User Guide

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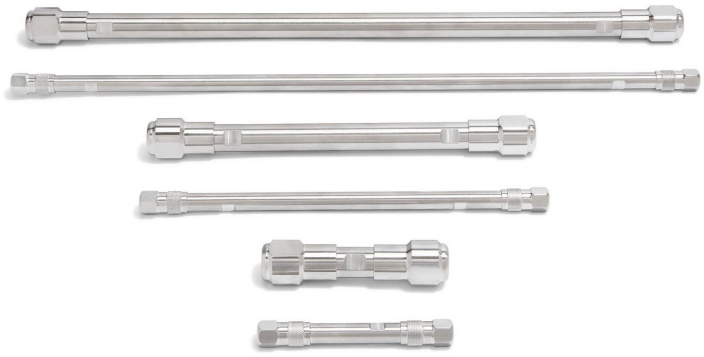
Agilent AdvanceBio SEC Columns

Size exclusion columns for analysis of biomolecules



Introduction

Agilent AdvanceBio SEC columns are designed and manufactured by Agilent for size exclusion chromatography of biomolecules. The innovative, high-porosity silica particles and unique hydrophilic bonding chemistry provide for exceptional stability with minimal nonspecific interactions.

AdvanceBio 2.7 µm SEC columns are available in four pore sizes: 130 Å for peptides and small therapeutic proteins; 300 Å for monoclonal antibodies (mAbs), antibody-drug conjugates (ADCs), and other proteins; a new 500 Å pore size for adeno-associated viruses (AAVs) and other large biotherapeutic proteins and oligonucleotides; and a new 1000 Å pore size for virus-like particles (VLPs) and other large biotherapeutics, such as oligonucleotides.

AdvanceBio 1.9 µm UHPLC SEC columns are optimized for high resolution, high-throughput separation, and characterization of size variants. They are offered in two pore sizes: AdvanceBio SEC 1.9 µm, 120 Å is best suited for the analysis of peptides and small therapeutic proteins, while AdvanceBio SEC 1.9 µm, 200 Å is designed for characterizing mAbs and ADCs.

Getting started

A column performance report, including a column-specific QC test chromatogram and a batch-specific protein and peptide separation, is enclosed with every Agilent AdvanceBio SEC column. The Agilent QC test system has been modified from a standard system to minimize dead volume, so it may vary from the system used in your lab. This modification assures a better evaluation of the column efficiency and assures a more consistent product. An optimized LC system will generate similar results to the chromatogram in the column performance report.

For the best chromatographic results with AdvanceBio SEC 1.9 µm columns, it is recommended that a low dispersion LC be used. You can optimize your LC for maximum resolution by minimizing tubing internal diameter (id) and length between the sample injector and the column, and between the column and detector. Low-volume micro UV detector flow cells may be necessary with 2.1 mm id columns.

Ensuring proper column connection is important. To avoid damage, Agilent recommends InfinityLab Quick Connect LC fittings (part number 5067-5966) or Bio-inert UHP-FF fittings (part number 5067-5695), particularly for use with PEEK-lined 2.1 mm id columns.

To monitor column and instrument performance, Agilent recommends regularly running a standard test mixture, such as Agilent AdvanceBio SEC standards.

All Agilent AdvanceBio SEC columns are recommended for use with UV, fluorescence, and light scattering detectors.

For SEC-MS, AdvanceBio 1.9 µm, AdvanceBio 2.7 µm 500 Å, or 1000 Å pore sizes are recommended.

If you have specific questions, contact Agilent technical support.

Important safety considerations

- All connection points in an LC system are potential sources of leaks. Users should be aware of the potential toxicity or flammability of their mobile phases.
- Do not remove the column end fittings.

Using your column

Installation

- Remove both end plugs, and ensure that your system's flow direction matches the arrow on the column. Do not use the column with the flow in the reverse direction.
- Use an Agilent InfinityLab Quick Connect LC fitting (part number 5067-5966) to quickly connect the column to your LC instrument. For PEEK-lined 2.1 mm id columns, Bio-inert UHP-FF fittings (part number 5067-5695) along with a tool to avoid over-tightening (part number 5043-0915) are recommended.

