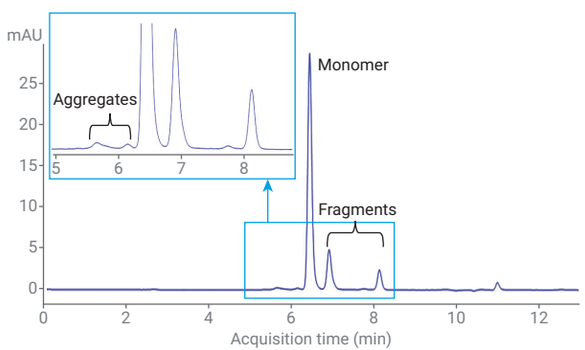
- Prepare your samples in the mobile phase, and ensure that they dissolve completely. Filter or centrifuge samples before injection.

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

Recommended starting conditions

Parameter	Value
Column	AdvanceBio SEC 200 Å, 4.6 × 300 mm, 1.9 µm (p/n PL1580-5201)
Instrument	Agilent 1260 Infinity II Bio-inert LC System
Flow Rate	0.35 mL/min
Mobile Phase	150 mM sodium phosphate, pH 7.0
Wavelength	280 nm
Column Temperature	25 °C
Sample	A stressed mAb (1 µg, injected onto column) mAb sample stressed in 100 mM sodium bicarbonate pH 9 and incubated overnight at 40 °C



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 replacing. 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 performance of the columns 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 adjust the flow rate to keep the pressure below 400 bar. 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

- **Shorter-term storage (less than two weeks):** store the column in the mobile phase.
- **Extended storage (longer than two weeks):** store in 100 mM sodium phosphate, pH 6.7 with 0.02% NaN_3 or 20% methanol in water for a minimum of 10 column volumes. Lower flow rates (0.1 mL/min) are recommended for 20% methanol flush to avoid over-pressuring the column due to high viscosity.
- Storage temperature is room temperature.

Ordering details

Description	Part Number
AdvanceBio SEC 120Å 1.9 µm, 4.6 × 300 mm	PL1580-5250
AdvanceBio SEC 120Å 1.9 µm, 4.6 × 150 mm	PL1580-3250
AdvanceBio SEC 120Å 1.9 µm guard, 4.6 × 30 mm	PL1580-1250
AdvanceBio SEC 200Å 1.9 µm, 4.6 × 300 mm	PL1580-5201
AdvanceBio SEC 200Å 1.9 µm, 4.6 × 150 mm	PL1580-3201
AdvanceBio SEC 200Å 1.9 µm guard, 4.6 × 30 mm	PL1580-1201
AdvanceBio SEC 120Å 1.9 µm, 2.1 × 150 mm PEEK	PL1980-3250PK
AdvanceBio SEC 120Å 1.9 µm, 2.1 × 50 mm PEEK	PL1980-1250PK
AdvanceBio SEC 200Å 1.9 µm, 2.1 × 150 mm PEEK	PL1980-3201PK
AdvanceBio SEC 200Å 1.9 µm, 2.1 × 50 mm PEEK	PL1980-1201PK

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Agilent AdvanceBio SEC 1.9 µm Column

User guide

Agilent size exclusion columns optimized for separation and characterization of size variants are offered in two pore sizes: Advance Bio SEC 120Å is best suited for analysis of peptides and small therapeutic proteins; AdvanceBio SEC 200Å was designed for characterizing mAbs and ADCs. In addition to 4.6 mm id stainless steel columns, AdvanceBio SEC 1.9 µm is also available in PEEK-lined stainless steel columns in 2.1 mm id. These bioinert columns provide a metal-free flow path and are particularly suited to SEC-MS applications.

Getting started

A column performance report, including a column-specific quality control (QC) test chromatogram and a batch-specific protein separation, is enclosed with every Agilent AdvanceBio SEC 200 Å 1.9 µm column. The 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 enables better evaluation of the column efficiency, and ensures 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, it is recommended that a low dispersion LC is 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.

