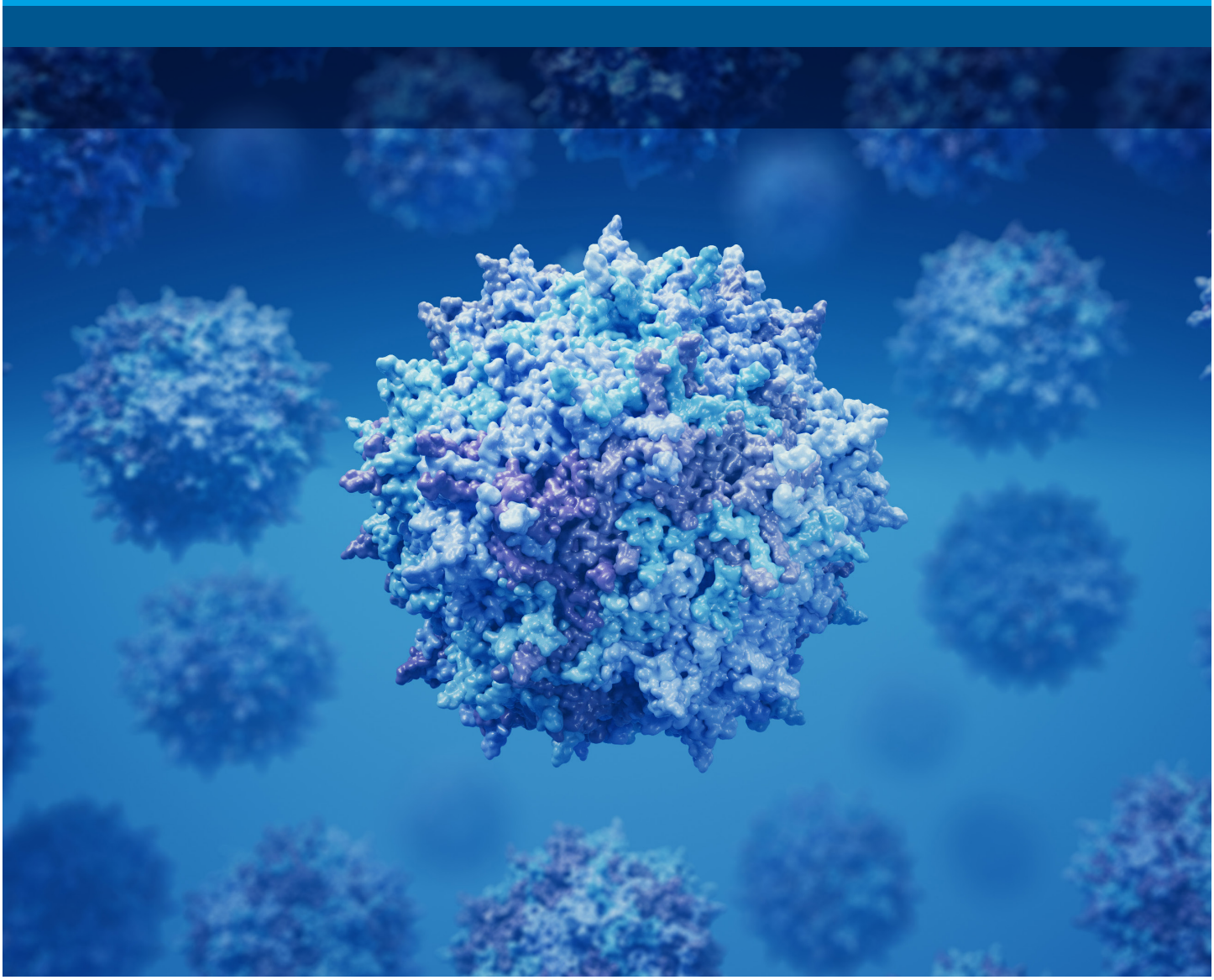
Agilent

Trusted Answers

High-Speed, High-Resolution Aggregate Analysis of Ultralarge Biotherapeutics

Agilent AdvanceBio SEC 2.7 µm columns with 500 and 1000 Å pores



Optimal pore sizes for your ultralarge biotherapeutics analyses

Adeno-associated viruses (AAV), virus-like particles (VLP), oligonucleotides, and lipid nanoparticles (LNP) represent the next generation of biotherapeutics for cell and gene therapy, as well as vaccines. Characterizing these novel and challenging biotherapeutics requires advanced and capable technology.

While maintaining all the best attributes of earlier Agilent AdvanceBio SEC columns, the new 500 and 1000 Å pore sizes now provide suitability for samples of larger diameter, making them ideal for your aggregation analyses of ultralarge biomolecules (> 1 MDa) such as AAVs and VLPs.

