Agilent Ion-Exchange BioHPLC Columns

CHARACTERIZE CHARGED VARIANTS OF PROTEINS WITH SPEED AND CONFIDENCE

The Measure of Confidence

Agilent Technologies

Now you have more *columns* and more *choices* to improve your charged-variant analysis of proteins

Ion-exchange (IEX) is a critical technique used to separate proteins and biotherapeutics. Like SEC, IEX can be used to separate proteins in their native state; it is also useful as a preparative technique to purify and isolate these proteins.

During production and purification, antibodies can exhibit changes in charge heterogeneity caused by amino acid substitutions, glycosylation, phosphorylation, and other post-translational or chemical modifications.

In protein analysis, charged variations at a given pH indicate a change in the primary molecular structure – resulting in additional forms of the protein in question. These are called isoforms (or charged variants), which can be resolved by IEX chromatography.

Because these changes can impact stability and activity – or cause immunologically adverse reactions – the analysis of charged variants is critical to biopharmaceuticals.

Agilent can help you separate and characterize biomolecules with our family of ion-exchange BioHPLC columns

Whether your goal is to characterize the next biopharmaceutical or isolate a target protein, Agilent can help you overcome the challenges of isolating and identifying charged variants of proteins.

Our ion-exchange BioHPLC columns are engineered and tested to give your lab:

- **High efficiency and reproducibility**
- **Fast, high-resolution separations**
- **More robust methods**
- **A wide range of pore sizes means you can effectively separate proteins and charged variants of any size**
- **Minimal chance of column contamination when capturing complex samples**

As a leading supplier to the biopharmaceutical industry, Agilent understands that quality and consistency are critical to providing safe, highly efficacious therapeutics. Agilent ion-exchange 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.

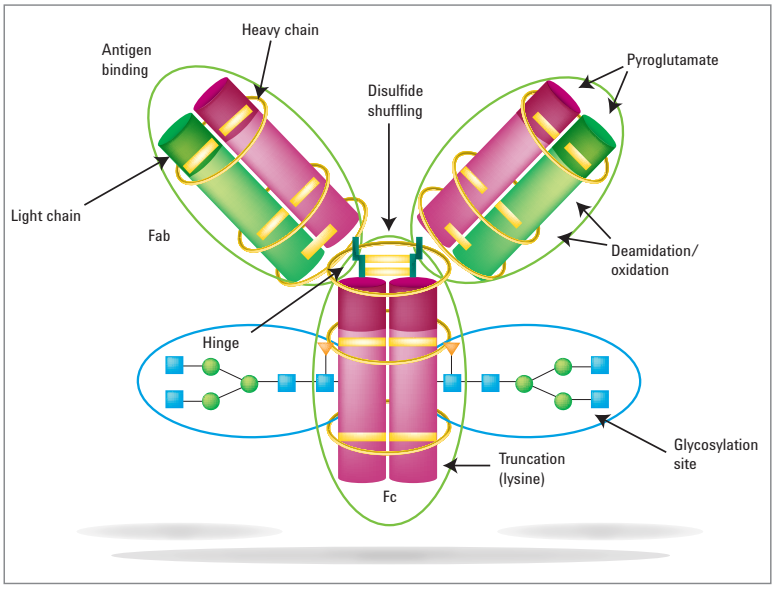


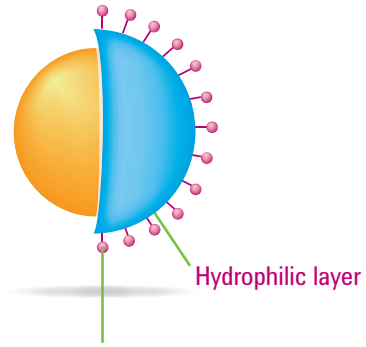
Figure 1. *Charged variants of monoclonal antibodies arise through different levels of glysoslation, deamidation, and oxidation of amino acids and through lysine truncation of heavy chains.*

To learn more about performing fast, high-resolution protein separations, visit **agilent.com/chem/AdvanceBio**

INSIDE: our complete portfolio of ion-exchange BioHPLC columns covering the very latest biomolecule applications

HIGH-RESOLUTION, CHARGED-BASED SEPARATIONS OF PROTEINS, PEPTIDES, AND MORE

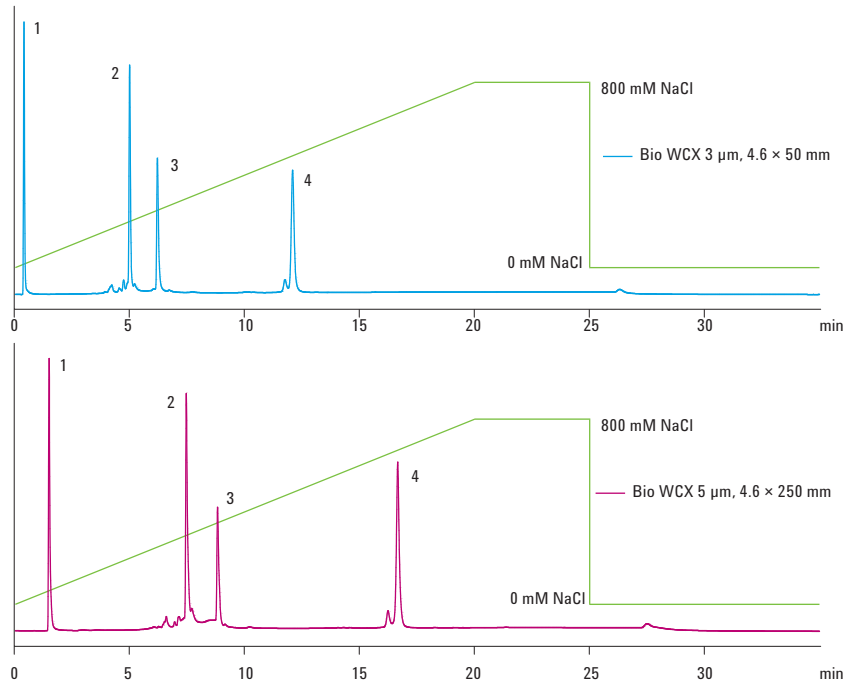
Bio IEX particle



Packed with polymeric, nonporous, ion-exchange particles, Agilent Bio IEX HPLC columns deliver high-recovery, high-efficiency separations of peptides, oligonucleotides, and proteins.

- **Accurate results:** no nonspecific binding because a hydrophilic, polymeric layer is grafted to highly cross-linked and rigid nonporous poly(styrene divinylbenzene) particles
- **Higher speed and resolution:** smaller particles, coating, and bonding are resistant to high pressures
- **Increased column capacity:** uniform, densely packed ion-exchange functional groups are chemically bonded to the hydrophilic layer
- **More robust methods** when used with Agilent Buffer Advisor software and the 1260 Infinity Bio-inert Quaternary LC
- **Choices to help you perfect your separation:** suitable for any protein separation, with four different types of ion-exchange – SCX, WCX, SAX, WAX

Achieve faster analysis time with smaller particles and shorter column lengths – speed up your separation by 30%



Columns:
Agilent Bio WCX 5 µm, 4.6 × 250 mm SS (p/n 5190-2445)
Agilent Bio WCX 3 µm, 4.6 × 50 mm SS (p/n 5190-2443)

