

Our measure is your success.



"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?" "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?"



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.

Visit www.agilent.com/chem/BioHPLC to learn more.

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

Column Characteristics

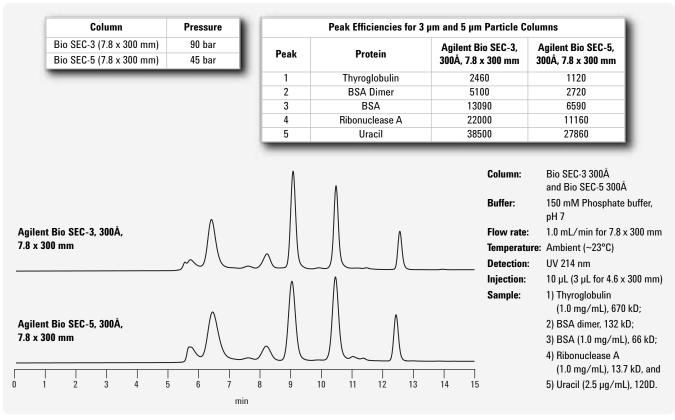
Agilent Bio SEC-3 HPLC columns are available in 100Å, 150Å and 300Å pore sizes to accommodate most peptide and protein size exclusion separations.

Column Phase	Size Exclusion
Packing	Spherical, high purity, porous silica with a hydrophilic polymeric coating
Particle size	3 μm
Pore structure	100Å, 150Å, 300Å
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
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



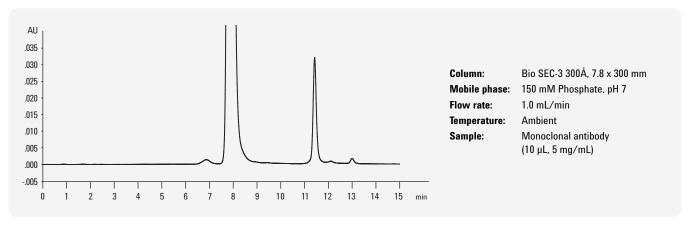
Recommended Applications

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

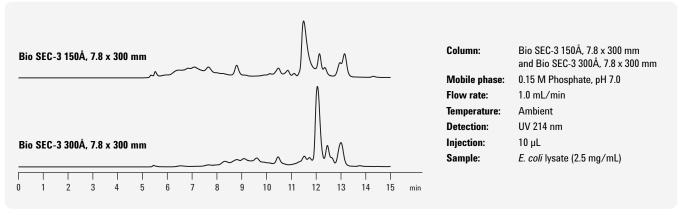


The combination of unique surface chemistry and small particles: This five-protein separation demonstrates how smaller particles deliver sharper peaks at higher flow rates, improving resolution and shortening runtimes.

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.



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:

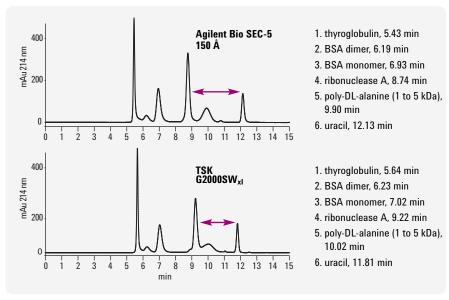
100Å, 150Å, 300Å, 500Å, 1000Å, and 2000Å pore sizes, offering a wide selection of exclusion limits.

Column Phase	Size Exclusion
Packing	Spherical, high purity, porous silica with a hydrophilic polymeric coating
Particle size	5 μm
Pore structure	100Å, 150Å, 300Å, 500Å, 1000Å, 2000Å
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

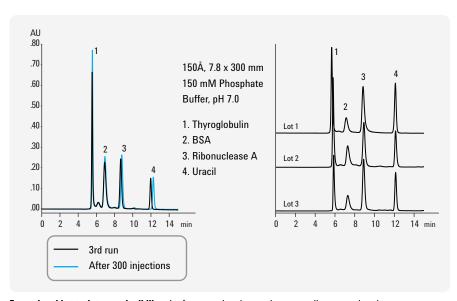
Agilent Bio SEC-5 HPLC columns

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.

