Application Note

Pharma and Biopharma

Glycan Peak Assignment Tool (GPAT)

Novel glucose unit-based software enabling more accurate peak assignments for InstantPC and 2-AB N-glycan HILIC/FLD analysis using the AdvanceBio Amide HILIC column and mobile phase

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Abstract

Glycan separation by hydrophilic interaction liquid chromatography (HILIC) coupled with fluorescence detection (FLD) is a popular and robust technique for the analysis of released and labeled N-glycans. However, retention time (RT) alone is often insufficient to confidently match the numerous possible glycan structures to the resulting chromatographic peaks. In such cases, it is possible to predict peak identities based on a library of glucose unit (GU)-normalized glycan retention times. For this purpose, the Glycan Peak Assignment Tool (GPAT) was developed. This tool contains libraries of GU values for over 100 InstantPC- and 2-AB-labeled N-glycans separated using the AdvanceBio Amide HILIC column and mobile phase. The tool is web-based, free of charge, and getting started requires only the input of retention times (RTs) acquired with an InstantPC or 2-AB-labeled ladder standard. Using the RTs from the ladder standard, the tool generates a list of predicted N-glycan RTs using the libraries, avoiding the need to input potentially sensitive sample information. In this note, we used the tool to annotate glycans present in the therapeutic antibody cetuximab and have characterized the prediction accuracy of GPAT on a variety of glycan structures. While the tool primarily relies on GU values and can be used effectively with a GU ladder calibration, it also features a novel refinement option. This option allows users to improve the tool RT predictions by providing the RTs of two known glycan structures. In both InstantPC and 2-AB glycan samples, this refinement feature enabled the tool to predict RTs for a variety of glycan structures with a sub-2% prediction error.

Introduction

N-glycosylation is a common post-translational modification that can impact the efficacy, stability, and safety of protein therapeutics.[1] Therefore, the N-glycan profiles of therapeutic antibodies are frequently examined as a critical quality attribute.[2] The most common approach to routine glycan profiling is to use HILIC coupled with fluorescence detection to analyze enzymatically released and labeled N-glycans. While this instrumental approach is relatively affordable and provides reliable quantitation, assigning glycan identities to peaks based on retention time alone can be challenging, particularly when analyzing unfamiliar samples.

Numerous techniques can be used to collect additional or orthogonal data to aid in deducing chromatographic peak identities, such as exoglycosidase digestions and/or mass spectrometry. Due to the variety of potential branching patterns and the existence of structural isomers, it is often necessary to combine data from multiple sources to verify or deduce the glycan structure associated with a particular peak. However, these approaches can add considerable time and cost to the analytical workflow.

An alternative approach to assigning peak identities is to reference a library of glycan GU values to annotate peaks in a fluorescence chromatogram. The approach of normalizing glycan retention times to a glucose unit scale has been used for several decades.[3,4,5] This approach aids in mitigating the effects of retention time shift, which can complicate chromatographic peak annotation. The relationship between GU and RT for a particular liquid chromatography system (LC) and HILIC column on a particular day can be established through the analysis of a glucose homopolymer. This relationship then allows previously measured GU values to be used for glycan RT prediction of runs on the same day.

To facilitate glycan peak assignments in HILIC data, a novel web-based GPAT was developed. This tool contains a library of GU values for over 100 common glycans with both the InstantPC (IPC) and 2-AB labels. By entering retention times collected from the analysis of InstantPC maltodextrin or 2-AB glucose homopolymer, users can generate a list of predicted glycan retention times to aid with peak assignments on their LC system (Figure 1). Since these retention time predictions are based on the analysis of a dextran ladder, there is no need for users to share potentially confidential sample information while using the tool. The tool uses a novel and optional refinement feature, which incorporates retention times from one neutral and one double-acidic glycan (also known as an "S2" glycan due to the presence of two nonreducing terminal sialic acid residues). This feature further improves the RT prediction accuracy. The tool is designed for use with data collected on the 2.1 × 150 mm version of the Agilent AdvanceBio Amide HILIC column[6] and is freely available through the Agilent website at the following link: www.agilent.com/biopharma/gpat.

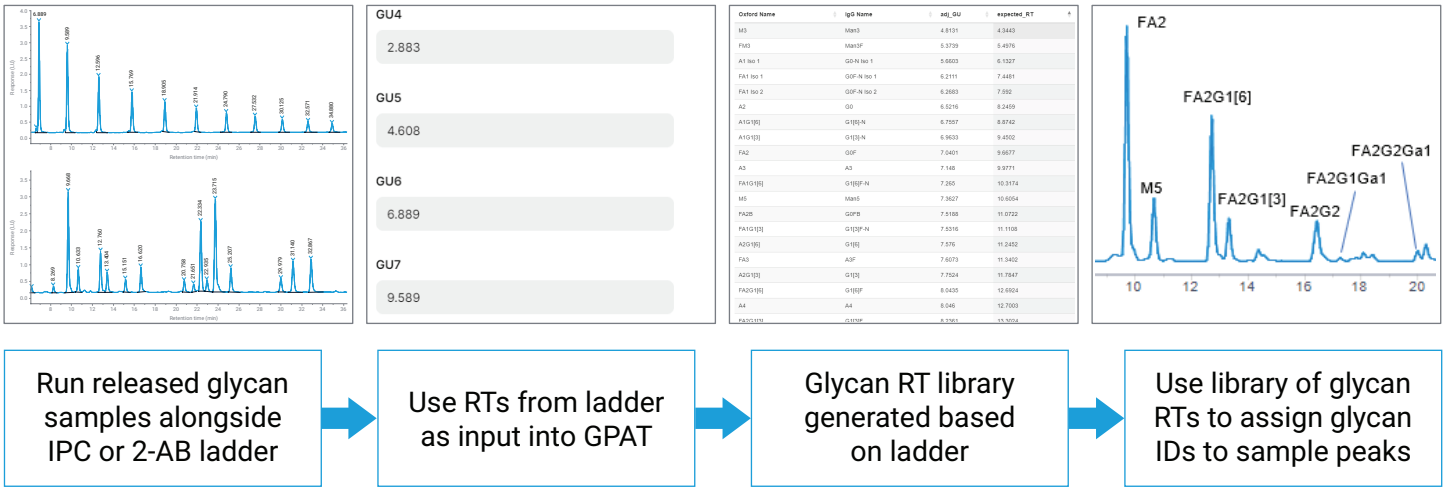

Figure 1. Peak assignment workflow using the GPAT.

