

ADVANCE YOUR GLYCAN MAPPING GET FAST, ACCURATE RESULTS WITH OUR END-TO-END SOLUTION

The Measure of Confidence

New AdvanceBio Workflow: Glycan Mapping Columns, Sample Preparation, and Standards

In the race to discover the next promising biotherapeutic or develop a reliable biosimilar, you cannot compromise on analytical accuracy and efficiency. Reducing process development time, having the ability to make procedural changes quickly, and minimizing variability in your analyses are just a few of the challenges you face every day.

Achieve new levels of resolution for glycan mapping – and reduce the time it takes to get there – with **Agilent AdvanceBio glycan-mapping columns, sample preparation, and standards**. AdvanceBio glycan mapping provides an optimized workflow designed to deliver reproducibility in the analysis of 2-AB labeled glycans, for accurate identification and quantification.



The Agilent AdvanceBio glycan mapping solution is part of the Agilent AdvanceBio Family. The family of AdvanceBio products are designed to deliver consistent, exceptional performance for the complete characterization of proteins, antibodies, conjugates, new biological entities and biopharmaceuticals.

The science behind AdvanceBio helps to advance the accuracy and productivity that support faster analysis and efficiency in your lab.

End-to-end solution provides optimum workflow efficiency.

! Problem	✓ Solved with AdvanceBio glycan-mapping workflow
Multiple vendor sourcing	One part number contains everything needed to prepare 2-AB labeled glycans from glycoproteins High-resolution, high-speed performance Unique bonding uses a HILIC mechanism to support glycan separations
Instrument limitations	AdvanceBio glycan-mapping columns provide speed and resolution from 400 bar to 1200 bar: <ul style="list-style-type: none">• 1.8 μm formats for premium UHPLC to 1200 bar• 2.7 μm superficially porous particles: stable to 600 bar High resolution with lower backpressure Ideal for all HPLC instruments
Difficulty achieving reproducible glycan results	Standards for use as sample preparation controls and for column and system performance verification Batch tested with the Agilent IgG glycan standard to ensure reproducibility



Agilent Technologies

Greater accuracy and efficiency for complex biopharmaceutical separations

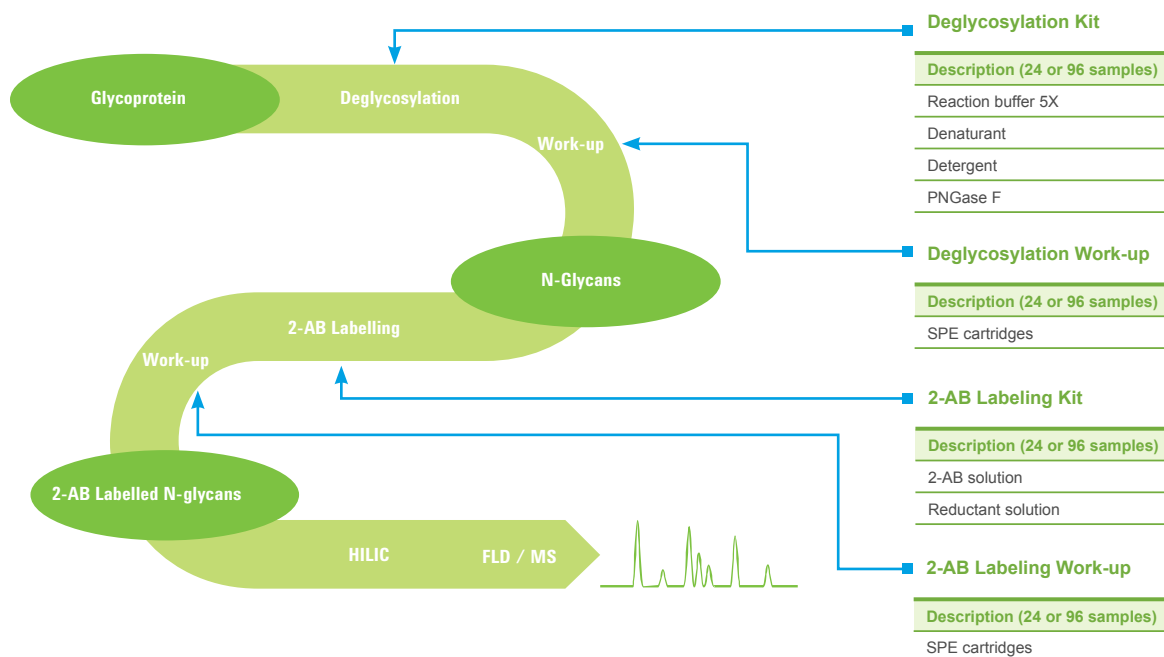
Before the chromatographic analysis, you must cleave and isolate the N-linked glycans from the reaction mix, then derivatize with 2-AB label before final cleanup and formatting for LC analysis.

