation Reagent and all other kit components listed in the table.*

Note: *Gly-X Vacuum Manifold spacer (GX100) is required, please contact ProZyme for more information.*

GX100, Gly-*X* Vacuum Manifold Spacer

EQUIPMENT & REAGENTS PROVIDED BY USER

- 96-well Thermocycler or two independent heat blocks, set for 90°C and 50°C. (e.g. 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)

  Note: *If you have a vacuum manifold other than the Millipore model suggested, please contact ProZyme at info@prozyme.com for setup instructions.*

- Gly-*X* Vacuum Manifold Spacer (ProZyme GX100)
- DI water
- 0.1% formic acid (for Protein A elution)
- 100% ethanol
- 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 50mM HEPES, pH 7.9.

Other sample considerations include:

- Amine buffer components (e.g. Tris, arginine, glycine, histidine) should be avoided as these will react with the InstantQ glycan labeling dye. 10 kDa molecular weight cut-off spin centrifugal filters are recommended for buffer exchange before the deglycosylation step for these samples. Water or non-interfering buffer 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 (~150mM salt) are compatible with the kit, however, higher salt concentrations may decrease the sensitivity of the method. 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) but some glycoproteins can be added in higher concentrations than 2 mg/ml.
- 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 3.2.
- For citrate-containing buffers, dilute sample with water or 50mM HEPES, pH 7.9 to reduce citrate below 20 mM or exchange buffer with molecular weight cut-off spin centrifugal filters.
- Use of Gly-*X* Blocker is recommended for smaller glycoproteins (<50 kDa) or complex sample matrices, see FAQs.
- Use of a more stringent clean up method is recommended for complex sample matrices, such as cell culture supernatants or biological fluids, see Appendix C.

If a precipitate is observed upon incubation at 90°C (Step 3.5), 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 ProZyme at info@prozyme.com.

Optional sample buffer exchange with 10kDa 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)

If you have questions on the compatibility of your sample buffer with the Gly-*X* protocol, please contact ProZyme at info@prozyme.com.

PROTOCOL

1) Getting Started

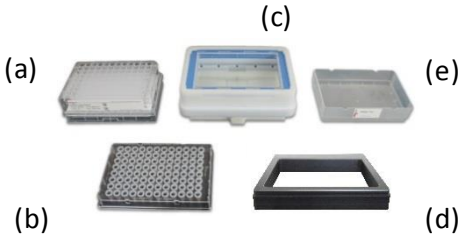
1. Prepare samples (see considerations above).
2. Set the thermocycler to 90°C, or set two independent heat blocks to 90°C and 50°C.
3. Prepare the working solutions in the table below.

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 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.
InstantQ Working Dye Solution	A) Warm to room temperature the InstantQ Dye vial, Dye Solvent and Activation Reagent. Remove from desiccant pouch. B) Add 400 µl of Dye Solvent to the InstantQ dye vial, vortex until dissolved. This can be stored at -20°C. C) Mix solubilized InstantQ Dye and Activation Reagent 1:1. Prepare a total volume of 20 µl per sample with ~20% overage. For 8 wells mix 96 µl InstantQ dye and 96 µl Activation Reagent, mix well. ***Note:*** *Use tubes or plates that are compatible with organic solvents (polystyrene is not compatible).*	20 µl of InstantQ Working Solution is required per sample, mix ~20% overage. Solubilized dye (without addition of Activation Reagent) is stable for 1 month at -20°C. Minimize exposure of InstantQ Dye to air. Final InstantQ Dye Working Solution is stable for 3 hours.

