Operating parameters

Parameter	Value
Mobile Phase Compatibility	150 mM phosphate buffer, pH 7.0 (recommended starting conditions) Other aqueous buffers with high and low salt can be used. Mixtures of water and acetonitrile can be used. (Check solubility of buffer components and system pressure.)
pH Stability	2 to 8.5
Operating Temperature	20–30 °C (recommended), 80 °C (maximum)
Typical Operating Pressure	< 200 bar (2,900 psi) (single column)
Maximum Pressure	400 bar (5,800 psi)
Recommended Flow Rate	0.1 to 2.0 mL/min for 7.8 mm i.d. columns 0.1 to 0.7 mL/min for 4.6 mm i.d. columns For two columns in series, lower flow rates may be necessary to ensure maximum pressure does not exceed 400 bar (5,800 psi).
Injection volume	5–10µL (recommended) Maximum 1% column volume

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

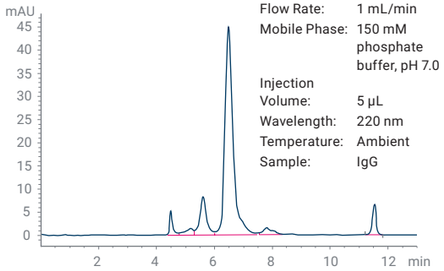
Column care and cleaning

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.

Recommended starting conditions

Agilent AdvanceBio SEC 300Å, 2.7 µm, 7.8 x 300 mm (p/n PL1180-5301)



High-resolution separation of an IgG sample, showing the monomer, aggregates, and degradation products.

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. 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 0.1 mL/min for 4.6 mm columns, and no more than 0.2 mL/min for 7.8 mm columns, while also ensuring the pressure remains below 200 bar.

Store columns at room temperature.

Ordering details

Description	Part Number
AdvanceBio SEC 300 Å, 2.7 µm, 7.8 x 300 mm	PL1180-5301
AdvanceBio SEC 300 Å, 2.7 µm, 7.8 x 150 mm	PL1180-3301
AdvanceBio SEC 300 Å, 2.7 µm guard, 7.8 x 50 mm	PL1180-1301
AdvanceBio SEC 300 Å, 2.7 µm, 4.6 x 300 mm	PL1580-5301
AdvanceBio SEC 300 Å, 2.7 µm, 4.6 x 150 mm	PL1580-3301
AdvanceBio SEC 300 Å, 2.7 µm guard, 4.6 x 50 mm	PL1580-1301
AdvanceBio SEC 130 Å, 2.7 µm, 7.8 x 300 mm	PL1180-5350
AdvanceBio SEC 130 Å, 2.7 µm, 7.8 x 150 mm	PL1180-3350
AdvanceBio SEC 130 Å, 2.7 µm guard, 7.8 x 50 mm	PL1180-1350
AdvanceBio SEC 130 Å, 2.7 µm, 4.6 x 300 mm	PL1580-5350
AdvanceBio SEC 130 Å, 2.7 µm, 4.6 x 150 mm	PL1580-3350
AdvanceBio SEC 130 Å, 2.7 µm guard, 4.6 x 50 mm	PL1580-1350
InfinityLab Quick Connect capillary, stainless steel, 150 mm, 0.12 mm, nonswaged Swagelok fitting	5500-1172
AdvanceBio SEC 130 Å protein standard, lyophilized, 1.5 mL	5190-9416
AdvanceBio SEC 300 Å protein standard, lyophilized, 1.5 mL	5190-9417

Please see www.agilent.com for PEG, PEO, and polysaccharide mol wt calibration standards.

www.agilent.com/chem/advancebio

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Agilent AdvanceBio SEC 2.7 µm Columns

User guide

Size exclusion columns for analysis of biomolecules, including monoclonal antibodies, proteins, and peptides.

Agilent AdvanceBio SEC columns are designed and manufactured by Agilent for size exclusion chromatography of biomolecules.

The innovative, high-porosity 2.7 µm silica particles and unique hydrophilic bonding chemistry provide for exceptional stability with minimal nonspecific interactions.

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.

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

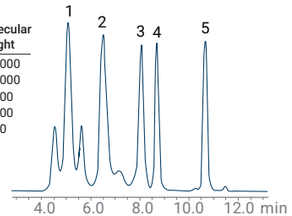
Agilent AdvanceBio SEC 2.7 µm columns are recommended for use with UV, DAD, and LS detectors.

Agilent AdvanceBio SEC 1.9 µm or Agilent Bio SEC-3 and Agilent Bio SEC-5 columns are recommended for SEC-MS with denaturing or aqueous mobile phases.

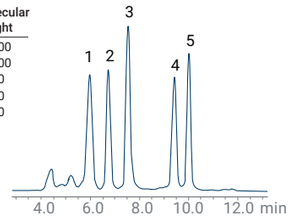
If you have specific questions, contact Agilent technical support at www.agilent.com/chem/techsupport.

Parameter	Value
Columns	AdvanceBio SEC 300Å, 4.6 × 300 mm, 2.7 µm (PL1580-5301) AdvanceBio SEC 130Å, 4.6 × 300 mm, 2.7 µm (PL1580-5350)
Flow Rate	0.35 mL/min
Mobile Phase	150 mM sodium phosphate, pH 7.0
Wavelength	220 nm
Column Temperature	30 °C

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



Agilent SEC 130Å Standard	Molecular Weight
1. Thyroglobulin	45,000
2. γ-Globulin	17,000
3. Ovalbumin	6,700
4. Myoglobin	1,700
5. Angiotensin II	1,000



Example chromatograms of protein and peptide batch-specific tests.

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 (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 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, 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.