

Agilent AdvanceBio Gly-X N-Glycan Prep with InstantPC kit

Simplified Workflow for Rapid FLD/MS
Glycan Analysis



Productivity Simplified and Standardized

The location and structure of N-linked glycans can play a critical role in the pharmacology of therapeutic proteins, potentially affecting immunogenicity, pharmacokinetics and pharmacodynamics.

Agilent AdvanceBio Gly-X (formerly ProZyme) is a next generation N-glycan preparation platform that provides a simplified in-solution workflow. Combined with Agilent InstantPC (formerly ProZyme) dye along with an efficient vacuum plate cleanup step to remove excess label and denaturant, samples are ready for UHPLC or LC/MS usually in 60 minutes or less.

Here's how you boost productivity:

- Samples ready for UHPLC or LC/MS in less than 1 hour
- 5 Min PNGase F digestion provides unbiased N-glycan release
- InstantPC dye for high UHPLC-FLD and MS signal
- Simple, ambient stable 96-well cleanup plate
- Supports rapid and high resolution analysis
- Modular format supports flexible sample throughput and eliminates waste

40 µg Glycoprotein

3 min Denaturation

5 min N-Glycanase digest

Released Glycans

1 min InstantPC Labeling

Labeled Glycans

96-well Cleanup Plate

UHPLC, MS/MS

Data Analysis



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.

Learn more:

[www.agilent.com/chem/
better-together](http://www.agilent.com/chem/better-together)

Deglycosylation

AdvanceBio Gly-X Technology

Gly-X N-glycan release and labeling are carried out in a single well of a 96-well plate. The 5-minute Gly-X digestion with N-Glycanase (PNGase F) is enabled by a proprietary denaturant that enhances exposure of N-glycan sites for rapid enzymatic cleavage.

Gly-X also provides conditions for unbiased PNGase F activity at elevated temperatures (50°C). Gly-X denaturant reagent is stable at room temperature, does not require special handling procedures and is MS-friendly. Gly-X achieves complete in-solution enzymatic deglycosylation of most proteins, up to 40 µg per well, in 5 minutes (Figure 1).