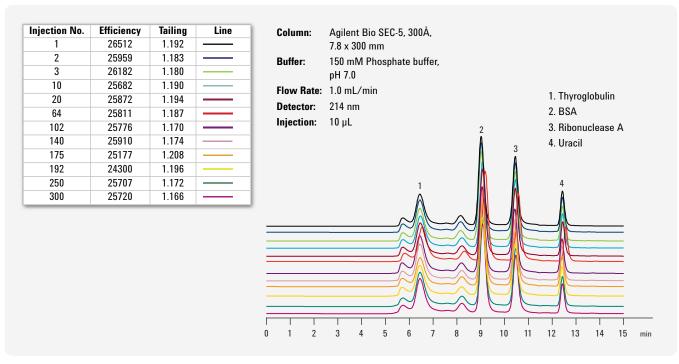


A side-by-side comparison: Separation of a protein mixture on an Agilent Bio SEC-5 HPLC column and a Tosoh TSK-Gel column. Notice the sharper peaks and better resolution on the Agilent Bio SEC-5 HPLC column.



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.

Agilent Bio MAb HPLC columns

High resolution ion exchange separations of monoclonal antibodies

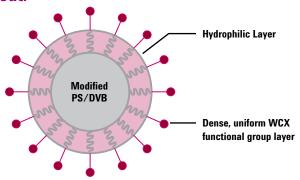
Thorough characterization of monoclonal antibodies includes the identification and monitoring of acidic and basic isoforms. The Agilent Bio MAb HPLC columns feature a unique resin specifically designed for high-resolution charge-based separations of monoclonal antibodies.

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.

Agilent Bio MAb 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
- A highly uniform, densely packed, weak cation exchange (WCX) layer chemically bonded to the hydrophilic, polymeric coating



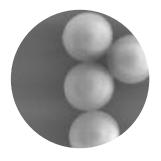




Particles available 1.7 μ m, 3 μ m, 5 μ m, and 10 μ m sizes, providing higher resolution with the smaller particles.

Column Phase	Weak Cation Exchange (carboxylate)
Packing	Non-porous, poly(styrene divinylbenzene) (PS/DVB), grafted hydrophilic coating and bonded with a uniform, weak cation exchange layer
Particle size	1.7, 3, 5, and 10 μm
Pore structure	Non-porous
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 2.1 mm I.D column. Always start at a low flow rate and default to the maximum hardware and particle pressures.



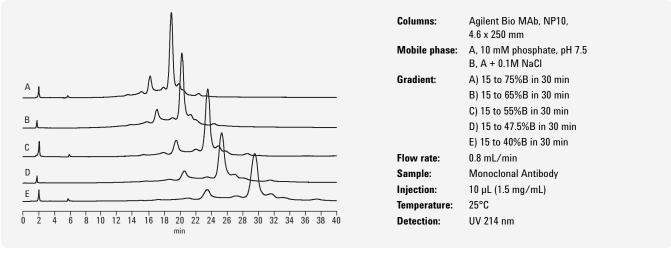


Agilent Bio MAb HPLC columns are packed with polymeric, non-porous, weak cation exchange particles (1.7 µm PS/DVD particles shown here). These particles are coated with a unique hydrophilic layer, improving recovery by dramatically reducing non-specific binding of antibody proteins.

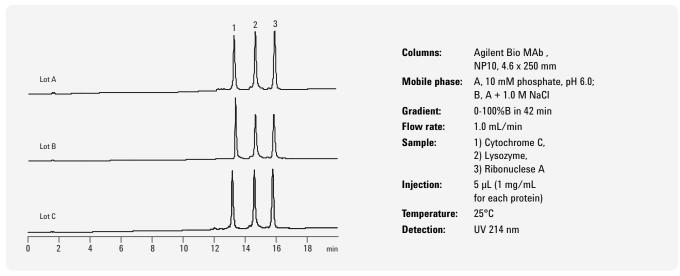
Agilent Bio MAb HPLC columns

Recommended Applications

Analytical separations of monoclonal antibody isoforms based on differences in their charge states.