Column conditioning

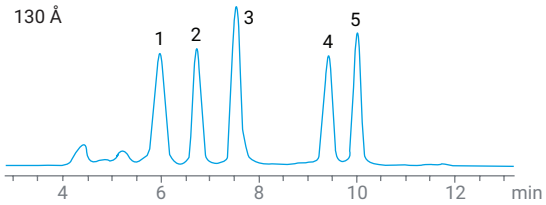
The columns must first be flushed into the mobile phase required for your separation. Ramp up the flow rate slowly from 0.0 mL/min to the intended operating flow rate over a period of several minutes. If possible, the maximum flow gradient should be set at 0.1 mL/min/min. Equilibrate the column by flushing for a minimum of 10 column volumes or until the baseline is stable.

Instructions for use

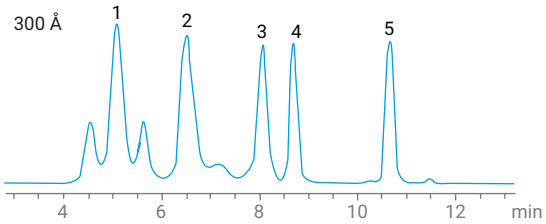
- Columns are compatible with commonly used aqueous buffers, including 150 mM sodium phosphate at pH 7.0, with or without the addition of other salts. The recommended salt concentration is ≤ 0.5 M. It is recommended that the percentage of organic solvent be less than 50%; close attention must be paid to solubility of buffer components and system pressure when using organic solvent. For native mode SEC-MS, ammonium acetate is recommended. Flush the column extensively before connecting to your MS detector in case nonvolatile mobile phase salts are still present. When changing eluents, consider the viscosity and risk of salt precipitation. If you are unsure, flush the column first with high-purity water before introducing a new eluent.

2.7 µm

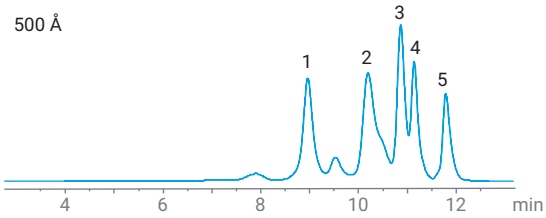
Agilent SEC AdvanceBio 130 Å Protein Standard	Molecular Weight (Da)
1. Ovalbumin	45,000
2. Myoglobin	17,000
3. Aprotinin	6,700
4. Neurotensin	1,700
5. Angiotensin II	1,000



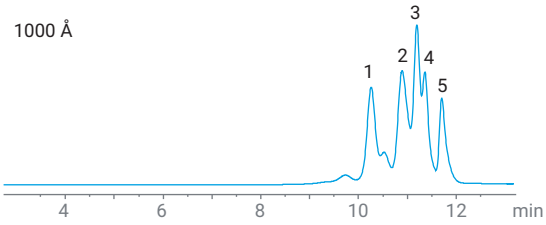
Agilent SEC AdvanceBio 300 Å Protein Standard	Molecular Weight (Da)
1. Thyroglobulin	670,000
2. γ-Globulin	150,000
3. Ovalbumin	45,000
4. Myoglobin	17,000
5. Angiotensin II	1,000



Agilent SEC AdvanceBio 300 Å Protein Standard	Molecular Weight (Da)
1. Thyroglobulin	670,000
2. γ-Globulin	150,000
3. Ovalbumin	45,000
4. Myoglobin	17,000
5. Angiotensin II	1,000

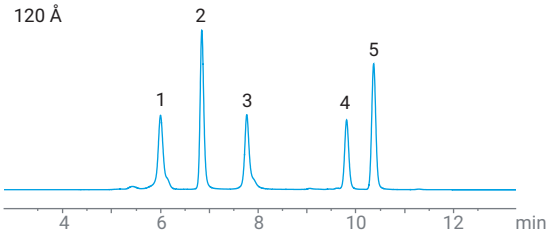


Agilent SEC AdvanceBio 300 Å Protein Standard	Molecular Weight (Da)
1. Thyroglobulin	670,000
2. γ-Globulin	150,000
3. Ovalbumin	45,000
4. Myoglobin	17,000
5. Angiotensin II	1,000

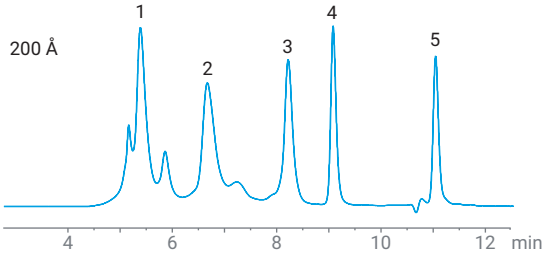


1.9 µm

Low Mol Wt Protein Mix	Molecular Weight (Da)
1. Ovalbumin	44,000
2. Myoglobin	17,000
3. Aprotinin	6,700
4. Neurotensin	1,700
5. Uridine	244



