Agilent AdvanceBio Gly-X Glycan Prep with 2-AB Express Kit

Simplified Workflow for Rapid FLD/MS Glycan Analysis

Traditional Dye, Advanced Workflow

The characterization of N-glycans is essential to biotherapeutic development. Typically, enzymatically-released glycans are derivatized with a tag to allow for fluorescence detection by LC/FLD and LC/MS. Sample preparation of labeled N-glycans often requires numerous hours or days to complete.

Agilent AdvanceBio Gly-X (formerly ProZyme) chemistry is a next generation N-glycan preparation platform that provides a simplified in-solution workflow. N-glycans are released from glycoproteins with N-glycanase in 5 minutes. When combined with next generation fluorescent dyes for example, Agilent AdvanceBio InstantPC (formerly ProZyme), the entire sample preparation protocol can be completed in less than 1 hour. While InstantPC has unsurpassed fluorescence signal and favorable properties for mass spectrometry allowing for the detection of low abundance glycans, 2-AB (2-aminobenzamide) dye has been used to generate N-glycan data for more than 20 years and is well established in many laboratories.

To answer the demand for a rapid Gly-X workflow with traditional 2-AB labeling, on-matrix Agilent AdvanceBio 2-AB Express (formerly ProZyme) labeling was developed. Express labeling eliminates the lengthy dry-down step of traditional workflows. Gly-X with 2-AB Express offers traditional reductive amination labeling without a dry down step, along with an efficient vacuum plate cleanup to remove excess label and denaturant. Samples are ready for UHPLC in just two hours.

40 µg Glycoprotein
3 min Denaturation
5 min N-Glycanase digest
Released Glycans
1 hr 2-AB Express Labeling (no dry down)
Labeled Glycans
96-well Cleanup Plate
UHPLC, MS/MS
Data Analysis

Here's how you boost productivity:

- 5 Min PNGase F digestion provides unbiased N-glycan release
- 2-AB Express labeling eliminates dry down and shortens the protocol
- Use of established 2-AB label assures data continuity for ongoing projects
- Modular format for flexible kit use

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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Deglycosylation

Gly-X N-Glycan Rapid Release 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-Glycanse (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).

