Confidently separate and characterize bio-molecules with Agilent BioHPLC columns

Size Exclusion BioHPLC columns

Ion Exchange BioHPLC columns

Our measure is your success.
"My team is under a lot of pressure to better characterize our lead protein therapeutics. Is there any new analytical technology out there that might help us?"

"It's a struggle to isolate and identify charge variants of our monoclonal antibodies. How can I better separate the protein's isoforms?"

“How can we be certain that our analytical methods will remain consistent in quality control?"

Whether your goal is to characterize the next biopharmaceutical or isolate a target protein, Agilent can help you overcome the many challenges involved in developing these analytical methods.
Introducing the Agilent Size Exclusion and Ion Exchange BioHPLC columns, featuring four new column families that enable highly reproducible and high resolution analytical separations of monoclonal antibodies, proteins, peptides, and other bio-molecules.

**Agilent Bio SEC-3 HPLC columns** (page 4) promote sharper peaks and faster size-based separations for bio-molecules and water-soluble polymers. They are packed with 3 µm porous silica particles coated with a proprietary, hydrophilic layer to maximize separation efficiency and resolution.

**Agilent Bio SEC-5 HPLC columns** (page 7) offer improved peak capacity and resolution for a broad range of size-based, bio-molecule separations. They are packed with 5 µm silica particles coated with a neutral hydrophilic layer decreasing secondary interactions.

**Agilent Bio MAb HPLC columns** (page 10) are specifically designed for high-resolution monoclonal antibody (MAb) separations. The columns are packed with polymeric, non-porous, weak cation exchange particles. The particles are coated with a hydrophilic layer and use a high density weak cation exchange layer, which offers unique selectivity for monoclonal antibodies.

**Agilent Bio IEX HPLC columns** (page 13) ensure high-resolution, high-recovery, and highly efficient separations of proteins, peptides, oligonucleotides, and other bio-molecules. The columns are packed with polymeric, non-porous particles coated with a unique hydrophilic layer that virtually eliminates non-specific interactions. Multiple ion exchange groups are attached at each bonding site offering excellent capacity and selectivity.

As a leading provider of analytical solutions to the biopharmaceutical industry, Agilent understands that quality and consistency are critical to providing safe and highly efficacious therapeutic products. **Agilent's analytical BioHPLC columns offer the speed, resolution and reproducibility you need to quickly and cost-effectively get life-changing products into the hands of those who need them.**

Agilent Bio SEC-3 HPLC columns (3 µm particle size)
High efficiency and high resolution size-based separations for bio-molecules

Agilent Bio SEC-3 HPLC columns are a unique technology for size exclusion chromatography (SEC). They are packed with spherical, narrowly dispersed 3 µm silica particles coated with a proprietary, hydrophilic layer. This thin polymeric layer is chemically bonded to pure, mechanically stable silica under controlled conditions, ensuring a highly efficient size exclusion particle.

Other column advantages include:
• Exceptional loading capacity, stability, and reproducibility for size-based, bio-molecule separations
• Sharper peaks, higher resolution, and better protein recovery
• Faster separations than large-particle SEC columns, in many cases
• Compatibility with most aqueous buffers
• Excellent stability in high and low salt conditions

Agilent Bio SEC-3 HPLC columns are available in 100Å, 150Å and 300Å pore sizes to accommodate most peptide and protein size exclusion separations.

<table>
<thead>
<tr>
<th>Column Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Packing</strong></td>
</tr>
<tr>
<td><strong>Particle size</strong></td>
</tr>
<tr>
<td><strong>Pore structure</strong></td>
</tr>
<tr>
<td><strong>Column exclusion limits (in Daltons)</strong></td>
</tr>
<tr>
<td><strong>pH stability</strong></td>
</tr>
<tr>
<td><strong>Operating temperature limit</strong></td>
</tr>
<tr>
<td><strong>Operating pressure limit</strong></td>
</tr>
<tr>
<td><strong>Mobile phase compatibility</strong></td>
</tr>
<tr>
<td><strong>Working flow rate</strong></td>
</tr>
</tbody>
</table>
Recommended Applications

Antibody and protein aggregation analysis, separation of proteins in cell lysates, separation of protein mixtures, natural polymers, nanomaterials, oligonucleotides, polysaccharides, and other bio-molecules.