Agilent SEC AdvanceBio 200 Å Protein Standard	Molecular Weight (Da)
1. Thyroglobulin	670,000
2. γ-Globulin	158,000
3. Ovalbumin	44,000
4. Myoglobin	17,000
5. Angiotensin II	1,000



Parameter	Value
Columns	AdvanceBio SEC 130 Å, 2.7 µm, 4.6 × 300 mm (PL1580-5350) AdvanceBio SEC 300 Å, 2.7 µm, 4.6 × 300 mm (PL1580-5301) AdvanceBio SEC 500 Å, 2.7 µm, 4.6 × 300 mm (PL1580-5325) AdvanceBio SEC 1000 Å, 2.7 µm, 4.6 × 300 mm (PL1580-5302) AdvanceBio SEC 120 Å, 1.9 µm, 4.6 × 300 mm (PL1580-5250) AdvanceBio SEC 200 Å, 1.9 µm, 4.6 × 300 mm (PL1580-5201)
Flow Rate	0.35 mL/min
Mobile Phase	150 mM sodium phosphate, pH 7.0
Wavelength	220 nm
Injection Volume	1 to 5 µL

Figure 1. Example separations of protein standard mixtures appropriate for system suitability testing.

– Mix your buffers freshly using high-purity components and ultrahigh purity water such as Milli-Q or Nanopure. Filter buffers through a 0.2 or 0.45 µm filter and degas before use. Filtering will remove particulates and help reduce the risk of bacterial growth, which will otherwise damage the column and your LC system. For light scattering detection, triply filtered (0.2 µm) mobile phase is recommended.
– Prepare your samples in the mobile phase, and ensure that they dissolve completely. Filter or centrifuge samples before injection.

Note: To maximize the lifetime of your column, we recommend using an Agilent AdvanceBio SEC guard column.

Operating parameters

Parameter	Value
Mobile Phase Compatibility	Aqueous buffers with high and low salt can be used. Mixtures of water and organic solvent can be used **with careful attention to solubility of buffer components and system pressure**.
pH Stability	2 to 8.5
Operating Temperature	20 to 30 °C (recommended), 80 °C (maximum)
Maximum Pressure	400 bar (5,800 psi) for 2.7 µm columns 620 bar (9,000 psi) for 1.9 µm columns
Recommended Flow Rates	0.1 to 2.0 mL/min for 7.8 mm id columns 0.1 to 0.7 mL/min for 4.6 mm id columns 0.05 to 0.1 mL/min for 2.1 mm id columns For two columns in series, lower flow rates may be necessary to ensure that maximum pressure is not exceeded.
Injection Volume	1 to 10 µL (recommended) Maximum 1% column volume

Note: Working at extremes of the operating parameters may reduce column lifetime.

Parameter	Value
Flow Rate	1 mL/min
Mobile Phase	150 mM Phosphate buffer, pH 7.0
Injection Volume	5 µL
UV Wavelength	220 nm
Temperature	Ambient
Sample	IgG

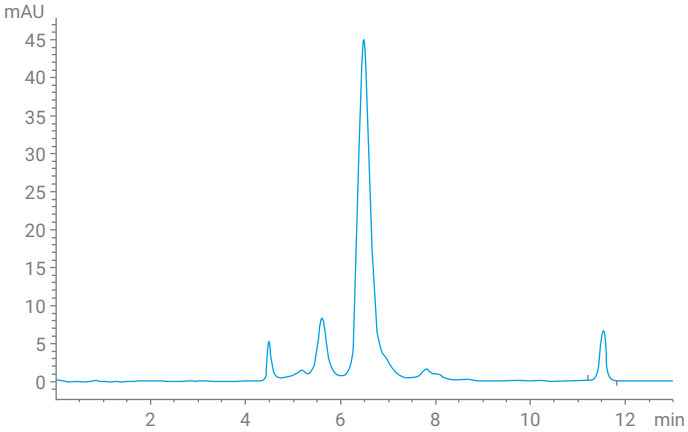


Figure 2. High-resolution separation of an IgG sample, showing the monomer, aggregates, and degradation products on an Agilent AdvanceBio SEC 300 Å, 7.8 × 300 mm, 2.7 µm column (part number PL1180-5301).