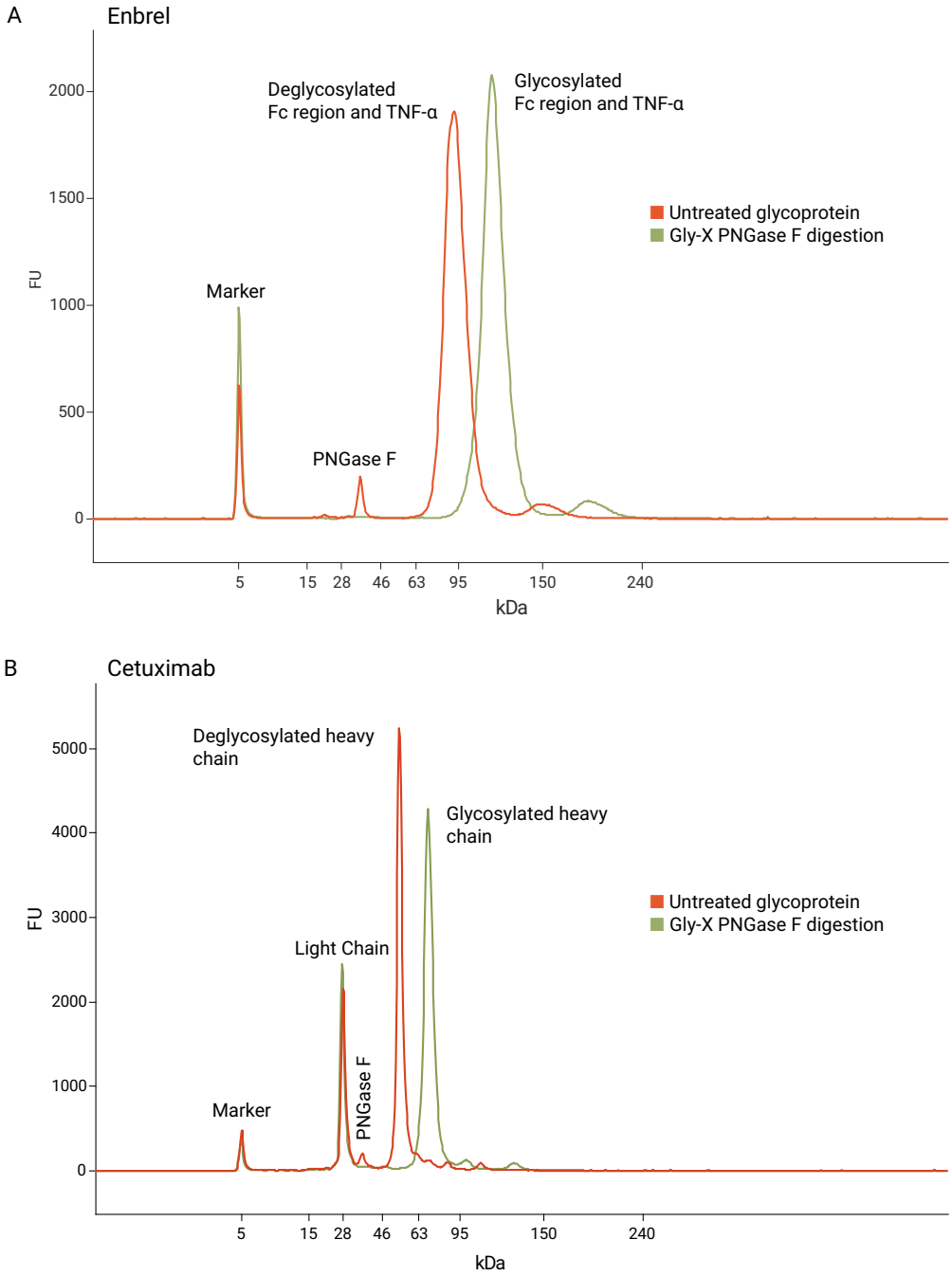

Figure 1: Agilent AdvanceBio Gly-X enzymatic deglycosylation efficiency. Enbrel (A) contains one N-linked glycosylation site in the IgG1 Fc region and two in the TNF-α component and Cetuximab (B), contains two heavy chain N-glycosylation sites, one in the Fc region, the second in the Fab region. Deglycosylation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

Labeling

Express Labeling of N-Glycans

Express labeling of released N-glycans is accomplished by rapid in-solution conversion of -NH_2 glycosylamine form to -OH free reducing end form, followed by sample loading onto a matrix and on-matrix N-glycan labeling through reductive amination reaction with 2-AB (Figure 2). The process of on-matrix labeling eliminates the need for sample dry down.

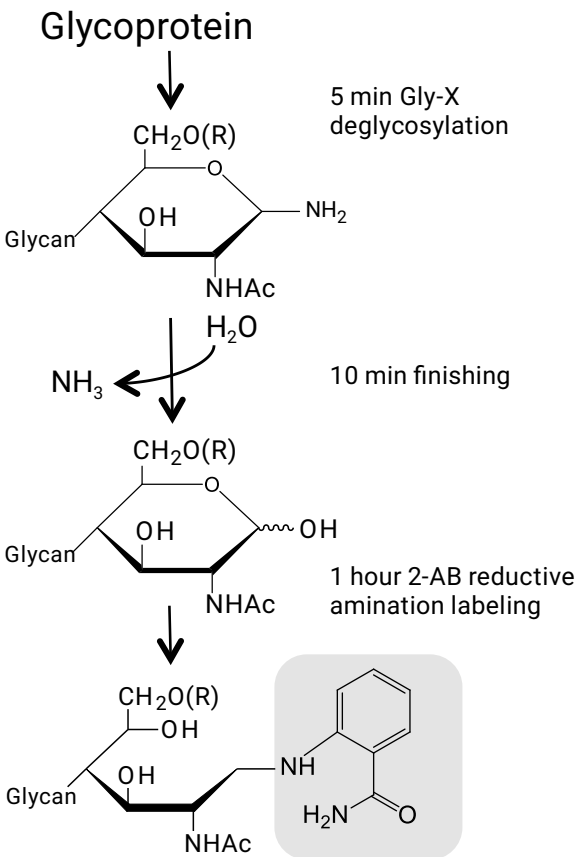

Figure 2: 2-AB Express labeling of N-glycans

Cleanup of 2-AB Express Labeled N-Glycans

After labeling, 2-AB labeled glycans are washed of free dye while bound to the matrix and eluted by changing polarity of the elution solvent.

A 96-well vacuum filtration manifold is used for all steps as follows:

- 3x wash with acetonitrile
- Elute with DI water

Gly-X 2-AB Express cleanup preserves more than 80-90% of labeled glycans (data not shown), while assuring no bias for individual glycans (Figure 3). The cleanup also preserves sialylated glycan species as shown in Figure 4.

