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**

**Main application areas**

<table>
<thead>
<tr>
<th>Biopharma</th>
<th>Easy aggregation and sizing studies for proteins and bioconjugates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio research</td>
<td>Accurate molecular weight, size, and aggregation information</td>
</tr>
<tr>
<td>Other</td>
<td>Metal-free analysis</td>
</tr>
<tr>
<td>Specific</td>
<td>Complete mAb characterization in a single instrument</td>
</tr>
</tbody>
</table>

**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 °C is a typical temperature for good separation. Column lifetime is optimized when used between 10 to 30 °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).

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**Bio SEC-3 Size Exclusion Column**

- 3 µm particle size
- Pore sizes: 100Å (0.1 to 100 kDa), 150Å (0.5 to 150 kDa), 300Å (5 to 1250 kDa)
- Feature: Highest resolution and efficiency
- Benefit: Faster SEC separation

**Bio SEC-5 Size Exclusion Column**

- 5 µm particle size
- Pore sizes: 100Å (0.1 to 100 kDa), 150Å (0.5 to 150 kDa), 300Å (5 to 1250 kDa), 500Å (15 to 5,000 kDa), 1000Å (50 to 7,500 kDa), 2000Å (>10,000 kDa)
- Feature: High stability and long column lifetime
- Benefit: Good reproducibility
The light scattering detector shows much greater sensitivity to the aggregation present in mAb.

**Instrument Control View**

**Analysis View**

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