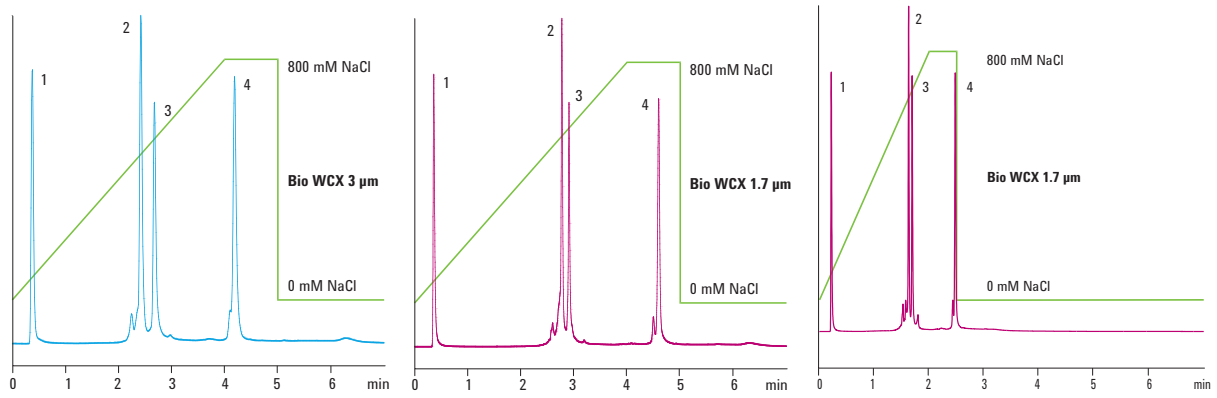
Mobile phase:
A: 20 mM sodium phosphate, pH 6.5
B: A + 1.6 M NaCl

Gradient: 0 to 50% B

Temperature:	Ambient
Injection volume:	10 µL
Sample:	Ovalbumin (1), ribonuclease A (2), cytochrome c (3), lysozyme (4)
Concentration:	0.5 mg/mL
Detection:	UV, 220 nm
Instrument:	Agilent 1260 Infinity Bio-inert Quaternary LC

Figure 2. *Protein separation on an Agilent Bio WCX 4.6 x 50 mm, 3 µm column vs. an Agilent Bio WCX 4.6 x 250 mm, 5 µm column (flow rate 1.0 mL/min). Faster analysis times were achieved through smaller particle size and shorter column length – samples eluted from the longer column in 17 minutes, and in just 12 minutes from the shorter column.*

Smaller particle sizes provide increased resolution



Left and middle: *Protein separation on an Agilent Bio WCX 3 µm column vs. an Agilent Bio WCX 1.7 µm column (flow rate 1.0 mL/min).*

Right: *By increasing the flow rate to 1.7 mL/min, we reduced the separation time to* ***less than 3 minutes****. (An Agilent Bio WCX column was used).*

Columns:
Agilent Bio WCX 3 µm, 4.6 × 50 mm SS (p/n 5190-2443)
Agilent Bio WCX 1.7 µm, 4.6 × 50 mm SS (p/n 5190-2441)

Mobile phase:
A: 20 mM sodium phosphate, pH 6.5
B: A + 1.6 M NaCl

Gradient: 0 to 50% B

Temperature: Ambient

Injection volume: 10 µL

Sample: Ovalbumin (1), ribonuclease A (2), cytochrome c (3), lysozyme (4)

Concentration: 0.5 mg/mL

Detection: UV, 220 nm

Instrument: Agilent 1260 Infinity Bio-inert Quaternary LC

Figure 3. *Reduce analysis time – without sacrificing peak shape and resolution – by increasing flow rate.*

To learn more about performing fast, high-resolution protein separations, visit **agilent.com/chem/AdvanceBio**

SHORTEN AND SIMPLIFY THE IEX WORKFLOW WITH BUFFER ADVISOR SOFTWARE

Use of dynamically mixed four-component gradients calculated with the **Agilent Buffer Advisor software** enables you to achieve a robust method through design-of-experiment principles. Using Buffer Advisor software results in a significantly shorter buffer preparation time, particularly when compared with manual preparation of buffers for premixed two-component gradients. You also get improved accuracy with your buffer preparation, which makes this a more robust method for transferring to other labs.

The **Agilent Buffer Advisor software** provides a wide range of prevalidated, user-selectable buffer systems for anion- and cation-exchange chromatography and delivers recipes for preparation of the most suitable stock solutions. With pH optimization by the software, greater accuracy and precision is achieved with the gradients in comparison to those generated using manually prepared buffer solutions. The Agilent Buffer Advisor software recalculates the four-component gradient and ensures that the concentrations of acidic and basic buffer components maintain the desired constant pH.

Initial screening of twenty experiments was achieved from just four mobile phase eluents instead of needing forty different solutions. The Agilent Buffer Advisor software automatically blends the buffers to create the desired pH and buffer strengths. The gradient timetable can then be programmed in the quaternary.

In 15 hours of unattended operation, the entire set of 20 experiments was completed and optimum results selected. The experiments required less than 1 L of eluent at a flow rate of 1.0 mL/min with a gradient of 0 to 500 mM NaCl.

Automated method development for optimized charged-variant separations

Columns:	Agilent Bio WCX 3 µm, 4.6 x 50 mm SS (p/n 5190-2443) Agilent Bio SCX 3 µm, 4.6 x 50 mm SS (p/n 5190-2423)
Mobile phase:	A: Water B: 1.5 M NaCl C: 40 mM NaH_2PO_4 D: 40 mM Na_2HPO_4 By combining predetermined proportions of C and D as determined by the Buffer Advisor software, buffer solutions at the desired pH range and strength were created.
Gradient:	Conditions for chromatograms shown: pH 5.0 to 7.0, 10 to 25 mM buffer strength 0 to 500 mM NaCl, 0 to 15 minutes 500 mM NaCl, 15 to 20 minutes DOE experiments pH 5.0 to 7.0 0 to 200 mM, 0 to 250 mM, and 0 to 300 mM
Flow rate:	1.0 mL/min
Temperature:	Ambient
Injection volume:	5 µL
Sample:	IgG monoclonal antibody
Concentration:	2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)
Detection:	UV, 220 nm
Instrument:	Agilent 1260 Infinity Bio-inert Quaternary LC

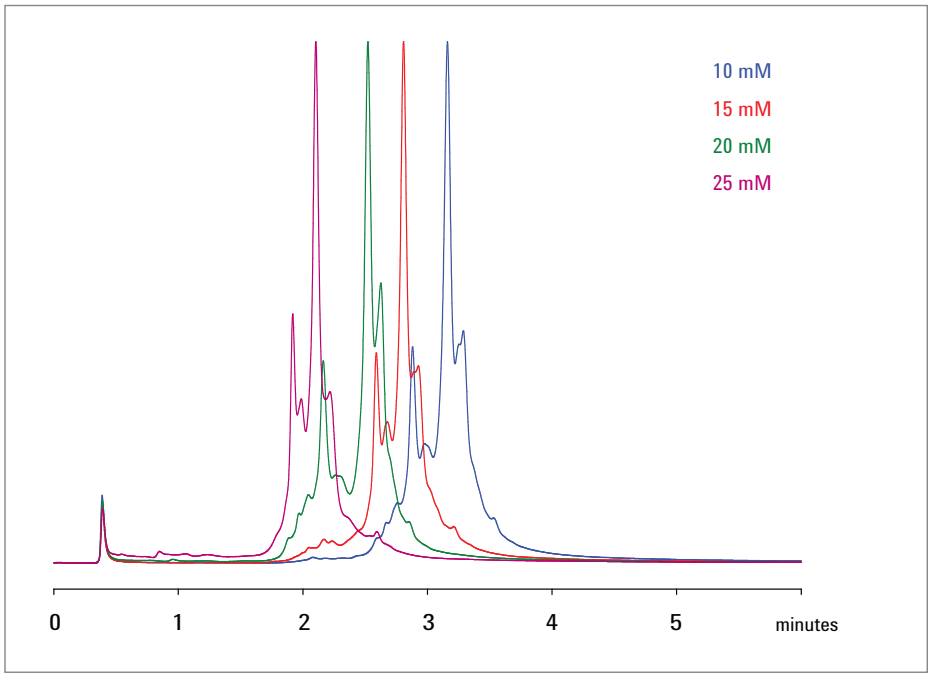


Figure 4. *Optimizing buffer strength at pH 6.5 from the screening chromatograms of a monoclonal IgG separation.*

Figure 4 shows four chromatograms from the set of twenty experiments all obtained at just one pH value, 6.5, selected from the Bio SCX 3 µm column separation series. To visualize the results of the entire series of experiments, the resolution factors between the main component peak and its earlier eluting major contaminant were calculated. The resulting data are shown in **Figures 5a** and **5b** for Bio SCX and Bio WCX columns, respectively.The **strong cation-exchange column** delivers a more robust method because the strong cation exchanger has a constant charge over a wide pH range.