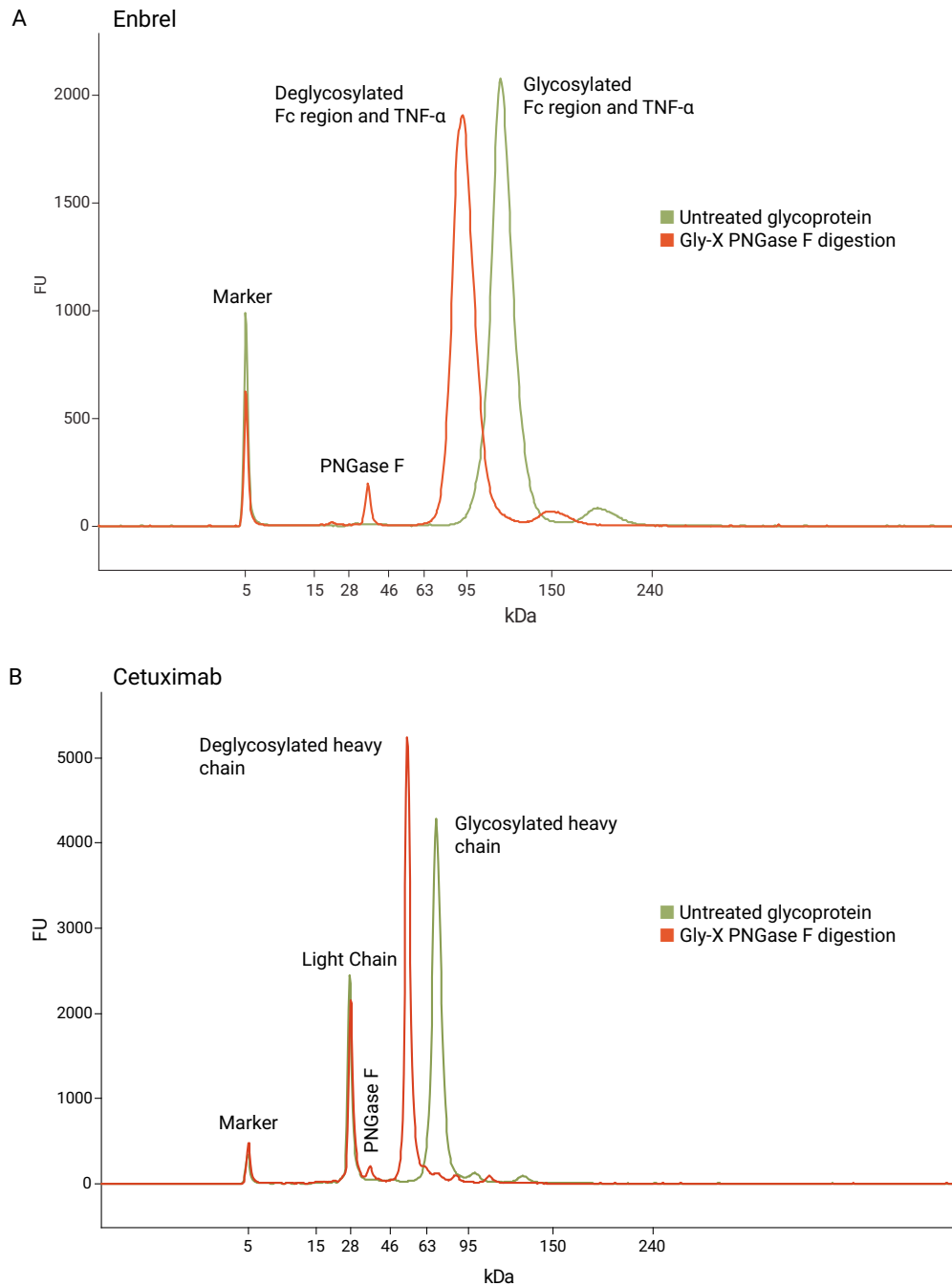


Figure 1: Agilent AdvanceBio Gly-X enzymatic deglycosylation efficiency. Enbrel (A) contains one N-linked glycosylation site in the IgG1 Fc region and two in the TNF-α component and Cetuximab (B), contains two heavy chain N-glycosylation sites, one in the Fc region, the second in the Fab region. Deglycosylation with Gly-X shifts the retention time of the target peak as detected on an Agilent Bioanalyzer.

Labeling

InstantPC Labeling of N-Glycans

In Gly-X the buffer components and denaturant are compatible with InstantPC dye, enabling a 1-minute labeling step immediately following PNGase F digestion. InstantPC dye (Figure 2) is a novel instant N-glycan label that provides markedly increased MS and HILIC/FLD sensitivity.

An activated form of procaine, InstantPC forms a stable urea linkage with glycosylamines released by PNGase F digestion and contains a tertiary amine which generates high MS signals in positive mode. InstantPC will add a monoisotopic mass of 261.14773 Da to a reducing end.

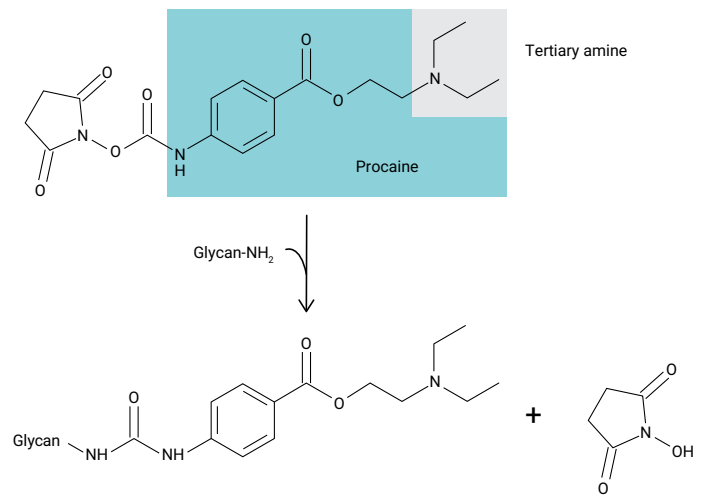


Figure 2: Structure of Agilent InstantPC, a novel glycan label.

Comparison of Agilent InstantPC to Other Glycan labels

Most commonly used labels for glycan analysis ionize poorly, so fluorescence is typically the only choice for detection of low abundance glycans. InstantPC has the highest LC/FLD signal of all glycan labels tested (Figure 3A).

The next best label for fluorescence was Procainamide, which was prepared by reductive amination, a more time-intensive workflow. In addition to high FLD signal in LC, InstantPC contains a tertiary amine which generates high MS signal in positive mode (Figure 3B).

