

SIZE EXCLUSION CHROMATOGRAPHY WORKFLOW

AGILENT BIO SEC SIZE EXCLUSION COLUMNS



In this document Agilent applications chemists share their recommendations for an optimum LC system and its configuration for characterizing biomolecules. They also offer guidance on a generic method to get you started, and how this method can be further optimized to meet your specific separation goals. Additional application information is available at www.agilent.com/chem/advancebio

Agilent 1260 Infinity Bio-Inert LC System

Mobile phases

Isocratic elution with phosphate buffered saline, pH 7.4. Use analytical grade reagents and filter through 0.2 μm before use.

Pump (G5611A)

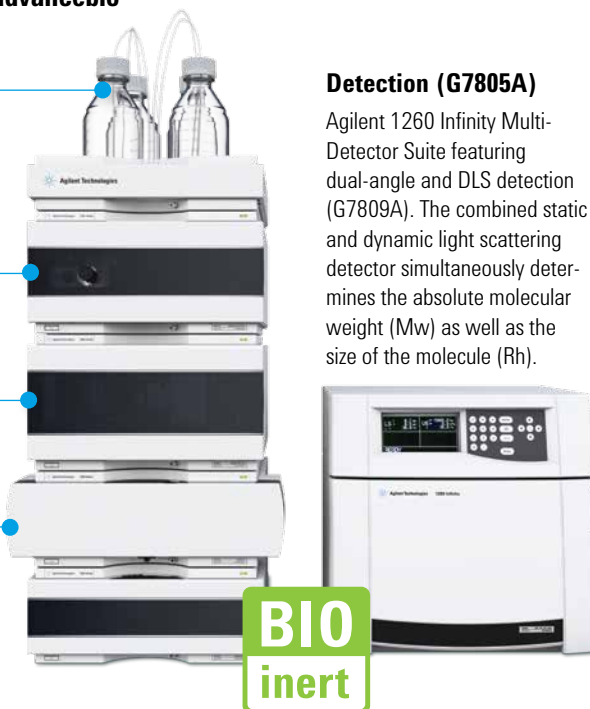
0.8 to 1.25 mL/min for 7.8 mm id
0.2 to 0.4 mL/min for 4.6 mm id

Sample injection (G5667A)

10 to 20 μL injection for samples containing 1 to 5 mg/mL mAb

Column compartment (G1316C)

25 $^{\circ}\text{C}$ is a typical temperature for good separation. Column lifetime is optimized when used between 10 to 30 $^{\circ}\text{C}$.



Detection (G7805A)

Agilent 1260 Infinity Multi-Detector Suite featuring dual-angle and DLS detection (G7809A). The combined static and dynamic light scattering detector simultaneously determines the absolute molecular weight (Mw) as well as the size of the molecule (Rh).

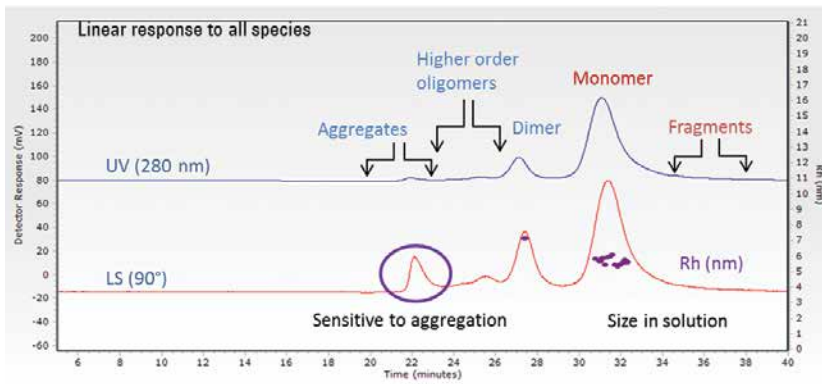
Main application areas

Biopharma	Easy aggregation and sizing studies for proteins and bioconjugates
Bio research	Accurate molecular weight, size, and aggregation information
Other	Metal-free analysis High salt resistance
Specific	Complete mAb characterization in a single instrument

	Bio SEC-3 Size Exclusion Column 3 μm particle size	Bio SEC-5 Size Exclusion Column 5 μm particle size
Pore sizes	100 \AA (0.1 to 100 kDa) 150 \AA (0.5 to 150 kDa) 300 \AA (5 to 1250 kDa)	100 \AA (0.1 to 100 kDa) 150 \AA (0.5 to 150 kDa) 300 \AA (5 to 1,250 kDa) 500 \AA (15 to 5,000 kDa) 1000 \AA (50 to 7,500 kDa) 2000 \AA (>10,000 kDa)
Feature	Highest resolution and efficiency	High stability and long column lifetime
Benefit	Faster SEC separation	Good reproducibility

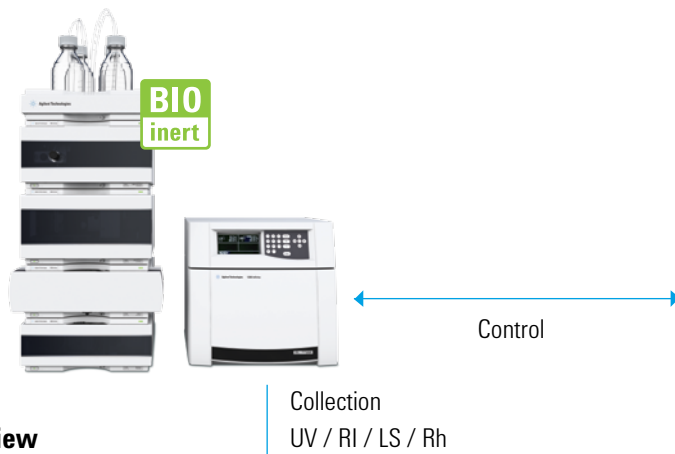


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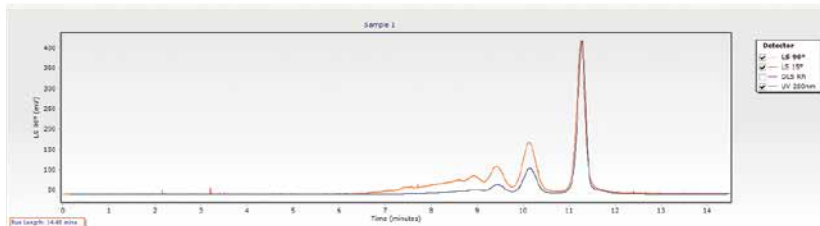
The light scattering detector shows much greater sensitivity to the aggregation present in mAb.

Instrument Control View

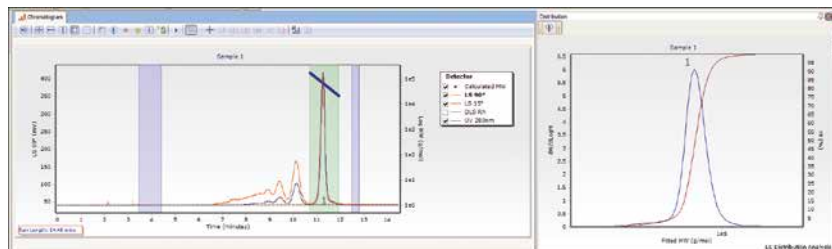


Agilent Bio SEC software is a dedicated chromatography data system for an easy, yet powerful, workflow-based solution.

Analysis View



Analysis



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