<table>
<thead>
<tr>
<th>Column</th>
<th>Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio SEC-3 (7.8 x 300 mm)</td>
<td>90 bar</td>
</tr>
<tr>
<td>Bio SEC-5 (7.8 x 300 mm)</td>
<td>45 bar</td>
</tr>
</tbody>
</table>

Peak Efficiencies for 3 µm and 5 µm Particle Columns

<table>
<thead>
<tr>
<th>Peak</th>
<th>Protein</th>
<th>Agilent Bio SEC-3, 300Å, 7.8 x 300 mm</th>
<th>Agilent Bio SEC-5, 300Å, 7.8 x 300 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Thyroglobulin</td>
<td>2460</td>
<td>1120</td>
</tr>
<tr>
<td>2</td>
<td>BSA Dimer</td>
<td>5100</td>
<td>2720</td>
</tr>
<tr>
<td>3</td>
<td>BSA</td>
<td>13090</td>
<td>6590</td>
</tr>
<tr>
<td>4</td>
<td>Ribonuclease A</td>
<td>22000</td>
<td>11160</td>
</tr>
<tr>
<td>5</td>
<td>Uracil</td>
<td>38500</td>
<td>27860</td>
</tr>
</tbody>
</table>

Column: Bio SEC-3 300Å and Bio SEC-5 300Å
Buffer: 150 mM Phosphate buffer, pH 7
Flow rate: 1.0 mL/min for 7.8 x 300 mm
Temperature: Ambient (~23°C)
Detection: UV 214 nm
Injection: 10 µL (3 µL for 4.6 x 300 mm)
Sample: 1) Thyroglobulin (1.0 mg/mL), 670 kDa;
2) BSA dimer, 132 kDa;
3) BSA (1.0 mg/mL), 66 kDa;
4) Ribonuclease A (1.0 mg/mL), 13.7 kDa, and
5) Uracil (2.5 µg/mL), 120D.

The combination of unique surface chemistry and small particles: This five-protein separation demonstrates how smaller particles deliver sharper peaks at higher flow rates, improving resolution and shortening runtimes.
Agilent Bio SEC-3 HPLC columns

Aggregation analysis of a humanized monoclonal antibody (MAb), the Agilent Bio SEC-3 HPLC columns provides baseline separation of the antibody aggregate and monomer peaks in 15 minutes.

Separation of an E. coli lysate on a 150Å and 300Å Agilent Bio SEC-3 HPLC column. Different proteins are well resolved when using columns with different exclusion limits.

Visit [www.agilent.com/chem/BioHPLC](http://www.agilent.com/chem/BioHPLC) to learn more, and order online.
Agilent Bio SEC-5 HPLC columns
(5 µm particle size)

Highly reproducible and high resolution sized-based separations of biological molecules

Agilent Bio SEC-5 HPLC columns are packed with 5 µm silica particles coated with a proprietary, neutral, hydrophilic layer for maximum efficiency and stability. The specially designed packing also promotes high pore volume, improving both peak capacity and resolution.

Other column advantages include:
• Maximum recovery for a broad range of size-based, bio-molecule separations
• Outstanding reproducibility and column lifetime
• Excellent stability, even under high and low salt conditions and other harsh buffer conditions
• Compatibility with most aqueous buffers

Particles available in:
100Å, 150Å, 300Å, 500Å, 1000Å, and 2000Å pore sizes, offering a wide selection of exclusion limits.