AdvanceBio glycan sample-prep kits provide all the reagents needed to prepare samples—and full documentation with tips and tricks for optimization.

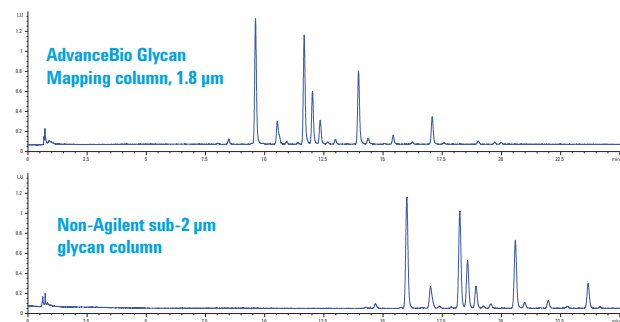
- One part number to order the full sample-prep workflow, including the enzyme to cleave and the 2-AB label.

- Detailed and comprehensive user guide with tips and tricks for preparing complex samples.
- Kits for each of the four steps of the workflow have separate part numbers for flexibility.
- Kits for 24 and 96 samples to avoid wasting expensive reagents.

AdvanceBio glycan mapping columns are compatible with mobile phases used for mass-selective and fluorescence detection.



Superior results – in 40% less time than the competition

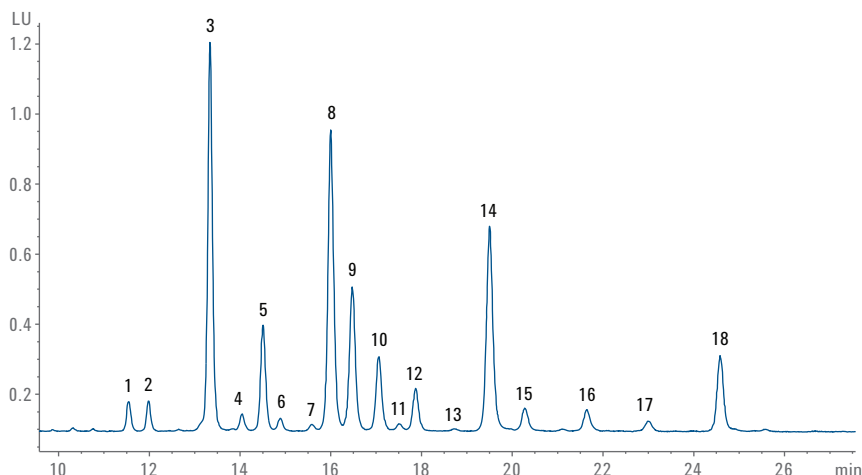


The Agilent AdvanceBio glycan mapping column delivers better resolution, narrower bands, and higher peak capacity than the non-Agilent sub-2 µm column in a 2.1 x 150 mm configuration.

Super-fast glycan analysis: Less than ten minutes with 1.8 µm particles

Parameter	Conditions
Column:	AdvanceBio glycan mapping Column 2.1 x 150 mm, 1.8 µm (P/N 859700-913)
Instrumentation:	Agilent 1290 Infinity LC with 1260 Infinity Fluorescence Detector (FLD)
Column temperature:	55 °C
Sample thermostat:	10 °C
Mobile phase A:	100 mM NH ₄ Formate, pH 4.5
Mobile phase B:	ACN
FLD:	Excitation = 260 Emission = 430
Injection Volume:	2 µL in 70:30 ACN: 100 mM NH ₄ Formate
Sample:	Agilent 2-AB labeled N-linked Human IgG glycan library (P/N 5190-6996)

Ultimate versatility: Speed and resolution, with column pressures of less than 400 bar



Here, a 2.7 μm AdvanceBio glycan mapping column enabled fast results with lower backpressure. The 2.7 μm AdvanceBio glycan-mapping media uses Agilent superficially porous particles to achieve high performance at low column back pressures. With the 100 mm and 150 mm column lengths, typical column pressures are less than 400 bar with the recommended methods for the analysis of 2-AB labeled glycans.

Chromatographic conditions

Parameter	Value
Gradient	min %A % B Flow rate (mL/min)
	0 85 15 0.5
	5 75 25 0.5
	35 64 36 0.5
	40 50 50 0.5
	42 50 50 0.5
	42.01 50 50 0.25
	43 0 100 0.25
	48 0 100 0.25
	50 85 15 0.25
	50.01 85 15 0.5
	51 85 15 0.5
Injection volume	2, 2.5 μL
Thermostat	6 $^{\circ}\text{C}$
Column temperature	60 $^{\circ}\text{C}$
FLD	Ex. 260 nm, Em. 430 nm

Peak	Oxford	Biopharma mAb style	Structure
1	FA1	G0-GlcNAc	
2	A2	G0	
3	FA2	G0F	
4	M5	Man5	
5	FA2B	G0FB	
6	A2G1	G1	
7	A2BG1	G1B	
8, 9	FA2G1	G1F	
10, 11	FA2BG1	G1FB	
12	A2G2	G2	
13	A2BG2	G2B	
14	FA2G2	G2F	
15	FA2BG2	G2FB	
16	FA2G1S1	G1FS1	
17	A2G2S1	A1	
18	FA2G2S1	A1F	



