

# High-Resolution, High-Throughput Size Exclusion Chromatography Analysis of Monoclonal Antibodies

Using an Agilent AdvanceBio SEC 200 Å 1.9 µm  
column

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## Abstract

This Application Note demonstrates the use of the Agilent AdvanceBio SEC 200 Å 1.9 µm column for high-resolution and high-throughput size exclusion chromatography (SEC) analysis of a monoclonal antibody (mAb). The optimized sub-2 µm particle enables faster separations and uncompromised high resolution for accurate quantitation.

## Introduction

Aggregates and fragments are critical quality attributes of biotherapeutic proteins that need to be well characterized. Size exclusion chromatography (SEC) is commonly used to analyze these size variants. There are cases where high-throughput SEC analysis is in high demand. Examples are in the early stage of drug development during clone selection, or process development, where large numbers of samples need to be analyzed daily. The AdvanceBio SEC 200 Å 1.9 µm column, with its unique, durable sub-2 µm particles, offers fast analysis with high resolution. These features significantly improve sample throughput, while delivering robust and accurate results.

## Experimental

### Materials

SILu Lite SigmaMAb universal antibody standard was purchased from MilliporeSigma and reconstituted with water to 1 mg/mL. Monobasic and dibasic sodium hydrogen phosphate and sodium chloride were purchased from MilliporeSigma. All chemicals used were ≥99.5 % pure. Water was purified from a Milli-Q A10 water purification system (Millipore). Solutions were prepared fresh daily, and filtered through a 0.22 µm membrane filter prior to use.

## Instrumentation

### LC system

An Agilent 1260 Infinity LC with the following configuration was used:

- Agilent 1260 Infinity II bio-inert quaternary pump (G5654A)
- Agilent 1260 Infinity II bio-inert multisampler (G5668A) with sample cooler (option #100)
- Agilent 1260 Infinity II multicolumn thermostat (G7116A) with bio-inert heat exchanger (option #019)
- Agilent 1260 Infinity II variable wavelength detector (G7114A)

### Instrument conditions

Parameter	1260 Infinity II LC
Column Temperature	25 °C
Mobile Phase	50 mM sodium phosphate, 200 mM NaCl, pH 7.0
Flow Rate	0.3 to 0.7 mL/min
Injection Volume	1 µL
Detection	UV at 220 nm

## Columns

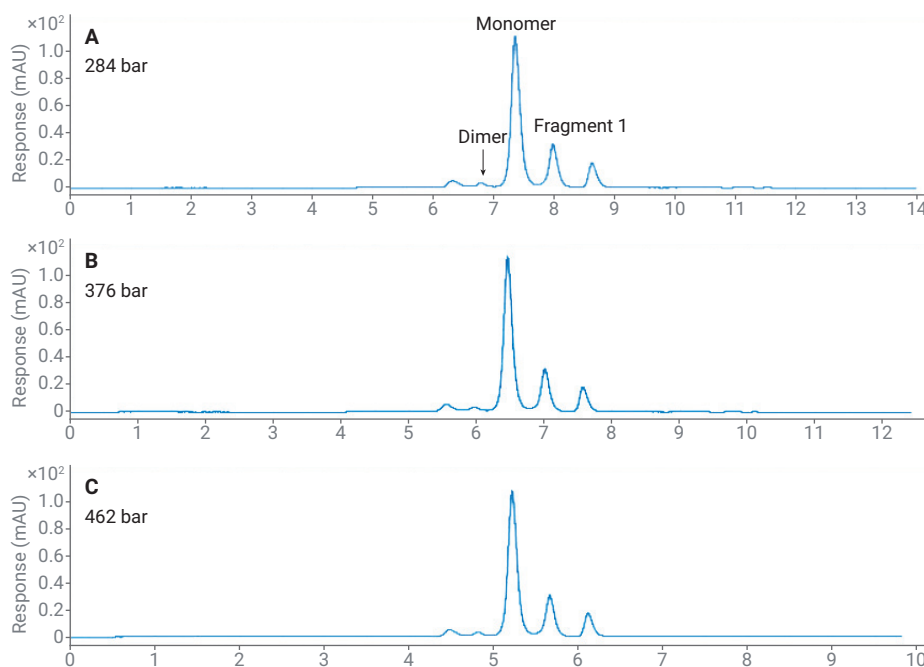
- Agilent AdvanceBio SEC 200 Å 1.9 µm, 4.6 × 300 mm (p/n PL1580-5201)
- Agilent AdvanceBio SEC 200 Å 1.9 µm, 4.6 × 150 mm (p/n PL1580-3201)