2) Prepare Cleanup Station

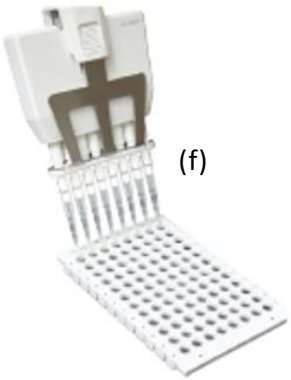
- Have at hand:

1. InstantQ Cleanup Plate A
2. InstantQ Collection Plate
3. Vacuum manifold connected to vacuum pump
4. Gly-*X* Manifold Spacer (ProZyme GX100)
5. Waste tray
6. Wash Solution: 100% Ethanol
7. Priming & Elution Solution: DI water



3) Gly-X Deglycosylation

1. Add 2 µl of Gly-*X* Denaturant (orange cap vial) to the bottom of the Gly-*X* Deglycosylation Plate (f), one well per sample.
2. **Optional:** Add 2 µl of Gly-*X* Blocker to each well.



Note: *Gly-X Blocker is not required for most biotherapeutics e.g. mAbs and Fc-fusions. See FAQs for further details.*

Note: *For samples with a pH of 5.5 or lower, also add 3 µl Gly-X Digestion Buffer (white cap vial), mix thoroughly with pipette.*

3. Add 20 µl of each glycoprotein sample (~2 mg/ml) to each well.
4. Mix well using a pipette, and tap on benchtop to collect samples at bottom of wells.
5. Incubate uncapped at 90°C for 3 min.

Note: *If a precipitate forms, review the sample buffer composition, avoid histidine and glycine in samples.*

6. Remove plate, place at room temp for 2 minutes.
7. Add 2 µl of N-Glycanase Working Solution to each sample. Mix well using a pipette.
8. Tap plate on benchtop to collect samples at bottom of wells (or spin).
9. Incubate uncapped at 50°C for 5 minutes. Do not exceed 5 minutes; longer incubation with enzyme can lead to reduced signal.
10. Remove plate from heat and proceed directly to InstantQ labeling.

4) InstantQ Labeling

1. Add 20 µl of InstantQ Dye Working Solution, prepared above, per sample. After each addition, mix thoroughly with a pipette. Repeat until InstantQ Dye Solution has been added to each sample.
2. Incubate uncapped at 50°C for 1 min.

Note: *The InstantQ Working Dye Solution must be used within 3 hours.*

5) InstantQ Cleanup

Note: Use of a more stringent clean up method is recommended for complex sample matrices, such as cell culture supernatants or biological fluids, see Appendix C.

LOAD

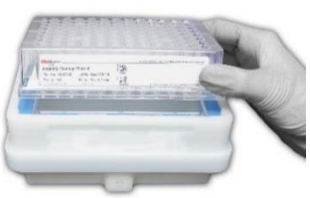
1. Mark the Gly-Q Cleanup Plate A (g) for the required number of wells.

Note: *Black cap strips should be placed on wells used in previous cleanup procedures to prevent re-use of wells.*

Note: *Cleanup Plate storage plate (i) 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.

4. Install InstantQ Cleanup Plate A on top of the manifold.



5. Prime InstantQ Cleanup Plate A
   a) Add 400 µl of DI water to each well and apply vacuum at 5 inHg until the wells are empty.
   b) Add 600 µl of 100% ethanol to each well and vacuum at 5 inHg until the wells are empty.
   c) Repeat Wash with 600 µl 100% ethanol.
   d) Empty the Waste Tray

Note: *If processing more than 72 samples, empty the Waste Tray after 2nd wash (Waste Tray holds approximately 120 ml).*

6. Load samples from Deglycosylation Plate into Cleanup Plate
   a) With a multichannel pipette, add 400 µl 100% ethanol to each well in the cleanup plate.

   Note: *There may be some loss of 100% ethanol due to pass through of the cleanup plate. Proceed to step 2 immediately or increase the 100% ethanol volume to 500 µl to allow for more time (≤2 min).*

   b) With a multichannel pipette, add 150 µl 100% ethanol to each sample in the Deglycosylation Plate. Mix with a pipette and transfer the entire sample (~190 µl) to the corresponding wells in the InstantQ Cleanup Plate A. Mix well with a pipette.
   c) Repeat until all samples have been transferred to the cleanup plate.
   d) Apply vacuum to 5 inHg until the wells are empty. Vacuum can be increased to 10 inHg if any wells are not empty after 2 minutes.