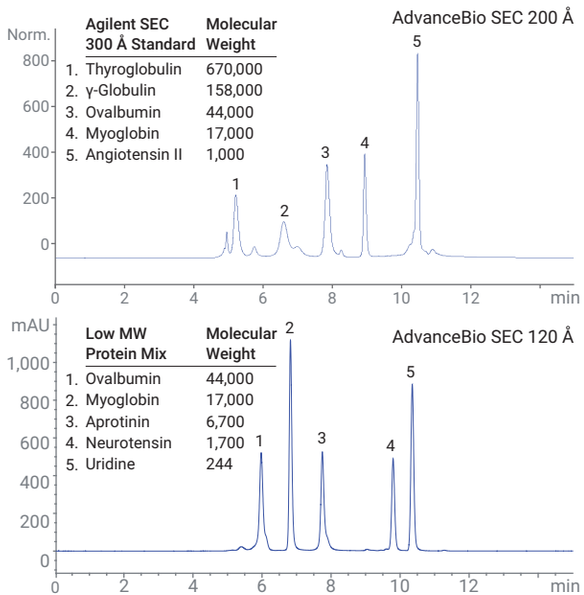
Ensuring proper column connection is important. Agilent recommends InfinityLab Quick Connect LC fittings (p/n 5067-5966), or Bio-inert UHP-FF fittings (p/n 5067-5695), particularly for use with PEEK-lined 2.1 mm id columns to avoid damage.

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

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

The AdvanceBio SEC 200 Å 1.9 µm columns are recommended for use with UV, DAD, LS detectors, and for SEC-MS applications under denaturing and native mode.

Parameter	Value
Columns	AdvanceBio SEC 200Å, 4.6 × 300 mm, 1.9 µm (PL1580-5201) AdvanceBio SEC 120Å, 4.6 × 300 mm, 1.9 µm (PL1580-5250)
Flow Rate	0.35 mL/min
Mobile Phase	150 mM sodium phosphate, pH 7.0
Wavelength	220 nm
Column Temperature	Ambient
Samples	AdvanceBio SEC 300 Å Protein Standard (p/n 5190-9417); Low MW Protein Mix (Ovalbumin 5.0 mg, Myoglobin 5.1 mg, Aprotinin 5.1 mg, Neurotensin 2.3 mg, and Uridine 2.4 mg) in 1 mL mobile phase



Important safety considerations

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

Using your column

Column specifications

Parameter	Value
Shipping Solvent/Long Term Storage Solution	pH 6.7 100 mM sodium phosphate buffer with 0.02 % NaN_3
Working Flow Rate	for 4.6 × 150 mm 0.1 to 0.7 mL/min for 4.6 × 300 mm 0.1 to 0.5 mL/min for 2.1 mm id columns 0.05 to 0.10 mL/min
Maximum Pressure	620 bar (9,000 psi)
pH Stability	2 to 8
Salt Concentration	≤0.5 M
Mobile Phase Compatibility	Compatible with all the SEC mobile phases for UV. Phosphate buffer, pH 7.0 with different salt concentrations and denaturing and native mode SEC-MS mobile phases.
Operating Temperature	20 to 40 °C (recommended), 80 °C (maximum)

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

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 (p/n 5067-5966) to quickly connect the column to your LC instrument, or Bio-inert UHP-FF fittings (p/n 5067-5695) for use with PEEK-lined 2.1 mm ID columns.

Column conditioning

- The columns are shipped in 100 mM sodium phosphate buffer, pH 6.7, containing 0.02% NaN_3, and must first be flushed into the mobile phase required for your separation. Ramp the flow rate slowly from 0.0 mL/min up 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. Salt concentration should not be more than 0.5 M. It is recommended that the percentage of organic solvent be less than 50%. 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, always take into account the viscosity and risk of salt precipitation. If you are unsure, flush the column first with high-purity water before introducing a new eluent.
- 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.