Unsurprisingly, the **weak cation-exchange sorbent** was unable to resolve the peaks at pH 5.0 since the weak cation-exchange sorbent was not charged under these conditions, and therefore did not function in an ion-exchange mode. As the pH was increased, the separation improved.

Another advantage of the Buffer Advisor software is the ability to mix different proportions of the stock solutions to create a linear pH gradient. This allows a similar separation, however, selectivity differences are observed (compare to **Figure 4**, for example) which may aid separation.

DOE experiment for robust method development

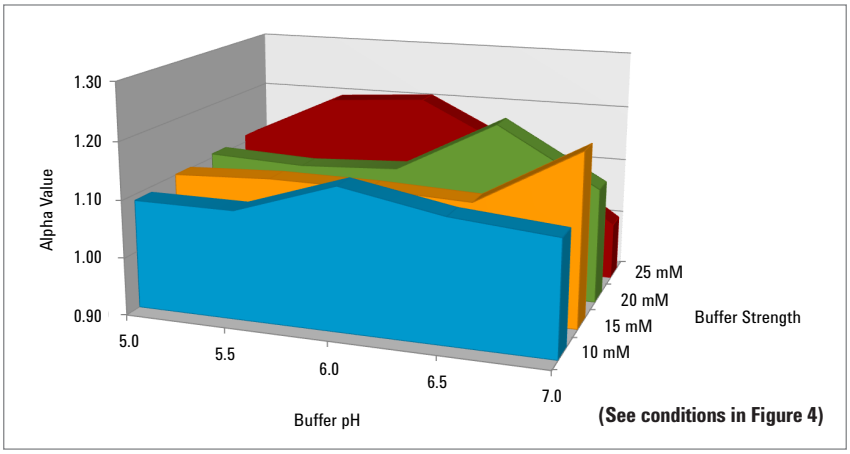


Figure 5a. *Alpha value plots for Bio SCX 3 µm, 4.6 x 50 mm column.*

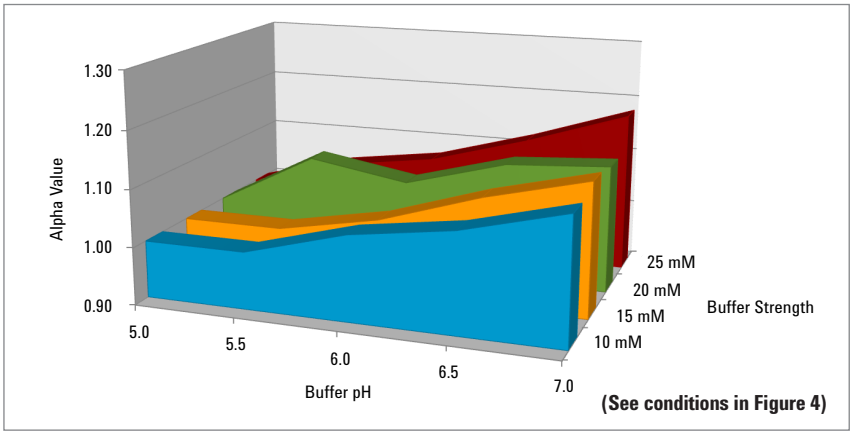


Figure 5b. *Alpha value plots for Bio WCX 3 µm, 4.6 x 50 mm column.*

Save time and money on buffer preparation

Manual preparation of buffers

Automated online dynamic mixing of buffers

Agilent Buffer Advisor software helps to automate the production of buffers. Dynamic mixing of only 4 stock solutions eliminates the need to prepare and titrate multiple buffer solutions.

Boost Performance: *4 stock solutions can be used to run more than 88 analyses, for faster results*

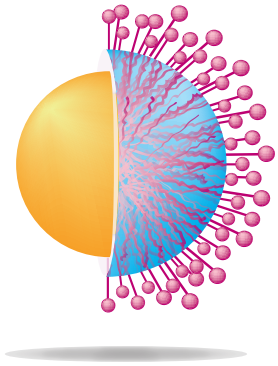
Save Time: *Quaternary mixing enables easy blending of multiple buffers of different pH, for less tedious buffer preparation*

Reduce Costs: *Evaluate conditions for pH scouting before running samples, for less sample waste and shorter analysis time*

To learn more about performing fast, high-resolution protein separations, visit **agilent.com/chem/AdvanceBio**

DESIGNED FOR HIGH-RECOVERY SEPARATIONS OF MONOCLONAL ANTIBODIES

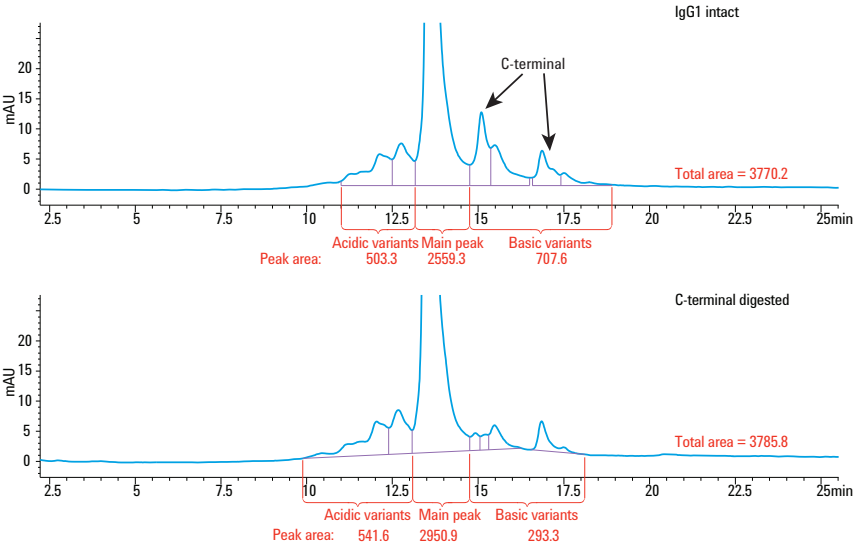
Bio MAb particle



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

Use Bio MAb to identify C-terminal truncation on heavy chains



Column:	Agilent Bio MAb 5 µm, 4.6 x 250 mm PEEK (p/n 5190-2407)
Mobile phase:	A. 10 mM Na phosphate buffer, pH 5.5 B. A + 0.5 M NaCl
Flow rate:	0.85 mL/min
Gradient:	10 to 35% B from 0 to 25 minutes (unless otherwise stated)
Detection:	UV 225 nm
Sample:	5 µL of 1 mg/mL of intact or C-terminal digested IgG1
Instrument:	Agilent 1260 Infinity Bio-inert Quaternary LC

Figure 6. *Calculation of C-terminal digested IgG1 from source A using an Agilent Bio MAb 5 µm column on the Agilent 1260 Infinity Bio-inert Quaternary LC. The column displayed high resolution, enabling better peak identification and accurate quantification.*

Effective analysis of antibodies requires both speed and precision. Agilent Bio MAb columns provide exclusive advantages that make them ideal for high-resolution antibody separations.