Experimental

N-Glycan sample preparation

Released N-glycan samples were prepared from the glycoprotein cetuximab (Erbitux, ImClone lot number IMF423) following the procedures given in the Agilent Gly-X InstantPC and 2-AB kits (part numbers GX96-IPC and GX96-2AB, respectively). An InstantPC N-glycan standard mix was prepared from the protein etanercept (Enbrel, Amgen lot number 1081237) as well as with part numbers GKPC-264, GKPC-321, and GKPC-325. A 2-AB N-glycan standard mix was prepared with part numbers GKSB-020 and GKSB-233. One vial each of InstantPC maltodextrin (part number GKPC-503) and 2-AB glucose homopolymer (part number GKSB-503) were dissolved in 30 µL 50 mM ammonium formate, pH 4.4, and used for instrument calibration as described in Table 1.

Instrumentation and sample analysis

Sample analysis was performed on an Agilent 1290 Infinity II LC system consisting of the following modules:

- Agilent 1290 Infinity II Bio multisampler (G7137A)
- Agilent 1290 Infinity II Bio high-speed pump (G7132A)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent 1260 fluorescence detector (G7121A)

Four chromatographic gradients were developed as part of the GPAT workflow. For each glycan label (InstantPC or 2-AB), a shorter gradient was designed for samples containing neutral, S1, and S2 glycans. An extended gradient was designed for samples containing neutral through S4 glycans (Tables 2 through 5).

Table 1. LC/FLD conditions for released glycan analysis

Parameter	Value
Column	Agilent AdvanceBio Amide HILIC, 2.1 × 150 mm, 1.8 µm (p/n 859750-913)
Column Temperature	60 °C
Mobile Phases	A) 50 mM ammonium formate, pH 4.4, prepared from p/n G3912-00000 B) Acetonitrile
Flow Rate	0.6 mL/min
Ladder Standards	AdvanceBio InstantPC Maltodextrin ladder (p/n GKPC-503) or AdvanceBio 2-AB Glucose homopolymer standard (p/n GKSB-503)
Injection Volume	0.5 µL for InstantPC maltodextrin; 1 µL for all other samples
Detection	InstantPC: λ_{Ex} 285 nm, λ_{Em} 345 nm 2-AB: λ_{Ex} 260 nm, λ_{Em} 430 nm

Table 2. LC gradient for InstantPC glycan samples containing neutral, S1, and S2 glycans.

Time (min)	%B
0	77
45	59
46	40
47	40
49	77
60	77

Table 3. LC gradient for InstantPC glycan samples containing neutral through S4 glycans.

Time (min)	%B
0	77
75	47
76	40
77	40
79	77
90	77

Table 4. LC gradient for 2-AB glycan samples containing neutral, S1, and S2 glycans.

Time (min)	%B
0	74
50	54
51	40
52	40
54	74
64	74

Table 5. LC gradient for 2-AB glycan samples containing neutral through S4 glycans.

Time (min)	%B
0	74
75	44
76	40
77	40
79	74
90	74

A sample sequence was prepared in Agilent OpenLab CDS with two initial blank runs followed by two replicate injections of InstantPC maltodextrin to verify system stability before running samples (Figure 2).

Software

The software used in this study was as follows:

- Agilent OpenLab CDS, version 2.7
- Agilent GPAT (www.agilent.com/biopharma/gpat)

Data processing in OpenLab CDS:
The chromatographic peaks were integrated automatically using the following ChemStation integration parameters. The integration of the maltodextrin and glucose homopolymer peaks was verified and any remaining peaks between GU4 and GU20 were integrated manually.

- Slope sensitivity: 0.500
- Peak width: 0.05
- Area reject: 1.00
- Height reject: 0.10
- Shoulder mode: Off
- Area% reject: 0.00

		Action	Sample name	Acq. method	Data file	Vial	Sample type
1	☑	Inject	Blank 1	Gradient 1 InstantPC.amx	Blank 1	D1F-A1	Sample
2	☑	Inject	Blank 2	Gradient 1 InstantPC.amx	Blank 2	D1F-A1	Sample
3	☑	Inject	InstantPC maltodextrin 1	Gradient 1 InstantPC.amx	InstantPC maltodextrin 1	D1F-A2	Sample
4	☑	Inject	InstantPC maltodextrin 2	Gradient 1 InstantPC.amx	InstantPC maltodextrin 2	D1F-A2	Sample
5	☑	Inject	InstantPC cetuximab	Gradient 1 InstantPC.amx	InstantPC cetuximab	D1F-A3	Sample
6	☑	Inject	InstantPC standard mix	Gradient 2 InstantPC.amx	InstantPC standard mix	D1F-B1	Sample
7	☑	Inject	Blank 3	Gradient 3 2-AB.amx	Blank 3	D1F-A1	Sample
8	☑	Inject	2-AB GHP 1	Gradient 3 2-AB.amx	2-AB GHP 1	D1F-A5	Sample
9	☑	Inject	2-AB GHP 2	Gradient 3 2-AB.amx	2-AB GHP 2	D1F-A5	Sample
10	☑	Inject	2-AB cetuximab	Gradient 3 2-AB.amx	2-AB cetuximab	D1F-A6	Sample
11	☑	Inject	2-AB standard mix	Gradient 4 2-AB.amx	2-AB standard mix	D1F-B3	Sample
12	☑	Inject	2-AB G2FS(6)2	Gradient 3 2-AB.amx	2-AB G2FS(6)2	D1F-B4	Sample

Figure 2. Example sequence used for data collection in OpenLab CDS.

Data entry into the GPAT: The GPAT supports three data entry options. In this example, data were entered through the manual data entry page, which contains text boxes for the retention time of each GU peak from GU4 to GU20 (Figure 3). If desired, data can also be uploaded as a .csv file or pasted as a comma-separated list on the corresponding data entry pages.

After selecting the appropriate label (InstantPC or 2-AB), retention times for two marker glycans were entered to refine the accuracy of the tool's retention time prediction (Figure 4). This step is optional but recommended. In this case, the glycans F(6)A2/G0F and A2G2S(3)2/G2S(3)2 were used for the InstantPC refinement since they were present in the samples. If needed, these compounds can be purchased as standards and injected separately. For the 2-AB data, the glycans F(6)A2/G0F and F(6)A2G2S(6)2/G2FS(6)2 were used as refinement values. Since F(6)A2G2S(6)2 was not present in either 2-AB sample, it was prepared as an individual standard (part number GKSB-313) and run as a separate injection.

