# 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


**Mobile phases**

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

**Sample injection (G5667A)**

1 to 5 µL injection for samples containing 1 to 5 mg/mL of mAb. Samples can be dissolved in $H_2O$ or mobile phase A. Columns can be injected up to 50 µL 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.

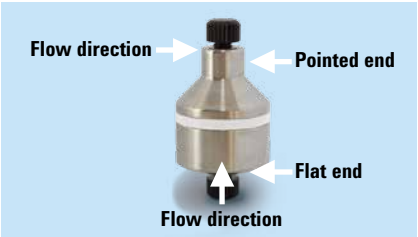
**Column compartment (G1316C)**

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

**Detection (G1315C)**

UV at 280 nm

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.

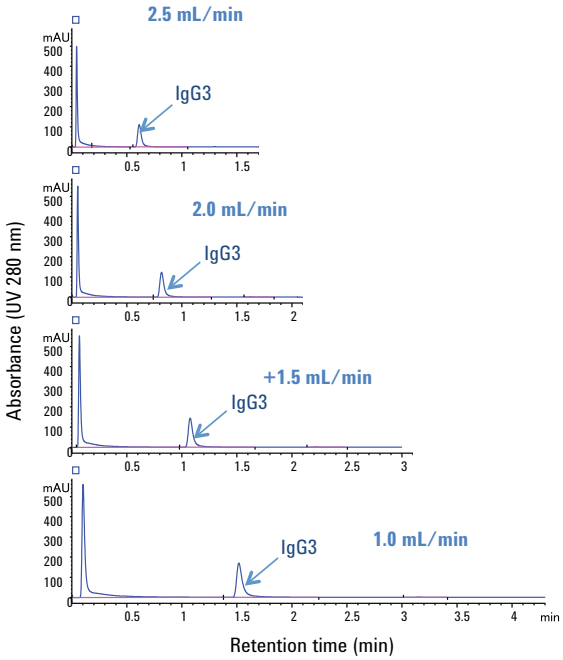
### Compatible eluting buffers

| 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 | |

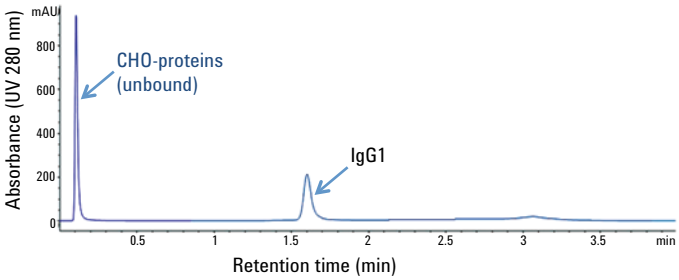
## Fast separation protocols

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 | | |
|---|---|---|
| Time (min) | % A | % B |
| 0 | 100 | 0 |
| 0.4 | 100 | 0 |
| 0.5 | 0 | 100 |
| 2.0 | 0 | 100 |
| 2.1 | 100 | 0 |
| 4.2 | 100 | 0 |

| 1.5 mL/min | | |
|---|---|---|
| Time (min) | % A | % B |
| 0 | 100 | 0 |
| 0.3 | 100 | 0 |
| 0.4 | 0 | 100 |
| 1.7 | 0 | 100 |
| 1.8 | 100 | 0 |
| 3.2 | 100 | 0 |

| 2.0 mL/min | | |
|---|---|---|
| Time (min) | % A | % B |
| 0 | 100 | 0 |
| 0.2 | 100 | 0 |
| 0.3 | 0 | 100 |
| 1.2 | 0 | 100 |
| 1.3 | 100 | 0 |
| 2.2 | 100 | 0 |

| 2.5 mL/min | | |
|---|---|---|
| Time (min) | % A | % B |
| 0 | 100 | 0 |
| 0.1 | 100 | 0 |
| 0.2 | 0 | 100 |
| 0.8 | 0 | 100 |
| 0.9 | 100 | 0 |
| 1.7 | 100 | 0 |

| Antibody | Antibody | Protein A | Protein G |
|---|---|---|---|
| **Human** | Human IgG1 | ++++ | ++++ |
| | Human IgG2 | ++++ | ++++ |
| | Human IgG3 | - | ++++ |
| | Human IgG4 | ++++ | ++++ |
| | Human IgA | ++ | - |
| | Human IgD | ++ | - |
| | Human IgE | ++ | - |
| | Human IgM | ++ | - |
| **Mouse** | Mouse IgG1 | + | ++ |
| | Mouse IgG2a | ++++ | ++++ |
| | Mouse IgG2b | +++ | +++ |
| | Mouse IgG3 | ++ | +++ |
| | Mouse IgM | +/- | - |

| Antibody Fragments | Protein A | Protein G |
|---|---|---|
| Human Fab | + | + |
| Human F(ab')2 | + | + |
| Human scFv | + | - |
| Human Fc | ++ | ++ |
| Human K | - | - |
| Human λ | - | - |

*Relative affinity of Protein A and 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


1. 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.
2. 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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