Parameter	Value
Flow Rate	0.35 mL/min
Mobile Phase	150 mM Phosphate buffer, pH 7.0
UV Wavelength	280 nm
Temperature	25 °C
Sample	Stressed mAb incubated overnight at pH 9 and 40 °C

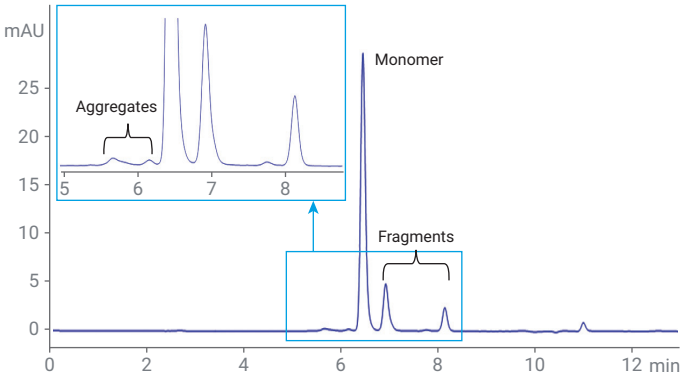


Figure 3. Example separation of a stressed IgG sample showing monomer, aggregates, and fragments on an Agilent AdvanceBio SEC 200 Å, 4.6 × 300 mm, 1.9 µm column (part number PL1580-5201).

Parameter	Value
Flow Rate	0.35 mL/min
Mobile Phase	50 mM Phosphate buffer, 400 mM NaCl, pH 7.2
Injection Volume	5 µL
Fluorescence Detection	Ex 280 nm, Em 343 nm
Temperature	Ambient
Sample	AAV9

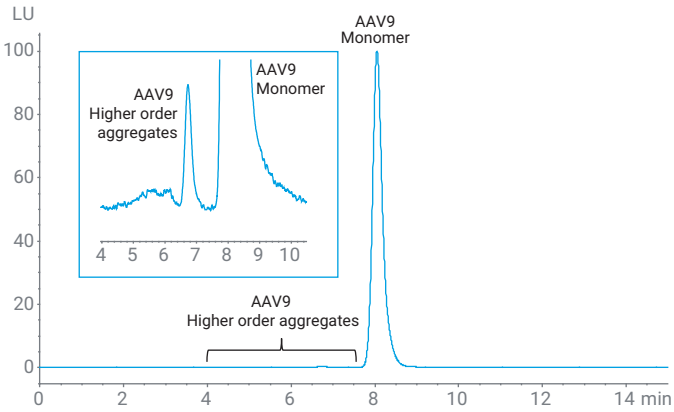


Figure 4. High resolution separation of AAV9 using an Agilent AdvanceBio SEC 500 Å, 4.6 × 300 mm, 2.7 µm column (part number PL1580-5325).

Column care and cleaning

Column care

An increase in backpressure and decrease in performance may occur over time. If the pressure has increased, first identify if this increase is due to a guard column that may need to be replaced. If the increase in pressure is in a system component, such as tubing or a filter, replace the component and retest.

Column cleaning instructions

It may be possible to restore column performance using one of the following cleaning solutions:

- **For strongly adsorbed contaminants:** high salt concentration at low pH (for example, 0.5 M Na_2SO_4, pH 3) or 0.5 M guanidine hydrochloride
- **Organic solvent for hydrophobic materials:** up to 50% methanol, ethanol, or isopropanol
- **Acidic reagents for basic contaminants:** 0.1% TFA, formic acid, or acetic acid in 15% acetonitrile

Always flush the column in the direction of the flow arrow, and lower the flow rate to keep the pressure below 200 bar for 2.7 µm columns or 400 bar for 1.9 µm columns. Rinse with at least five column volumes of ultrapure water before and after flushing with at least 20 column volumes of the cleaning solution.

It is not recommended to use all three cleaning buffers sequentially. Choose the most appropriate buffer for your probable contaminant. Take care to avoid precipitation of buffer salts, and avoid overpressuring the column due to mobile phase viscosity differences.

Recommended storage

Short-term storage (less than two weeks): store the column in the mobile phase.

Extended storage (longer than two weeks): store the column in filtered 100 mM sodium phosphate, pH ≤ 7, with or without 0.02% NaN_3, or 20% methanol in water. Flush the column with a minimum of 10 column volumes. To switch to or from 20% methanol, column flushing must be done at low flow rates to avoid overpressuring the column due to high viscosity. Starting at a lower flow rate, flush at no more than 0.05 mL/min for 2.1 mm id columns, 0.1 mL/min for 4.6 mm id columns, and 0.2 mL/min for 7.8 mm id columns, while also ensuring the pressure remains below 200 bar for 2.7 µm columns or 400 bar for 1.9 µm columns.