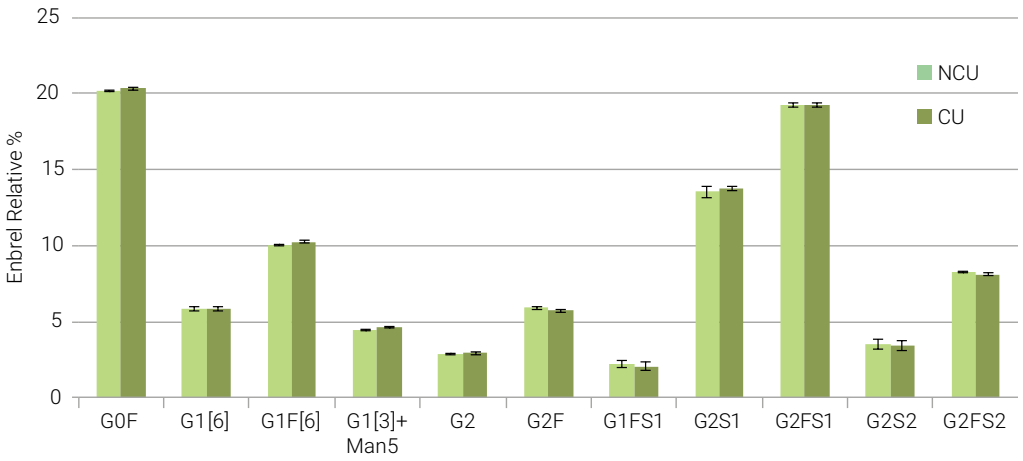

Figure 3: Glycan recovery. Enbrel N-glycans were measured by UPLC with (■) and without (■) cleanup. Data show no bias in relative % area of individual glycan species; N = 8.

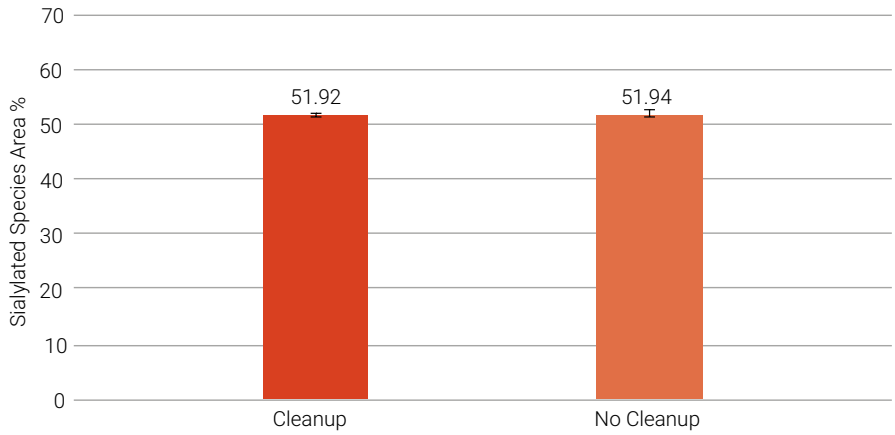

Figure 4: Preservation of sialylated species. Enbrel N-glycans were measured by UPLC and total sialylated species area was compared with (■) and without (■) cleanup. Graph shows no loss of sialylated glycans.

Analysis

HILIC-FLD Profiles of 2-AB Express Labeled N-Glycans

HILIC elution profile for Enbrel 2-AB N-glycans is shown in Figure 5. The elution order is similar to other glycan labels such as InstantAB and InstantPC (data not shown).

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 (data not shown).