## Software

Agilent OpenLab 2.2 CDS.

## Results and discussion

Figure 1 shows SEC chromatograms of mAb with aggregates and fragments using 300 mm columns with flow rates at 0.35, 0.4, and 0.5 mL/min. Excellent resolution of both dimer/monomer and monomer/fragment1 was achieved even at 0.5 mL/min (Table 1) by saving 28 % of run time versus 0.35 mL/min flow rate without compromising resolution.

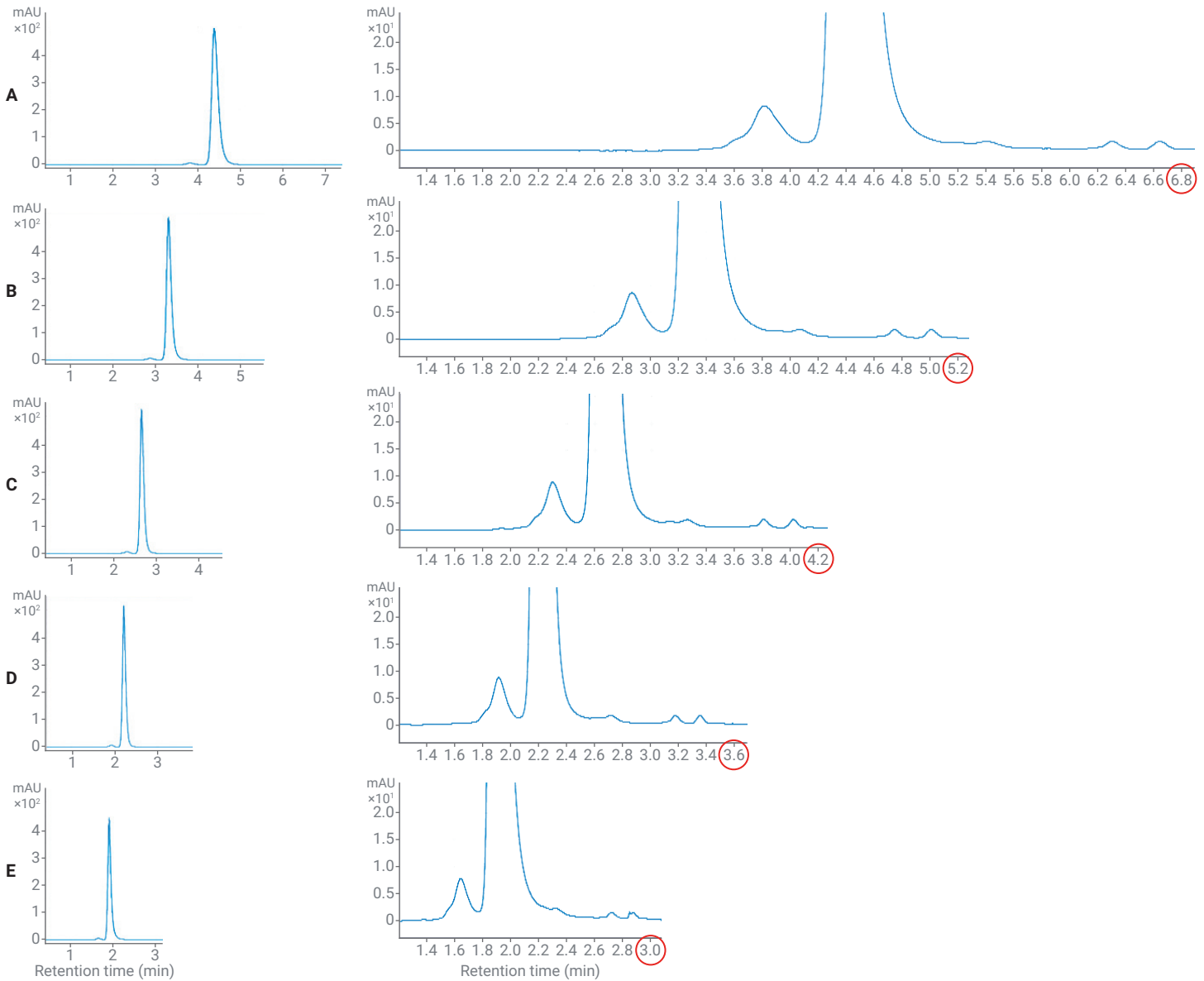


**Figure 1.** Size exclusion chromatograms of SigmaMAb (mixed with its F(ab')<sub>2</sub> and Fc fragments) using 4.6 × 300 mm SEC columns running with 50 mM sodium phosphate, 200 mM NaCl, pH 7.0 at A) 0.35 mL/min; B) 0.4 mL/min; C) 0.5 mL/min.

Shorter 150 mm columns offer a solution for fast, high-throughput SEC analysis, which is commonly used in the early stage of biotechnological development or where rapid analysis times are essential (for instance, during process monitoring). Figure 2 shows that different flow rates up to 0.7 mL/min were tested on a 150 mm AdvanceBio SEC 200 Å 1.9 µm column.

**Table 1.** Peak tailing factor and resolution under different flow rates.

Flow Rate (mL/min)	Tailing Factor (Monomer)	Resolution (Dimer/Monomer)	Resolution (Monomer/Fragment 1)
0.35	1.18	1.98	2.37
0.4	1.16	1.96	2.36
0.5	1.14	1.91	2.29