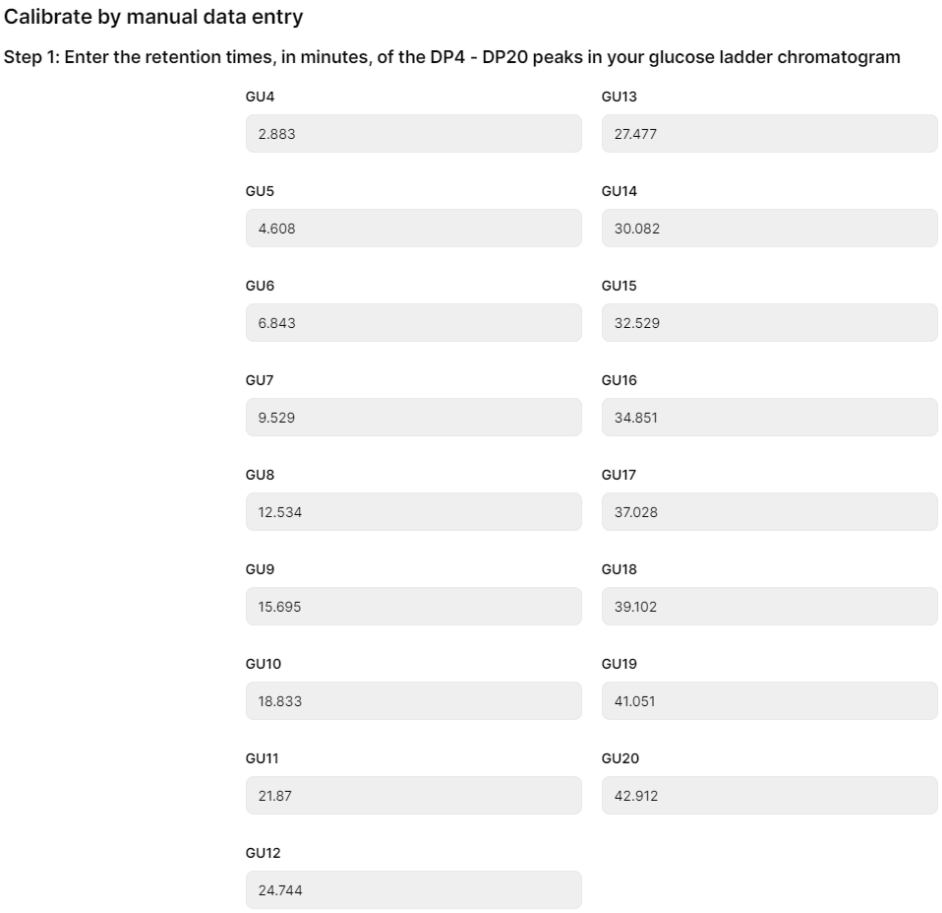

Figure 3. InstantPC maltodextrin ladder retention time entry into the GPAT.

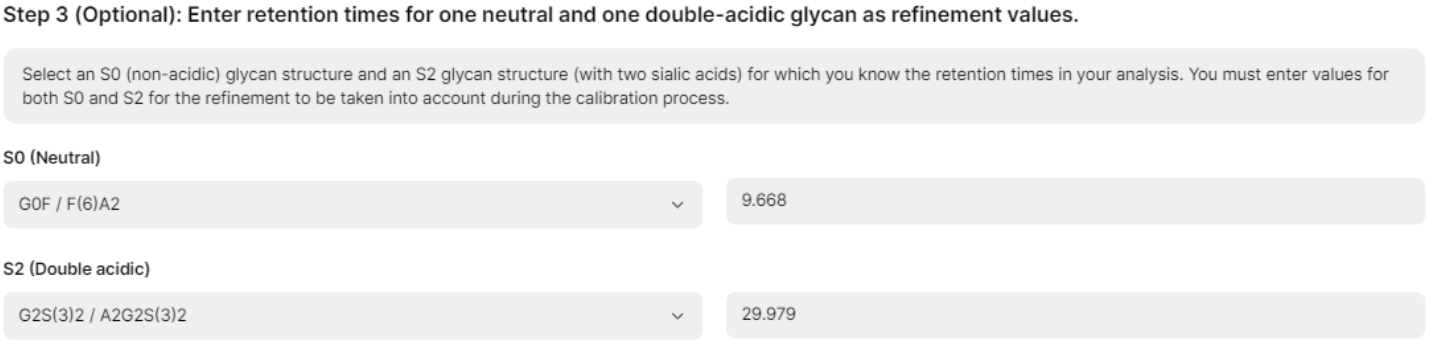

Figure 4. Entry of one neutral and one acidic glycan retention time to enable the GPAT optional refinement feature.

Results and discussion

Using the retention time predictions of the GPAT, a total of 13 glycan identities were assigned in the InstantPC cetuximab sample and nine structures were assigned in the 2-AB cetuximab sample. The InstantPC and 2-AB workflows provided comparable chromatographic results. However, the stronger signal intensity provided by the InstantPC label resulted in more low-abundance glycan structures being visible in the InstantPC chromatogram (Figures 5 and 6).

For maximum flexibility and convenience, the tool is designed to work with any of the four gradients shown in Tables 2 through 5. For each glycan label, a shorter gradient is available for analyzing the maltodextrin or glucose homopolymer calibrant sample as well as samples containing neutral, S1, and S2 glycans. Since glycans with three and four sialic acid residues typically elute at much later retention times, longer gradients are available to analyze samples containing these glycans.