   Note: *This step loads the sample to the InstantQ Cleanup Plate A matrix.*

WASH

1. Wash with 600 µl of Wash Solution (100% Ethanol) and apply vacuum to 5 inHg, collecting wash in the Waste Tray.
2. Repeat with a second 600 µl ethanol wash.
3. Repeat with a third 600 µl ethanol wash.

   Note: *Make sure the wells are completely empty, if not, after 2 minutes, increase the vacuum to 10 inHg.*

   Note: *If processing more than 48 samples, empty the Waste Tray after 2nd wash (Waste Tray holds approximately 120 ml)*

4. Release the vacuum, remove the Clean-up Plate A and rest on the Storage Plate (i). Empty the Waste Tray.

ELUTE

1. Remove the Waste Tray from manifold.
2. Place Gly-*X* Manifold Spacer (ProZyme GX100) into manifold, with the cut-away edge of the spacer facing up.
3. Place Collection Plate on top of the Spacer Plate and reassemble vacuum manifold.
4. Install the Gly-*X* InstantQ Cleanup Plate A on top of the vacuum manifold.

1 2 3 4

5. Add 150 µl of DI water to each well. Using vacuum, (≤5 inHg) elute samples into the Collection Plate.
6. Maintain the vacuum for at least 1 minute to collect the eluent.

   Note: *All wells should be completely empty, if not, keep the vacuum at <5 inHg for another 1-2 minutes. Do not increase vacuum during the elution step.*

7. Release the vacuum, remove the Cleanup Plate and rest on the Storage Plate.
8. Remove the collection plate. Optional: seal the Collection Plate with pre-slit sealing film included in the kit, recommended for overnight use of Gly-Q to prevent evaporation of samples.
9. 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.*

10. Add Black 'Used Well' Sealing Caps to used Cleanup Plate A wells, return Cleanup Plate to bag and store at RT.
11. InstantQ labeled glycan samples are ready for analysis. Samples may be stored at -20°C for at least one month or 4°C for up to 5 days.

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

    Note: *Cleanup Plate storage (round bottom) plate (i) should be used to store Cleanup Plate after use. Store in foil bag provided.*

(h)

(i)

ANALYSIS OF LABELED GLYCANS

GLY-Q & GLY-Q MANAGER STARTUP

1. Open Gly-Q Manager software.
2. Turn on the Gly-Q instrument.
3. Turn on the Gly-Q vacuum pump.
4. Prepare Gly-Q Cartridge and insert into instrument (see Appendix)
    a) Each cartridge can be used for up to 120 injections, see the Gly-Q Manager info bar display for the number of Cartridge Runs.
5. Prepare a Gly-Q Reagent Tray by adding 4 ml each of Park, Wash, Separation and Clean solutions to their respective reservoirs.
    a) Park: 4 ml DI water, layer on mineral oil to cover if cartridge is stored on system >5 hours.
    b) Wash & Clean: 4 ml DI water
    c) Separation: 4 ml Gly-Q separation buffer (provided with kit)

SETTING UP A SAMPLE SEQUENCE

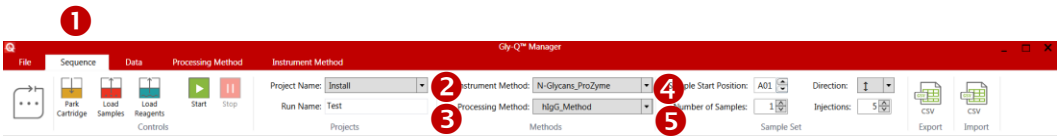
1. In Gly-Q Manager, navigate to the Sequence Tab (default start up tab).
2. Select/ or enter a Project Name.
3. Name your Run.
4. Select Instrument Method.
5. Select Processing Method.

Note: Pre-installed Methods are recommended.

Note: The Instrument Method defines the location of the Migration Standards (upper and lower) (MS) and the GU ladder (GU). To change these positions, or to use additional standards, navigate to the Instrument Methods Tab, create a new method, save, and then select the new method in the Sequence Tab.

Note: An Instrument Method can only be modified prior to data acquisition.