**Figure 2.** Size exclusion chromatograms of SigmaMAb using 4.6 × 150 mm SEC columns running with 50 mM sodium phosphate, 200 mM NaCl, pH 7.0 at A) 0.3 mL/min; B) 0.4 mL/min; C) 0.5 mL/min; D) 0.6 mL/min; E) 0.7 mL/min.

The unique particles in this column enable excellent stability at much higher flow rates with high resolution of dimer/monomer for accurate quantitation of the dimer peak area (Table 2).

Table 2 calculates the effect of flow rate on sample throughput. When increasing the flow rate from 0.3 to 0.7 mL/min, 480 samples can be analyzed per day, which is a 2.3-fold improvement in throughput. Compared to running the 300 mm column at 0.3 mL/min, which can only analyze 105 samples per day, the throughput increases 4.6-fold.

## Conclusion

This study demonstrates the ability of the AdvanceBio SEC 200 Å 1.9 µm column to be used for fast analysis of mAb aggregates. The durable particles enable running at a higher flow rate without loss of high resolution. By reducing column length from 300 to 150 mm, and by increasing flow rate from 0.3 to 0.7 mL/min, we can enhance sample throughput 4.6-fold.

**Table 2.** Effect of flow rate on resolution, monomer area percentage, and sample throughput.

Flow Rate (mL/min)	Run time (min)	Backpressure (bar)	Resolution (Dimer/Monomer)	Dimer Area %	Samples Per Hour	Samples Per Day (24 hours)
0.3	6.8	164	1.81	2.33	8-9	211
0.4	5.2	218	1.79	2.35	11-12	276
0.5	4.2	272	1.78	2.35	14	342
0.6	3.6	324	1.77	2.39	16-17	400
0.7	3.0	380	1.58	2.30	20	480

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