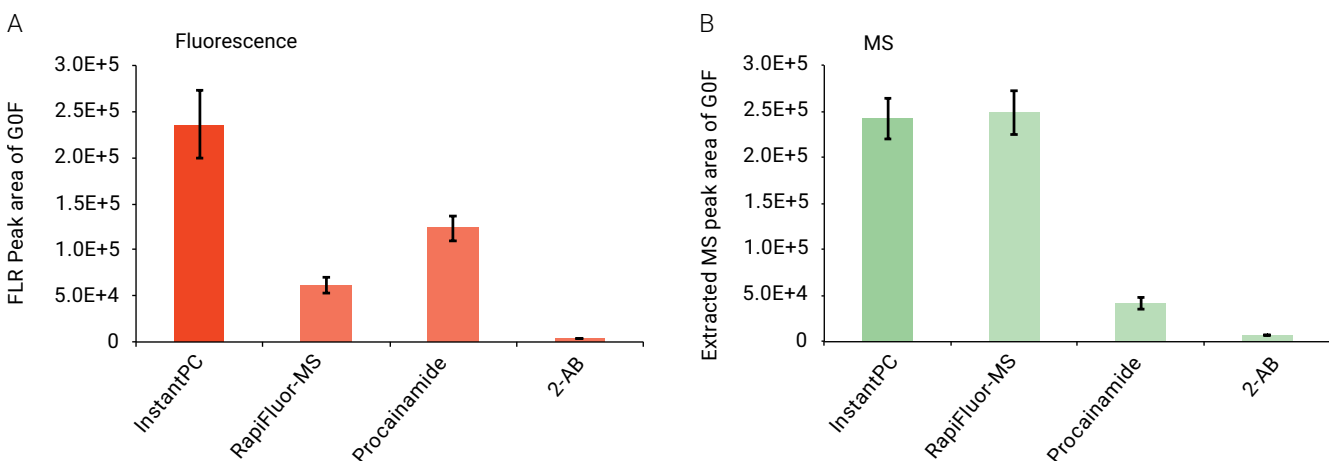


Figure 3: Comparison of FLD (A) and MS (B) Response. Glycans from equal amounts glycoprotein samples were labeled with Agilent InstantPC, RapiFluor-MS, procainamide and 2-AB according to manufacturer instructions, and measured by UHPLC. Bars represent peak area of GOF N-glycan species.

Cleanup

Cleanup of Agilent InstantPC-labeled N-Glycans

The Agilent AdvanceBio Gly-X workflow includes a cleanup step to remove free dye and denaturant from labeled glycans.

The samples are loaded onto a 96-well clean up plate optimized to first capture labeled glycans and then release them by changing polarity of the elution solvent.

A common vacuum filtration manifold is used for all steps as follows:

1. Load samples
2. 2x wash with Load/Wash solution
3. Elute with 160 mM Ammonium formate/10% Acetonitrile

Gly-X cleanup preserves more than 95% of labeled glycans (data not shown). Sialylated glycan species are preserved as shown in Figure 4. In addition to removing free label, the cleanup also removes denaturant from the labeled glycans.

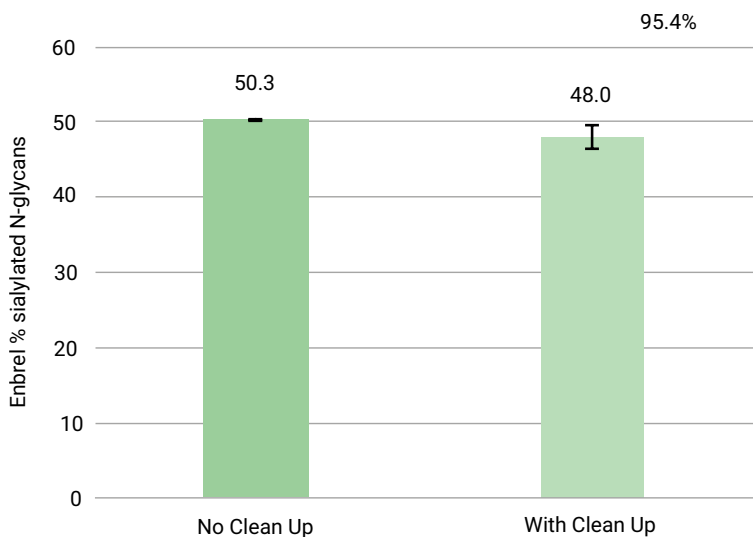


Figure 4: Agilent AdvanceBio Gly-X Cleanup. The relative % area of sialylated Enbrel N-glycans was calculated for glycans prepared using Gly-X with Agilent InstantPC kit with and without the final cleanup step.

Analysis

HILIC/FLD Profiles of Agilent InstantPC-Labeled N-Glycans

HILIC elution profiles for Enbrel InstantPC N-glycans are shown in Figure 5. The elution order is similar to other glycan labels such as 2-AB. HILIC methods of varying length can be used depending on the complexity of the glycan profile.

Figure 5 shows Enbrel N-glycans separated with a 60-minute method. The free dye peak is minimal, as Gly-X cleanup removes more than 99.97% of free dye. The relative % peak area data obtained from sample prepared with Gly-X is highly reproducible (Table 1).