Store columns at room temperature.

Ordering details

Description	Part Number
AdvanceBio SEC 120 Å, 1.9 µm, 4.6 × 300 mm	PL1580-5250
AdvanceBio SEC 120 Å, 1.9 µm, 4.6 × 150 mm	PL1580-3250
AdvanceBio SEC 120 Å, 1.9 µm, 4.6 × 30 mm, guard	PL1580-1250
AdvanceBio SEC 120 Å, 1.9 µm, 2.1 × 150 mm, PEEK-lined SS	PL1980-3250PK
AdvanceBio SEC 120 Å, 1.9 µm, 2.1 × 50 mm, PEEK-lined SS	PL1980-1250PK
AdvanceBio SEC 200 Å, 1.9 µm, 4.6 × 300 mm	PL1580-5201
AdvanceBio SEC 200 Å, 1.9 µm, 4.6 × 150 mm	PL1580-3201
AdvanceBio SEC 200 Å, 1.9 µm, 4.6 × 30 mm, guard	PL1580-1201
AdvanceBio SEC 200 Å, 1.9 µm, 2.1 × 150 mm, PEEK-lined SS	PL1980-3201PK
AdvanceBio SEC 200 Å, 1.9 µm, 2.1 × 50 mm, PEEK-lined SS	PL1980-1201PK
AdvanceBio SEC 130 Å, 2.7 µm, 7.8 × 300 mm	PL1180-5350
AdvanceBio SEC 130 Å, 2.7 µm, 7.8 × 150 mm	PL1180-3350
AdvanceBio SEC 130 Å, 2.7 µm, 7.8 × 50 mm, guard	PL1180-1350
AdvanceBio SEC 130 Å, 2.7 µm, 4.6 × 300 mm	PL1580-5350
AdvanceBio SEC 130 Å, 2.7 µm, 4.6 × 150 mm	PL1580-3350
AdvanceBio SEC 130 Å, 2.7 µm, 4.6 × 50 mm, guard	PL1580-1350
AdvanceBio SEC 300 Å, 2.7 µm, 7.8 × 300 mm	PL1180-5301
AdvanceBio SEC 300 Å, 2.7 µm, 7.8 × 150 mm	PL1180-3301
AdvanceBio SEC 300 Å, 2.7 µm, 7.8 × 50 mm, guard	PL1180-1301
AdvanceBio SEC 300 Å, 2.7 µm, 4.6 × 300 mm	PL1580-5301
AdvanceBio SEC 300 Å, 2.7 µm, 4.6 × 150 mm	PL1580-3301
AdvanceBio SEC 300 Å, 2.7 µm, 4.6 × 50 mm, guard	PL1580-1301
AdvanceBio SEC 500 Å, 2.7 µm, 7.8 × 300 mm	PL1180-5325
AdvanceBio SEC 500 Å, 2.7 µm, 7.8 × 50 mm, guard	PL1180-1325
AdvanceBio SEC 500 Å, 2.7 µm, 4.6 × 300 mm	PL1580-5325
AdvanceBio SEC 500 Å, 2.7 µm, 4.6 × 150 mm	PL1580-3325
AdvanceBio SEC 500 Å, 2.7 µm, 4.6 × 50 mm, guard	PL1580-1325
AdvanceBio SEC 1000 Å, 2.7 µm, 7.8 × 300 mm	PL1180-5302
AdvanceBio SEC 1000 Å, 2.7 µm, 7.8 × 50 mm, guard	PL1180-1302
AdvanceBio SEC 1000 Å, 2.7 µm, 4.6 × 300 mm	PL1580-5302
AdvanceBio SEC 1000 Å, 2.7 µm, 4.6 × 150 mm	PL1580-3302
AdvanceBio SEC 1000 Å, 2.7 µm, 4.6 × 50 mm, guard	PL1580-1302
AdvanceBio SEC 130 Å protein standard, lyophilized, 1.5 mL Peptides and proteins from 1 to 45 kDa	5190-9416
AdvanceBio SEC 300 Å, protein standard, lyophilized, 1.5 mL Peptides and proteins from 1 to 670 kDa	5190-9417

See www.agilent.com for PEG, PEO, and polysaccharide molecular weight calibration standards.

www.agilent.com/chem/advancebio-sec

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