GLYCAN MAPPING WORKFLOW
AGILENT ADVANCEBIO GLYCAN MAPPING 2.7 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 1260 Infinity Bio-Inert LC System

Mobile phases

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

Pump (G5611A)

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.

Sample injection (G5667A)

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.

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.

Detection (G1316C)

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

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	20%	80%	0.7 mL/min
12	40%	60%	0.7 mL/min
12.5	80%	20%	0.5 mL/min
13.5	80%	20%	0.5 mL/min
16	20%	80%	0.5 mL/min
17	20%	80%	0.7 mL/min
20	20%	80%	0.7 mL/min

AdvanceBio Glycan Mapping, 2.7 µm, stable to 600 bar

Description	Part Number
4.6 x 100 mm	685975-913
4.6 x 150 mm	683975-913
4.6 x 250 mm	680975-913

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

AdvanceBio Glycan Mapping, 2.7 µm, 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 Guard, 2.1 mm, 2.7 µm	821725-906

* Recommended initial column size

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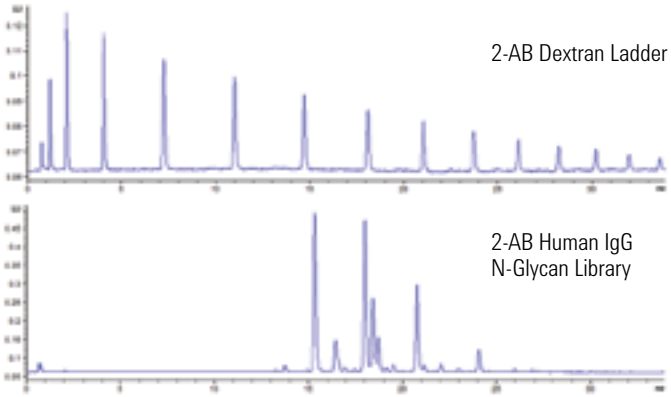
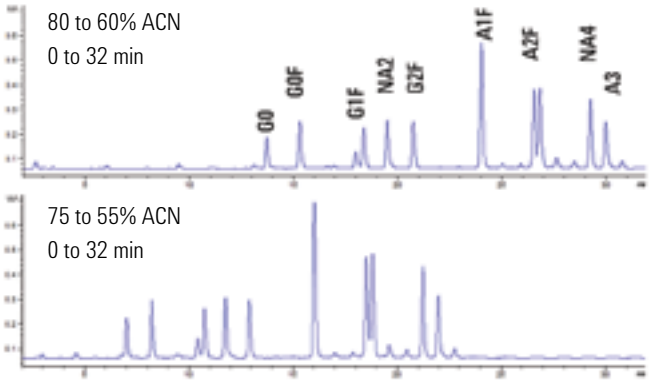


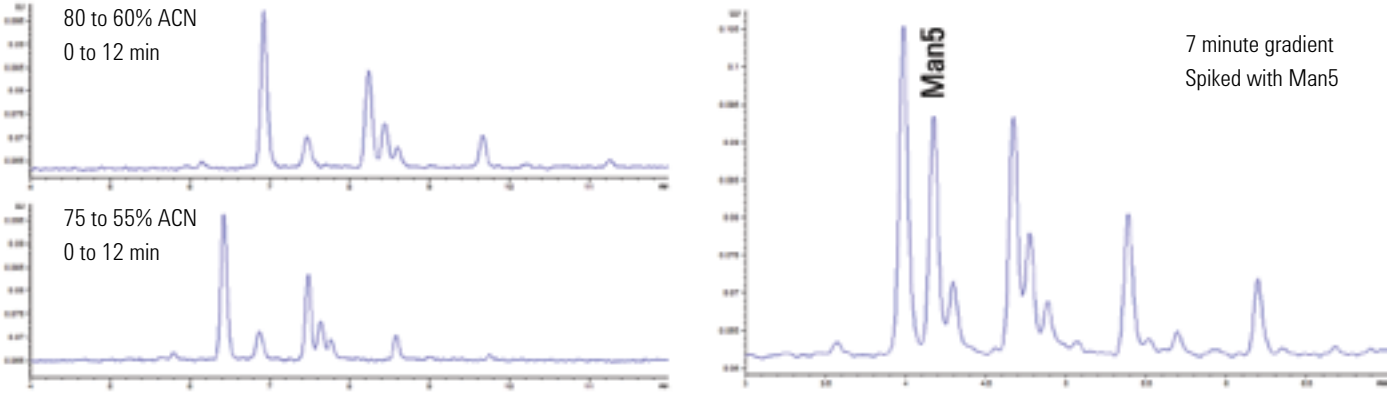
Time	Eluent A	Eluent B	Flow
0	20%	80%	0.5 mL/min
32	40%	60%	0.5 mL/min
33	80%	20%	0.5 mL/min
35	80%	20%	0.5 mL/min
36	20%	80%	0.5 mL/min
45	20%	80%	0.5 mL/min

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	25%	75%	0.5 mL/min
32	45%	55%	0.5 mL/min
33	80%	20%	0.5 mL/min
35	80%	20%	0.5 mL/min
36	25%	75%	0.5 mL/min
45	25%	75%	0.5 mL/min

Effect of gradient adjustment on larger (later eluting) 2-AB Labeled N-Glycans.



High speed and very high speed separations of 2-AB Labeled Human IgG N-Glycan Library (p/n 5190-6996).

High speed separations can be more difficult to achieve due to instrument parameters such as pump gradient dwell time, and extra column dead volume through use of wider bore (lower pressure) capillaries.

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