Note: A Processing Method can be changed prior to and after data acquisition.



6. Select starting position of the 96-well plate (first sample to be analyzed).
7. Enter number of samples.
8. Select direction (rows vs columns) and number of injections per well.
9. Select number of injections per sample.

Note: Sample names can be edited on the Sequence Preview table.

Note: Sample lists can be imported & exported using the Import/Export Sequence functions. Supported file types are.xls and .csv.

CHECKING/EDITING REAGENT LOT INFORMATION

1. In Gly-Q Manager, navigate to the File Tab.
2. Under Cartridge & Reagents verify Reagent Kit Lot Number and Reagent Kit Expiration Date. Edit if needed.

   Note: Cartridge Serial Number, Expiration Date, Injection Counter and Last Run Date are updated automatically.

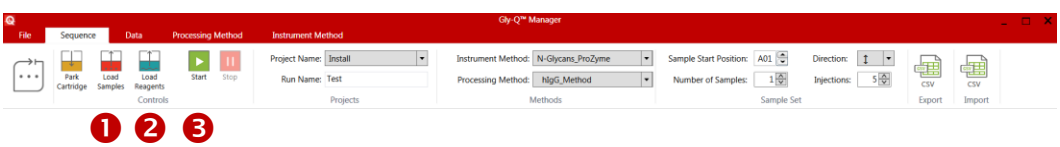
STARTING A RUN

1. Select the Load Samples button at the top left of the Sequence Tab. This will prompt the instrument to rotate the sample plate holder to the front of the instrument window. Insert sample plate.
2. Select the Load Reagents button, at the top left of the Sequence Tab. This will rotate the reagent wells to the front of the instrument window. Transfer 50 µl each of Migration Standards (MS, blue dot on cap) and GU Ladder (GU, yellow dot on cap), into the color-coded PCR tubes provided. Pipette 30 µl of mineral oil on top of both the Migration Standard and the GU Ladder. Place into the appropriate locations as shown in the Sequence Tab Reagents and Standards diagram (2 below). Install the Reagents Tray with Park, Wash, Separation and Clean buffer solutions (prepared above).

   Note: *Remember to close the Gly-Q instrument door after loading samples and reagents.*

3. Select Start from Sequence Tab screen to begin sample sequence.

   Note: *Instrument first runs a long high voltage prime before injection of the GU ladder.*



COMPLETING A RUN

1. After the samples have been processed, the system Cartridge will return to Park.
2. Use the "Load Sample" and "Load Reagents" buttons to remove sample plate and reagent tray. Migration Standard and GU Ladder tubes can be capped and stored at -20°C directly in PCR tubes. For re-use, thaw and centrifuge briefly to bring mineral oil to the surface.
3. Store the cartridge.
    a) To store on the instrument: Press the Park button before shutting down the system. The cartridge can remain on the system for up to 3 days. Mineral Oil must be layered onto the Park solution.
    b) For longer term storage (>3 days) remove the cartridge from the instrument and place tape over the cartridge cap (to cover the pin hole). Return it to the original clamshell packaging. The cartridge tip must be in contact with the gel block inside the package. Place inside the foil pouch and store vertically at 4°C.

APPENDIX A

CARTRIDGE PREPARATION

1. Open clamshell container (keep this for storage between uses).
2. Remove cartridge and pin (red) from package.

   Note: *The pin is used to puncture the top of the cartridge prior to first use; it is stored in the clamshell container.*
3. Remove tape from cartridge cap.

4. Use pin to puncture the top of the cartridge prior to initial use. Push pin all the way in, a slight resistance should be encountered when performing this step.
5. Gently wipe the tip of the cartridge.

