Quick Start Guide

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Size Exclusion Chromatography

Agilent AdvanceBio SEC 1.9 µm column

In this document, Agilent applications chemists share their recommendations for an optimum LC system and its configuration for analyzing biomolecule aggregation by size exclusion chromatography (SEC), as well as a generic method to get you started, and tips for optimization.

More application information is available at www.agilent.com/chem/advancebiosec

Operating guidelines

- Target application: dimer/monomer, lower molecular weight mAb fragments.
- Protein aggregation is impacted by various environmental factors, including pH, ionic strength, and temperature. To quantify levels of aggregation, use a mobile phase that does not affect the sample. Typically, start with 150 mM phosphate buffer at pH 7.0.
- Before use, ensure all components of the mobile phase are soluble and have been filtered at 0.2 µm.
- Ideally, samples should be dissolved in the mobile phase. If a sample is cloudy, it may be necessary to change the mobile phase conditions.
- Use a 300 mm column for higher resolution and sensitivity, or use a 150 mm column and increase the flow rate to increase sample throughput.
- SEC is a noninteractive LC technique, meaning small injection volumes must be used to achieve efficient separations. Sample size should be ≤1% of the total column volume.
- AdvanceBio 1.9 µm SEC columns are recommended for SEC/DAD, SEC/UV, SEC/MS native and denature applications.
- An Agilent 1290 Infinity II Bio LC system is recommended.

Please refer to the user guide at www.agilent.com/chem/biocolumn-userguides for column conditioning, use, and storage.

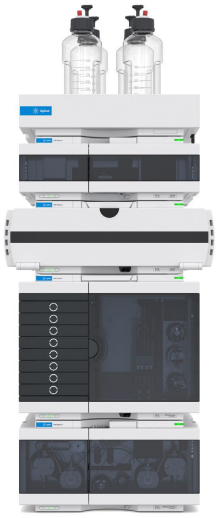


Figure 1. An Agilent 1290 Infinity II Bio LC system.

Please note:
Use ultralow-dead-volume capillaries to increase the performance.

Mobile phases (HPLC grade or higher)

Isocratic elution with freshly prepared aqueous or aqueous/organic buffers

Detection (G7117B)

DAD with a bio-inert standard flow-cell

Column compartment (G7116B)

20 to 30 °C is the typical temperature range used for SEC of biologically active proteins

Sample injection (G7167B)

1 to 5 µL injection for samples containing 1 to 5 mg/mL of protein

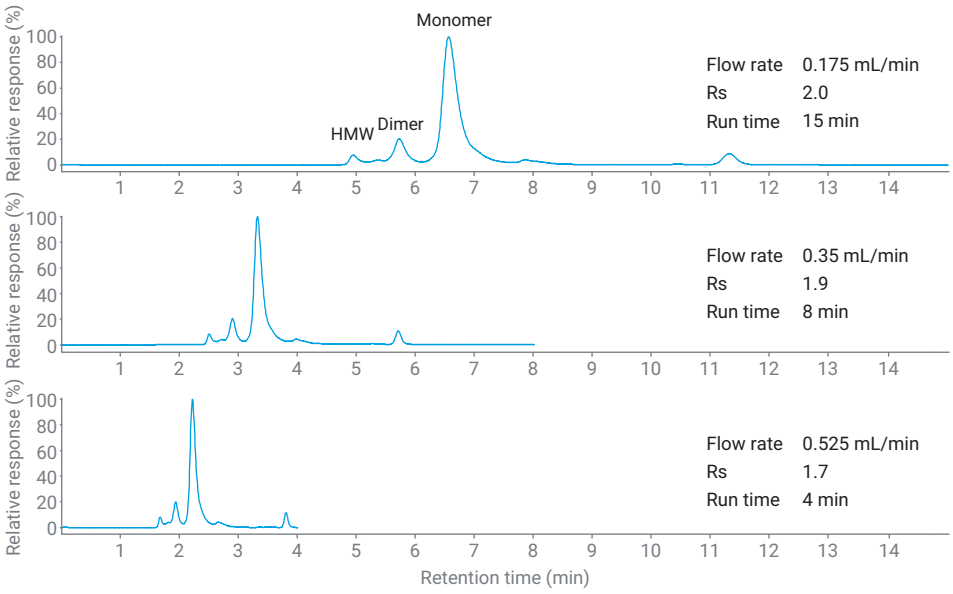
Pump (G7120A)

