Automation of Agilent AdvanceBio Gly-X Glycan Prep with InstantDyes

Walk away sample preparation with AdvanceBio Gly-X kits

Walk Away N-Glycan Sample Prep with Agilent AdvanceBio Gly-X

Automation can reduce hands-on time, user error and data variability. Each step of the Agilent Gly-X Glycan Prep with InstantDye (formerly ProZyme) protocol, including rapid deglycosylation, labeling and cleanup are carried out in 96-well format plates. This makes the Gly-X kits directly compatible with most liquid handling workstations, without modification or the need for special kit components or kit formats.

Gly-X N-glycan sample preparation can be performed manually or on an automation workstation providing flexibility and efficient use of the kit modules based on throughput and workflow needs.

40 µg Glycoprotein
3 min Denaturation
5 min N-Glycanase digest
Released Glycans
1 min InstantPC Labeling
Labeled Glycans
96-well Cleanup Plate
UHPLC, LC/MS, CE
Data Analysis

Here's how you boost productivity:

- Gly-X kits support both manual and automated methods
- Automated method produces data consistent with manual workflow
- Flexible use of kit modules between methods
- Demonstrated for both LC/MS and CE labels

Advancing glycosciences, together.

With the addition of ProZyme products and services, Agilent provides a single source offering for instruments and consumables, from sample to trusted answer.
Our expertise now covers the complete glycan analysis workflow, so you can easily get the reliable, reproducible results you need.

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General Guidelines

Gly-X N-glycan deglycosylation and InstantDye labeling is carried out in a 96-well plate. The cleanup is performed in a 96-well vacuum plate. To automate the Gly-X kits, the automation platform requirements for pipetting are volumes between 4–400 µL. The platform should support locations for vials, troughs, and 96-well plates. The platform should also support moving the 96-well plates between stations on the deck.

Gly-X deglycosylation and labeling requires two incubation temperatures (50 °C and 90 °C) and are carried out in a 96-well PCR plate. These steps are usually performed with two on-deck heater blocks or an on-deck thermocycler.

Gly-X cleanup is performed in a 96-well vacuum plate for which an on-board automated positive pressure manifold is recommended. An on-board vacuum manifold can also be used.

Labeled N-glycans are eluted from the cleanup plate into a PCR plate, also performed in the positive pressure manifold.

Automation Workflow

The automation workflow (Figure 1) begins with the system transferring required volumes of three stock reagents (Gly-X Denaturant, N-Glycanase and selected InstantDye) from vials into a 96-well 'source' plate. The number of wells for each transferred reagent matches the number of samples to be processed. The glycoprotein samples are then moved from the sample plate into the wells of the source plate that contain transferred reagents, mixed and returned to the sample plate. The sample plate is then incubated at the specified temperature.

In this manner, all incubations are done in the sample plate, and Multichannel pipettes can be used to transfer the samples from sample plate to source plate and back to the sample plate.

After addition of Gly-X Denaturant, samples are incubated at 90 ºC for 3 minutes. Following the transfer of N-Glycanase, the sample plate is then incubated at 50 °C for 5 minutes. Deglycosylation is followed by the addition of labeling reagents and incubation for 1 min at 50 ºC. Samples are then transferred to the Gly-X Cleanup plate, and washed using the Hamilton [MPE]2 positive pressure module on the Nimbus deck using a pressure gradient. Elution buffer for InstantPC-labeled N-glycans is included in the kit, elution for InstantQ-labeled glycans is DI water. Labeled N-glycans are eluted with 100 µL of eluent into a PCR plate and are ready for analysis by LC/MS (InstantPC) or CE (InstantQ) using the Agilent Gly-Q Glycan Analysis System (formerly ProZyme).