<table>
<thead>
<tr>
<th>Column Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Column Phase</strong></td>
</tr>
<tr>
<td>Packing</td>
</tr>
<tr>
<td>Particle size</td>
</tr>
<tr>
<td>Pore structure</td>
</tr>
</tbody>
</table>
| Column exclusion limits (in Daltons) | 100 Å MW range: 100 to 100,000
150 Å MW range: 500 to 150,000
300 Å MW range: 5,000 to 1,250,000
500 Å MW range: 15,000 to 5,000,000
1000 Å MW range: 50,000 to 7,500,000
2000 Å MW range: >10,000,000 |
| pH stability | 2 to 8.5 |
| Operating temperature limit | Recommended range: 10 to 30°C, maximum: 80°C |
| Operating pressure limit | Recommended operating pressure: 135 bar (2,000 psi)
Maximum pressure: 240 bar (3,500 psi) |
| Mobile phase compatibility | Recommended: 150 mM phosphate buffer, pH 7.0, other aqueous buffers with high and low salt can be used |
| Working flow rate | 0.1 to 1.25 mL/min for 7.8 mm I.D. columns
0.1 to 0.4 mL/min for 4.6 mm I.D. columns |
Agilent Bio SEC-5 HPLC columns

Antibody and protein aggregation analysis, separation of proteins in cell lysates, separation of protein mixtures, natural polymers, nanomaterials, oligonucleotides, polysaccharides, and other bio-molecules.


Exceptional lot-to-lot reproducibility: the four protein mixture shows excellent retention time reproducibility over 300 injections and on three columns from different manufacturing lots.
Even after 300 injections of a five protein mixture, the Agilent Bio SEC-5 HPLC columns provide reproducible results with minimal peak tailing over time.

<table>
<thead>
<tr>
<th>Injection No.</th>
<th>Efficiency</th>
<th>Tailing</th>
<th>Line</th>
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<td>26512</td>
<td>1.192</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>25989</td>
<td>1.183</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>26182</td>
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<td>10</td>
<td>25682</td>
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<td>20</td>
<td>25872</td>
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<tr>
<td>175</td>
<td>25177</td>
<td>1.208</td>
<td></td>
</tr>
<tr>
<td>192</td>
<td>24300</td>
<td>1.196</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>25707</td>
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<td></td>
</tr>
<tr>
<td>300</td>
<td>25720</td>
<td>1.166</td>
<td></td>
</tr>
</tbody>
</table>

**Column:** Agilent Bio SEC-5, 300Å, 7.8 x 300 mm

**Buffer:** 150 mM Phosphate buffer, pH 7.0

**Flow Rate:** 1.0 mL/min

**Detector:** 214 nm

**Injection:** 10 µL

1. Thyroglobulin
2. BSA
3. Ribonuclease A
4. Uracil

Visit [www.agilent.com/chem/BioHPLC](http://www.agilent.com/chem/BioHPLC) to learn more, and order online.
Thorough characterization of monoclonal antibodies includes the identification and monitoring of acidic and basic isoforms. The Agilent Bio MAb HPLC columns feature a unique resin specifically designed for high-resolution charge-based separations of monoclonal antibodies.

The unique particle design includes:
• A packing support composed of a rigid, spherical, highly cross-linked polystyrene divinylbenzene (PS/DVB) non-porous bead
• Particles grafted with a hydrophilic, polymeric layer, virtually eliminating nonspecific binding of antibody proteins, increasing efficiency and recoveries
• Bio MAb particles use an optimized process to layer the weak cation exchange phase to the particle, making it a higher ligand density than the Agilent Bio WCX column particles.
Particles available 1.7 µm, 3 µm, 5µm, and 10 µm sizes, providing higher resolution with the smaller particles.

