

# GLYCAN MAPPING WORKFLOW

## AGILENT ADVANCEBIO GLYCAN MAPPING 1.8 $\mu\text{m}$ 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](http://www.agilent.com/chem/advancebio)

### Agilent 1290 Infinity UHPLC System

AdvanceBio Glycan Mapping products include sample preparation, labelled and unlabelled standards and 1.8  $\mu\text{m}$  and 2.7  $\mu\text{m}$  columns.

#### AdvanceBio Glycan Mapping, 1.8 $\mu\text{m}$ , 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 $\mu\text{m}$	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.

#### 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  $\mu\text{L}$  cell

#### Column compartment (G1316C)

40  $^{\circ}\text{C}$  gives longer column life; 60  $^{\circ}\text{C}$  gives sharper peaks but significantly reduces lifetime. Selectivity and resolution may change with temperature.

#### Sample injection (G4226A)

1 to 2  $\mu\text{L}$  injection for maximum resolution. Samples should first be dissolved in  $\text{H}_2\text{O}$  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.



#### Suggested gradient for resolution

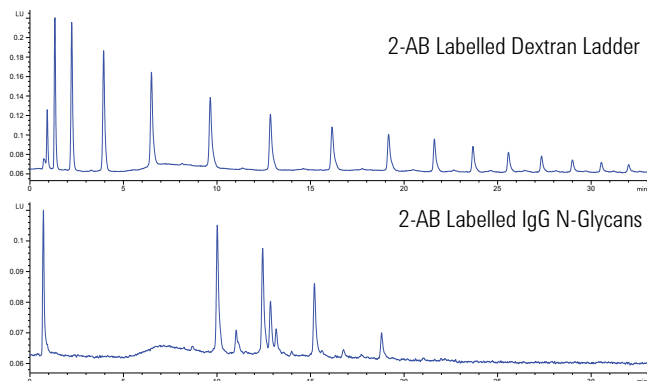
Time	Eluent A	Eluent B	Flow
0	20%	80%	0.5 mL/min
32	40%	60%	0.5 mL/min
33	80%	20%	0.5 mL/min
35	80%	20%	0.5 mL/min
36	20%	80%	0.5 mL/min
45	20%	80%	0.5 mL/min

#### 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

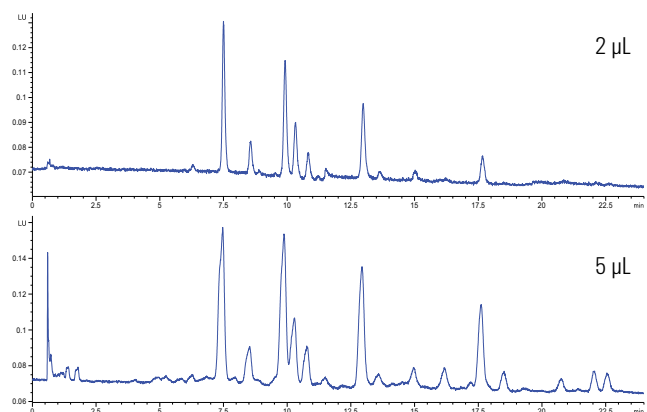


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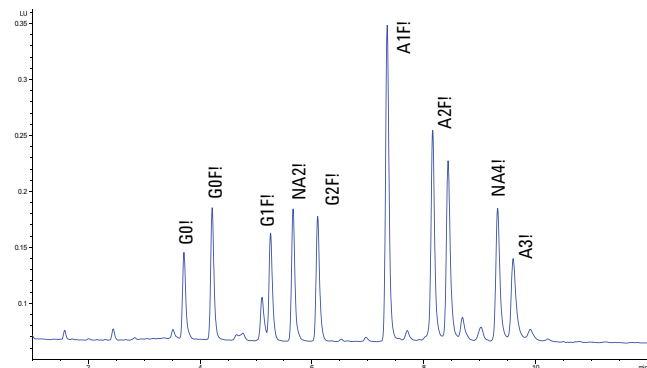
High-resolution separation of 2-AB Labeled Dextran Ladder (p/n 5190-6998) and 2-AB Labeled Human IgG N-Glycan Library (p/n 5190-6996).

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



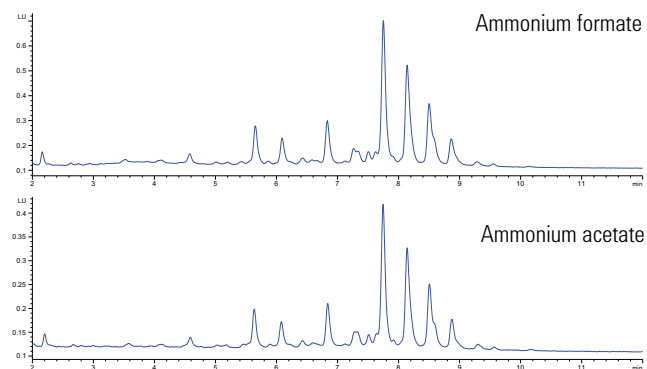
Over-injection of 2-AB Labeled Human IgG N-Glycan Library (2 μL vs. 5 μL).

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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