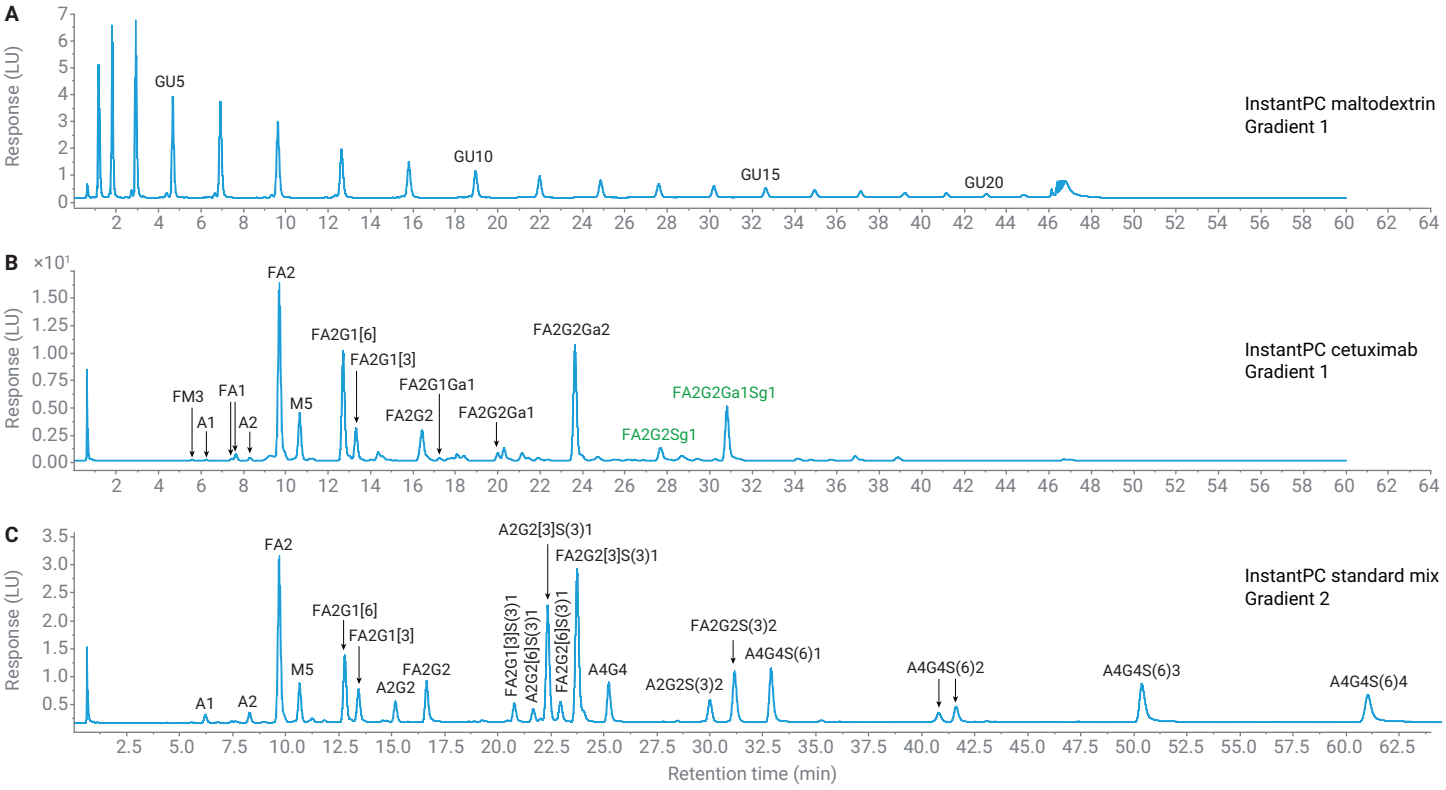

Figure 5. InstantPC chromatographic data used for retention time prediction in the GPAT. Compound names in green represent glycans which are not present in the tool's library and were assigned by alternate methods.

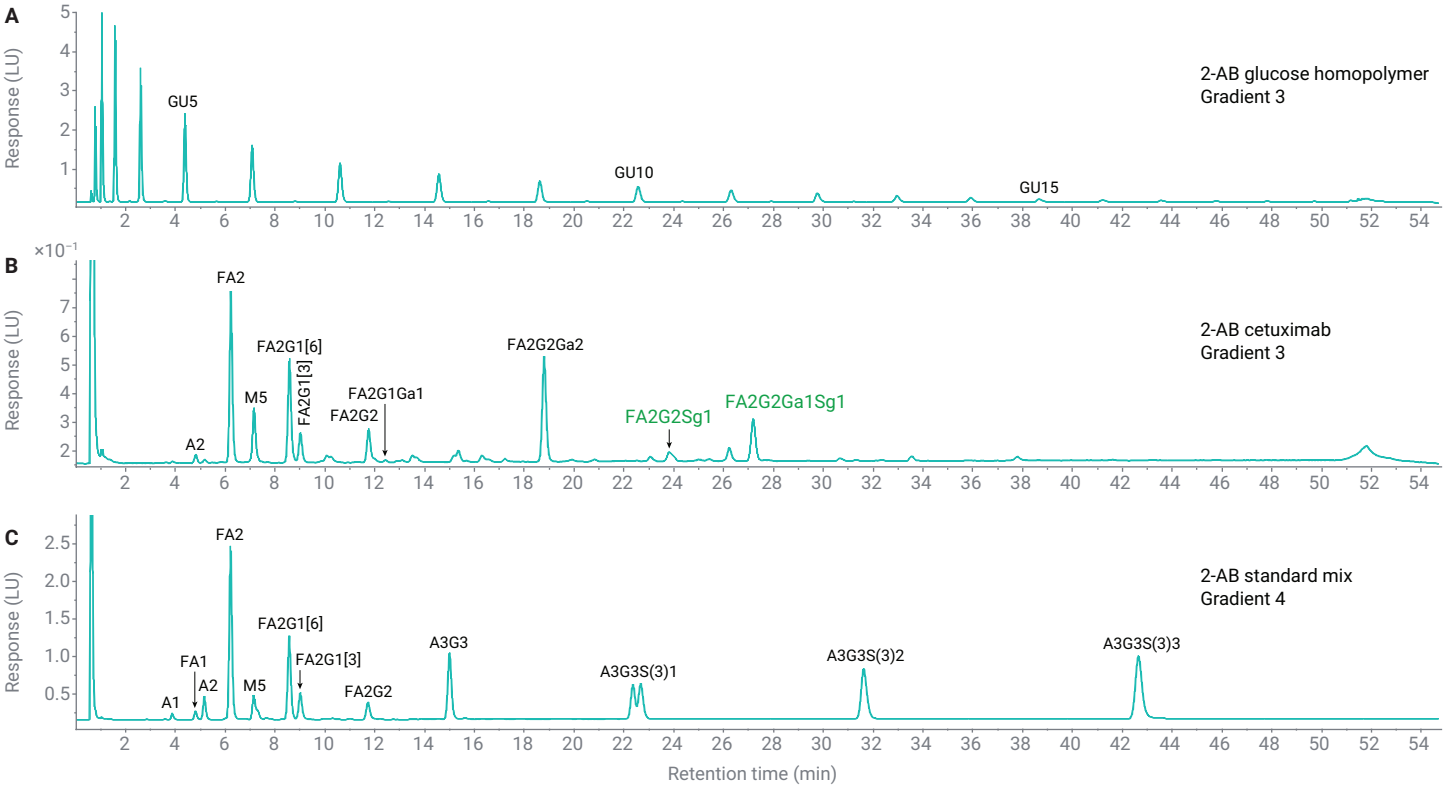

Figure 6. 2-AB chromatographic data used for retention time prediction in the GPAT. Compound names in green represent glycans which are not present in the tool's library and were assigned by alternate methods.