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



Agilent Bio IEX HPLC columns

High resolution charge-based analytical separations of proteins, peptides, and other biological molecules

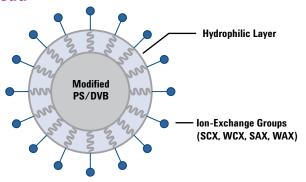
Agilent Bio IEX HPLC columns are packed with polymeric, nonporous, ion exchange particles and are designed for high resolution, high recovery and highly efficient separations of proteins, peptides, oligonucleotides, and other bio-molecules.

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.



Agilent Bio IEX HPLC columns

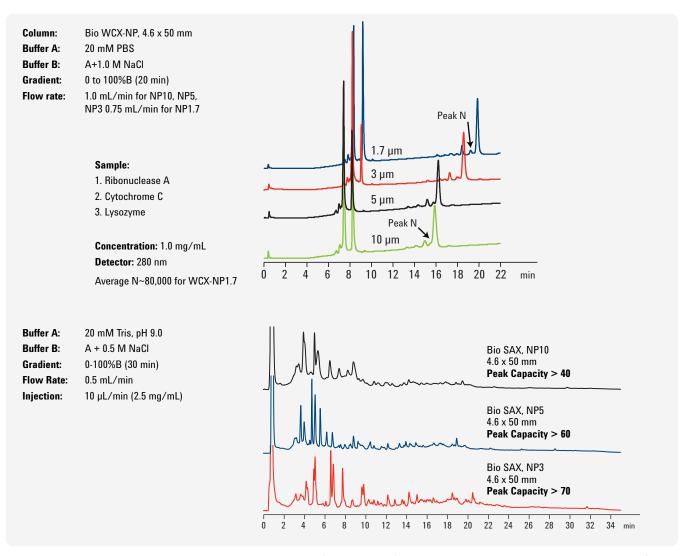
Column Characteristics

All phases available in 1.7 $\mu m,\,3~\mu m,\,5~\mu m,$ 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 $_2$ H5) $_2$
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, NP3: 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.





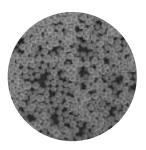


The separations above demonstrate how a smaller particle size gives you the flexibility to push for sharper peaks and better resolution. Note the sharpness of the 1.7 µm particle size peaks.

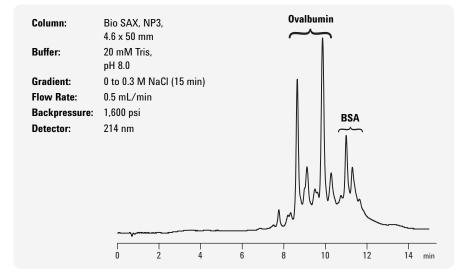
Agilent Bio IEX HPLC columns

Recommended Applications

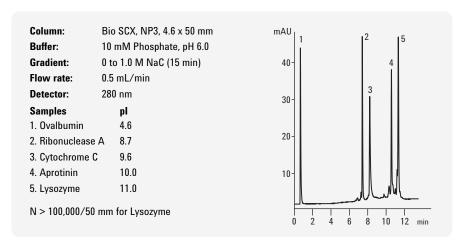
Analytical separations of peptides, proteins, carbohydrates, oligonucleotides, polynucleotides, cell lysates, and multi-dimensional separations.



Highly crosslinked and rigid nonporous polystyrene divinylbenzene particles (5 µm PS/DVD shown here) are grafted with a hydrophilic, polymeric layer, eliminating nonspecific binding, increasing efficiency and recoveries.



Using an Agilent Bio SAX, NP3 (3 μ m 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 (pl).



Agilent BioHPLC columns for starting your method development and validation:

Use the charts on the next pages to identify which BioHPLC columns are best for your specific application.