Achieve reliable results with time to spare

Working in discovery, biomolecule characterization, and quality control of biopharmaceuticals, you need a robust column that provides high-resolution results, fast.

Our AdvanceBio SEC columns containing small 2.7 µm particles with large pore volume enable high-resolution separations on a shorter column (Figure 1), which increases throughput and frees up more time—so you can run more samples or move onto your next project.

	HPLC Conditions
Column	AdvanceBio SEC 500 Å, 2.7 µm, 4.6 x 150 mm (p/n PL1580-3325)
Mobile Phase	50 mM sodium phosphate + 400 mM NaCl, pH 7.2
Flow Rate	0.35 mL/min
Detection	Fluorescence λex 280 nm, λem 348 nm
HPLC System	Agilent 1290 Infinity II Bio LC System with Binary High-Speed Pump
Sample	AAV8, 5 µL injection

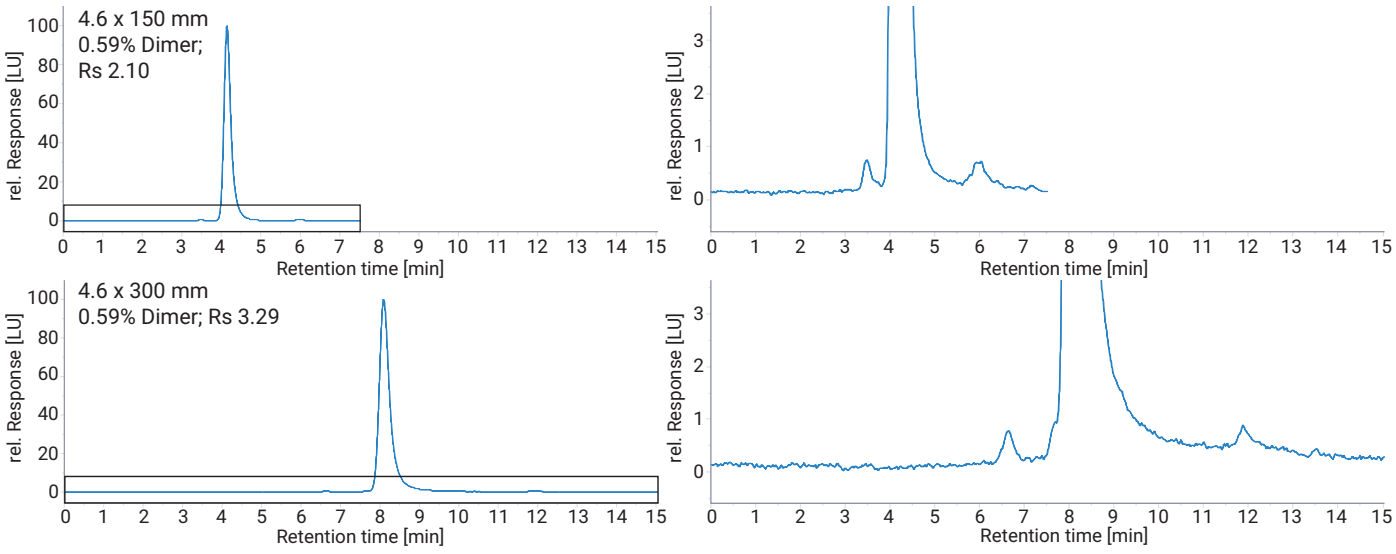


Figure 1. Higher resolution under typical operating conditions with a 300 mm Agilent AdvanceBio SEC 2.7 µm, 500 Å column enables users to switch to a shorter 150 mm column and still have sufficient resolution for a reliable measurement while completing the analysis in half the time.

Minimize interactions for good peak shape and state-of-the-art resolution

Equipped with the familiar hydrophilic chemistry of our previous AdvanceBio SEC columns, secondary interactions are minimized between the sample and stationary phase. This provides good peak shape that lends confidence to size measurements, and helps ensure good resolution (Figure 2).

	HPLC Conditions
Column	AdvanceBio SEC 500 Å, 2.7 µm, 4.6 x 300 mm (p/n PL1580-5325)
Mobile Phase	50 mM sodium phosphate + 400 mM NaCl, pH 7.2
Flow Rate	0.35 mL/min
Detection	Fluorescence λex 280 nm, λem 348 nm
HPLC System	Agilent 1290 Infinity II Bio LC System with Binary High-Speed Pump

