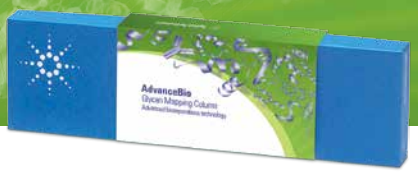
GLYCAN MAPPING WORKFLOW
AGILENT ADVANCEBIO GLYCAN MAPPING 1.8 µm COLUMNS



In this document Agilent applications chemists share their recommendations for an optimum LC system and its configuration for characterizing biomolecules. They also offer guidance on a generic method to get you started, and how this method can be further optimized to meet your specific separation goals.
Additional application information is available at **www.agilent.com/chem/advancebio**

Agilent 1290 Infinity UHPLC System

Mobile phases

Eluent A: 100 mM ammonium formate, pH 4.5
Eluent B: acetonitrile
(mass spec compatible)

Detection (G1321B)

Agilent 1260 Infinity Fluorescence Detector, ex 260 nm, em 430 nm, 8 µL cell

Column compartment (G1316C)

40 °C gives longer column life; 60 °C gives sharper peaks but significantly reduces lifetime. Selectivity and resolution may change with temperature.

Sample injection (G4226A)

1 to 2 µL injection for maximum resolution. Samples should first be dissolved in H_2O then made up to 70:30 ACN:Water. Chiller should be used.

Pump (G4220A)

0.5 mL/min for high resolution separations; up to 1.0 mL/min for high speed. High aqueous clean up should ALWAYS be run at reduced flow rate.

AdvanceBio Glycan Mapping products include sample preparation, labelled and unlabelled standards and 1.8 µm and 2.7 µm columns.

AdvanceBio Glycan Mapping, 1.8 µm, stable to 1200 bar

Description	Part Number
2.1 x 100 mm	858700-913
2.1 x 150 mm *	859700-913
Fast Guard, 2.1 mm, 1.8 µm	651750-913

* Recommended initial column size

Both gradients provide 1.25%/mL slope. It may be necessary to adjust the start and end point to obtain highest resolution for samples containing different types of glycan. Larger glycan structures may require 75 to 55% acetonitrile gradient for optimum results for example.

Suggested gradient for resolution

Time	Eluent A	Eluent B	Flow
0	20%	80%	0.5 mL/min
32	40%	60%	0.5 mL/min
33	80%	20%	0.5 mL/min
35	80%	20%	0.5 mL/min
36	20%	80%	0.5 mL/min
45	20%	80%	0.5 mL/min

Suggested gradient for speed

Time	Eluent A	Eluent B	Flow
0	25%	75%	1.0 mL/min
12	40%	60%	1.0 mL/min
12.5	80%	20%	0.5 mL/min
13.5	80%	20%	0.5 mL/min
14	25%	75%	0.5 mL/min
15	25%	75%	1.0 mL/min
20	25%	75%	1.0 mL/min

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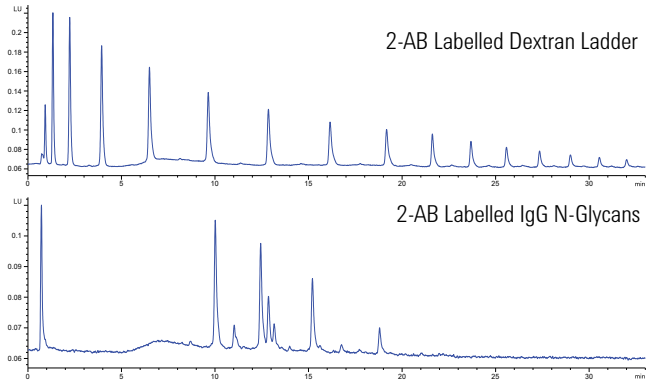
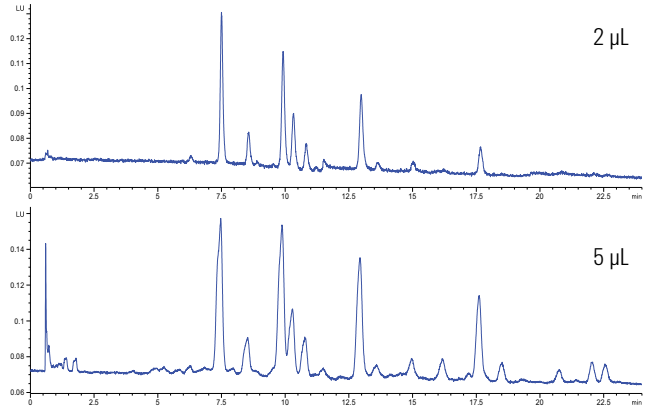