6. Prime the Cartridge for use as directed in the Gly-Q System User Manual.

Note: *Handle the cartridge tip with care. The glass capillary extends beyond the metal tip. Do not allow the tip to contact hard surfaces.*

APPENDIX B

PEAK ASSIGNMENT WITH SIALIDASE A

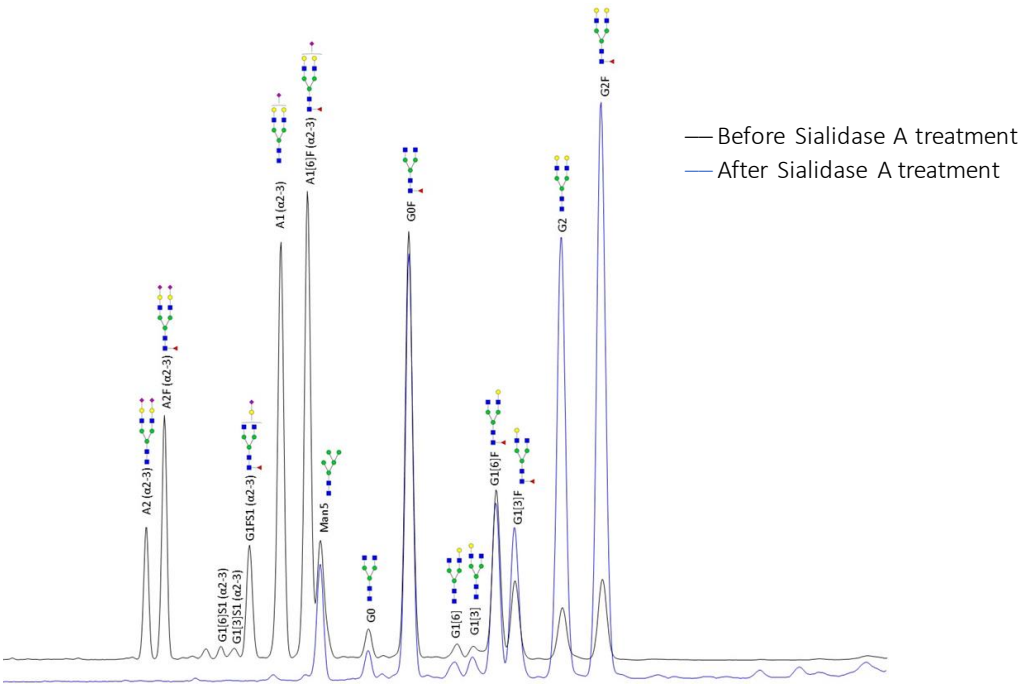
Glyko® Sialidase A™ is available to aid in Gly-Q peak assignment. Sialidase A(GK80040) releases α(2,3)-, α(2,6)-, α(2,8)-, and α(2,9)-linked sialic acid sialic acids from InstantQ labeled glycans causing the peaks on Gly-Q electropherogram to move from sialylated to neutral GU windows. Other exoglycosidases are available and digestion conditions may vary depending on the enzyme, please contact ProZyme for details. For example, Sialidase S (GK80021) releases α(2,3)-linked sialic acid from glycans.

Desialylation with Sialidase A

Sialidase A (GK80040) releases α(2,3)-, α(2,6)-, α(2,8)-, and α(2,9)-linked sialic acid from InstantQ labeled glycans.

1. Add 1 µl of Sialidase A to PCR plate wells.
2. Transfer 75 µl of InstantQ labeled glycan to each well.
3. Incubate at 50°C for 10 minutes.
4. Load the plate onto Gly-Q and run with the same Instrument and Processing methods as untreated samples.

Example Electropherogram: Enbrel N-Glycans



APPENDIX C

ALTERNATIVE CLEANUP PROTOCOL FOR COMPLEX SAMPLE MATRICES

The following more stringent cleanup method has been developed for complex sample matrices, such as biological fluids. The method enables better residual salt and protein removal that can result in higher signal and improved baseline for these sample types. However, total fluorescence signal may be more variable. For glycans from purified proteins, the cleanup protocol on page 10 is optimal.

PREPARE LOAD AND WASH SOLUTIONS

Note: *A total of 1200 µl TFA/ACN solution and 90% ACN Solution is needed per sample; make up 1440 µl of each solution per sample (including 20% overage).*

1. Prepare ACN/TFA Solution; mix acetonitrile (ACN), trifluoroacetic acid (TFA) and water in a ratio 87:2:11 (v/v).
2. Prepare 90% ACN Solution (ACN:water, 90:10 v/v).