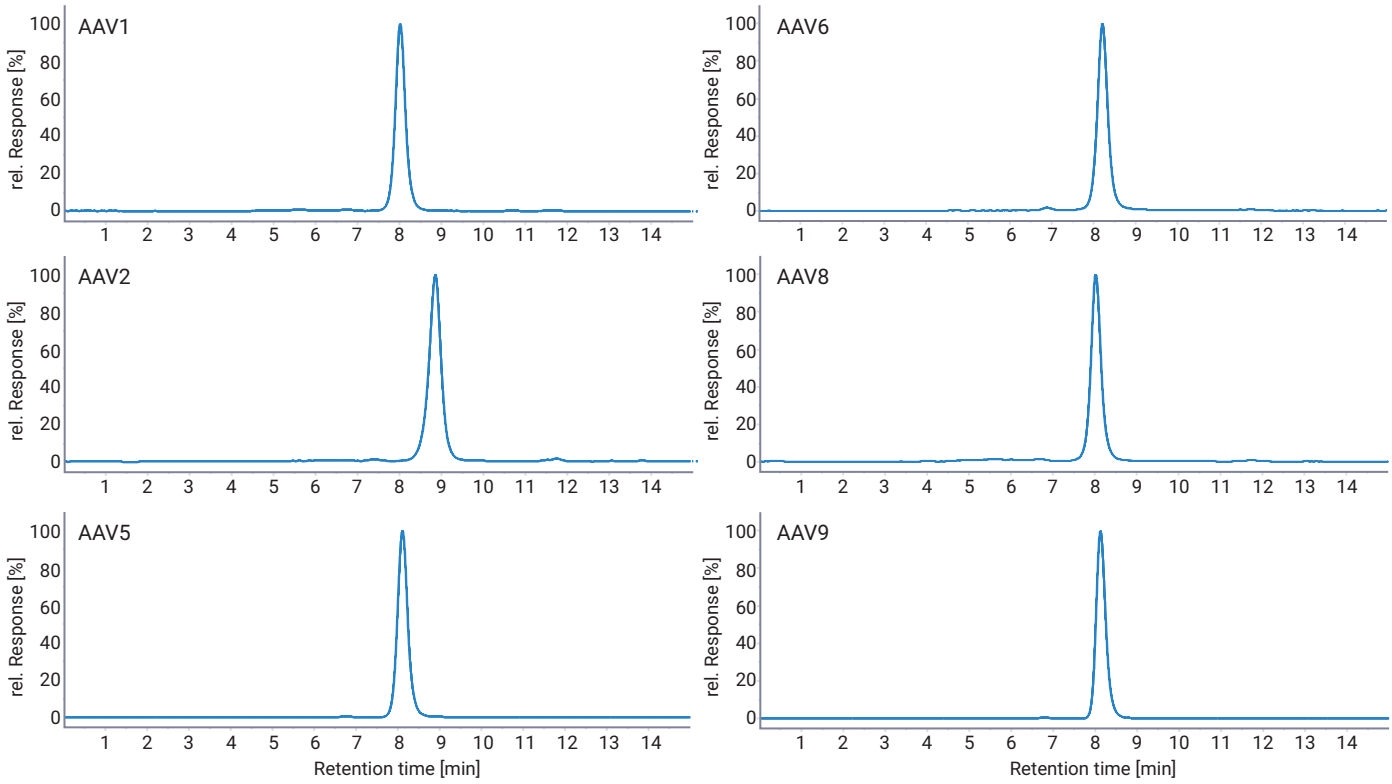


Figure 2. Agilent AdvanceBio SEC 2.7 µm, 500 Å columns enable high-resolution separations of AAVs for a variety of serotypes.

Have more confidence in your results with batch-to-batch reproducibility

AdvanceBio SEC columns have high batch-to-batch reproducibility, providing you with reliable results every time, as well as more reproducible peak integration (Figures 3 and 4).

PEG/PEO calibration curves illustrate the consistency of the pore size and structure from batch to batch, while testing with AAV or VLP ensures that consistency translates to relevant biological samples.