### Column Characteristics

<table>
<thead>
<tr>
<th>Column Phase</th>
<th>Weak Cation Exchange (carboxylate)</th>
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</thead>
<tbody>
<tr>
<td>Packing</td>
<td>Non-porous, poly(styrene divinylbenzene) (PS/DVB), grafted hydrophilic coating and bonded with a uniform, weak cation exchange layer</td>
</tr>
<tr>
<td>Particle size</td>
<td>1.7, 3, 5, and 10 µm</td>
</tr>
<tr>
<td>Pore structure</td>
<td>Non-porous</td>
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<tr>
<td>pH stability</td>
<td>2 to 12</td>
</tr>
<tr>
<td>Operating temperature limit</td>
<td>80°C</td>
</tr>
<tr>
<td>Column hardware operating pressure limit</td>
<td>600 bar (8,700 psi) for stainless steel column hardware 400 bar (5,800 psi) for PEEK column hardware</td>
</tr>
<tr>
<td>Particle operating pressure limit</td>
<td>275 bar (4,000 psi) for 10 µm particles 413 bar (6,000 psi) for 5 µm particles 551 bar (8,000 psi) for 3 µm particles 689 bar (10,000 psi) for 1.7 µm particles</td>
</tr>
<tr>
<td>Mobile phase compatibility</td>
<td>Compatible with aqueous solution buffers, acetonitrile/acetone/methanol and water mixtures. Commonly used buffers: phosphate, tris, MES and acetate</td>
</tr>
<tr>
<td>Working flow rate</td>
<td>Typical 0.1 to 1.0 mL/min for a 4.6 mm or 2.1 mm I.D column. Always start at a low flow rate and default to the maximum hardware and particle pressures.</td>
</tr>
</tbody>
</table>

Agilent Bio MAb HPLC columns are packed with polymeric, non-porous, weak cation exchange particles (1.7 µm PS/DVB particles shown here). These particles are coated with a unique hydrophilic layer, improving recovery by dramatically reducing non-specific binding of antibody proteins.
Optimization of method conditions for the isoform characterization of a monoclonal antibody. Changes in the gradient conditions sharpen peaks and increase resolution of acidic and basic isoforms.

The combination of well-controlled resin production, column surface chemistry, and column packing virtually eliminates retention time variations from column to column and lot to lot.

Visit www.agilent.com/chem/BioHPLC to learn more, and order online.
Agilent Bio IEX HPLC columns
High resolution charge-based analytical separations of proteins, peptides, and other biological molecules

Agilent Bio IEX HPLC columns are packed with polymeric, nonporous, ion exchange particles and are designed for high resolution, high recovery and highly efficient separations of proteins, peptides, oligonucleotides, and other bio-molecules.

Unique features of these columns include:
• Highly crosslinked and rigid nonporous poly(styrene divinylbenzene) (PS/DVB) particles are grafted with a hydrophilic, polymeric layer, eliminating nonspecific binding, increasing efficiency and recoveries
• Uniform, densely packed ion exchange functional groups are chemically bonded to the hydrophilic layer (multiple ion exchange groups per anchoring) to increase column capacity
• Includes strong cation exchange (SCX), weak cation exchange (WCX), strong anion exchange (SAX) and weak anion exchange (WAX) phases. Bio WCX uses a bonding process to attach the phase; the Bio MAb columns use a layering process.

Agilent Bio IEX HPLC columns: superior performance from the inside out.

• Particles, coating and bonding are resistant to high pressures, promoting higher resolution and faster separations.
• Hydrophilic coating eliminates most non-specific interactions.
• Multiple ion-exchange groups are captured on one anchoring to increase capacity.
## Column Characteristics

All phases available in 1.7 µm, 3 µm, 5 µm, and 10 µm non-porous particles sizes and a variety of column dimensions