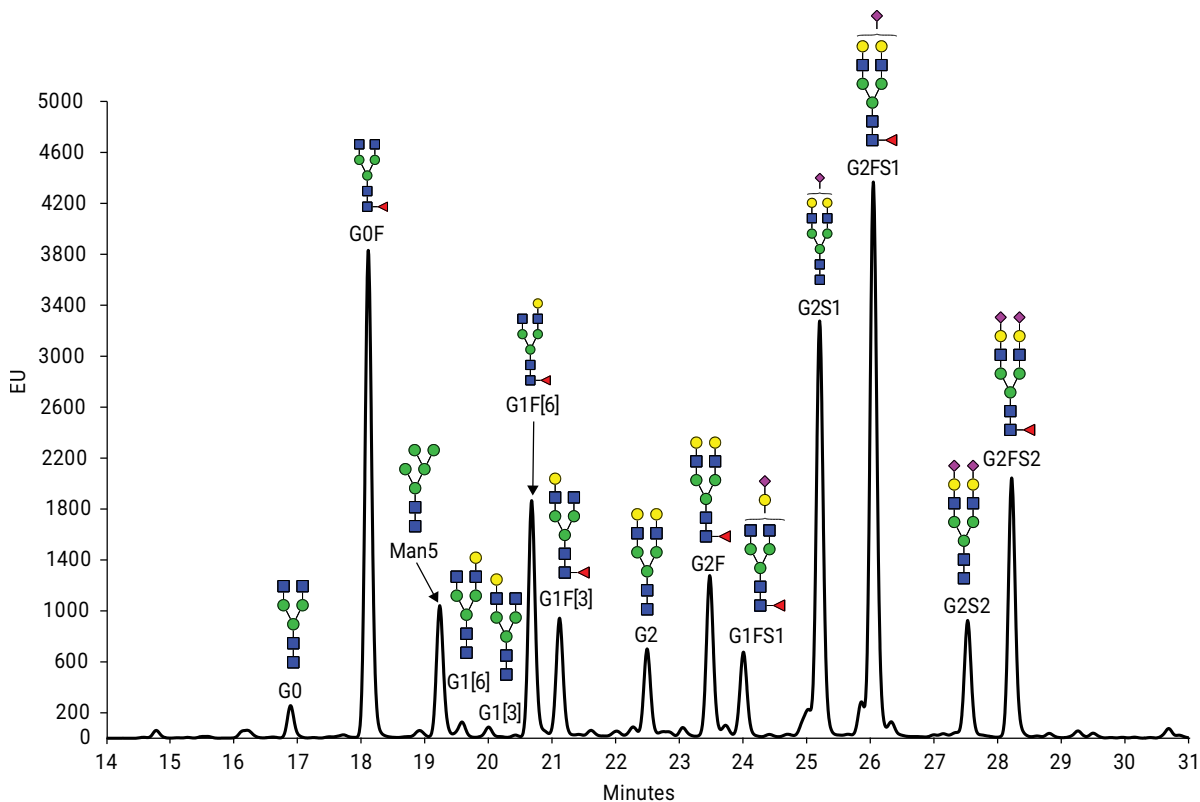


Figure 5: HILIC/FLD profile of Enbrel InstantPC-labeled N-glycans utilizing the Gly-X with InstantPC kit. A 60-minute gradient.

Table 1. Relative % peak area for N-glycans with >2% total peak area prepared from Enbrel, n = 8.

Glycan	% Area	% Area CV	Glycan	% Area	% Area CV
G0F-N	19.8	2.1	G1F[3]	4.1	0.9
G0	4.7	1.5	G2F	13.7	2.5
G0F	9.4	1.5	G2FS1[3]	4.8	0.3
Man5	4.8	2.4	G2FS1[6]	20.3	0.9
G1[6]	2.5	2.4	G2FS2	10.7	2.2
G1F[6]	4.5	1.0			

Specifications	Agilent AdvanceBio Gly-X with InstantPC
Protein load range	1–40* µg
Deglycosylation efficiency	Complete for most proteins
Label	Optimized for FLD and MS detection, instant chemistry
Residual denaturing reagent	Not detectable by LC-MS (Q-TOF)
Glycan recovery	>95%
Free dye removal	>99%
Preservation of sialylated species	>95%
Precision (well-to-well, day-to-day)	<5% CV or relative % peak area of major glycans
Total workflow time, including reagent preparation	42 min (16 samples), 99 min (96 samples)
Sample stability on autosampler	Up to 6 days (pierceable caps not provided)
Long-term sample stability	Up to 30 days at -20 °C in Gly-X Eluent
Kit configuration	Reagents sufficient to prepare 1-96 samples per run. Cleanup plate is reusable, and stored at room temp





























*More than 40 µg glycoprotein may be used, depending on the extent of glycosylation.

Ordering Information

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
Gly-X InstantPC Cleanup Module (96-ct)	GX96-102
AssayMAP PA50 protein A affinity purification kit (96-ct)	G5524-60010 KIT

* GX24-IPC contains a 96-ct cleanup plate. Store the cleanup plate at room temp and order refills of GX24-201PC.

Ordering Information

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

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

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