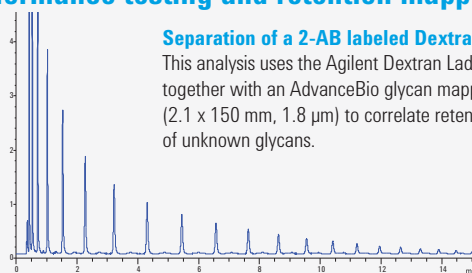
Ensure consistent results with glycan standards for performance testing and retention mapping



Missed information in the early stages of development can cause major setbacks downstream. What's more, production process inconsistency may lead to changes in glycosylation, which can negatively impact immunogenicity and efficacy. Agilent reference

standards help you make sure that critical data have been captured, and that every workflow component is working optimally. Choose from two options:

- **Dextran ladder standard** facilitates data analysis and reporting
- **IgG N-linked glycan standard** confirms workflow efficiency



Separation of a 2-AB labeled Dextran Ladder

This analysis uses the Agilent Dextran Ladder Standard, together with an AdvanceBio glycan mapping column (2.1 x 150 mm, 1.8 μm) to correlate retention times of unknown glycans.

Mobile phase A:	100 mM NH_4F pH 4.5	Time	%A	%B	Flow rate
Mobile phase B:	ACN				mL/min
FLD:	Ex = 260, Em = 430	0	25	75	1.0
Sample:	Agilent 2-AB (P/N 5190-6998) labeled Dextran Ladder	12	40	60	1.0
		12.15	60	40	0.5
		12.5	60	40	0.5
Injection Volume:	2 μL (10 pmol total glycan/1 μL 75/25ACN/water)	12.9	25	75	0.5
		13.05	25	75	1.0
		15	25	75	1.0

Flexible, high-performance LC instruments and supplies for your protein analysis



Robust and easy to use: The 100% bio-inert Agilent 1260 Infinity Bio-inert Quaternary LC delivers outstanding results with its low surface activity, corrosion resistance, active seal wash, and quaternary buffer mixing.



New Benchmarks in Efficiency: The Agilent 1290 Infinity II LC system is the next generation in UHPLC, providing maximum analytical, instrument & laboratory efficiency, with pressures up to 1300bar and flows up to 5mL/min.

Better efficiency and interaction-free results: Agilent Bio-inert LC supplies improve chromatographic reliability with sharper peaks and high reproducibility. Learn more at agilent.com/chem/biosupplies



Ordering Information

AdvanceBio Glycan Mapping Columns

1.8 μ m, stable to 1200 bar

Description	Part Number
2.1 x 100 mm	858700-913
2.1 x 150 mm	859700-913
Fast Guards, 2.1 mm, 1.8 μ m	821725-905

2.7 μ m superficially porous, stable to 600 bar

Description	Part Number
2.1 x 100 mm	685775-913
2.1 x 150 mm	683775-913
2.1 x 250 mm	651750-913
Fast Guards, 2.1 mm, 2.7 μ m	821725-906
4.6 x 100 mm	685975-913
4.6 x 150 mm	683975-913
4.6 x 250 mm	680975-913

Glycan Standards

Description	Part Number
Dextran ladder standard, 10 μ g, 0.5 mL vial	5190-6997
2-AB labeled dextran ladder standard, 200 pmol	5190-6998
IgG N-linked glycan library, 20 μ g, 0.5 mL	5190-6995
2-AB labeled IgG N-linked glycan library, 200 pmol	5190-6996

AdvanceBio Glycan Sample Prep Kits

Description	Part number
AdvanceBio N-glycan sample preparation kit (24 samples)	5190-8000
AdvanceBio N-glycan deglycosylation kit (24 samples)	5190-8001
AdvanceBio N-glycan deglycosylation cleanup cartridges (24 samples)	5190-8002
AdvanceBio 2-AB glycan labeling kit (24 samples)	5190-8003
AdvanceBio 2-AB glycan labeling cleanup cartridges (24 samples)	5190-8004
AdvanceBio N-glycan sample preparation kit (96 samples)	5190-8005
AdvanceBio N-glycan deglycosylation kit (96 samples)	5190-8006
AdvanceBio N-glycan deglycosylation cleanup cartridges (96 samples)	5190-8007
AdvanceBio 2-AB glycan labeling kit (96 samples)	5190-8008
AdvanceBio 2-AB glycan labeling cleanup cartridges (96 samples)	5190-8009
96 well plate for deglycosylation and labeling	5190-8010

Visit agilent.com/chem/AdvanceBio to learn more and to see HPLC posters and Application Notes.

This information is subject to change without notice.

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