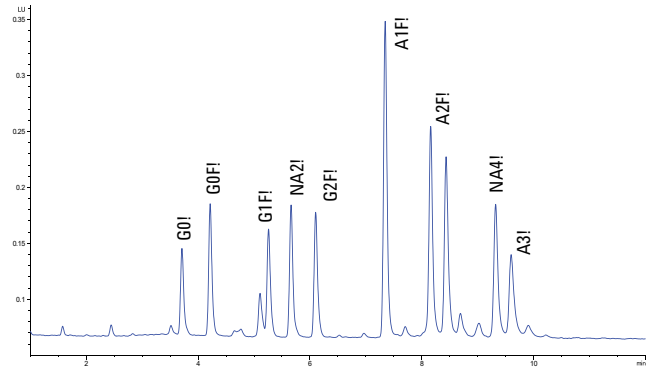
High-resolution separation of 2-AB Labeled Dextran Ladder (p/n 5190-6998) and 2-AB Labeled Human IgG N-Glycan Library (p/n 5190-6996).

Time	Eluent A	Eluent B	Flow
0	20%	80%	0.5 mL/min
32	40%	60%	0.5 mL/min
33	80%	20%	0.5 mL/min
35	80%	20%	0.5 mL/min
36	20%	80%	0.5 mL/min
45	20%	80%	0.5 mL/min



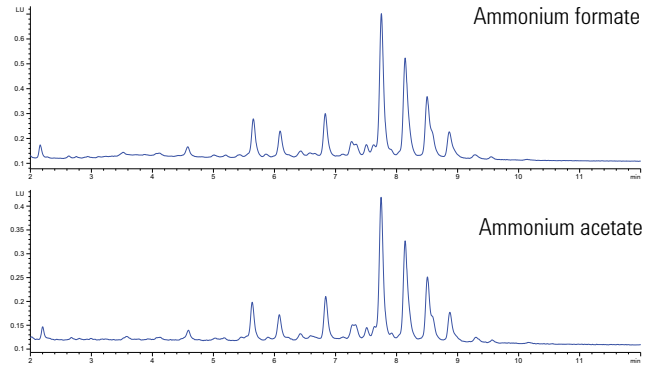
Over-injection of 2-AB Labeled Human IgG N-Glycan Library (2 µL vs. 5 µL).

Samples should be prepared by dissolving in water and then adding acetonitrile to give a final composition 30:70 water:acetonitrile. The small column dimension, 2.1 x 150 mm, still requires small injection volumes. The figure on the left demonstrates the outcome from injecting 5 µL – peaks become broader and resolution is lost – compared to 2 µL injection.



High speed separation of 2-AB Labeled N-Glycans (tentative peak assignment).

Time	Eluent A	Eluent B	Flow
0	25%	75%	1.0 mL/min
12	40%	60%	1.0 mL/min
12.5	80%	20%	0.5 mL/min
13.5	80%	20%	0.5 mL/min
14	25%	75%	0.5 mL/min
15	25%	75%	1.0 mL/min
20	25%	75%	1.0 mL/min



Glycans, such as those found in bovine fetuin, can be eluted with ammonium formate or ammonium acetate mobile phases.

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