To characterize the prediction accuracy of the tool, the actual retention times of known glycans in the samples were compared with the retention times predicted by the tool, both with and without the use of refinement values. The resulting prediction accuracy values are shown in Tables 6 through 9. The refinement value option allows users to enter an RT for one neutral and one acidic glycan, if desired, to fine-tune the accuracy of RT predictions. This adjustment is helpful in minimizing the prediction error for S2, S3, and S4 glycans, which elute at late RTs and are more heavily influenced by column age and mobile phase concentration effects. For example, Table 6 demonstrates that the prediction error for the InstantPC glycans FA2G2S(3)2 and A4G4S(6)2 Isomer 1 were each reduced by more than 1% upon implementing the adjustment value feature. Also, several other acidic glycan prediction errors

Table 6. RT prediction accuracy of the InstantPC glycan standard mix.

Glycan	Actual RT (min)	Predicted RT without Refinement (min)	Predicted RT with Refinement (min)	% Error without Refinement	% Error with Refinement
A1	6.193	6.181	6.133	−0.19%	−0.97%
A2	8.269	8.302	8.246	0.40%	−0.28%
FA2	9.668	9.728	9.668	0.62%	0.00%
M5	10.663	10.669	10.605	0.05%	−0.54%
FA2G1[6]	12.760	12.760	12.692	0.00%	−0.53%
FA2G1[3]	13.404	13.370	13.302	−0.25%	−0.76%
A2G2	15.151	15.013	14.945	−0.91%	−1.36%
FA2G2	16.620	16.493	16.425	−0.76%	−1.17%
FA2G1[3]S(3)1	20.758	20.936	20.606	0.86%	−0.73%
A2G2[6]S(3)1	21.651	21.833	21.509	0.84%	−0.65%
A2G2[3]S(3)1	22.334	22.536	22.217	0.91%	−0.52%
FA2G2[6]S(3)1	22.935	23.152	22.836	0.94%	−0.43%
FA2G2[3]S(3)1	23.715	23.945	23.635	0.97%	−0.34%
A4G4	25.207	25.259	25.200	0.20%	−0.03%
A2G2S(3)2	29.979	30.523	29.979	1.81%	0.00%
FA2G2S(3)2	31.140	31.699	31.168	1.80%	0.09%
A4G4S(6)1	32.867	33.138	32.881	0.82%	0.04%
A4G4S(6)2 Iso 1	40.772	41.346	40.933	1.41%	0.40%
A4G4S(6)2 Iso 2	41.590	42.184	41.785	1.43%	0.47%
A4G4S(6)3	50.354	51.319	50.843	1.92%	0.97%
A4G4S(6)4	61.013	62.547	62.081	2.51%	1.75%

were reduced by more than 0.5%. No InstantPC-labeled glycan predicted RT differed from the actual RT by more than 2% when using the refinement feature, and the majority of glycans differed by less than 1%. The RT prediction accuracy for 2-AB-labeled glycans was similarly improved by the refinement feature.

By characterizing the accuracy that is possible on a particular LC system, users can reasonably infer a retention time window around a sample peak for which candidate matches from the GPAT should be considered. This approach is useful when deducing identities for peaks that appear to have numerous potential matches in the tool output. For example, the average prediction error after refinement for the standard mix described in Table 6 is 0.57%, with most prediction errors below 1.5%. For a peak in an InstantPC chromatogram with unknown identity, it is logical to therefore consider any glycan whose predicted RT is within 1 to 2% of the peak RT as a potential match. Then, putative matches with an RT that differs by > 2% can be eliminated.

Table 7. RT prediction accuracy of InstantPC-labeled cetuximab N-glycans.

Glycan	Actual RT (min)	Predicted RT without Refinement (min)	Predicted RT with Refinement (min)	% Error without Refinement	% Error with Refinement
FM3	5.545	5.544	5.498	-0.03%	-0.85%
A1	6.226	6.181	6.133	-0.72%	-1.50%
FA1 Iso 1	7.436	7.502	7.448	0.88%	0.16%
FA1 Iso 2	7.620	7.646	7.592	0.34%	-0.37%
A2	8.281	8.302	8.246	0.25%	-0.42%
FA2	9.674	9.728	9.668	0.56%	-0.07%
M5	10.635	10.669	10.605	0.32%	-0.28%
FA2G1[6]	12.682	12.760	12.692	0.61%	0.08%
FA2G1[3]	13.288	13.370	13.302	0.62%	0.11%
FA2G2	16.409	16.493	16.425	0.51%	0.10%
FA2G1Ga1 Iso 1	17.246	17.354	17.287	0.63%	0.24%
FA2G2Ga1 Iso 1	19.975	20.057	19.991	0.41%	0.08%
FA2G2Ga2	23.614	23.741	23.680	0.54%	0.28%

Table 8. RT prediction accuracy of the 2-AB glycan standard mix.

Glycan	Actual RT (min)	Predicted RT without Refinement (min)	Predicted RT with Refinement (min)	% Error without Refinement	% Error with Refinement
A1	3.852	3.878	3.856	0.66%	0.10%
FA1	4.782	4.769	4.746	-0.27%	-0.76%
A2	5.139	5.154	5.130	0.30%	-0.18%
FA2	6.194	6.220	6.193	0.42%	-0.02%
M5	7.125	7.127	7.098	0.03%	-0.38%
FA2G1[6]	8.546	8.592	8.559	0.53%	0.15%
FA2G1[3]	8.990	9.042	9.008	0.58%	0.20%
FA2G2	11.705	11.820	11.780	0.98%	0.64%
A3G3	14.981	15.176	15.133	1.30%	1.02%
A3G3S(3)1 Iso 1	22.340	22.756	22.261	1.86%	-0.35%
A3G3S(3)1 Iso 2	22.652	23.073	22.583	1.86%	-0.31%
A3G3S(3)2	31.602	32.364	31.580	2.41%	-0.07%
A3G3S(3)3	42.631	43.791	42.904	2.72%	0.64%

Table 9. RT prediction accuracy of 2-AB-labeled cetuximab N-glycans.