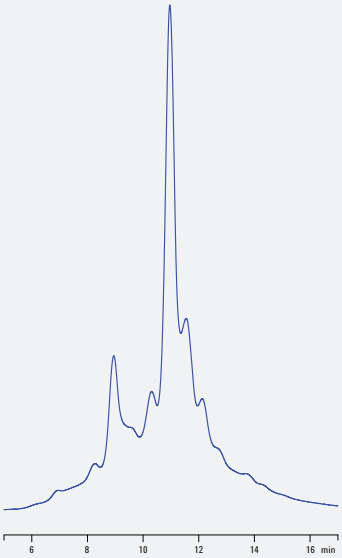
- **Higher accuracy:** no nonspecific binding because a hydrophilic, polymeric layer is grafted to highly cross-linked and rigid nonporous poly(styrene divinylbenzene) particles
- **Superior MAb analysis:** a higher density outer layer made with an optimized process layers the weak cation-exchange phase to the particle
- **Method robustness:** proven performance that delivers precise, consistent results
- **Choices to help you perfect your separation:** a range of particle sizes for the highest resolution antibody separations

pH Gradients - a powerful alternative for charged-variant analysis

An alternative technique to conventional ion-exchange methods for charged variants is emerging, and that technique uses a pH gradient.

Conventional ion-exchange methods use an eluent of increasing ionic strength to elute and separate proteins and variants from the IEX column. As the salt increases, the interactions between the protein and IEX column are disrupted and the separation is achieved.

A pH gradient approach can be used with cation-exchange chromatography. The increase in pH will cause proteins to become neutral (or negatively charged) and elute from the column. This approach is growing in popularity because of the high resolution achieved and the ease of running these methods with newer HPLCs.



Analysis of a IgG monoclonal antibody using a pH gradient of 6.5 to 7.5 (0-20 min), 50 mM, Agilent Bio MAb 5 µm, 4.6 x 50 mm

The chart below compares the two methods.

	Salt-based IEX
Advantages	• *Established method* • *Uses standard HPLC instrumentation* • *Ability to collect fractions directly*
Disadvantages	• *Intolerant to salt and pH extremes of sample matrixes* • *Method development required, often time-consuming*

	pH-based IEX
Advantages	• *Multiproduct option* • *Tolerant to changes in salt concentration and pH of sample* • *Uses common HPLC instruments and expertise* • *Capable of analyzing in-process samples* • *Can be quick and easy with tools like Buffer Advisor software*
Disadvantages	• *Relatively new*

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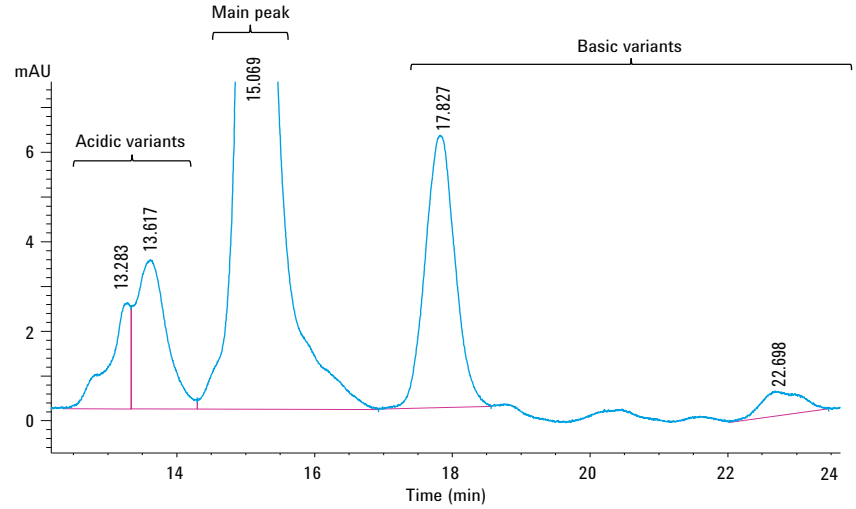
REPRODUCIBILITY AND PRECISION

The unique resin of Agilent Bio MAb columns make them ideal for the charge-based separation of monoclonal antibodies. Agilent Buffer Advisor software will help you to complete the pH gradient.

Tables 1 and 2 show the average retention times and area RSDs from six replicates of an IgG1 injection. The Bio MAb columns were key to a method with excellent reproducibility and precision as shown by the low RSDs.

A variation of injection volume by ±10% compared to the actual method caused the area RSD to deviate significantly; however, this deviation is expected due to the load on the ion-exchange column. There were no further significant changes in the chromatographic pattern when deliberate variations were made in experimental conditions, thus showing the method to be robust.

Bio MAb columns enable precise quantitation, robust methods



Column:	Agilent Bio MAb PEEK, 4.6 × 250 mm, 5 µm (p/n 5190-2407)
Mobile phase A:	10 mM sodium phosphate buffer, pH 6.0
Mobile phase B:	10 mM sodium bicarbonate buffer, pH 9.5
Gradient:	Time (min) Mobile phase (% B) 0 0 25 100 27 100 30 0
Injection volume:	10 µL (needle with wash, flush port active for 7 seconds)
Flow rate:	1.0 mL/min
Data acquisition:	214 and 280 nm
Acquisition rate:	20 Hz
Flow cell:	60 mm path
Column temperature:	30 °C
Sample thermostat:	5 °C
Post time:	5 minutes

Figure 7. *pH gradient-based cation-exchange chromatogram of an IgG1 separation using an Agilent Bio MAb PEEK, 4.6 x 250 mm, 5 µm column.*

COUNT ON RELIABLE, ROBUST PERFORMANCE

The realities of your lab demand that your column delivers a consistent separation, even with small variations in your method. We test our columns to ensure they provide the robust performance you need.

Table 1. Charged-variant quantification by area %, n=6.

	RT (min)A	Area%
Acidic variants	13.28	9.87
	13.61	
Main peak	15.058	76.92
Basic variants	17.82	13.21
	22.69	

Table 2. Retention time and area RSD (%), n=6 for main peak.

	Retention time	Peak area
Mean (min)	15.058	1172
RSD	0.1061	1.60

Table 3. Method robustness evaluation – varying key parameters.

		RT deviation (limit: ± 3.0 %)	Area deviation (limit ± 5.0 %)
Parameters	**Variation**	**Main peak**	
Variation in injection volume (10 µL ± 10%)	– 1 µL	– 0.19	10.49
	+ 1 µL	0	– 9.89
Variation in column temperature (30 °C ± 5%)	– 5%	– 1.19	2.73
	+ 5%	0.66	2.13
Variation in buffer pH (6.0 ± 0.2)	– 0.2	0.199	– 0.68
	+ 0.2	0.99	– 0.08
Variation of flow rate (1.0 ± 2%)	– 2%	0.66	2.73
	+ 2%	0	– 1.10

To evaluate the robustness of this method, four critical parameters of the optimized method were varied – injection volume, column temperature, buffer pH, and flow rate. The RSD deviations were all as expected.

Easy, reliable pH testing, designed for chromatographers

Agilent now offers a full line of pH meters and electrodes. Designed for chromatographers, these pH meters offer intuitive user design and exceptional ruggedness for your lab. Agilent CrossLab electrodes are available for non Agilent pH meters. Learn more at **agilent.com/chem/AgilentpH**

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AGILENT BIO-MONOLITH COLUMNS

POLYMER-BASED, MONOLITH COLUMNS FOR MACRO-BIOMOLECULE SEPARATIONS

Agilent Bio-Monolith ion-exchange HPLC columns provide rapid, high-resolution separations of antibodies (IgG, IgM), plasmid DNA, viruses, phages, and other macro-biomolecules.

