

# SIZE EXCLUSION CHROMATOGRAPHY WORKFLOW

## AGILENT BIO SEC-3 AND BIO SEC-5 HPLC 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](http://www.agilent.com/chem/advancebio)

### Agilent 1260 Infinity Bio-Inert LC System

#### Mobile phases

Isocratic elution: 150 mM sodium phosphate, pH 7.0 or select sample buffer in which the protein is soluble and stable. Bio SEC-3 and Bio SEC-5 can tolerate up to 30% polar organics in the mobile phase.

#### Pump (G5611A)

0.8 to 1 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  $\mu$ L injection for samples containing 1 to 5 mg/mL of protein.

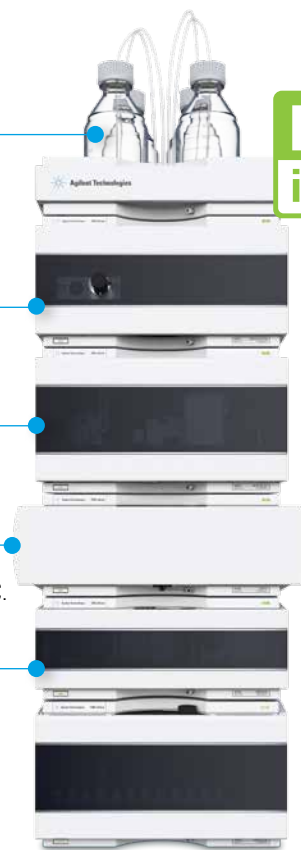
#### Column compartment (G1316C)

25 °C is a typical temperature for good separation. Column lifetime is optimized when used from 10 to 30 °C.

#### Detection (G1315D)

Agilent 1260 Infinity DAD VL with bio-inert standard flow cell, 10 mm

**BIO  
inert**



### Suggested gradient for speed and resolution

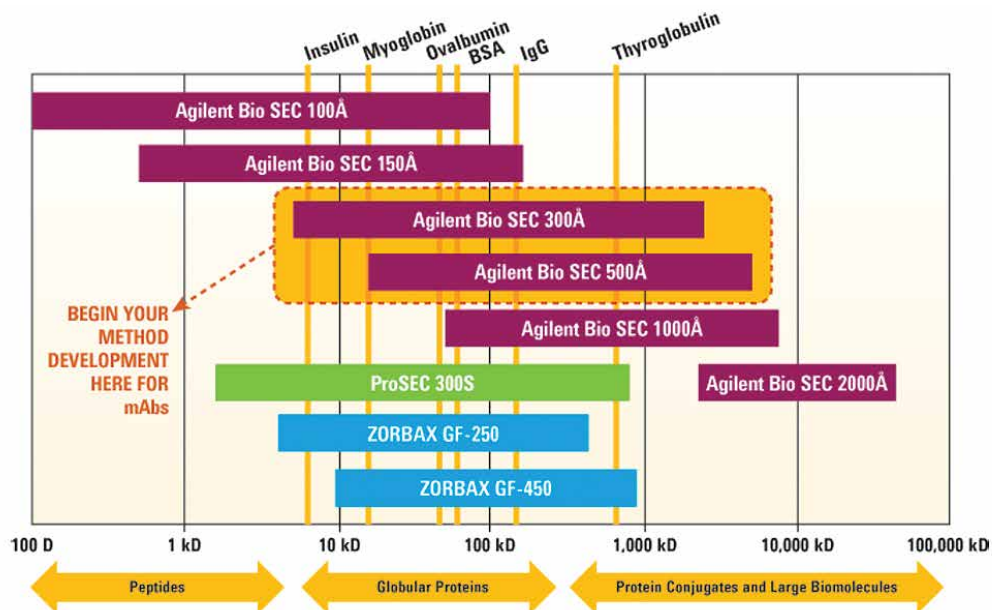
Time	Mobile Phase	Flow
0-15 min	Isocratic elution using mobile phase	0.8 to 1 mL/min

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



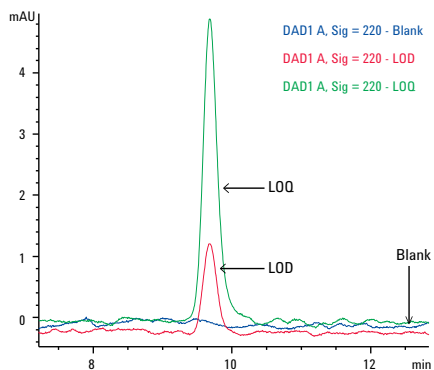
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# Which Agilent SEC column is right for your application?

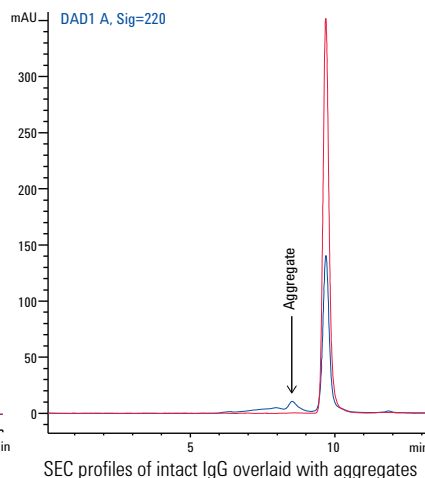
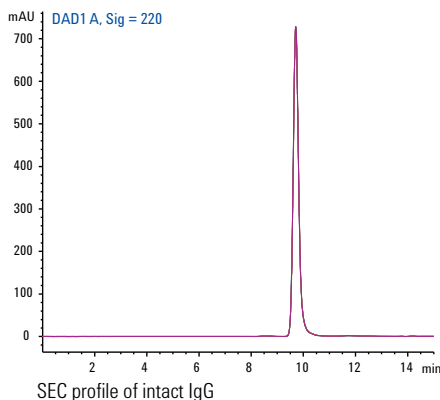


## SEC of monoclonal antibody (IgG)

Column: Bio SEC-3, 300Å,  
7.8 x 300 mm, 3 µm  
Sample: IgG1 (1 mg/mL)  
Injection: 10 µL  
Mobile Phase: 50 mM sodium  
phosphate buffer,  
pH 7.4 + 150 mM NaCl  
Flow rate: 0.8 mL/min  
Run Time: 15 min



SEC of IgG. LOD (12.5 µg/mL) and LOQ (25 µg/mL) overlaid with blank



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