

BIOMOLECULE CHARACTERIZATION WORKFLOW

AGILENT BIO-MONOLITH PROTEIN A AND PROTEIN G AFFINITY COLUMNS

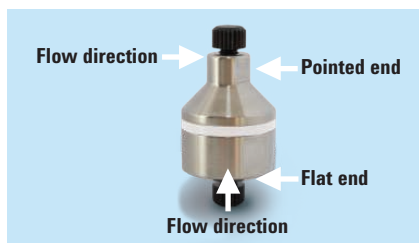


In this document Agilent applications chemists share their recommendations for an optimum LC system and its configuration required for characterizing biomolecules. They also offer guidance on a generic method to get you started, and how this method can be further optimized to meet your specific separation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

Agilent 1260 Infinity Bio-Inert LC System

Agilent Bio-Monolith Protein columns are compatible with all HPLC/UHPLC systems

HCl has a lower refractive index compared to other eluents. If the low concentration sample is used and baseline noise and artifact peaks are of concern, HCl can be used as an eluent.



The Bio-Monolith Protein A column has a white band and Bio-Monolith Protein G has a yellow band around the column.

Mobile phases

Binding buffer: 50 mM sodium phosphate buffer, pH 7.4. Eluting buffer: See table below.

Sample injection (G5667A)

1 to 5 μ L injection for samples containing 1 to 5 mg/mL of mAb. Samples can be dissolved in H₂O or mobile phase A. Columns can be injected up to 50 μ L or up to 400 to 500 mg mAb/injection.

Pump (G5611A)

1.0 to 3.0 mL/min for high speed.
1.0 mL/min gives shaper and taller peaks and better signal-to-noise.

Column compartment (G1316C)

25 °C is a typical temperature for successful separations. Columns can be operated from 4 to 40 °C.

Detection (G1315C)

UV at 280 nm

**BIO
inert**



Compatible eluting buffers

Column	Eluting Buffer	Concentration	pH
Bio-Monolith Protein A	Citric acid	0.1 M	2.5 to 3.0
	Glycine	0.1 M	2.5 to 3.0
	Acetic acid	5 to 20 %	
	HCl	12 mM to 0.1 M	
Bio-Monolith Protein G	Citric acid	0.1 M	2.0 to 2.5
	Glycine	0.1 M	2.0 to 2.5
	Acetic acid	5 to 20 %	
	HCl	12 mM to 0.1 M	

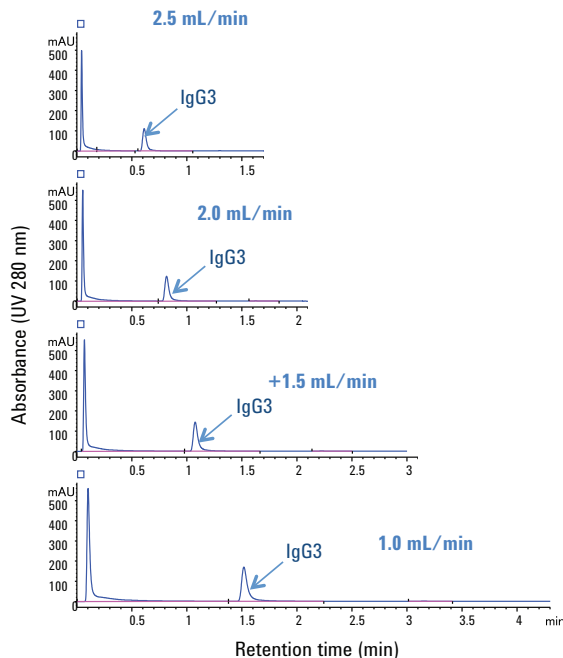


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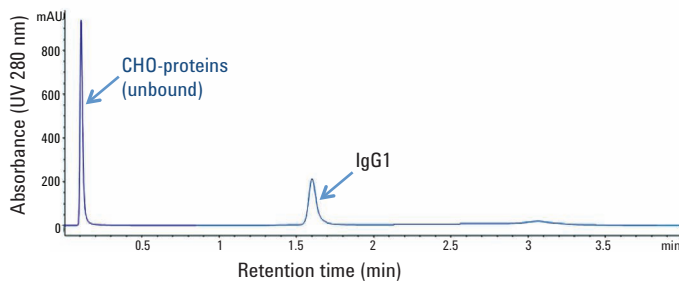
Fast separation protocols

The columns can be operated up to 3 mL/min. Gradient tables can be used for Bio-Monolith Protein A as well, just adjust the eluting buffer (mobile phase B) to pH 2.5 to 3.0.

Column: Bio-Monolith Protein G
 Sample: IgG3 (2 mg/mL)
 Injection: 5 µL
 Mobile phase A: 50 mM sodium phosphate buffer, pH 7.4
 Mobile phase B: 0.1 M citric acid, pH 2.0
 Temp.: 25 °C
 HPLC: Agilent 1260 Bio-Inert Quaternary LC
 Detection: UV at 280 nm



Column: Bio-Monolith Protein A c
 Sample: CHO-cell host cell protein and IgG1 (7 mg/mL CHO cell spiked with 2 mg/mL IgG1)
 Injection: 5 µL
 Mobile phase A: 50 mM sodium phosphate buffer, pH 7.4
 Mobile phase B: 0.1 M citric acid, pH 2.8
 Flow rate: 1.0 mL/min (see gradient table below)



1.0 mL/min			1.5 mL/min			2.0 mL/min			2.5 mL/min		
Time (min)	% A	% B	Time (min)	% A	% B	Time (min)	% A	% B	Time (min)	% A	% B
0	100	0	0	100	0	0	100	0	0	100	0
0.4	100	0	0.3	100	0	0.2	100	0	0.1	100	0
0.5	0	100	0.4	0	100	0.3	0	100	0.2	0	100
2.0	0	100	1.7	0	100	1.2	0	100	0.8	0	100
2.1	100	0	1.8	100	0	1.3	100	0	0.9	100	0
4.2	100	0	3.2	100	0	2.2	100	0	1.7	100	0

Antibody	Antibody	Protein A	Protein G	Antibody Fragments	Protein A	Protein G
Human	Human IgG1	++++	++++	Human Fab	+	+
	Human IgG2	++++	++++	Human F(ab') ₂	+	+
	Human IgG3	-	++++	Human scFv	+	-
	Human IgG4	++++	++++	Human Fc	++	++
	Human IgA	++	-	Human K	-	-
	Human IgD	++	-	Human λ	-	-
	Human IgE	++	-			
	Human IgM	++	-			
Mouse	Mouse IgG1	+	++			
	Mouse IgG2a	++++	++++			
	Mouse IgG2b	+++	+++			
	Mouse IgG3	++	+++			
	Mouse IgM	+/-	-			

Relative affinity of Protein A and Protein G for respective antibodies.
 ++++ = Strong affinity
 +++ = Moderate affinity
 ++ = Weak affinity
 + = Slight affinity
 - = No affinity

Column selection guidelines. Binding affinities of Protein A and Protein G to antibodies [1, 2]

References

- Richman, D. D.; Cleveland, P. H.; Oxman, M. N.; Johnson, K. M. The binding of 1. Staphylococci protein A by the sera of different animal species. *J. Immunol.* **1982**, *128*, 2300-2305.
- Frank, M. B. Antibody Binding to Protein A and Protein G beads; 5. In *Molecular Biology Protocols*; Frank, M. B., Ed.; Oklahoma Medical Research Foundation, Oklahoma City, USA, **1997**.

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