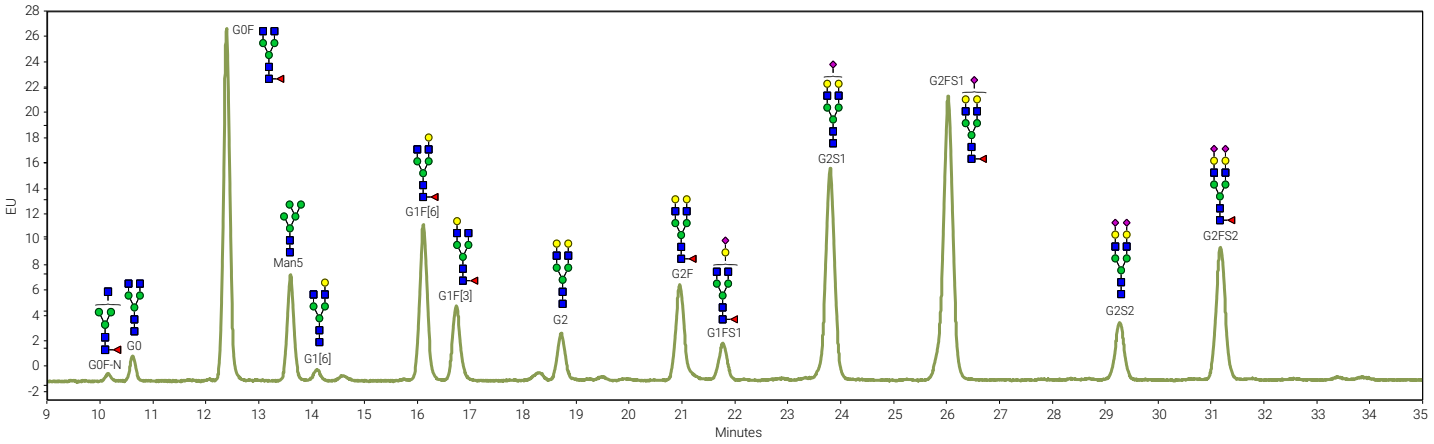

Figure 5: HILIC-FLD profile of Enbrel 2-AB labeled N-glycans utilizing the Agilent AdvanceBio Gly-X with 2-AB Express Labeling Kit. A 60-minute gradient.

Specifications	Gly-X with 2-AB Express
Protein load range	1–40 µg (or more, depending on protein glycosylation)
Deglycosylation efficiency	>99% for most proteins
Glycan recovery	>80%
Free dye removal	>99%
Preservation of sialylated species	>99%
Precision (well-to-well, day-to-day)	<5% CV or relative % peak area of major glycans
Total workflow time	130 min (24 samples)
Sample stability	At least 6 months at -20 °C, 5 days at 4–8 °C

Ordering Information

Description	Part Number
Gly-X with 2-AB Express Kit (96 ct)	GX96-2AB
Gly-X with 2-AB Express Kit (24 ct)	GX24-2AB
Gly-X with 2-AB Express Deglycosylation and Labeling Module Set (96 ct)	GX96-401AB
Gly-X with 2-AB Express Deglycosylation and Labeling Module Set (24 ct)	GX24-401AB
Gly-X 2-AB Express Labeling Module (96 ct)	GX96-401
Gly-X 2-AB Express Labeling Module (24 ct)	GX24-401
Gly-X Cleanup Module for 2-AB Express (96 ct)	GX96-402
Gly-X 2-AB Express Starter Kit (Gly-X Vacuum Manifold Spacer, and 2 heater block lids)	GX400
AssayMAP PA50 protein A affinity purification kit (96 ct)	G5524-60010 KIT

2-AB Labeled N-Glycan Libraries	Part Number
Human IgG N-Linked Glycan Library	GKSB-005
Biantennary and High Mannose Partitioned Library	GKSB-520
Human α1-acid glycoprotein N-Linked Glycan Library	GKSB-001
Bovine Fetuin N-linked Glycan Library	GKSB-002
Glucose Homopolymer Standard	GKSB-503
α(2-3) Sialylated Biantennary Library	GKSB-232
α(2-6) Sialylated Biantennary Library	GKSB-262
α(2-3) Sialylated Triantennary Library	GKSB-233
α(2-6) Sialylated Triantennary Library	GKSB-263
α(2-3) Sialylated Tetraantennary Library	GKSB-234
α(2-6) Sialylated Tetraantennary Library	GKSB-264

Ordering Information

2-AB Labeled Individual Glycan Standards	Part Number
G0-N	GKSB-401
G0	GKSB-301
G0F-N	GKSB-402
G0F	GKSB-302
G0FB	GKSB-303
G1	GKSB-317
G1F	GKSB-316
G2	GKSB-304
G2F	GKSB-305
G2FB	GKSB-306
G2F w/1 α-gal	GKSB-318
G2S1 α(2,6)	GKSB-311
G2FS1 α(2,6)	GKSB-315
G2S2 α(2,6)	GKSB-312
G2FS2 α(2,6)	GKSB-313
A3	GKSB-307
G3	GKSB-308
G3S3 α(2,6)	GKSB-314

2-AB Labeled Individual Glycan Standards	Part Number
A4	GKSB-309
G4	GKSB-310
HYBR	GKSB-111
NN	GKSB-100
Man3	GKSB-101
Man3F	GKSB-102
Man5	GKSB-103
Man6	GKSB-104
Man7	GKSB-105
Man8	GKSB-106
Man9	GKSB-107
GalGalNAc	GKSB-201
3'-SLN	GKSB-203
6'-SLN	GKSB-204

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