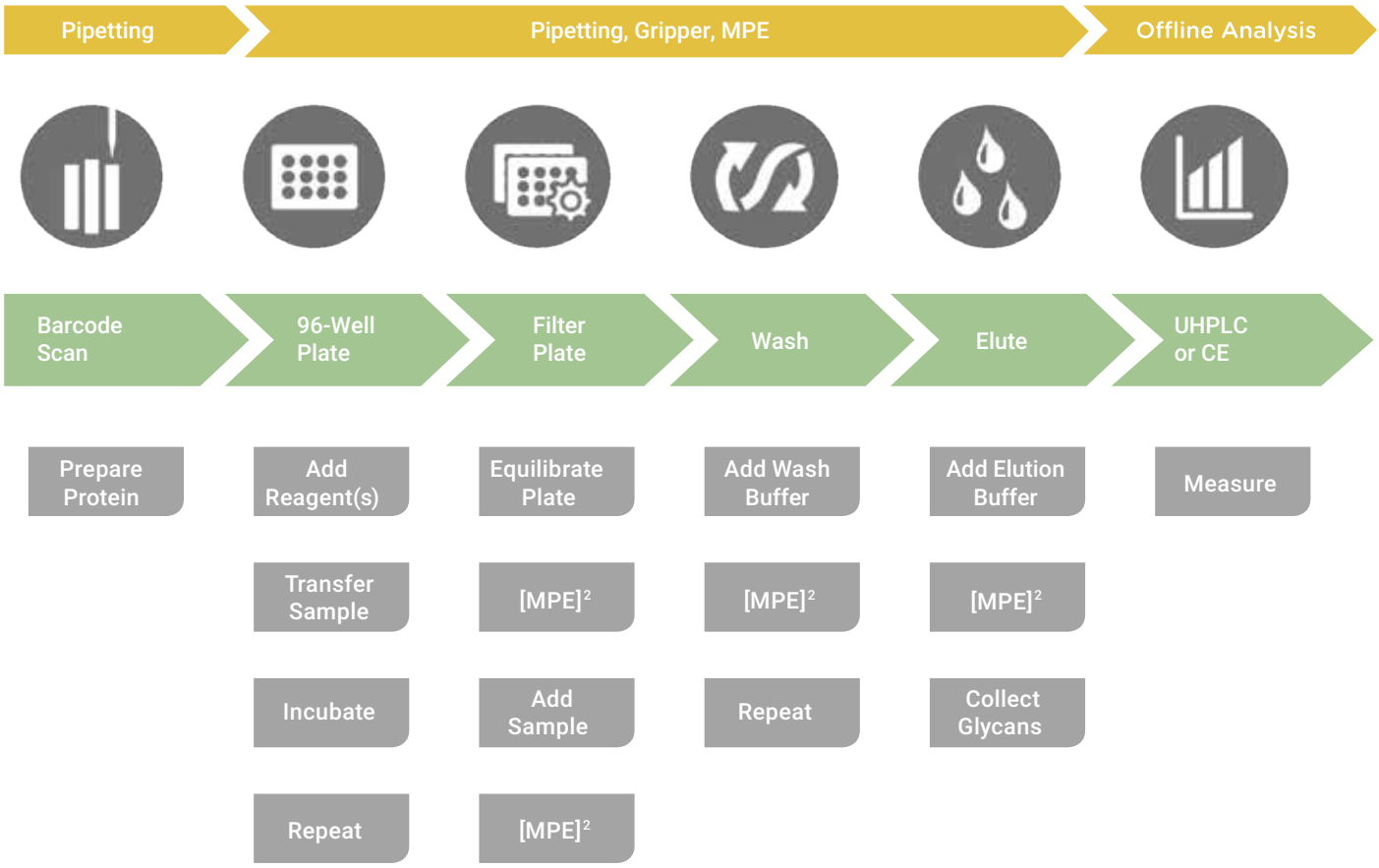


Figure 1: Automation overview: Workflow on Hamilton NIMBUS for Agilent AdvanceBio Gly-X N-Glycan Prep with InstantPC and with InstantQ kits.

Deck Setup

Load Consumables and Kit Components

- Load tips, 50 µL and 1000 µL
- Load reagent source plate: PCR plate, semi-skirted
- Load Gly-X Cleanup plate stack
- Load Gly-X Collection plate or skirted PCR plate

Load Kit Reagents

Gly-X Denaturant –
Dilute 1:1 with DI water into a 0.5 mL vial

- Place in reagent block

N-Glycanase Working Solution – Prepare with Digestion Buffer according to kit instructions, then dilute the mix 1:1 with DI water into a 0.5 mL vial

- Place in reagent block

InstantDyes – Prepare according to kit instructions

- Place in a 0.5 mL vial in reagent block

Load Required Buffers

Load/Wash buffer

- For InstantPC, prepare 100 mL of Load/Wash buffer and place in trough on deck
- For InstantQ, place ethanol in trough on deck

Elution reagents

- For InstantPC, add Gly-X InstantPC eluent provided in the kit in trough on deck
- For InstantQ, place water in trough on deck

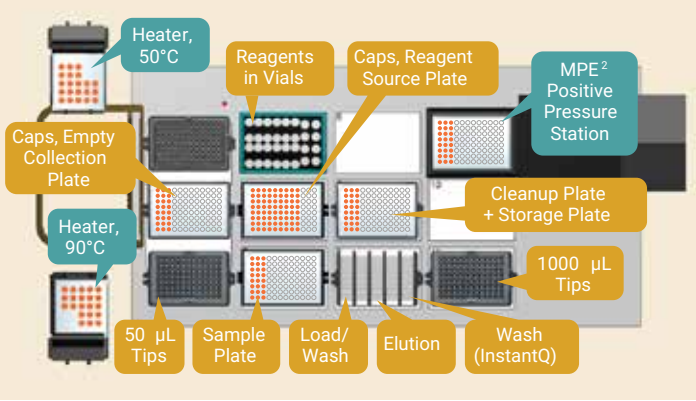


Figure 2: Hamilton NIMBUS deck layout. Location of plates, vials, troughs, heaters and MPE positive pressure manifold.