- **Improved accuracy:** less chance of contamination when capturing complex samples. A monolith disk (5.2 x 4.95 mm, 100 µL) with continuous channels eliminates diffusion mass transfer
- **Fast separations:** the absence of diffusion, pores, and void volume allows for rapid transport between mobile and stationary phases
- **Efficient method development:** extremely fast separations decrease method development time and costs. Save time and buffer by locking in method parameters
- **Choices to help you perfect your separation:** includes strong cation-exchange phases as well as strong and weak anion-exchange phases. Bio-Monolith HPLC columns are compatible with HPLC and preparative LC systems, including Agilent 1200 Infinity Series

Agilent Bio-Monolith HPLC Column Selection Guide

Column	Description	Key Applications
Bio-Monolith QA	The quaternary amine bonded phase (strong anion-exchange) is fully charged over a working pH range of 2 to 13, binding negatively charged biomolecules.	• Adenovirus process monitoring and quality control • IgM purification monitoring and quality control • Monitoring DNA impurity removal • Monitoring endotoxin removal • HSA purity
Bio-Monolith DEAE	The diethylaminoethyl bonded phase (weak anion-exchange) offers increased selectivity of biomolecules with negative charge over a working pH range of 3 to 9.	• Process monitoring and quality control of bacteriophage manufacturing and purification • Process monitoring and quality control of plasmid DNA purification
Bio-Monolith SO_3	The sulfonyl bonded phase (strong cation-exchange) is fully charged over a working pH range of 2 to 13, binding positively charged biomolecules.	• Fast and high resolution analytical separations of large molecules such as proteins and antibodies • Hemoglobin A1c fast analytics

Samples were taken from the bioreactor at 36, 158, and 191 minutes, shown in **Figure 8**. Peak 1 represents phage, media, and host cells, peak 2 represents the intact gDNA, and peak 3 represents the fragmented gDNA. During phage proliferation, genomic DNA (gDNA) concentration increases as the host cells are lysed. During the late stages of fermentation, gDNA begins to degrade into fragments, which cannot be easily removed by purification media. Therefore, it is critical to stop the fermentation cycle prior to the degradation of the genomic DNA.

These complex samples contain cell debris, which can clog the HPLC column and limit the ability for quick fermentation monitoring. The open pore structure of the Bio-Monolith DEAE column reduces clogging and allows efficient real-time process monitoring.

Bio-Monolith DEAE columns enable efficient process monitoring for maximum product yield

Bio-Monolith DEAE column monitors phage production during fermentation

Column:	Bio-Monolith DEAE, 5.2 x 4.95 mm (p/n 5069-3636)
Mobile phase:	A: 125 mM Phosphate buffer, pH 7.0 B: 125 mM Phosphate buffer + 1 M NaCl, pH 7.0
Flow rate:	1 mL/min
Gradient:	100% buffer A (2.5 min) 0 to 100% buffer B (10 min) 100% buffer A (2 min)
Detector:	UV at 280 nm
Instrument:	High pressure gradient HPLC system, Agilent 1200 Infinity Series

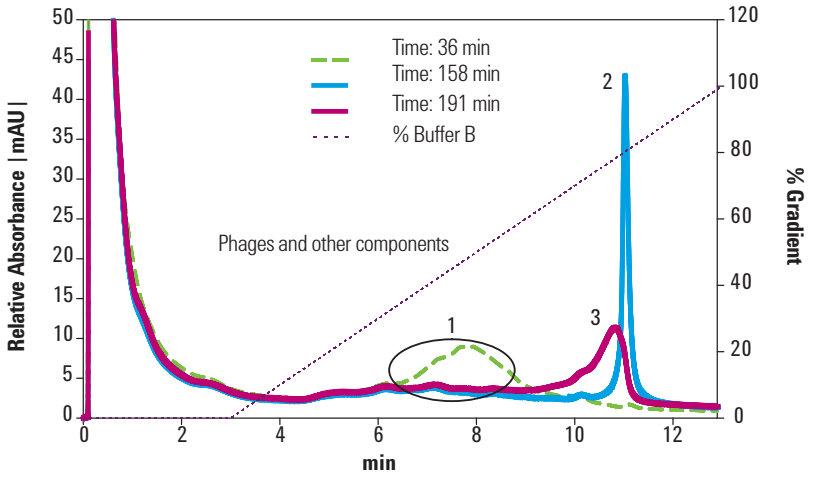


Figure 8. *As phage proliferation progresses, the genomic DNA (gDNA) concentration increases as the host cells are being lysed. In the late stages of fermentation, gDNA begins to degrade into fragments. These gDNA fragments cannot be easily removed by purification media, therefore it is critical to stop the fermentation cycle prior to the degradation of the genomic DNA. The chromatogram above represents three samples taken from the bioreactor at 36, 158, and 191 minutes. Peak 1 represents phage, media and host cells, peak 2 the intact gDNA, and peak 3 the fragmented gDNA.*

To learn more about performing fast, high-resolution protein separations, visit **agilent.com/chem/AdvanceBio**

AGILENT PL-SAX COLUMNS

RELIABLE SEPARATIONS OF SYNTHETIC OLIGONUCLEOTIDES

Agilent PL-SAX columns are well suited for anion-exchange HPLC separations of proteins, peptides, and deprotected synthetic oligonucleotides. Denaturing conditions of temperature, organic solvent, and high pH levels are used for the oligonucleotide separations.

- **Long column lifetime:** the **strong anion-exchange** functionality ensures exceptional chemical and thermal stability, even with sodium hydroxide eluents
- **Excellent chromatographic performance** through small particle sizes
- **End-to-end analytical options:** 5 µm for analytical, 10 and 30 µm for purification
- **Flexible method development:** IEX across a wide pH range. Strong anion-exchange functionality – covalently linked to a fully porous chemically stable polymer – extends the operating pH range
- **Suitable for purification** to conserve biological activity in proteins

High resolution of oligonucleotides

High resolution separation of a poly-t-oligonucleotide size standard spiked with 10 mer, 15 mer, 30 mer and 50 mer (main peaks)

Column:	PL-SAX 1000Å, 4.6 x 50 mm, 8 µm, (p/n PL1551-1802)
Mobile phase:	A: 7:93 v/v ACN: 0.1 M TEAA, pH 8.5 B: 7:93 v/v ACN: 0.1 M TEAA, 1 M ammonium chloride, pH 8.5
Gradient:	0 to 40% B in 10 min, followed by 40 to 70% B in 14 min and 70 to 100% B in 25 min
Flow rate:	1.5 mL/min
Temperature:	60 °C
Detector:	UV, 220 nm

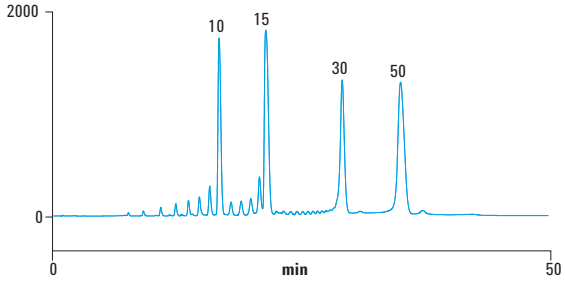


Figure 9. *High-resolution separation of poly-t-oligonucleotide. With the gradient used here, baseline separation of the n-1 from n was easily obtained up to the 15 mer.*

High resolution separations of oligonucleotides can be achieved using the PL-SAX strong anion-exchanger with separations of n-1 from n being achievable. **Figure 9** shows the separation of a poly-t-oligonucleotide size standard spiked with a 10 mer, 15 mer, 30 mer, and 50 mer (main peaks). Acetonitrile was added to the eluent to suppress the formation of secondary structures that would have a detrimental effect on the separation.

The chemical stability of PL-SAX packing is demonstrated in our analysis of choline kinase (**Figure 10**), where enzyme activity was successful stabilized. Here, the 75-mL sample of partially purified choline kinase from a liver cytosol contained approximately 1 mg of the enzyme at molecular weight 160,000.

In **Figure 11**, the enzyme amyloglucosidase was fractionated from *Aspergillus niger* cell culture filtrate. The enzyme occurs in two forms with molecular weights of 99 kD and 112 kD (peaks 1 and 2, respectively), which have the same amino acid composition but different carbohydrate content. Using the PL-SAX 4000Å 4.6 x 50 mm, 8 µm column, it is possible to purify 3.6 mg of the isoenzymes or 20 mg of total enzyme in under two minutes.

