Operating Parameters

Operating Parameters	
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)
Working flow rate	0.1 to 2.0 mL/min for 7.8 mm i.d. columns (recommended) 0.1 to 0.7 mL/min for 4.6 mm i.d. columns (recommended) *For two columns in series, lower flow rates may be necessary to ensure maximum pressure does not exceed 400 bar (5,800 psi).*
Working at extremes of the operating parameters is likely to reduce column lifetime.	

Recommended Starting Conditions

Column:	Agilent AdvanceBio SEC 300Å, 2.7 µm, 7.8 x 300 mm (p/n PL1180-5301)		
Flow rate:	1 mL/min		
Mobile phase:	150 mM phosphate buffer, pH 7.0		
Wavelength:	220 nm	Injection volume:	5 µL
Temperature:	ambient	Sample:	IgG

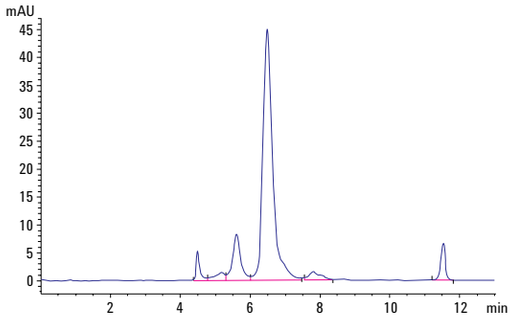

Figure 2: *High-resolution separation of an IgG sample, showing the monomer, aggregates and degradation products.*

Column Care

An increase in back pressure and decrease in performance may occur over time. If the pressure has increased, first identify if this is due to the instrument or columns. If the increase in pressure is in a system component, such as tubing or filter, replace and retest.

Column Cleaning Instructions

Where the performance has deteriorated below the acceptance criteria, test each column individually to identify which column has deteriorated. It may be possible to restore performance of the identified columns using one of the cleaning solutions detailed below.

- Chaotropes for strongly adsorbed contaminants – 500 mM Na_2SO_4 or 500 mM 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 200 bar. Rinse with at least 5 column volumes of ultra-pure 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 over-pressuring of the column.

Recommended Storage Conditions

Whenever a column is not installed on the LC tightly seal both ends of the column with the removable end plugs supplied with the column. For short term storage of less than one week, store the column in the mobile phase. For extended storage of longer than one week, first flush the column with water to remove buffer salts, and then flush the column with water containing 0.02 % NaN_3 (sodium azide) or 20 % ethanol for a minimum of 10 column volumes. Recommended storage temperature is 4 to 35°C.

Ordering Details

Part Number	Description
PL1180-5301	AdvanceBio SEC 300Å, 2.7 µm, 7.8 x 300 mm
PL1180-3301	AdvanceBio SEC 300Å, 2.7 µm, 7.8 x 150 mm
PL1180-1301	AdvanceBio SEC 300Å, 2.7 µm, 7.8 x 50 mm
PL1580-5301	AdvanceBio SEC 300Å, 2.7 µm, 4.6 x 300 mm
PL1580-3301	AdvanceBio SEC 300Å, 2.7 µm, 4.6 x 150 mm
PL1580-1301	AdvanceBio SEC 300Å, 2.7 µm, 4.6 x 50 mm
PL1180-5350	AdvanceBio SEC 130Å, 2.7 µm, 7.8 x 300 mm
PL1180-3350	AdvanceBio SEC 130Å, 2.7 µm, 7.8 x 150 mm
PL1180-1350	AdvanceBio SEC 130Å, 2.7 µm, 7.8 x 50 mm
PL1580-5350	AdvanceBio SEC 130Å, 2.7 µm, 4.6 x 300 mm
PL1580-3350	AdvanceBio SEC 130Å, 2.7 µm, 4.6 x 150 mm
PL1580-1350	AdvanceBio SEC 130Å, 2.7 µm, 4.6 x 50 mm
5500-1172	A-Line capillary, stainless steel, 150 mm, 0.12 mm, non-swaged Swagelok fitting
5190-9416	AdvanceBio SEC 130Å Protein Standard, lyophilized, 1.5 mL
5190-9417	AdvanceBio SEC 300Å Protein Standard, lyophilized, 1.5 mL

Please see **www.agilent.com** for PEG, PEO, and polysaccharide MW calibration standards.