Glycan	Actual RT (min)	Predicted RT without Refinement (min)	Predicted RT with Refinement (min)	% Error without Refinement	% Error with Refinement
FA2	6.208	6.220	6.193	0.19%	-0.24%
M5	7.137	7.127	7.098	-0.14%	-0.55%
FA2G1[6]	8.555	8.592	8.559	0.43%	0.04%
FA2G1[3]	8.995	9.042	9.008	0.52%	0.14%
FA2G2	11.732	11.820	11.780	0.75%	0.41%
FA2G1Ga1	12.411	12.550	12.510	1.12%	0.80%
FA2G2Ga2	18.775	18.885	18.843	0.59%	0.36%

Figure 7 shows examples of how this approach can be implemented with the peaks representing InstantPC-labeled Man5 and FA2G1[3]S(3)1. In this example, only one glycan is predicted to elute within a 2% window around the InstantPC Man5 peak, making the peak assignment straightforward. However, for InstantPC FA2G1[3]S(3)1, three structures are predicted to elute within 2% of the measured RT. If multiple candidate glycans still match closely with an unknown peak, it may be necessary to rely on prior knowledge or data generated by orthogonal analytical methods, such as mass spectrometry data, to unambiguously assign peak identities. If needed, analytical N-glycan standards labeled with InstantPC or 2-AB can be purchased and run side by side with a glycan sample to verify peak identities. These standards are available from Agilent Technologies (Glycan Standards Flyer).

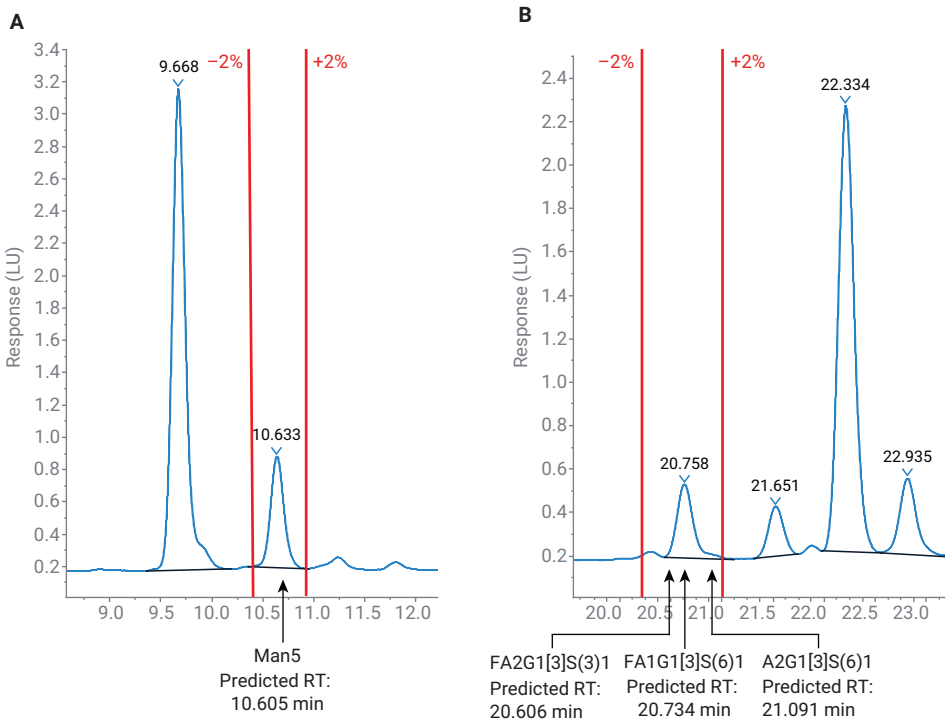

Figure 7. Examples of retention time windows for which putative peak identities generated by the GPAT should be considered. The InstantPC glycan peak at 10.663 minutes only has one match within a 2% tolerance window based on the GPAT output (A). The peak at 20.758 minutes has three matches within the same relative tolerance window, reflecting the greater number of glycans with similar GU values in this region (B).

Conclusion

A GU database containing over 100 InstantPC and 2-AB-labeled glycans was generated and made freely available through the GPAT. The tool uses nonconfidential data to calibrate an LC system for glycan retention time predictions on the AdvanceBio Amide HILIC column and aids in determining peak identities in fluorescence chromatograms. The GPAT uses a unique adjustment value feature to improve the accuracy of retention time predictions, thereby decreasing the number of candidate structures for a chromatographic peak. The tool is freely accessible through the Agilent website. Complete lists of the glycans contained in the InstantPC and 2-AB libraries and their corresponding GU values are provided in Appendix Tables 10 and 11.

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Appendix

Table 10. InstantPC GU library.