Purify biologically active proteins

Analysis of choline kinase on PL-SAX 4000Å

Column:	PL-SAX 4000Å, 4.6 x 50 mm, 8 µm (p/n PL1551-1803)
Mobile phase:	A: 20 mM Tris 5% ethylene glycol, pH 7.5 (The following are required to retain enzyme activity) 1.0 mM Ethylene glycol tetraacetic acid 2.0 mM ß-Mercaptoethanol 0.2 mM Phenylmethylsulfonyl fluoride B: A + 1 M KCl
Flow rate:	3.0 mL/min
Detector:	UV, 280 nm

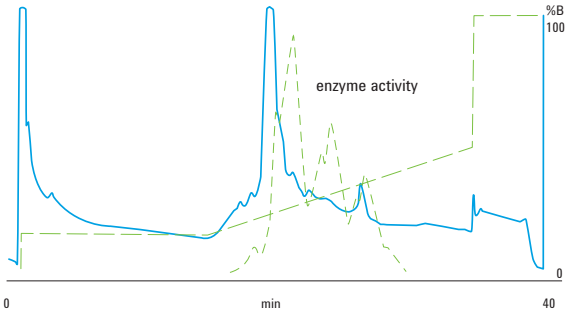


Figure 10. *In this analysis of choline kinase, enzyme activity was maintained, and active proteins were still present at the end of the separation.*

Maximize sample loading

Column:	PL-SAX 4000Å, 4.6 x 50 mm, 8 µm (p/n PL1551-1803)	Gradient:	Linear 0 to 100% B in 2 min
Eluent A:	0.01M Tris HCl, pH 8	Flow rate:	4.0 mL/min
Eluent B:	A + 0.5M NaCl, pH 8	Detector:	UV, 280 nm

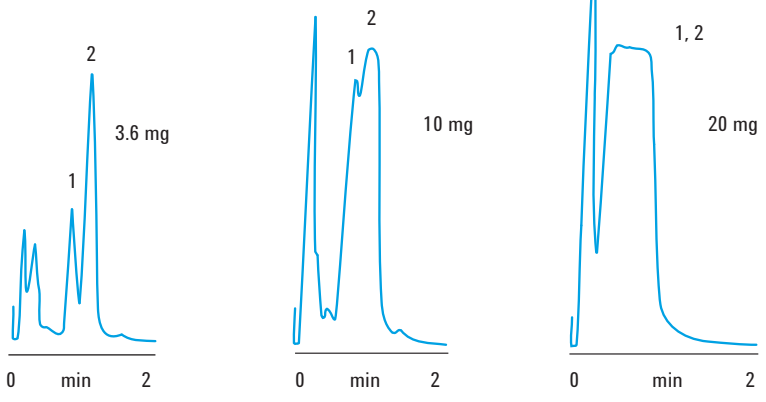


Figure 11. *High-speed purification of amyloglucosidase at different enzyme concentrations. An Agilent PL-SAX 4000Å 8 µm column made it possible to purify 3.6 mg of the isoenzymes – or 20 mg of the total enzyme – in under two minutes.*

To learn more about performing fast, high-resolution protein separations, visit **agilent.com/chem/AdvanceBio**

AGILENT PL-SCX COLUMNS

SEPARATE AND PURIFY DIVERSE BIOMOLECULES

PL-SCX is a macroporous PS/DVB matrix with a very hydrophilic coating and strong cation-exchange functionality. The manufacturing process is controlled to provide optimum density of strong cation-exchange moieties for analyzing, separating, and purifying a wide range of biomolecules – from small peptides to large proteins.

- **High resolution:** 5 µm media delivers reliable higher-resolution separations, while the 10 and 30 µm media is ideally used for medium-pressure LC
- **Choices to optimize your separation:** two pore sizes (1000Å and 4000Å) that provide good mass transfer characteristics for a range of solute sizes
- **Exceptional stability** for long column lifetime

Exceptional chemical stability for the widest range of eluents and clean-up procedures for purification

Column: PL-SCX 1000Å, 4.6 x 50 mm, 8 µm (p/n PL1545-1802)

Eluent A: 0.02 M KH_2PO_4, pH 6

Eluent B: A + 0.5 M NaCl, pH 6

Gradient: Linear 0 to 100% B in 20 min

Detection: UV, 280 nm

Wash solution (approx 40 column volumes)	R_2 Factor (peaks 2 and 3)	Protein capacity (mg lysozyme/mL CV)
Initial run	1.5	33
0.2 M HCl	1.7	31
0.2 M NaOH	2.0	33
6 M Urea	1.6	33
1% TFA	1.6	33
10% Acetic acid	1.7	28
100% Methanol	1.6	31
0.5 M HCl	1.5	31
2 M NaOH	2.0	33

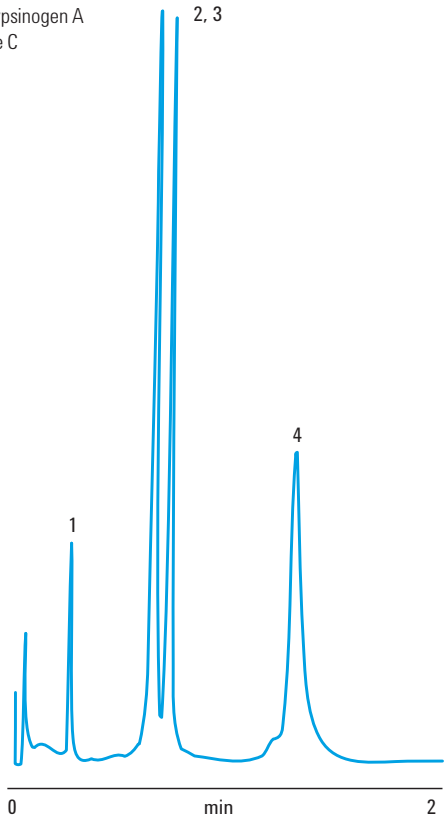
Table 4. *Comparison of protein separations on an Agilent PL-SCX column before and after washing with strong acids, alkalis, and organics.*

Captiva Low Protein Binding Filters

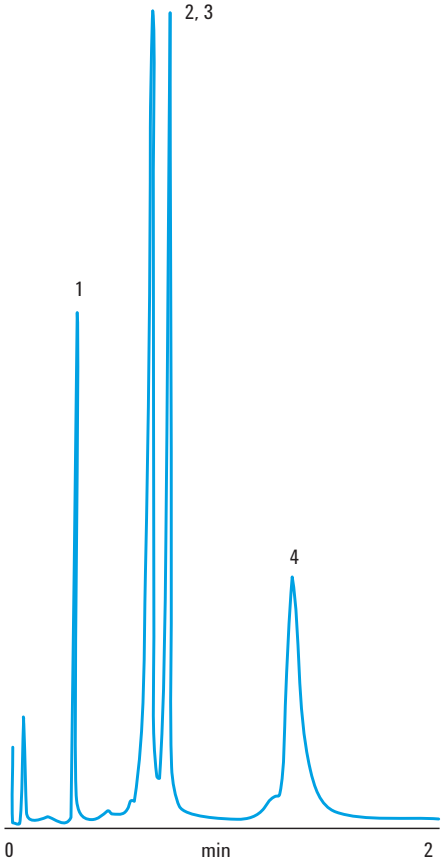
Agilent PES filters provide superior and consistent low protein binding for protein-related filtration.

Learn more at
agilent.com/chem/filtration

Peak Identification
1. Myoglobin
2. α-Chymotrypsinogen A
3. Cytochrome C
4. Lysozyme



Initial chromatogram of a protein separation on an Agilent PL-SCX column.



Final chromatogram of the same protein solution on the same column after washing with forty column volumes of strong eluents.