LOAD

Note: *No priming is required.*

1. With a multichannel pipette, add 450 µl ACN/TFA Solution to one column of wells in the Gly-*X* Cleanup Plate. Do not apply vacuum.

   Note: *There will be some loss of ACN/TFA Solution due to pass through of the cleanup plate. Proceed to step 2 immediately or increase the ACN/TFA Solution volume to 500 µl to allow for more time (≤2 min).*

2. With a multichannel pipette, add 150 µl of ACN/TFA 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.
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 ACN/TFA Solution and apply vacuum to <5 inHg, collecting wash in the Waste Tray.
2. Wash twice with 600 µl of 90% ACN Solution applying vacuum to <5 inHg and collecting wash in the Waste Tray.

   Note: *All wells should be completely empty, if not, after 2 minutes, increase the vacuum to 10 inHg.*

Note: *If processing more than 48 samples, empty the Waste Tray after 2nd 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 (ProZyme GX100) into manifold, with the cut-away edge of the spacer facing up.
3. Place Collection Plate on top of the Spacer Plate and reassemble vacuum manifold
4. Install the Gly-*X* InstantQ Cleanup Plate A on top of the vacuum manifold.
5. Add 150 µl of DI water to each well. Using vacuum, (≤5 inHg) elute samples into the Collection Plate.
6. Maintain the vacuum for at least 1 minute to collect the eluent.
7. Release the vacuum, remove the Cleanup Plate and rest on the Storage Plate.
8. Remove the collection plate.
9. Vortex to mix, and spin/tap to collect samples at the bottom of the wells, or mix with a pipette.

FAQS

Q: When do I need to use the Gly-*X* Blocker with my deglycosylation reaction? What does the Blocker do?

A: The Gly-*X* Blocker is optional. It is not required for most biotherapeutics e.g. IgGs, Fc-fusion proteins. However, if you observe an uneven/rounded baseline during analysis we suggest running the protocol with and without the Blocker to test whether it is required. Potential scenarios that may require the Blocker are:

a) if your glycoprotein is <50 kDa;

b) if the glycoprotein contains a large proportion of basic amino acids;

c) if your sample is a complex matrix such as a biofluid.

The Blocker ensures that labeled protein does not interfere with N- glycan analysis by blocking free amine groups on the glycoprotein before they can be labeled with InstantQ dye.

Using the blocker may cause the reaction to turn yellow, particularly when there is a lot of protein material. This is caused by Maillard reaction products and is not a cause for concern. Please contact ProZyme for further details.

Q. Why should I use 0.1% formic acid to elute samples from Protein A ready for Gly-*X* with InstantQ, rather than a low pH glycine solution?

A. Glycine has a primary amine which will react with the InstantQ label, so we suggest that 0.1% formic acid is used as a protein eluent. Alternatively, glycine may be used but samples must be desalted before deglycosylation.

Q. Can I use ProZyme AssayMAP Protein A cartridges to purify proteins ready for Gly-*X* with InstantQ, and elute with 0.1% formic acid?

A. Yes. AssayMAP PA50 Protein A Cartridges are available from ProZyme in 24-count (G5524-60001) and 96-count (G5524-60010) kits, and 0.1% formic acid may be used as an eluent.

Q. What is the lower limit of glycoprotein I can use with the Gly-*X*/InstantQ protocol?

A. Each laboratory will need to establish the lower limit for each specific protein. 0.125 mg/ml can be used as a starting point.

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

A: It depends on the protein. When using >40 µg protein the user should check that relative percent area data maintains linearity.

Q: Can I use Eppendorf microtubes for the Denaturation and Digestion Steps, rather than a PCR plate?

A: Yes, leave the tubes open for the heating steps, and ensure that material does not get in the lid during mixing.

Q: Can I run glycans labeled with InstantQ dye on other CE systems?