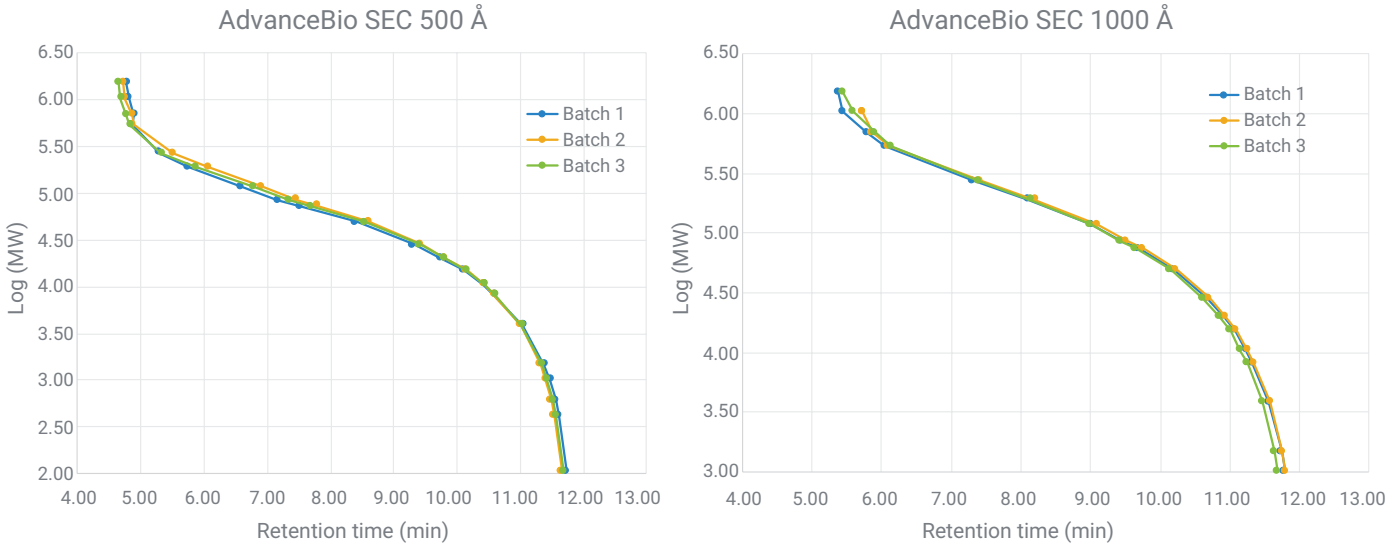
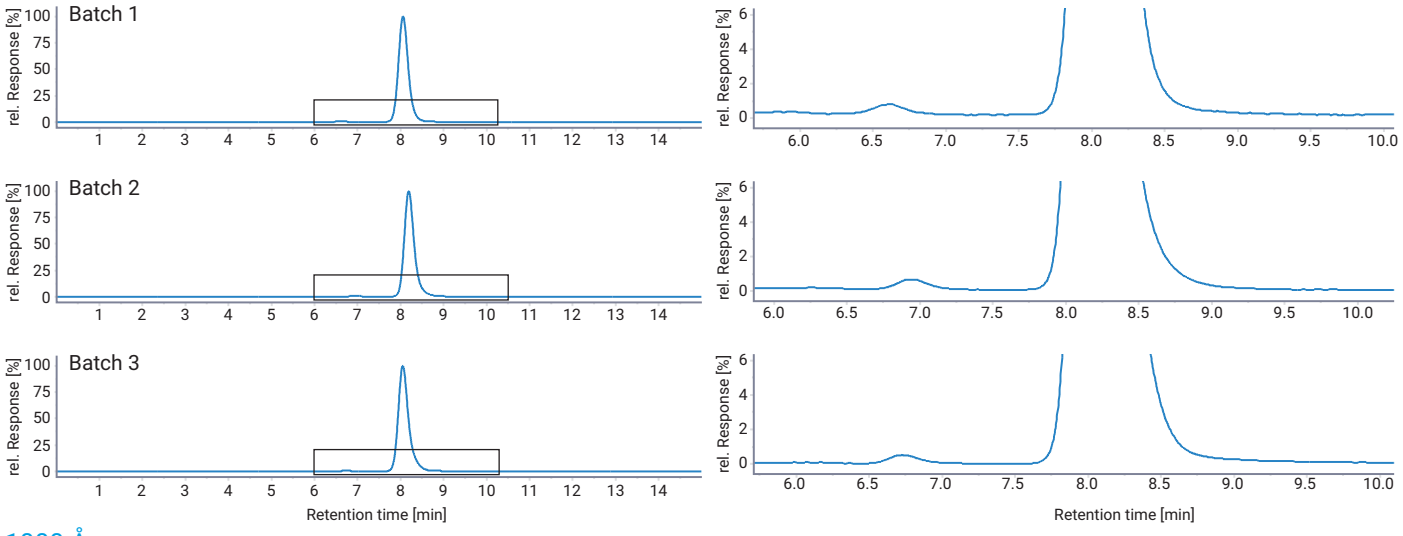


Figure 3. A series of PEG/PEO polymer standards was used to probe the separation range of the Agilent AdvanceBio SEC 500 Å and 1000 Å pores. Overlaying the resulting curves demonstrates excellent batch-to-batch reproducibility.