Oxford Name	IgG Name	GU Value
M3	Man3	4.83
FM3	Man3F	5.40
A1 Iso 1	G0-N Iso 1	5.68
FA1 Iso 1	G0F-N Iso 1	6.23
FA1 Iso 2	G0F-N Iso 2	6.29
A2	G0	6.54
A1G1[6]	G1[6]-N	6.78
A1G1[3]	G1[3]-N	6.98
FA2	G0F	7.06
A3	A3	7.17
FA1G1[6]	G1[6]F-N	7.29
M5	Man5	7.38
FA2B	G0FB	7.54
FA1G1[3]	G1[3]F-N	7.55
A2G1[6]	G1[6]	7.60
FA3	A3F	7.63
A2G1[3]	G1[3]	7.77
FA2G1[6]	G1[6]F	8.06
A4	A4	8.07
FA2G1[3]	G1[3]F	8.26
FA2BG1[6]	G1[6]FB	8.42
FA4	A4F	8.46
FA2BG1[3]	G1[3]FB	8.59
M6	Man6	8.63
M5A1B	H5N4 hybrid bisect	8.73
A2G2	G2	8.77
A1G1[6]S(3)1	G1[6]S(3)1-N	9.01
FA2G2	G2F	9.23
A1G1[3]S(3)1	G1[3]S(3)1-N	9.44
FA2BG2	G2FB	9.45
FA1G1[6]S(3)1	G1[6]FS(3)1-N	9.48
FA2G1Ga1	G1FGa1 Iso 1	9.50
A1G1[6]S(6)1	G1[6]S(6)1-N	9.65
M7D2	Man7D2	9.66
A2G1[6]S(3)1	G1[6]S(3)1	9.75
M7D3	Man7D3	9.83
M7D1	Man7D1	9.83
FA1G1[3]S(3)1	G1[3]FS(3)1-N	10.01
A1G1[3]S(6)1	G1[3]S(6)1-N	10.10
A2G1[3]S(3)1	G1[3]S(3)1	10.14
FA1G1[6]S(6)1	G1[6]FS(6)1-N	10.18
FA2G1[6]S(3)1	G1[6]FS(3)1	10.20
FA2G2Ga1 Iso 1	G2FGa1 Iso 1	10.36
A2G1[6]S(6)1	G1[6]S(6)1	10.38
A3G3	G3	10.39
FA2G1[3]S(3)1	G1[3]FS(3)1	10.65
FA1G1[3]S(6)1	G1[3]FS(6)1-N	10.69
FA3G3	G3F	10.78
A2G1[3]S(6)1	G1[3]S(6)1	10.81
M8D2D3	Man8D2D3	10.81
M8D1D2	Man8D1D2	10.87
FA2G1[6]S(6)1	G1[6]FS(6)1	10.89
A2G2[6]S(3)1	G2[6]S(3)1	10.95
M8D1D3	Man8D1D3	11.04
A2G2[3]S(3)1	G2[3]S(3)1	11.18
FA2G1[3]S(6)1	G1[3]FS(6)1	11.33
FA2G2[6]S(3)1	G2[6]FS(3)1	11.40
A2G2[6]S(6)1	G2[6]S(6)1	11.59
FA2G2Ga2	G2FGa2	11.60
FA2G2[3]S(3)1	G2[3]FS(3)1	11.67
A2G2[3]S(6)1	G2[3]S(6)1	11.88
M9	Man9	12.03
FA2G2[6]S(6)1	G2[6]FS(6)1	12.11
A4G4	G4	12.14
FA2G2[3]S(6)1	G2[3]FS(6)1	12.39
FA4G4	G4F	12.46
A3G3S(3)1 Iso 1	G3S(3)1 Iso 1	12.61
A3G3S(3)1 Iso 2	G3S(3)1 Iso 2	12.79
FA2BG2[3]S(6)1	G2[3]FBS(6)1	12.79
FA2G2Ga1S(3)1 Iso 1	G2FGa1S(3)1 Iso 1	12.90
FA3G3S(3)1 Iso 1	G3FS(3)1 Iso 1	13.18
A3G3S(6)1 Iso 1	G3S(6)1 Iso 1	13.29
A3G3S(6)1 Iso 2	G3S(6)1 Iso 2	13.43
FA2G2Ga1S(6)1 Iso 1	G2FGa1S(6)1 Iso 1	13.63
A2G2S(3)2	G2S(3)2	14.16
A4G4S(3)1 Iso 1	G4S(3)1 Iso 1	14.35
A4G4S(3)1 Iso 2	G4S(3)1 Iso 2	14.60
FA2G2S(3)2	G2FS(3)2	14.64
FA4G4S(3)1 Iso 1	G4FS(3)1 Iso 1	14.89
A4G4S(6)1 Iso 1	G4S(6)1 Iso 1	15.24
FA4G4S(6)1 Iso 1	G4FS(6)1 Iso 1	15.56
A3G3S(3)2 Iso 1	G3S(3)2 Iso 1	15.72
A2G2S(6)2	G2S(6)2	15.79
A3G3S(3)2 Iso 2	G3S(3)2 Iso 2	15.99
FA3G3S(3)2 Iso 1	G3FS(3)2 Iso 1	16.09
FA2G2S(6)2	G2FS(6)2	16.34
FA3G3S(3)2 Iso 2	G3FS(3)2 Iso 2	16.39
FA2BG2S(6)2	G2FBS(6)2	16.47
A3G3S(6)2 Iso 1	G3S(6)2 Iso 1	17.31
A4G4S(3)2 Iso 1	G4S(3)2 Iso 1	17.50
A3G3S(6)2 Iso 2	G3S(6)2 Iso 2	17.62
A4G4S(3)2 Iso 2	G4S(3)2 Iso 2	17.74
FA4G4S(3)2 Iso 1	G4FS(3)2 Iso 1	17.77
FA3G3S(6)2 Iso 1	G3FS(6)2 Iso 1	17.78
FA4G4S(3)2 Iso 2	G4FS(3)2 Iso 2	18.05
A4G4S(6)2 Iso 1	G4S(6)2 Iso 1	19.10
FA4G4S(6)2 Iso 1	G4FS(6)2 Iso 1	19.45
A4G4S(6)2 Iso 2	G4S(6)2 Iso 2	19.55
FA4G4S(6)2 Iso 2	G4FS(6)2 Iso 2	19.78
A3G3S(3)3	G3S(3)3	20.17
FA3G3S(3)3	G3FS(3)3	20.56
A4G4S(3)3 Iso 1	G4S(3)3 Iso 1	21.68
FA4G4S(3)3 Iso 1	G4FS(3)3 Iso 1	21.89
A4G4S(3)3 Iso 2	G4S(3)3 Iso 2	21.95
FA4G4S(3)3 Iso 2	G4FS(3)3 Iso 2	22.21
A4G4S(3)3 Iso 3	G4S(3)3 Iso 3	22.23
FA4G4S(3)3 Iso 3	G4FS(3)3 Iso 3	22.48
A3G3S(6)3	G3S(6)3	23.32
FA3G3S(6)3	G3FS(6)3	23.72
A4G4S(6)3 Iso 1	G4S(6)3 Iso 1	25.13
FA4G4S(6)3 Iso 1	G4FS(6)3 Iso 1	25.34
A4G4S(3)4	G4S(3)4	28.70
FA4G4S(3)4	G4FS(3)4	28.94
A4G4S(6)4	G4S(6)4	34.21
FA4G4S(6)4	G4FS(6)4	34.24

Table 11. 2-AB GU library.