| Column Phases | SCX (Strong cation exchange) – SO$_3$H  
|               | WCX (Weak cation exchange) – COOH  
|               | SAX (Strong anion exchange) – N(CH$_3$)$_3$  
|               | WAX (Weak anion exchange) – N(C$_2$H$_5$)$_2$  |
| Packing       | Non-porous, poly(styrene divinylbenzene) (PS/DVB), grafted hydrophilic coating and bonded with a uniform, ion exchange layer  |
| Particle size | 1.7, 3, 5, and 10 µm  |
| Pore structure| Non-porous  |
| Dynamic Binding Capacity | SCX NP3: 53 mg/mL, NP5: 38 mg/mL, NP10: 20 mg/mL  
|               | WCX NP3: 19 mg/mL, NP5: 15 mg/mL, NP10: 10 mg/mL  
|               | SAX NP3: 35 mg/mL, NP5: 28 mg/mL, NP5: 17 mg/mL  
|               | WAX NP3: 26 mg/mL, NP5: 18 mg/mL, NP5: 12 mg/mL  |
| pH stability  | 2 to 12  |
| Operating temperature limit | 80°C  |
| Column hardware operating pressure limit | 600 bar (8,700 psi) for stainless steel column hardware  
|               | 400 bar (5,800 psi) for PEEK column hardware  |
| Particle operating pressure limit | 275 bar (4,000 psi) for 10 µm particles  
|               | 413 bar (6,000 psi) for 5 µm particles  
|               | 551 bar (8,000 psi) for 3 µm particles  
|               | 689 bar (10,000 psi) for 1.7 µm particles  |
| Mobile phase compatibility | Compatible with aqueous solution buffers, acetonitrile/acetone/methanol and water mixtures. Commonly used buffers: phosphate, tris, MES and acetate  |
| Working flow rate | Typical 0.1 to 1.0 mL/min for a 4.6 mm or 2.1mm I.D. column. Always start at a low flow rate and default to the maximum hardware and particle pressures.  |
The separations above demonstrate how a smaller particle size gives you the flexibility to push for sharper peaks and better resolution. Note the sharpness of the 1.7 µm particle size peaks.

**Column:** Bio WCX-NP, 4.6 x 50 mm

**Buffer A:** 20 mM PBS

**Buffer B:** A + 1.0 M NaCl

**Gradient:** 0 to 100% B (20 min)

**Flow rate:** 1.0 mL/min for NP10, NP5, NP3 0.75 mL/min for NP1.7

**Sample:**
1. Ribonuclease A
2. Cytochrome C
3. Lysozyme

**Concentration:** 1.0 mg/mL

**Detector:** 280 nm

Average N~80,000 for WCX-NP1.7

**Buffer A:** 20 mM Tris, pH 9.0

**Buffer B:** A + 0.5 M NaCl

**Gradient:** 0-100% B (30 min)

**Flow Rate:** 0.5 mL/min

**Injection:** 10 µL/min (2.5 mg/mL)
Recommended Applications

Analytical separations of peptides, proteins, carbohydrates, oligonucleotides, polynucleotides, cell lysates, and multi-dimensional separations.

Using an Agilent Bio SAX, NP3 (3 µm particle) column, the isoforms and impurities of both ovalbumin and BSA can easily be resolved.

Exceptional separating power: the hydrophilic, polymeric layer and densely packed ion exchange functional groups provides extremely sharp peak shapes and high resolution of a mixture of proteins with a broad range of isoelectric points (pI).

Visit www.agilent.com/chem/BioHPLC to learn more, and order online.
Use the charts on the next pages to identify which BioHPLC columns are best for your specific application.

### Agilent Bio SEC-3 HPLC Columns

<table>
<thead>
<tr>
<th>Description</th>
<th>Size (mm)</th>
<th>Particle Size (µm)</th>
<th>Part No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio SEC-3, 100Å</td>
<td>7.8 x 300</td>
<td>3</td>
<td>5190-2501</td>
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<tr>
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</tr>
<tr>
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<td>7.8 x 50</td>
<td>3</td>
<td>5190-2512</td>
</tr>
<tr>
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<td>4.6 x 300</td>
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### Agilent Bio SEC-5 HPLC Columns

<table>
<thead>
<tr>
<th>Description</th>
<th>Size (mm)</th>
<th>Particle Size (µm)</th>
<th>Part No.</th>
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</thead>
<tbody>
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### Agilent Bio MAb HPLC Columns

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### Agilent Bio IEX HPLC Columns

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