Reliable Results

Fusion protein Enbrel and water blanks were placed into the sample plate in a checkerboard layout to verify well to well performance, and to test for any crossover. The test was preformed for both InstantPC dye (by UHPLC) and InstantQ dye (by CE with Gly-Q system). The chart below shows that similar data is obtained regardless of method used during development of the automation protocol.

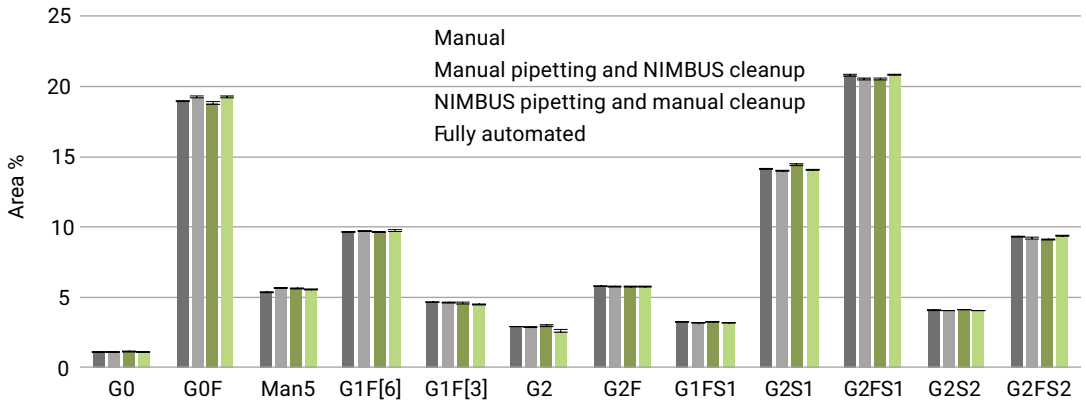


Figure 3: Enbrel InstantPC-labeled N-glycans prepared by various methods (n=8).

Ordering Information

AdvanceBio Gly-X with InstantPC

Kits and Modules

Description	Part Number
AdvanceBio Gly-X with InstantPC Kit (96 ct)	GX96-IPC
AdvanceBio Gly-X with InstantPC Kit (24 ct)	GX24-IPC
AdvanceBio Gly-X with InstantPC Deglycosylation and Labeling Module Set (96 ct)	GX96-201PC
AdvanceBio Gly-X with InstantPC Deglycosylation and Labeling Module Set (24-ct)	GX24-201PC
AdvanceBio Gly-X InstantPC Labeling Module (96-ct)	GX96-101
AdvanceBio Gly-X InstantPC Labeling Module (24-ct)	GX24-101
AdvanceBio Gly-X InstantPC Cleanup Module for InstantPC (96-ct)	GX96-102
AdvanceBio Gly-X Vacuum Manifold Spacer (2 pack)	GX100
AssayMAP PA50 Protein A Affinity Purification Kit (96 ct)	G5524-60010 KIT

AdvanceBio Gly-X with InstantQ

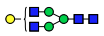
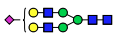
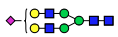
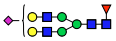
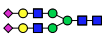
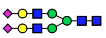
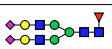
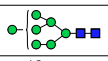
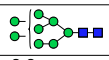
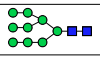
Kits and Modules

Description	Part Number
AdvanceBio Gly-X with InstantQ Kit (96 ct)	GX96-IQ
AdvanceBio Gly-X with InstantQ Kit (24 ct)	GX24-IQ
AdvanceBio Gly-X with InstantQ Deglycosylation and Labeling Module Set (96 ct)	GX96-302IQ
AdvanceBio Gly-X with InstantQ Deglycosylation and Labeling Module Set (24-ct)	GX24-302IQ
AdvanceBio Gly-X InstantQ Labeling Module (96-ct)	GX96-301
AdvanceBio Gly-X InstantQ Labeling Module (24-ct)	GX24-301
AdvanceBio Gly-X InstantPC Cleanup Module for InstantPC (96-ct)	GX96-302
AdvanceBio Gly-X Vacuum Manifold Spacer (2 pack)	GX100
AssayMAP PA50 Protein A Affinity Purification Kit (96 ct)	G5524-60010 KIT
Gly-Q Cartridge Module	GQ103
Sialidase A	GK80040
Sialidase S	GK80021