	HPLC Conditions
Column	AdvanceBio SEC 500 Å, 2.7 µm, 4.6 x 150 mm (p/n PL1580-3325)
Mobile Phase	50 mM sodium phosphate + 400 mM NaCl, pH 7.2
Flow Rate	0.35 mL/min
Detection	Fluorescence λex 280 nm, λem 348 nm
HPLC System	Agilent 1290 Infinity II Bio LC System with Binary High-Speed Pump

500 Å



1000 Å

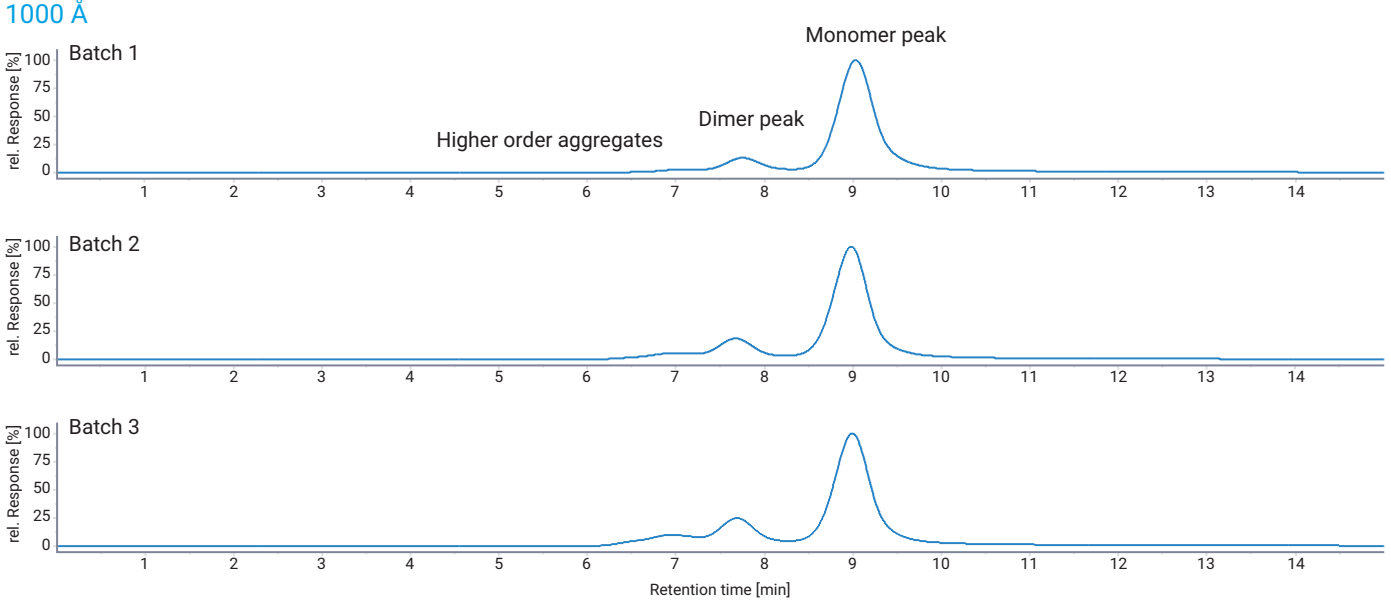


Figure 4. Batch-to-batch reproducibility was tested with AAV2 on the Agilent AdvanceBio SEC 500 Å column and a ~ 50 nm diameter VLP on the AdvanceBio SEC 1000 Å column.

Save costs by replacing columns less often

AdvanceBio SEC columns are complete with robust particles and uniform packing, extending column lifetime—so you can save money and hassle by replacing them less frequently (Figure 5). Column lifetime was tested by injecting a uridine standard, and column efficiency and system backpressure were maintained through the test up to at least 1000 injections.

	HPLC Conditions
Column	AdvanceBio SEC 500 Å, 2.7 µm, 4.6 x 300 mm (p/n PL1580-5325) AdvanceBio SEC 1000 Å, 2.7 µm, 4.6 x 300 mm (p/n PL1580-5302)
Mobile Phase	150 mM sodium phosphate, pH 7
Flow Rate	0.35 mL/min
Detection	UV 220 nm
HPLC System	Agilent 1260 Infinity II Bio LC System
Sample	2 µL injection of 1 mg/mL uridine