0.1 to 0.7 mL/min for 4.6 mm id
0.05 to 0.1 mL/min for 2.1 mm id

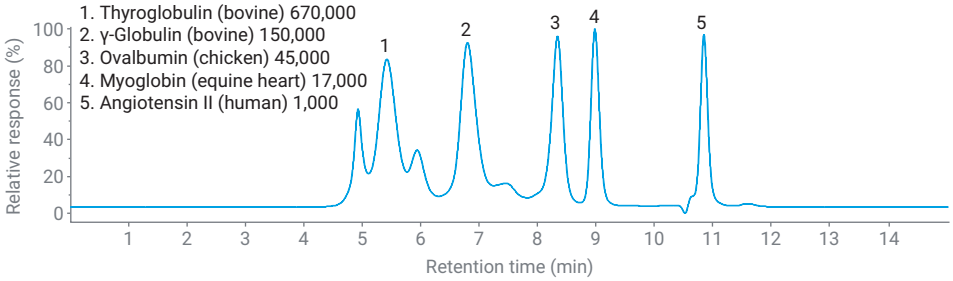
Important: Ramp up the flow rate slowly from 0.0 mL/min to the intended operating flow rate over a period of several minutes.

Reducing analysis time

Choosing the best particle size for your application

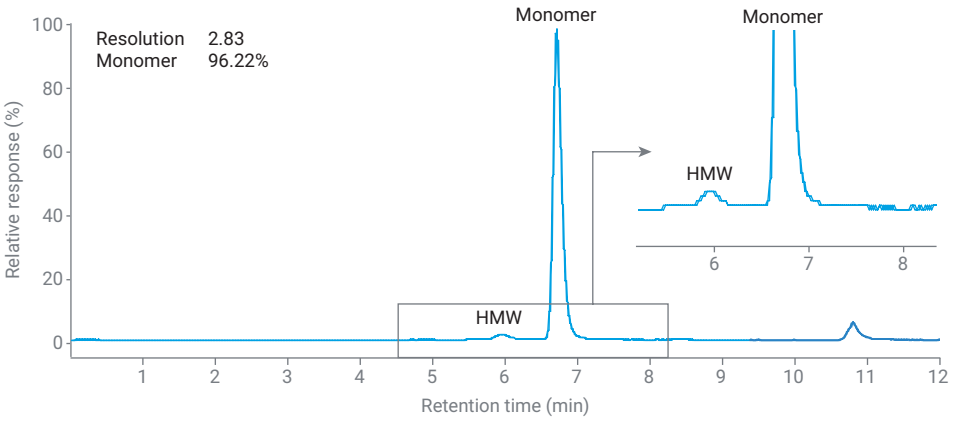


Column	Agilent AdvanceBio SEC 200 Å, 4.6 × 150 mm, 1.9 µm
Sample	Bovine IgG
Mobile Phase	150 mM sodium phosphate, pH 7.0

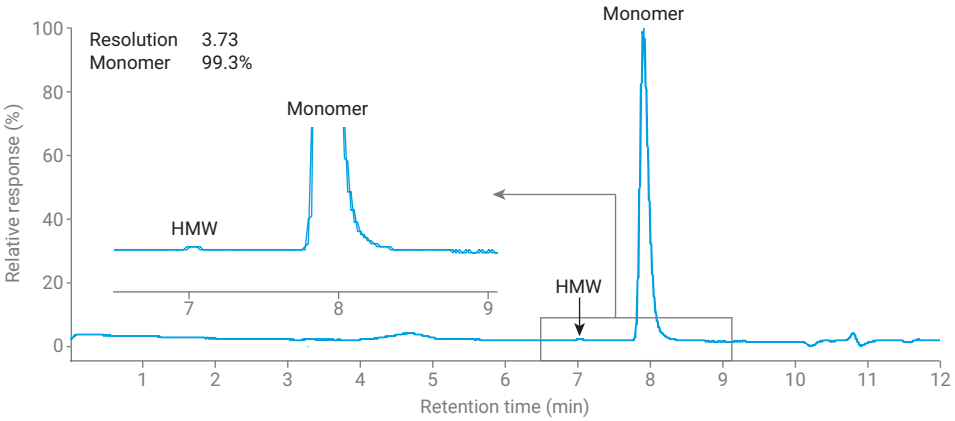


Column	Agilent AdvanceBio SEC 300 Å, 4.6 × 300 mm
Sample	Agilent AdvanceBio SEC 300 Å Protein Standard, Lyophilized, 1.5 mL vial (p/n 5190-9417)
Mobile Phase	150 mM sodium phosphate, pH 7.0
Run Time	15 min

Aggregate analysis for intact proteins



Column	Agilent AdvanceBio SEC 200 Å, 4.6 × 300 mm
Sample	Agilent NIST mAb
Mobile Phase	150 mM sodium phosphate, pH 7.0



Column	Agilent AdvanceBio SEC 120 Å, 4.6 × 300 mm
Sample	Porcine Insulin
Mobile Phase	200 mL of anhydrous acetic acid, 300 mL of acetonitrile, and 400 mL of water, adjusted to pH 3.0 with concentrated ammonia, and diluted to 1,000 mL with water

Learn more about this column at www.agilent.com/chem/advancebiosec

Learn more about more Agilent biocolumns for SEC at www.agilent.com/chem/aggregate-fragment-analysis

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