Agilent Bio SEC-3 HPLC Columns

Description	Size (mm)	Particle Size (µm)	Part No.
Bio SEC-3, 100Å	7.8 x 300	3	5190-2501
Bio SEC-3, 100Å	7.8 x 150	3	5190-2502
Bio SEC-3, 100Å	4.6 x 300	3	5190-2503
Bio SEC-3, 100Å	4.6 x 150	3	5190-2504
Bio SEC-3, 100Å, Guard	7.8 x 50	3	5190-2505
Bio SEC-3, 150Å	7.8 x 300	3	5190-2506
Bio SEC-3, 150Å	7.8 x 150	3	5190-2507
Bio SEC-3, 150Å	4.6 x 300	3	5190-2508
Bio SEC-3, 150Å	4.6 x 150	3	5190-2509
Bio SEC-3, 150Å, Guard	7.8 x 50	3	5190-2510
Bio SEC-3, 300Å	7.8 x 300	3	5190-2511
Bio SEC-3, 300Å	7.8 x 150	3	5190-2512
Bio SEC-3, 300Å	4.6 x 300	3	5190-2513
Bio SEC-3, 300Å	4.6 x 150	3	5190-2514
Bio SEC-3, 300Å, Guard	7.8 x 50	3	5190-2515



Agilent BioHPLC columns (continued)

Agilent Bio SEC-5 HPLC Columns

Description	Size (mm)	Particle	Part No.
		Size (µm)	
Bio SEC-5, 100Å	7.8 x 300	5	5190-2516
Bio SEC-5, 100Å	7.8 x 150	5	5190-2517
Bio SEC-5, 100Å	4.6 x 300	5	5190-2518
Bio SEC-5, 100Å	4.6 x 150	5	5190-2519
Bio SEC-5, 100Å, Guard	7.8×50	5	5190-2520
Bio SEC-5, 150Å	7.8 x 300	5	5190-2521
Bio SEC-5, 150Å	7.8 x 150	5	5190-2522
Bio SEC-5, 150Å	4.6 x 300	5	5190-2523
Bio SEC-5, 150Å	4.6 x 150	5	5190-2524
Bio SEC-5, 150Å, Guard	7.8 x 50	5	5190-2525
Bio SEC-5, 300Å	7.8 x 300	5	5190-2526
Bio SEC-5, 300Å	7.8 x 150	5	5190-2527
Bio SEC-5, 300Å	4.6 x 300	5	5190-2528
Bio SEC-5, 300Å	4.6 x 150	5	5190-2529
Bio SEC-5, 300Å, Guard	7.8 x 50	5	5190-2530
Bio SEC-5, 500Å	7.8 x 300	5	5190-2531
Bio SEC-5, 500Å	7.8 x 150	5	5190-2532
Bio SEC-5, 500Å	4.6 x 300	5	5190-2533
Bio SEC-5, 500Å	4.6 x 150	5	5190-2534
Bio SEC-5, 500Å, Guard	7.8 x 50	5	5190-2535
Bio SEC-5, 1000Å	7.8 x 300	5	5190-2536
Bio SEC-5, 1000Å	7.8 x 150	5	5190-2537
Bio SEC-5, 1000Å	4.6 x 300	5	5190-2538
Bio SEC-5, 1000Å	4.6 x 150	5	5190-2539
Bio SEC-5, 1000Å, Guard	7.8 x 50	5	5190-2540
Bio SEC-5, 2000Å	7.8 x 300	5	5190-2541
Bio SEC-5, 2000Å	7.8 x 150	5	5190-2542
Bio SEC-5, 2000Å	4.6 x 300	5	5190-2543
Bio SEC-5, 2000Å	4.6 x 150	5	5190-2544
Bio SEC-5, 2000Å, Guard	7.8 x 50	5	5190-2545

Agilent Bio MAb HPLC Columns

Description	Size (mm)	Particle Size (µm)	Part No.
Bio MAb, stainless steel	4.6 x 50	1.7	5190-2401
Bio MAb, stainless steel guard	4 x 10	1.7	5190-2402
Bio MAb, stainless steel	4.6 x 50	3	5190-2403
Bio MAb, stainless steel guard	4 x 10	3	5190-2404
Bio MAb, stainless steel	4.6 x 250	5	5190-2405
Bio MAb, stainless steel guard	4 x 10	5	5190-2406
Bio MAb, PEEK	4.6 x 250	5	5190-2407
Bio MAb, PEEK guard	4.6 x 50	5	5190-2408
Bio MAb, PEEK	2.1 x 250	5	5190-2411
Bio MAb, PEEK guard	2.1 x 50	5	5190-2412
Bio MAb, stainless steel	4.6 x 250	10	5190-2413
Bio MAb, stainless steel guard	4 x 10	10	5190-2414
Bio MAb, PEEK	4.6 x 250	10	5190-2415
Bio MAb, PEEK guard	4.6 x 50	10	5190-2416
Bio MAb, PEEK	2.1 x 250	10	5190-2419
Bio MAb, PEEK guard	2.1 x 50	10	5190-2420





