

# Agilent AdvanceBio SEC 1.9 $\mu\text{m}$ Columns



## Introduction

Agilent UPLC size exclusion columns optimized for separation and characterization of size variants are offered in two pore sizes: AdvanceBio SEC 120 Å is best suited for analysis of peptides and small therapeutic proteins; AdvanceBio SEC 200 Å was designed for characterizing mAbs and ADCs. In addition to 4.6 mm id stainless steel columns, AdvanceBio SEC 1.9  $\mu\text{m}$  is also available in PEEK-lined stainless steel columns in 2.1 mm id. These bio-inert 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 1.9  $\mu\text{m}$  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.

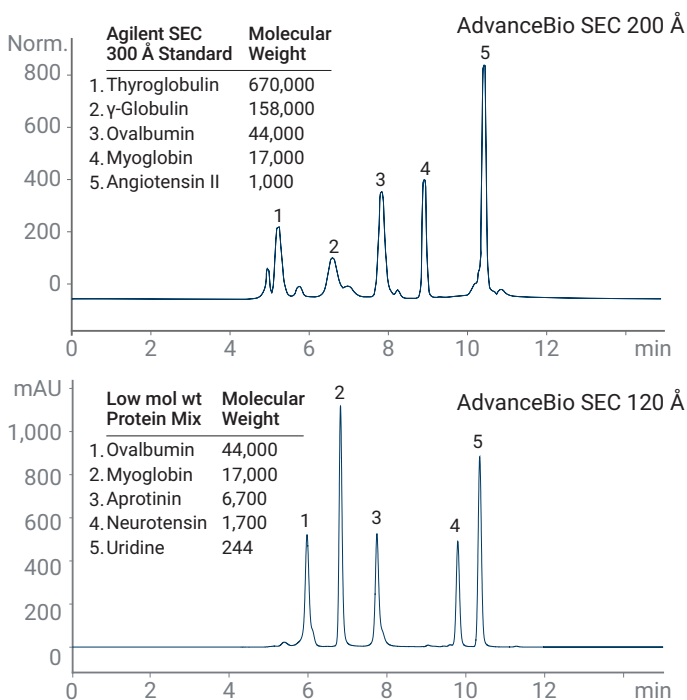
Ensuring proper column connection is important. Agilent recommends InfinityLab Quick Connect LC fittings (p/n 5067-5966), or Bio-inert UHP-FF fittings (p/n 5067-5695), particularly for use with PEEK-lined 2.1 mm id columns to avoid damage.

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

The AdvanceBio SEC 1.9  $\mu\text{m}$  columns are recommended for use with UV, DAD, LS detectors, and for SEC-MS applications under denaturing and native mode.

If you have specific questions, contact Agilent technical support at [www.agilent.com/chem/techsupport](http://www.agilent.com/chem/techsupport).

Parameter	Value
Columns	AdvanceBio SEC 200Å, 4.6 × 300 mm, 1.9 $\mu\text{m}$ (PL1580-5201) AdvanceBio SEC 120Å, 4.6 × 300 mm, 1.9 $\mu\text{m}$ (PL1580-5250)
Flow Rate	0.35 mL/min
Mobile Phase	150 mM sodium phosphate, pH 7.0
Wavelength	220 nm
Column Temperature	Ambient



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

## 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

### Column specifications

Parameter	Value
Shipping Solvent/Long-Term Storage Solution	pH 6.7 100 mM sodium phosphate buffer with 0.02% NaN <sub>3</sub>
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
Injection Volume	1 to 5 µL (recommended) Maximum 1% column volume
Maximum Pressure	620 bar (9,000 psi)
pH Stability	2 to 8.5
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 (part number 5067-5966) to quickly connect the column to your LC. 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 are shipped in 100 mM sodium phosphate buffer, pH 6.7, containing 0.02% NaN<sub>3</sub>, and 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. Salt concentration should not be more than 0.5 M. It is recommended that the percentage of organic solvent

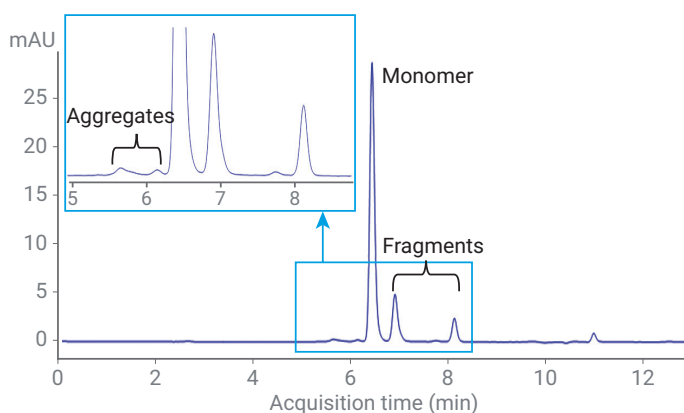
be less than 50%. For native mode SEC-MS, ammonium acetate is recommended. Flush the column extensively before connecting to your MS detector in case nonvolatile mobile phase salts are still present. When changing eluents, always consider the viscosity and risk of salt precipitation. If you are unsure, flush the column first with high-purity water before introducing a new eluent.

- 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.
- 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.

## Recommended starting conditions

Parameter	Value
Column	Agilent 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



**Figure 2.** Example separation of a stressed IgG sample, showing monomer, aggregates, and fragments.

## Column care and cleaning

### Column care

An increase in backpressure and a 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<sub>2</sub>SO<sub>4</sub>, 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

**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<sub>3</sub>, 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 0.1 mL/min for 4.6 mm columns, and no more than 0.05 mL/min for 2.1 mm columns, being sure to keep the pressure below 400 bar.

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

Please see [www.agilent.com](http://www.agilent.com) for PEG, PEO, and polysaccharide mol wt calibration standards.

[www.agilent.com/en/product/biopharma-hplc-analysis/aggregate-fragment-analysis/advancebio-sec](http://www.agilent.com/en/product/biopharma-hplc-analysis/aggregate-fragment-analysis/advancebio-sec)

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