Oxford Name	IgG Name	GU Value
M3	Man3	4.22
FM3	Man3F	4.57
A1 Iso 1	G0-N Iso 1	4.71
FA1 Iso 1	G0F-N Iso 1	5.11
A2	G0	5.27
A1G1[6]	G1[6]-N	5.50
A1G1[3]	G1[3]-N	5.61
A2B	G0B	5.63
FA2	G0F	5.68
A3	A3	5.73
FA1G1[6]	G1[6]F-N	5.93
M5	Man5	6.01
FA2B	G0FB	6.05
FA1G1[3]	G1[3]F-N	6.06
A2G1[6]	G1[6]	6.07
FA3	A3F	6.13
A2G1[3]	G1[3]	6.19
A2BG1[6]	G1[6]B	6.37
A4	A4	6.44
FA2G1[6]	G1[6]F	6.48
FA2G1[3]	G1[3]F	6.62
FA2BG1[6]	G1[6]FB	6.75
FA4	A4F	6.80
FA2BG1[3]	G1[3]FB	6.87
M6	Man6	6.97
A2G2	G2	7.00
M5A1B	H5N4 hybrid bisect	7.03
A2BG2	G2B	7.18
FA2G2	G2F	7.38
FA2BG2	G2FB	7.53
FA2G1Ga1 Iso 1	G1FGa1 Iso 1	7.56
A1G1[6]S(3)1	G1[6]S(3)1-N	7.58
A1G1[3]S(3)1	G1[3]S(3)1-N	7.70
M7 D2	Man7 D2	7.74
M7 D3	Man7 D3	7.85

Oxford Name	IgG Name	GU Value
M7 D1	Man7 D1	7.88
FA1G1[6]S(3)1	G1[6]FS(3)1-N	7.92
A2G1[6]S(3)1	G1[6]S(3)1	8.08
A1G1[6]S(6)1	G1[6]S(6)1-N	8.08
FA1G1[3]S(3)1	G1[3]FS(3)1-N	8.12
A2G1[3]S(3)1	G1[3]S(3)1	8.18
A3G3	G3	8.19
A1G1[3]S(6)1	G1[3]S(6)1-N	8.20
FA2G2Ga1 Iso 1	G2FGa1 Iso 1	8.21
FA2G1[6]S(3)1	G1[6]FS(3)1	8.40
FA3G3	G3F	8.50
M8 D2D3	Man8 D2D3	8.54
A2G1[6]S(6)1	G1[6]S(6)1	8.55
FA2G1[3]S(3)1	G1[3]FS(3)1	8.56
FA1G1[6]S(6)1	G1[6]FS(6)1-N	8.58
M8 D1D2	Man8 D1D2	8.61
FA1G1[3]S(6)1	G1[3]FS(6)1-N	8.63
A2G1[3]S(6)1	G1[3]S(6)1	8.68
M8 D1D3	Man8 D1D3	8.72
A2G2[6]S(3)1	G2[6]S(3)1	8.92
A2G2[3]S(3)1	G2[3]S(3)1	8.92
FA2G1[6]S(6)1	G1[6]FS(6)1	8.92
FA2G2Ga2	G2FGa2	9.07
FA2G1[3]S(6)1	G1[3]FS(6)1	9.08
FA2G2S(3)1 Iso 1	G2FS(3)1 Iso 1	9.29
M9	Man9	9.40
A4G4	G4	9.42
A2G2[6]S(6)1	G2[6]S(6)1	9.43
A2G2[3]S(6)1	G2[3]S(6)1	9.43
FA4G4	G4F	9.69
FA2G2[3]S(6)1	G2[3]FS(6)1	9.83
A3G3S(3)1 Iso 1	G3S(3)1 Iso 1	10.03
A3G3S(3)1 Iso 2	G3S(3)1 Iso 2	10.11
FA2G2Ga1S(3)1	G2FGa1S(3)1	10.15
FA3G3S(3)1 Iso 1	G3FS(3)1 Iso 1	10.35

Oxford Name	IgG Name	GU Value
FA3G3S(3)1 Iso 2	G3FS(3)1 Iso 2	10.41
A3G3S(6)1 Iso 1	G3S(6)1 Iso 1	10.55
FA2G2Ga1S(6)1	G2FGa1S(6)1	10.74
A4G4S(3)1 Iso 1	G4S(3)1 Iso 1	11.28
A4G4S(3)1 Iso 2	G4S(3)1 Iso 2	11.43
FA4G4S(3)1 Iso 1	G4FS(3)1 Iso 1	11.57
A2G2S(3)2	G2S(3)2	11.61
FA4G4S(3)1 Iso 2	G4FS(3)1 Iso 2	11.71
A4G4S(6)1 Iso 1	G4S(6)1 Iso 1	11.89
FA2G2S(3)2	G2FS(3)2	11.97
FA4G4S(6)1 Iso 1	G4FS(6)1 Iso 1	12.18
A3G3S(3)2 Iso 1	G3S(3)2 Iso 1	12.83
A2G2S(6)2	G2S(6)2	13.02
FA3G3S(3)2 Iso 1	G3FS(3)2 Iso 1	13.16
FA2G2S(6)2	G2FS(6)2	13.46
A4G4S(3)2 Iso 1	G4S(3)2 Iso 1	14.16
A3G3S(6)2 Iso 1	G3S(6)2 Iso 1	14.24
FA4G4S(3)2 Iso 1	G4FS(3)2 Iso 1	14.45
FA3G3S(6)2 Iso 1	G3FS(6)2 Iso 1	14.55
A4G4S(6)2 Iso 1	G4S(6)2 Iso 1	15.75
FA4G4S(6)2 Iso 1	G4FS(6)2 Iso 1	15.80
FA4G4S(6)2 Iso 2	G4FS(6)2 Iso 2	15.97
A3G3S(3)3	G3S(3)3	17.11
FA3G3S(3)3	G3FS(3)3	17.38
A4G4S(3)3 Iso 1	G4S(3)3 Iso 1	18.55
FA4G4S(3)3 Iso 1	G4FS(3)3 Iso 1	18.71
FA4G4S(3)3 Iso 2	G4FS(3)3 Iso 2	18.79
A3G3S(6)3	G3S(6)3	20.39
FA3G3S(6)3	G3FS(6)3	20.56
A4G4S(6)3 Iso 1	G4S(6)3 Iso 1	21.66
FA4G4S(6)3 Iso 1	G4FS(6)3 Iso 1	21.77
A4G4S(3)4	G4S(3)4	26.34
FA4G4S(3)4	G4FS(3)4	26.36
FA4G4S(6)4	G4FS(6)4	32.14
A4G4S(6)4	G4S(6)4	32.39

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