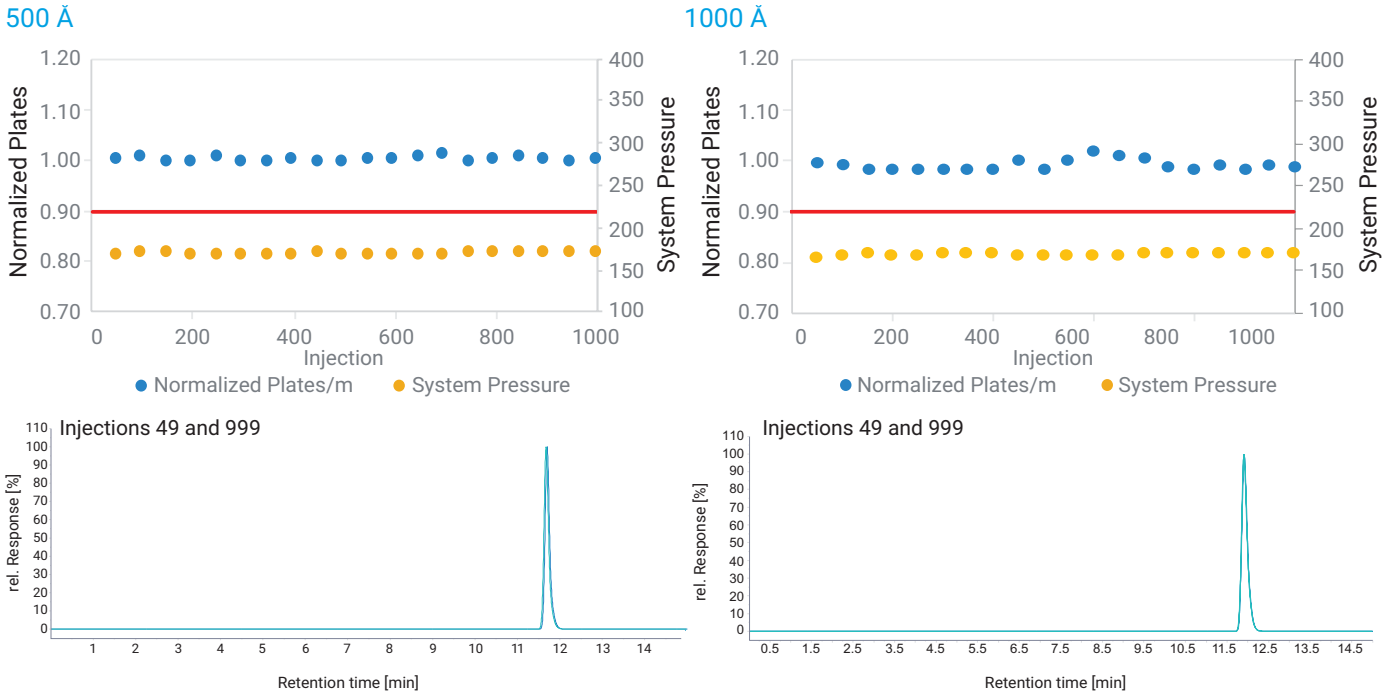


Figure 5. Agilent AdvanceBio SEC columns are mechanically robust with a stable hydrophilic coating that minimizes secondary interactions. Even after 1,000 injections, column performance is maintained. The red line indicates a 10% decrease in efficiency.

Pair your AdvanceBio SEC columns with a variety of detection techniques

While UV detection is commonly used for traditional SEC analysis, new and challenging ultralarge biotherapeutics like AAVs and VLPs usually require the higher sensitivity of fluorescence detection, or the additional molecular weight and size information available from light scattering detection.

Light scattering is particularly sensitive to background noise, such that many columns require extensive flushing in addition to highly filtered mobile phases. However, AdvanceBio SEC columns show suitably low background levels after standard column conditioning with the operating mobile phase. This makes them ideal for light scattering detection, as well as suitable for other detection techniques such as fluorescence or mass spectrometry (Figure 6).

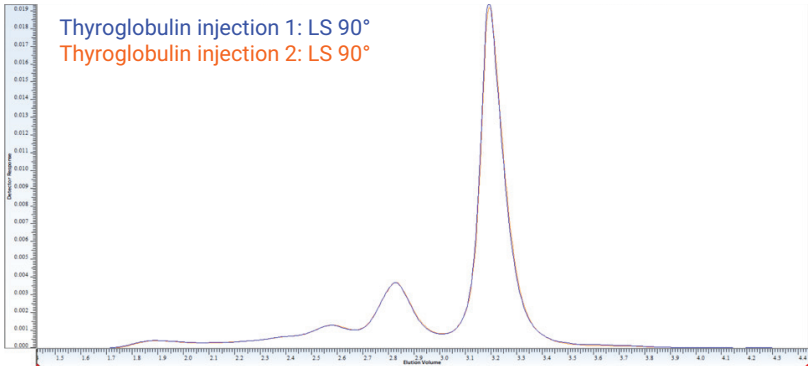


Figure 6. With appropriately filtered mobile phase, Agilent AdvanceBio SEC columns show low background signal, making them compatible with detection techniques such as light scattering, which is shown above with low background noise for two replicate injections of thyroglobulin on a 4.6 x 300 mm AdvanceBio SEC 500 Å column on an Agilent 1260 Infinity II bio-inert LC with multi-angle light scattering detection.

Maximize data analysis robustness and results reliability

Compared to competitor columns, AdvanceBio SEC columns demonstrate excellent resolution and tailing (Figure 7). This state-of-the-art resolution enables more robust data analysis and reliable results.