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USER GUIDE

for Agilent AdvanceBio SEC Columns

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

Agilent Technologies

Agilent Technologies

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 enables a better evaluation of the column efficiency and assures a more consistent product. An optimized LC system will generate similar results to the chromatogram on your Column Performance Report.

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

The Agilent AdvanceBio SEC columns are recommended for use with UV, DAD and LS detectors but not for SEC-MS applications.

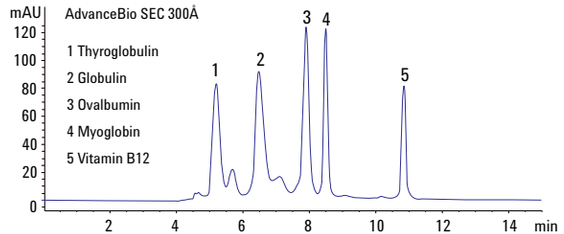
The Agilent Bio SEC-3 and Agilent Bio SEC-5 columns are recommended for SEC-MS with denaturing and aqueous mobile phases.

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

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 fittings.

Column: Agilent AdvanceBio SEC 300Å, 2.7 µm, 4.6 x 300 mm (p/n PL1580-5301)
Flow rate: 0.35 mL/min
Mobile phase: 150 mM phosphate buffer, pH 7.0
Wavelength: 220 nm
Temperature: 30 °C
Injection volume: 5 µL
Sample concentration: 3.6 mg/mL



Column: Agilent AdvanceBio SEC 130Å, 2.7 µm, 4.6 x 300 mm, (p/n PL1580-5350)
Flow rate: 0.35 mL/min
Mobile phase: 150 mM phosphate buffer, pH 7.0
Wavelength: 220 nm
Temperature: 30 °C
Injection volume: 5 µL
Sample concentration: 3.6 mg/mL

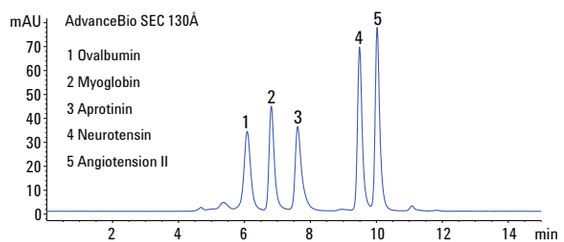


Figure 1: *Example chromatograms of protein and peptide batch-specific tests .*

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 Agilent A-Line Quick Connect fittings to quickly connect the column to your HPLC or UHPLC instrument. No tools are needed to make leak-free connections that fit the column perfectly.

Column Conditioning

The columns are shipped with water containing 0.02 % sodium azide and must first be flushed into the mobile phase required for your separation. Starting at a low flow rate such as 0.1 mL/min and gradually increase the flow until you reach the intended operating flow rate. Equilibrate the column by flushing for a minimum of ten column volumes (approximately 2.5 hours) or until the baseline is stable.

Instructions for Use

Agilent AdvanceBio SEC columns are compatible with commonly used aqueous buffers, including 150 mM sodium phosphate at pH 7.0 or PBS (phosphate buffered saline). When changing eluents, always take the viscosity and risk of salt precipitation into account. 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 prior to use. This will remove particulates and help reduce the risk of bacterial growth, which will otherwise damage the column and your UHPLC or HPLC system.

Prepare your samples in the mobile phase and make sure they dissolve completely. Filter or centrifuge samples before injection..

To maximize the lifetime of your column, we recommend to use an Agilent AdvanceBio SEC guard column.