Getting Started

A Column Performance Report, including a column-specific QC test chromatogram and a batch-specific glycan separation, is enclosed with every Agilent column.

Our QC test system may vary slightly from the system used in your lab. We modified our system to minimize dead volume, which allows us to evaluate column efficiency and ensure product consistency.

An optimized LC system will generate similar results to the chromatogram on your QC Performance Report.

Important safety notes

- All points of connection in an LC system are potential leak sources. Always be aware of the potential toxicity or flammability of mobile phases.
- These columns are mechanically stable and have been tested to the recommended maximum operating pressure to ensure safe lab operation on many LC instruments.
- Because of the small particle size, dry AdvanceBio Glycan Mapping packings are respirable. Opening columns is strongly discouraged due to the safety risk and likelihood of reducing column performance. If you must, open columns only in a well-ventilated area.

Other tips

- Flow direction is marked on the column.
- Small injection volumes are best for HILIC separations. Injection volumes > 1 µL will compromise peak shape and resolution.

If you have specific questions, contact our Technical Support team at www.agilent.com/chem/techsupport

Column Operating Parameters

pH, temperature, and pressure

- Maximum operating pressure is 1200 bar for 1.8 µm columns, and 600 bar for 2.7 µm columns.
- Optimal column life is achieved by operating only up to 80% of the maximum pressure.
- The typical operating temperature is 40 °C. Higher temperatures can be used, but will shorten column lifetime.
- The operating pH range is 2 to 7.

Note: Using the column above pH 7 and 40 °C reduces column lifetime. AdvanceBio Glycan Mapping columns are silica-based with a HILIC amide phase. All silica has some solubility in pH> 6 aqueous mobile phases, and solubility is increased at elevated temperatures.

Shipping solvents and compatibility

AdvanceBio Glycan Mapping columns are shipped with acetonitrile:water and are ready to use for HILIC separations. HILIC columns require more equilibration than reversed-phase columns. They are compatible with buffers and acetonitrile, which are most commonly used for glycan analysis.

A typical mobile phase for glycan analysis is acetonitrile: ammonium formate buffer pH 4.5. You can use this phase with fluorescence or MS detection.

Glycan Standards Support Your Data Reporting and Workflow Efficiency

Description	Part No.
Dextran ladder standard, 10 µg, 0.5 mL vial	5190-6997
2-AB labeled dextran ladder standard, 200 pmol	5190-6998
IgG N-linked glycan library, 20 µg, 0.5 mL	5190-6995
2-AB labeled IgG N-linked glycan library, 200 pmol	5190-6996

Additional glycan standards, including standards with other labels, are available at www.prozyme.com

Published in the USA, May 9, 2019
Part Number: 820120-007

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Confidently Characterize Protein Glycosylation

Agilent AdvanceBio Glycan Mapping column user guide

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Using Your Column

Installation

Attach columns to the instrument using a short 3/8 in wrench. This will help prevent over-tightening of the end fittings, which can damage the column.

Column conditioning

Agilent AdvanceBio Glycan Mapping columns are designed for separating N-linked glycans cleaved from glycoproteins and glycopeptides. For the HILIC mechanism to work effectively, you must fully equilibrate the column before use.

We recommend that you first flush the column in this sequence:

1. 100% acetonitrile for at least 10 column volumes
2. Aqueous phase containing 15% acetonitrile for 10 column volumes
3. Mobile phase used at the start of your analysis for 20 column volumes

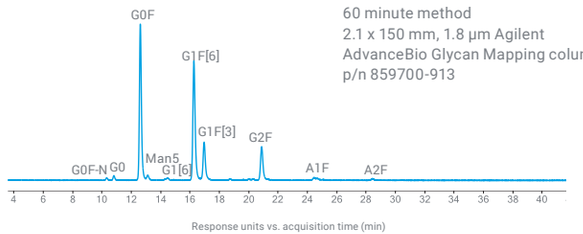
Check that the column is fully equilibrated by performing two to three analytical runs to confirm reproducibility.

Before use

Filter samples before injection into the column. The column inlet frit is nominally 0.5 µm for 1.8 µm columns, and 2 µm for 2.7 µm columns. Samples should be filtered through a 0.2 µm sample filter.

Recommended Starting Gradients

High resolution analysis of 2-AB labeled glycans from MabThera

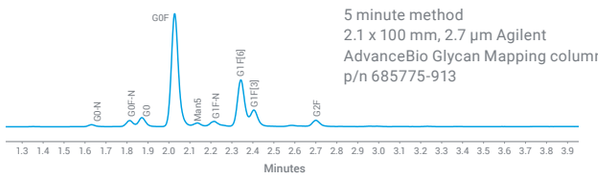


60 minute method
2.1 x 150 mm, 1.8 µm Agilent AdvanceBio Glycan Mapping column
p/n 859700-913

Run conditions

Mobile phase A:	50 mM ammonium formate, pH 4.5
Mobile phase B:	Acetonitrile
Column temperature:	40 °C
Injection volume:	1 µL
Sample:	2-AB labeled N-glycans released from 0.4 µg of MabThera in H_2O
Detection:	Fluorescence; 260/430 nm

High throughput screening of InstantPC labeled glycans from Herceptin



5 minute method
2.1 x 100 mm, 2.7 µm Agilent AdvanceBio Glycan Mapping column
p/n 685775-913

Run conditions

Mobile phase A:	100 mM ammonium formate, pH 4.4
Mobile phase B:	Acetonitrile
Column temperature:	35 °C
Injection volume:	1 µL
Sample:	InstantPC labeled N-glycans released from 0.4 µg of Trastuzumab in Gly-X InstantPC Eluent (160 mM ammonium formate, 10% (v/v) acetonitrile)
Detection:	Fluorescence; 285/345 nm

Column Care

Extend column life through proper cleaning

- *If the solvent flow appears restricted* (unusually high column backpressure), check to see that the flow is unobstructed up to the column inlet.
- *If the restriction is before the column*, replace the piece of tubing or filter that is plugged.
- *If the column is plugged*, replace the column. **Do not backflush.**

Remove particulates before sample analysis to avoid blockage of the column inlet frit. If this is not possible, use guard columns or an inline filter to protect the column and increase its lifetime. Be sure to filter samples before injecting them onto any column.

For more information about guard columns, go to www.agilent.com/chem/advancebio-glycan-mapping

Proper column storage

We recommend acetonitrile:water (95:5) as the long-term storage solvent. You may flush the column with 60% acetonitrile:40% water to remove buffer before switching to the storage solvent. Before storing the column, tightly cap the end fittings with end plugs to prevent the packing from drying out.

Columns can be safely stored for short periods in the mobile phases. However, you should remove salts from the instrument and column by purging the column with the same mobile phase without the buffer. Example: Use 90:10 ACN:H_2O to remove a 90:10 ACN:0.01 M formate buffered mobile phase.

For short-term storage, re-equilibration is faster when you store the column in 80% ACN:20% 5 mM ammonium formate. Three to six injections should be made to verify column equilibration.