	HPLC Conditions
Column	AdvanceBio SEC 500 Å, 2.7 µm, 4.6 x 300 mm (p/n PL1580-5325) AdvanceBio SEC 1000 Å, 2.7 µm, 4.6 x 300 mm (p/n PL1580-5302) Competitor 1 700 Å, 3 µm, 4.6 x 300 mm Competitor 2 450 Å, 2.5 µm, 4.6 x 300 mm Competitor 2 1000 Å, 3 µm, 4.6 x 300 mm
Mobile Phase	50 mM sodium phosphate + 400 mM NaCl, pH 7.2
Flow Rate	0.35 mL/min
Detection	Fluorescence λex 280 nm, λem 348 nm
HPLC System	Agilent 1260 Infinity II Bio LC System with Binary High-Speed Pump

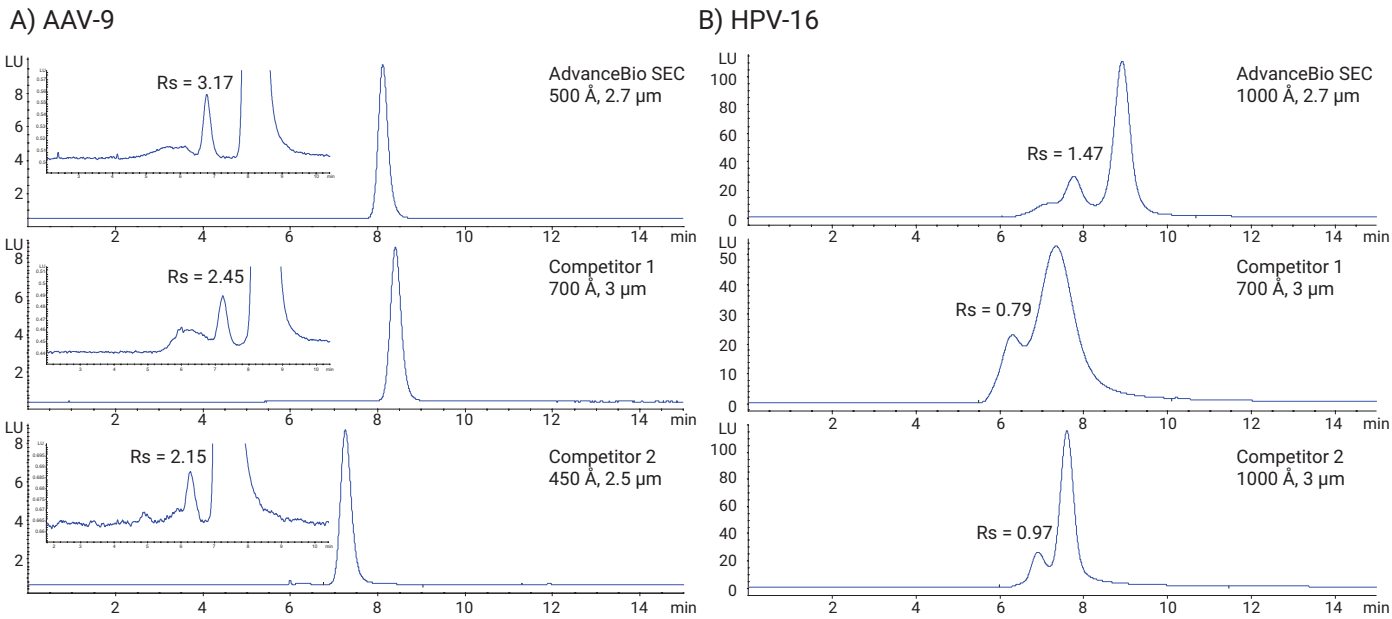


Figure 7. Agilent AdvanceBio SEC columns show enhanced resolution and tailing versus competitor columns.

Choosing an SEC column for your application

Choosing the correct pore size is often the most critical aspect of SEC column selection. While molecular weight is commonly used to determine a suitable pore size depending on analyte size, it is ultimately the hydrodynamic radius—the size of the sample in solution—that matters. Oligonucleotides, however, are much more elongated than proteins of the same molecular weight, requiring a larger pore size.

AdvanceBio SEC 2.7 µm columns are an extension of the AdvanceBio SEC portfolio. The recommendations in Figure 8 can help you choose which AdvanceBio SEC column is most suitable for your sample.