A: The LIF detector typically used on the Beckman PA800-plus for APTS glycans is not compatible with the InstantQ Dye excitation wavelength. Suitability on other systems may be determined by the user.

RESOURCES AND REFERENCES

Visit ProZyme's website for additional information, downloadable posters, publications and tech notes:

www.prozyme.com/pages/resources

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Virtual Patent Marking

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TECHNICAL ASSISTANCE

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

If you have any questions or experience difficulties regarding any aspect of our products, please contact us:

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ProZyme values customer opinions and we 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-IQ	Gly-*X* with InstantQ Kit (96 ct)
GX24-IQ*	Gly-*X* with InstantQ Kit (24 ct)
GX96-302IQ	Gly-*X* with InstantQ Deglycosylation and Labeling Module Set (96 ct)
GX24-302IQ	Gly-*X* with InstantQ Deglycosylation and Labeling Module Set (24 ct)
GX96-301	Gly-*X* InstantQ Labeling Module (96 ct)
GX24-301	Gly-*X* with InstantQ Labeling Module (24 ct)
GX96-302	Gly-*X* InstantQ Cleanup Module (96 ct)
GX100	Gly-*X* Vacuum Manifold Spacer (2 pack)
G5524-60010 KIT	AssayMAP PA50 protein A affinity purification kit (96 ct)
G5524-60001 KIT	AssayMAP PA50 protein A affinity purification kit (24 ct)
GQ103	Gly-Q Cartridge Module
GK80040	Glyko Sialidase A
GK80021	Glyko Sialidase S

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

Gly-Q System

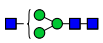
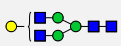
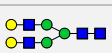
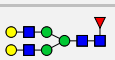
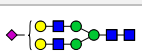
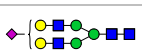
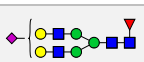
Product Code	Description
GQ2100	Gly-Q Glycan Analysis System*
GQ001	Cartridge storage rack
GQ2050	Gly-Q Computer (Windows OS)

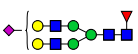
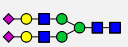
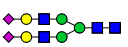
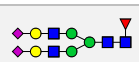
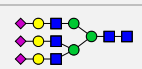
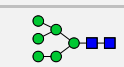
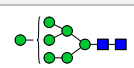
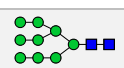
* *Gly-Q System includes instrument, pressure pump, priming station, Gly-Q Manager software (GQSW2100), 1-year warranty.*

Ladder and Migration Standards

Product Code	Description
GKSQ-503	Gly-Q GU Ladder
GKSQ-500	Gly-Q Migration Standards (upper and lower)
GKSQ-505	Gly-Q Alignment Standards Set (GKSQ-500 & GKSQ-503)

N-Glycan Standards

Product Code	Description
GKSQ-401	G0-N 
GKSQ-301	G0 
GKSQ-402	G0F-N 
GKSQ-302	G0F 
GKSQ-317	G1 
GKSQ-316	G1F 
GKSQ-304	G2 
GKSQ-305	G2F 
GKSQ-311	A1 (α2,6) 
GKSQ-321	A1 (α2,3) 
GKSQ-315	A1F (α2,6) 

Product Code	Description
GKSQ-325	A1F (α2,3) 
GKSQ-312	A2 (α2,6) 
GKSQ-322	A2 (α2,3) 
GKSQ-313	A2F (α2,6) 
GKSQ-323	A2F (α2,3) 
GKSQ-314	A3 (α2,6) 
GKSQ-103	Man5 
GKSQ-104	Man6 
GKSQ-105	Man7 
GKSQ-106	Man8 
GKSQ-107	Man9 

N-Glycan Libraries

Product Code	Description
GKSQ-005	Human IgG N-Linked Glycan Library
GKSQ-009	RNase B N-Linked Glycan Library
GKSQ-233	α(2,3) Sialylated Triantennary Library
GKSQ-234	α(2,3) Sialylated Tetraantennary Library
GKSQ-263	α(2,6) Sialylated Triantennary Library
GKSQ-264	α(2,6) Sialylated Tetraantennary Library

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