See conditions on page 16

Figure 12. *As you can see in the above examples, PL-SCX material is extremely stable when exposed to high pressure, strong acids, and strong alkalis.*

To learn more about performing fast, high-resolution protein separations, visit **agilent.com/chem/AdvanceBio**

ORDERING INFORMATION

Agilent Bio IEX columns for a wide range of protein and peptide characterizations

Agilent Bio IEX HPLC Columns, PEEK

Size (mm)	Particle Size (µm)	Bio SCX	Bio WCX	Bio SAX	Bio WAX
4.6 x 250	10	5190-2435	5190-2455	5190-2475	5190-2495
4.6 x 50	10	5190-2436	5190-2456	5190-2476	5190-2496
4.6 x 250	5	5190-2427	5190-2447	5190-2467	5190-2487
4.6 x 50	5	5190-2428	5190-2448	5190-2468	5190-2488
2.1 x 250	10	5190-2439	5190-2459	5190-2479	5190-2499
2.1 x 50	10	5190-2440	5190-2460	5190-2480	5190-2500
2.1 x 250	5	5190-2431	5190-2451	5190-2471	5190-2491
2.1 x 50	5	5190-2432	5190-2452	5190-2472	5190-2492

Agilent Bio IEX HPLC Columns, Stainless Steel

Size (mm)	Particle Size (µm)	Bio SCX	Bio WCX	Bio SAX	Bio WAX
21.2 x 250	5	5190-6879	5190-6881	5190-6883	5190-6877
10 x 250	5	5190-6878	5190-6880	5190-6882	5190-6876
4.6 x 250	10	5190-2433	5190-2453	5190-2473	5190-2493
4.6 x 150	3				5190-6875
4.6 x 250	5	5190-2425	5190-2445	5190-2465	5190-2485
4.6 x 50	3	5190-2423	5190-2443	5190-2463	5190-2483
4.6 x 50	1.7	5190-2421	5190-2441	5190-2461	5190-2481
4.0 x 10, Guard	10	5190-2434	5190-2454	5190-2474	5190-2494
4.0 x 10, Guard	5	5190-2426	5190-2446	5190-2466	5190-2486
4.0 x 10, Guard	3	5190-2424	5190-2444	5190-2464	5190-2484
4.0 x 10, Guard	1.7	5190-2422	5190-2442	5190-2462	5190-2482

To find parts and supplies to support high-performance bioseparations, such as stainless steel-coated PEEK capillaries for high-pressure bio-inertness and robustness, visit **agilent.com/chem/LCsupplies**

To receive a copy of the Infinity Supplies calalog, and other key resources, visit **agilent.com/chem/GetGuides**

Agilent Bio MAb columns for monoclonal antibodies

Agilent Bio MAb HPLC Columns

Size (mm)	Particle Size (µm)	Bio MAb PEEK	Bio MAb Stainless Steel
21.2 x 250	5		5190-6885
10 x 250	5		5190-6884
4.6 x 250	10	5190-2415	5190-2413
4.6 x 50	10	5190-2416	
4.6 x 250	5	5190-2407	5190-2405
4.6 x 50	5	5190-2408	
4.6 x 50	3		5190-2403
4.6 x 50	1.7		5190-2401
4.0 x 10, Guard	10		5190-2414
4.0 x 10, Guard	5		5190-2406
4.0 x 10, Guard	3		5190-2404
4.0 x 10, Guard	1.7		5190-2402
2.1 x 250	10	5190-2419	
2.1 x 50	10	5190-2420	
2.1 x 250	5	5190-2411	
2.1 x 50	5	5190-2412	

Agilent Bio-Monolith columns for macro-biomolecule separations

Agilent Bio-Monolith HPLC Columns

Column	Part No.
Bio-Monolith QA	5069-3635
Bio-Monolith DEAE	5069-3636
Bio-Monolith SO_3	5069-3637

To learn more about performing fast, high-resolution protein separations, visit **agilent.com/chem/AdvanceBio**

ORDERING INFORMATION

Agilent PL-SAX columns for synthetic oligonucleotides

PL-SAX Strong Anion-Exchange Columns

Size (mm)	Particle Size (µm)	PL-SAX 1000Å	PL-SAX 4000Å
1.0 x 50	5	PL1351-1502	PL1351-1503
2.1 x 50	5	PL1951-1502	PL1951-1503
4.6 x 50	5	PL1551-1502	PL1551-1503
2.1 x 50	8	PL1951-1802	PL1951-1803
2.1 x 150	8	PL1951-3802	PL1951-3803
4.6 x 50	8	PL1551-1802	PL1551-1803
4.6 x 150	8	PL1551-3802	PL1551-3803
4.6 x 250	10	PL1551-5102	PL1551-5103
4.6 x 150	10	PL1551-3102	PL1551-3103
25 x 50	10	PL1251-1102	PL1251-1103
25 x 150	10	PL1251-3102	PL1251-3103
50 x 150	10	PL1751-3102	PL1751-3103
100 x 300	10	PL1851-2102	PL1851-2103
4.6 x 250	30	PL1551-5702	PL1551-5703
4.6 x 150	30	PL1551-3702	PL1551-3703
25 x 150	30	PL1251-3702	PL1251-3703
50 x 150	30	PL1751-3702	PL1751-3703
100 x 300	30	PL1851-3102	PL1851-3103

PL-SAX Strong Anion-Exchange Bulk Media

Weight	Particle Size (µm)	PL-SAX 1000Å	PL-SAX 4000Å
100 g	10	PL1451-4102	PL1451-4103
1 kg	10	PL1451-6102	PL1451-6103
100 g	30	PL1451-4702	PL1451-4703
1 kg	30	PL1451-6702	PL1451-6703

Agilent PL-SCX columns for a wide range of biomolecules and solutes

PL-SCX Strong Cation-Exchange Columns

Size (mm)	Particle Size (µm)	PL-SCX 1000Å	PL-SCX 4000Å
1.0 x 50	5	PL1345-1502	PL1345-1503
2.1 x 50	5	PL1945-1502	PL1945-1503
4.6 x 50	5	PL1545-1502	PL1545-1503
2.1 x 50	8	PL1945-1802	PL1945-1803
2.1 x 150	8	PL1945-3802	PL1945-3803
4.6 x 50	8	PL1545-1802	PL1545-1803
4.6 x 150	8	PL1545-3802	PL1545-3803
4.6 x 250	10	PL1545-3102	PL1545-3103
4.6 x 150	10	PL1545-5102	PL1541-5103
25 x 50	10	PL1245-1103	PL1245-1103
25 x 150	10	PL1245-3103	PL1245-3103
50 x 150	10	PL1745-3103	PL1745-3103
100 x 300	10	PL1845-2103	PL1845-2103
4.6 x 250	30	PL1545-3702	PL1545-3703
4.6 x 150	30	PL1545-5703	PL1545-5703
25 x 150	30	PL1245-3702	PL1245-3703
50 x 150	30	PL1745-3703	PL1745-3703
100 x 300	30	PL1845-3102	PL1845-3103

PL-SCX Strong Cation-Exchange Bulk Media

Weight	Particle Size (µm)	PL-SCX 1000Å	PL-SCX 4000Å
100 g	10	PL1445-4102	PL1445-4102
1 kg	10	PL1445-6102	PL1445-6103
100 g	30	PL1445-4702	PL1445-4703
1 kg	30	PL1445-6702	PL1445-6703

To learn more about performing fast, high-resolution protein separations, visit **agilent.com/chem/AdvanceBio**

AGILENT 1260 INFINITY BIO-INERT QUATERNARY LC: INFINITELY BETTER BIOMOLECULE ANALYSIS

From solvent delivery that is free from iron and steel... to metal-free sample-flow-path components... the Agilent 1260 Infinity Bio-inert Quaternary LC sets new standards in performance and reliability.