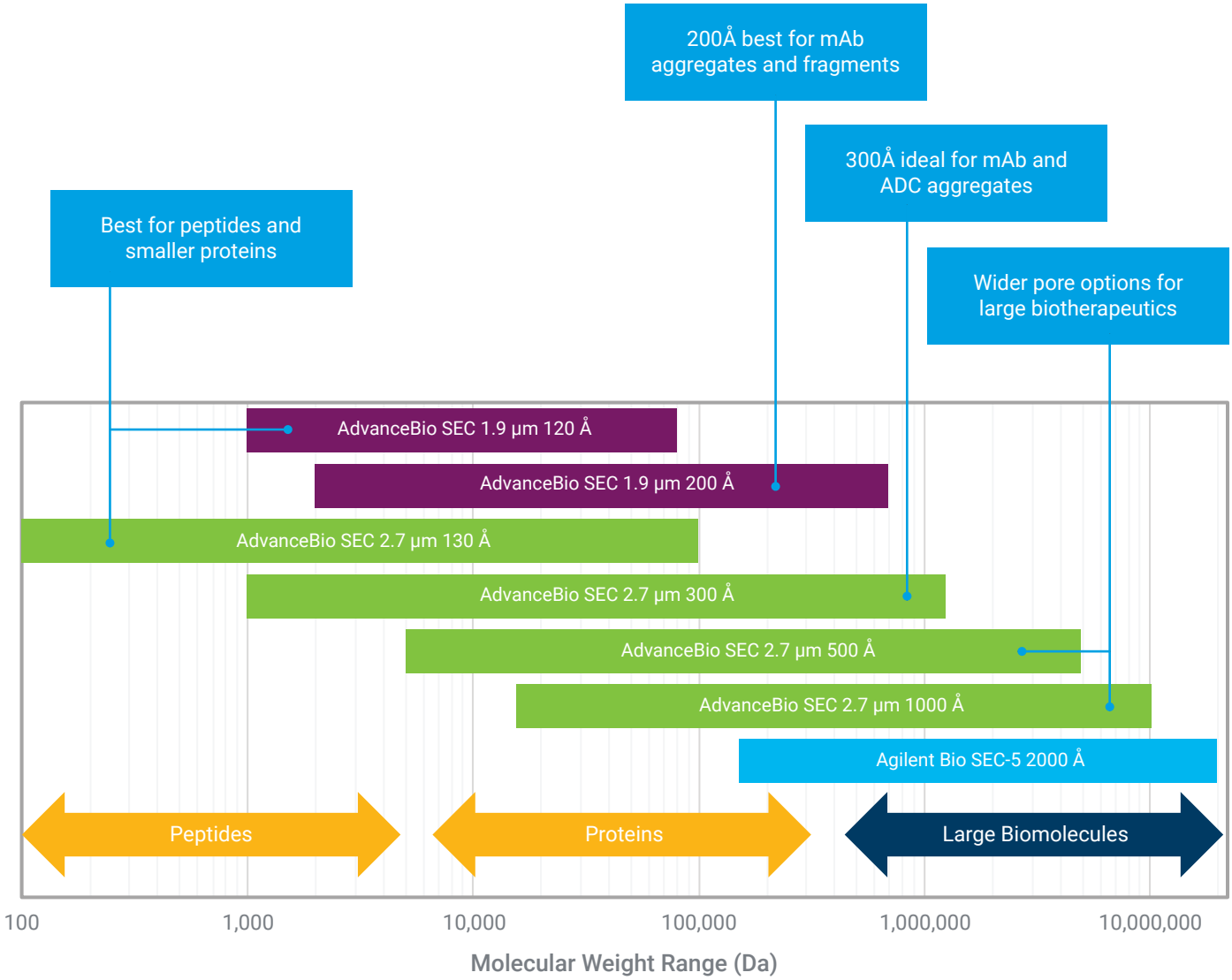


Figure 8. Agilent AdvanceBio SEC column recommendations based on your application.

Ordering information

Description	Part Number
AdvanceBio SEC 500 Å, 2.7 µm, 7.8 x 300 mm	PL1180-5325
AdvanceBio SEC 500 Å, 2.7 µm, 7.8 x 50 mm, guard	PL1180-1325
AdvanceBio SEC 500 Å, 2.7 µm, 4.6 x 300 mm	PL1580-5325
AdvanceBio SEC 500 Å, 2.7 µm, 4.6 x 150 mm	PL1580-3325
AdvanceBio SEC 500 Å, 2.7 µm, 4.6 x 50 mm, guard	PL1580-1325
AdvanceBio SEC 1000 Å, 2.7 µm, 7.8 x 300 mm	PL1180-5302
AdvanceBio SEC 1000 Å, 2.7 µm, 7.8 x 50 mm, guard	PL1180-1302
AdvanceBio SEC 1000 Å, 2.7 µm, 4.6 x 300 mm	PL1580-5302
AdvanceBio SEC 1000 Å, 2.7 µm, 4.6 x 150 mm	PL1580-3302
AdvanceBio SEC 1000 Å, 2.7 µm, 4.6 x 50 mm, guard	PL1580-1302

Please see www.agilent.com/chem/advancebio-sec for other AdvanceBio SEC pore sizes.

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U.S. and Canada
1-800-227-9770
agilent_inquiries@agilent.com

Europe
info_agilent@agilent.com

Asia Pacific
inquiry_lsca@agilent.com

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