Ordering Information

AdvanceBio Gly-X with InstantPC

Standards and Controls

InstantPC Labeled Individual Glycan Standards		Part Number
G0-N		GKPC-401
G0		GKPC-301
G0F-N		GKPC-402
G0F		GKPC-302
G1		GKPC-317
G1F		GKPC-316
G2		GKPC-304
G2F		GKPC-305
G1F + 1aGal		GKPC-403
G2F + 1aGal		GKPC-404
G2F + 2aGal		GKPC-318
G1S1 (α2,3)		GKPC-329
G1S1 (α2,6)		GKPC-319
G1FS1 (α2,3)		GKPC-330
G1S1 (α2,6)		GKPC-320
G2S1 (α2,3)		GKPC-321
G2S1 (α2,6)		GKPC-311
G2FS1 (α2,3)		GKPC-325
G2FS1 (α2,6)		GKPC-315
G2S2 (α2,3)		GKPC-322
G2S2 (α2,6)		GKPC-312
G2FS2 (α2,3)		GKPC-323
G2FS2 (α2,6)		GKPC-313
Man5		GKPC-103
Man6		GKPC-104
Man7		GKPC-105
Man8		GKPC-106
Man9		GKPC-107

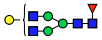
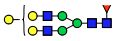
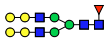
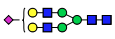
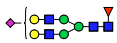
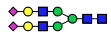
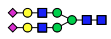
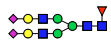
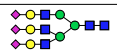
Standards and Controls

InstantPC Labeled N-Glycan Libraries	Part Number
AdvanceBio Human IgG N-Linked Glycan Library	GKPC-005
Glucose Homopolymer	GKPC-503
CHO mAb N-Linked Glycan Library	GKPC-020
CHO mAb N-Linked Glycan Library + CHO mAb Glycoprotein	GKPC-020-P
α(2-3) Sialylated Triantennary Library	GKPC-233
α(2-6) Sialylated Triantennary Library	GKPC-263
α(2-3) Sialylated Tetraantennary Library	GKPC-234
α(2-6) Sialylated Tetraantennary Library	GKPC-264

Ordering Information

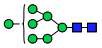
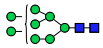
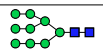
AdvanceBio Gly-X with InstantQ

Standards and Controls

InstantQ Labeled Individual N-Glycan Standards		Part Number
G0-N		GKSQ-401
G0		GKSQ-301
G0F-N		GKSQ-402
G0F		GKSQ-302
G1		GKSQ-317
G1F		GKSQ-316
G2		GKSQ-304
G2F		GKSQ-305
G1F + 1aGal		GKSQ-403
G2F + 1aGal		GKSQ-404
G2F + 2aGal		GKSQ-318
A1 (α2,3)		GKSQ-321
A1 (α2,6)		GKSQ-311
A1F (α2,3)		GKSQ-325
A1F (α2,6)		GKSQ-315
A2 (α2,3)		GKSQ-322
A2 (α2,6)		GKSQ-312
A2F (α2,3)		GKSQ-323
A2F (α2,6)		GKSQ-313
A3 (α2,6)		GKSQ-314

AdvanceBio Gly-X with InstantQ

Standards and Controls

InstantQ Labeled Individual N-Glycan Standards		Part Number
Man5		GKSQ-103
Man6		GKSQ-104
Man7		GKSQ-105
Man8		GKSQ-106
Man9		GKSQ-107

InstantQ N-Glycan Libraries	Part Number
Human IgG N-glycan Library	GKSQ-005
RNase B N-Linked Glycan Library	GKSQ-009
CHO mAb N-Linked Glycan Library	GKSQ-020
CHO mAb N-Linked Glycan Library + CHO mAb Glycoprotein	GKSQ-020-P
α(2-3) Sialylated Triantennary Library	GKSQ-233
α(2-3) Sialylated Triantennary Library	GKSQ-234
α(2-6) Sialylated Triantennary Library	GKSQ-263
α(2-6) Sialylated Tetraantennary Library	GKSQ-264

InstantQ Ladder and Migration Standards	Part Number
Gly-Q GU Ladder	GKSQ-503
Gly-Q Migration Standards (upper and lower)	GKSQ-500
Gly-Q Alignment Standards Set (GKSQ-500 & GKSQ-503)	GKSQ-505

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