Agilent Bio IEX HPLC Columns

Description	Size (mm)	Particle Size (μm)	Bio SCX Part No.	Bio WCX Part No.	Bio SAX Part No.	Bio WAX Part No.
Agilent Bio IEX, stainless steel	4.6 x 50	1.7	5190-2421	5190-2441	5190-2461	5190-2481
Agilent Bio IEX, stainless steel guard	4 x 10	1.7	5190-2422	5190-2442	5190-2462	5190-2482
Agilent Bio IEX, stainless steel	4.6 x 50	3	5190-2423	5190-2443	5190-2463	5190-2483
Agilent Bio IEX, stainless steel guard	4 x 10	3	5190-2424	5190-2444	5190-2464	5190-2484
Agilent Bio IEX, stainless steel	4.6 x 250	5	5190-2425	5190-2445	5190-2465	5190-2485
Agilent Bio IEX, stainless steel guard	4 x 10	5	5190-2426	5190-2446	5190-2466	5190-2486
Agilent Bio IEX, PEEK	4.6 x 250	5	5190-2427	5190-2447	5190-2467	5190-2487
Agilent Bio IEX, PEEK guard	4.6 x 50	5	5190-2428	5190-2448	5190-2468	5190-2488
Agilent Bio IEX, PEEK	2.1 x 250	5	5190-2431	5190-2451	5190-2471	5190-2491
Agilent Bio IEX, PEEK guard	2.1 x 50	5	5190-2432	5190-2452	5190-2472	5190-2492
Agilent Bio IEX, stainless steel	4.6 x 250	10	5190-2433	5190-2453	5190-2473	5190-2493
Agilent Bio IEX, stainless steel guard	4 x 10	10	5190-2434	5190-2454	5190-2474	5190-2494
Agilent Bio IEX, PEEK	4.6 x 250	10	5190-2435	5190-2455	5190-2475	5190-2495
Agilent Bio IEX, PEEK guard	4.6 x 50	10	5190-2436	5190-2456	5190-2476	5190-2496
Agilent Bio IEX, PEEK	2.1 x 250	10	5190-2439	5190-2459	5190-2479	5190-2499
Agilent Bio IEX, PEEK guard	2.1 x 50	10	5190-2440	5190-2460	5190-2480	5190-2500



Agilent BioHPLC Columns



Whether you are characterizing monoclonal antibodies, separating a target protein or monitoring your process scale purifications, Agilent BioHPLC tools can easily be integrated into your workflow for high-quality results, every time.

Our complete line of BioHPLC columns includes:

Size Exclusion BioHPLC columns

- · Agilent Bio SEC-3
- Agilent Bio SEC-5
- ZORBAX GFC-250/450

Ion Exchange BioHPLC columns

- · Agilent Bio MAb
- · Agilent Bio IEX
- · Bio-Monolith

Reverse Phase BioHPLC columns

- Poroshell 300
- Poroshell 120
- StableBond 300Å
- Extend 300Å

Proteomics and LC/MS BioHPLC columns

- ZORBAX Capillary, Nano and MicroBore
- Multiple Affinity Removal System
- mRP High-Recovery Protein

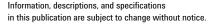
Remember, too, that all Agilent columns are backed by over 40 years of chromatography expertise, the industry's best technical support, and a 90-day warranty from the date of shipment.

For more information

To learn more about Agilent BioHPLC columns, visit us online at www.agilent.com/chem/BioHPLC

Or call toll free: 1-800-227-9770, option 3, then option 3 again (in the U.S. and Canada) $\frac{1}{2}$

In other countries, please call your local Agilent Representative or Agilent Authorized Distributor or visit us online at www.agilent.com/chem/contactus



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