This robust system stands up to the challenging solvent conditions commonly used for analyzing proteins and biotherapeutics and it also minimizes problems associated with nonspecific binding. Paired with Agilent ion-exchange BioHPLC columns, you can achieve the highest resolution time.

100% bio-inert sample flow path

All capillaries and fittings throughout the autosampler, column compartment, and detectors are completely metal-free, so biomolecules only come into contact with ceramics or PEEK. This helps you avoid the pitfalls of peak tailing, low recovery, and decreased column life by minimizing secondary interaction of proteins and peptides with metallic surfaces.

True UHPLC performance

Power range of up to 600 bar, capable of handling the higher pressures demanded by emerging column technologies with smaller particles. It's the perfect match for all SEC and IEX columns with particle sizes down to 1.7 µm.

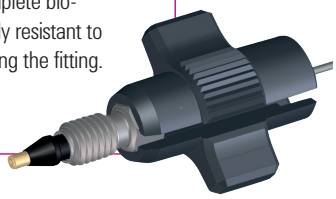
Capillary and fitting technology for robust and secure operation – day in, day out.

With the 1260 Infinity Bio-inert LC, Agilent uses capillary and fitting technology that facilitates the unique combination of metal-free bio-inertness and high pressure operation. Three different types of capillaries are deployed:

- Highly corrosion resistant titanium capillaries for the solvent delivery lines
- Metal-clad PEEK capillaries in the autosampler and column compartment
- PEEK capillaries in the low pressure parts of the system downstream of the separation column

The metal-clad PEEK capillaries feature a unique connection system for complete bio-inertness at every connection. The mechanically interlocked PEEK tip is highly resistant to lateral or rotational tension, eliminating torque at the capillary while tightening the fitting.

To learn more visit **agilent.com/chem/LCcapillaries**



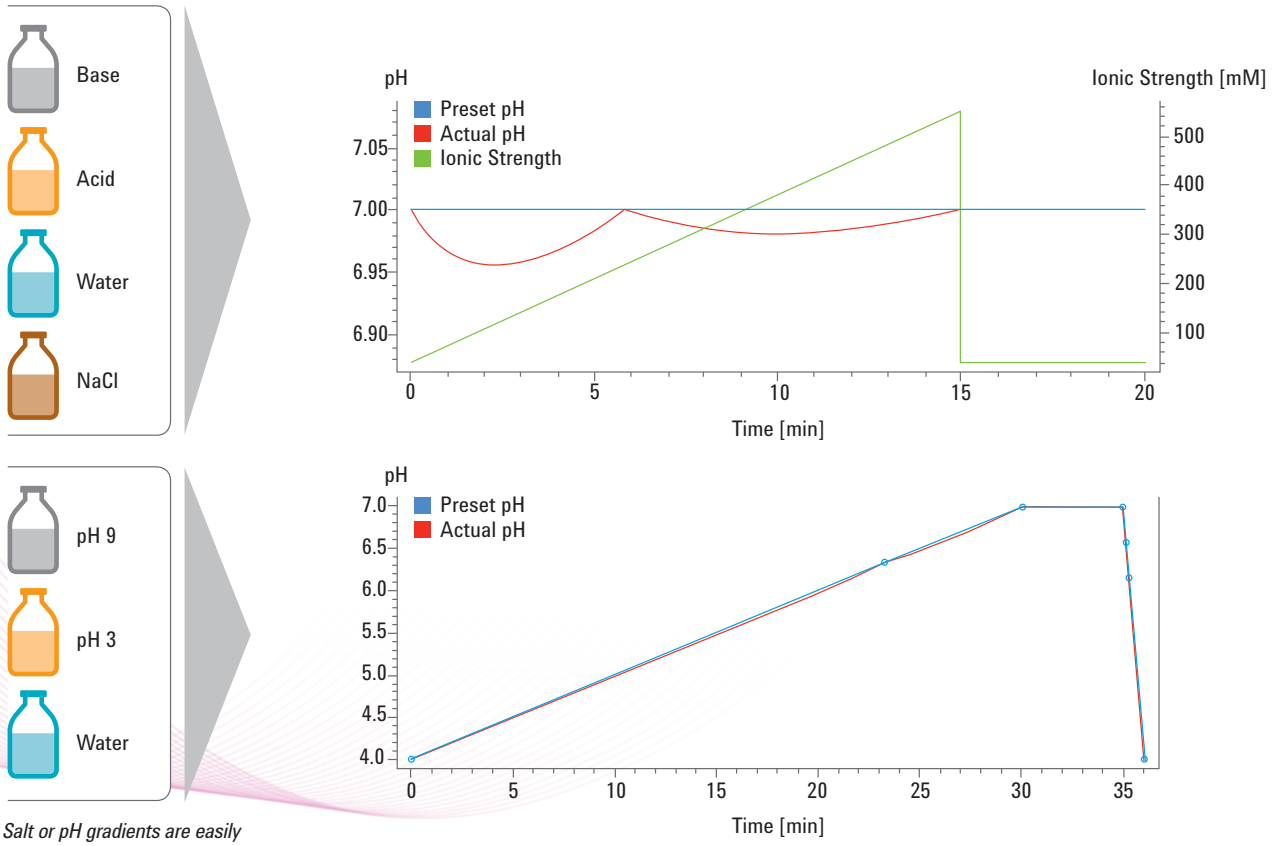
To learn more about the Agilent 1260 Infinity Bio-inert LC, visit **agilent.com/chem/1200BioLC**

SIMPLIFY YOUR BIO-ANALYSIS WORKFLOW

Straightforward buffer blending and pH scouting

Utilizing the mixing principle of the 1260 Infinity Bio-inert Quaternary Pump, the Buffer Advisor software facilitates dynamic mixing of solvents from only four stock solutions, simplifying your bio-analysis workflow and significantly reducing the time required for buffer preparation.

First, theoretical modeling helps you to find the best salt or pH conditions for your protein separation. The optimized gradient conditions are saved in an XML-format file for later import in Agilent OpenLAB CDS. This file sets the solvent blending in the timetable of the 1260 Infinity Bio-inert Quaternary 4-channel mixing Pump. Four stock solutions are all you need to prepare; acidic buffer, basic buffer, water, salt. To create a salt gradient, an increasing amount of salt solution from channel D is mixed with the acidic and basic buffer components from channels A and B, and with water for dilution from channel C.



Salt or pH gradients are easily created from stock solutions.

To learn more about the time-saving benefits of Buffer Advisor software, visit

agilent.com/chem/infinity-bufferadvisor

A FAMILY OF BIOCOLUMNS TO GIVE YOU THE MOST CHOICE AND CONTROL

Agilent ion-exchange BioHPLC columns are designed and tested to ensure excellent resolution and biochromatographic performance – making them ideal for charged-variant studies.

Our broad selection includes:

- **Agilent Bio IEX columns** for proteins and peptides
- **Agilent Bio MAb columns** for monoclonal antibodies
- **Agilent Bio-Monolith columns** for preparative LC systems
- **Agilent PL-SAX columns** for synthetic oligonucleotides
- **Agilent PL-SCX columns** for biomolecules and solutes

From sample simplification to analysis, Agilent ion-exchange BioHPLC columns are easy to integrate into your workflow for reproducible, high-quality results and easy method scale-up.

You can count on Agilent for all of your BioHPLC column needs, including size exclusion, reversed-phase, HILIC, and affinity columns.

Trust Agilent AdvanceBio columns for faster, more consistent biopharmaceutical analysis

Agilent AdvanceBio HPLC columns are designed to deliver consistent, exceptional performance for separating and characterizing peptides and proteins. The science behind this state-of-the-art column family advances accuracy, increases productivity, and eliminates interferences that can impede your analytical progress.

For ultimate confidence, AdvanceBio columns are rigorously tested to ensure great results, and are backed by Agilent's 60-day full satisfaction warranty.

For more information

To learn more about Agilent ion-exchange BioHPLC columns, visit
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