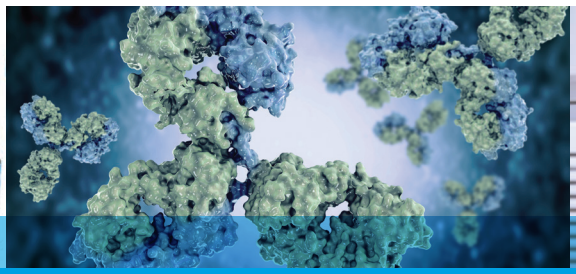
Agilent CrossLab
From Insight to Outcome

Agilent BioHPLC Columns

Your essential resource for biomolecule analysis



Agilent

Agilent CrossLab
From Insight to Outcome

Who We Are. What We Do.

Across the lab, around the world, with you every step of the way.

CrossLab is an Agilent capability that integrates services, consumables and lab-wide resource management to help laboratories improve efficiency, optimize operations, increase instrument uptime, develop user skill, and more.

Agilent CrossLab supports Agilent and select non-Agilent instruments and provides consultative support for workflow enablement, lab analytics, compliance, inventory management and asset management, including relocation services.

Learn more about Agilent CrossLab, and see examples of insight that leads to great outcomes, at **www.agilent.com/crosslab**

Look out for CrossLab stories from the lab to find out how we can help.

Real stories from the lab

Agilent CrossLab service engineers strive to deliver insight at every interaction, helping users improve efficiency, optimize resources, increase instrument uptime, and develop user skill. Learn how Agilent CrossLab has brought insight and value to customers around the world.

www.agilent.com/chem/crosslabstories

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Tips and tools

For applications examples spanning the biocolumn portfolio, please see the Critical Quality Attributes Application Compendium at **www.agilent.com/chem/cqa-applications**

Biocolumn Selection Guidelines

Biotherapeutics have enormous potential to improve human health. The number of approved protein and antibody therapeutics continues to grow around the world as this important therapeutic class addresses unmet medical needs. But discovery and development of biopharmaceuticals is difficult. Scientists face many challenges and must not only stay abreast of advances in knowledge and improvements in technology, but also navigate the maze of shifting government regulations. Making good decisions fast is critical. At every stage in the process, from disease research to QA/QC and manufacturing, Agilent can help you make the right choices for moving therapeutics successfully to market. And it's not just because we build reliable instruments and consumables that provide accurate, reproducible results. We understand the biopharmaceutical workflow and provide families of products that work together seamlessly—as engines of research, discovery, and development—to move candidate biopharmaceuticals forward.

Given that protein biopharmaceuticals are very heterogeneous, they will require a number of chromatographic methods to accurately characterize the active pharmaceutical ingredient (API). Methods include size exclusion chromatography for the quantitation of dimers and aggregates, and ion-exchange chromatography for charge variant analysis. As part of the full characterization, it will be necessary to look at primary amino acid sequence and any post-translational modification to the sequence that may occur during purification or formulation steps. To enable complete, reproducible, and high-quality analysis for key characterization workflows, Agilent provides a broad range of columns and supplies.

This comprehensive guide will help you find the right column for your characterization workflow. We have also included advice and tips on method development, solvent choice, mobile phase modification, optimization, and many example separations, all to assist you in column selection and method development.

Agilent has complete solutions for your needs. These include the Agilent 1260 Infinity II bio-inert LC with a metal-free sample path and the Agilent 1290 Infinity II LC, designed to provide highest speed, resolution, and ultrasensitivity for UHPLC applications. Biomolecules may be complex in structure, but their analysis is simplified by using Agilent HPLC columns, systems, and supplies.

What is a biomolecule?

Biomolecules are compounds made by living organisms. They can range in size from amino acids and small lipids to large polynucleotides, such as DNA or RNA.

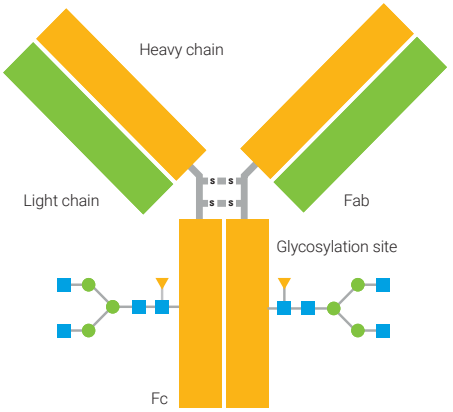
In this section, we deal with the separation of:

Proteins—separation based on size with size exclusion chromatography, charge with ion-exchange chromatography, and hydrophobicity with reversed-phase or hydrophobic interaction chromatography.

Peptides—biocolumns for the analysis and purification of the full range of peptides, including hydrophobic, hydrophilic, basic, and acidic peptides across the full size range. Also, columns for peptide mapping by HPLC and UHPLC.

DNA/RNA oligonucleotides—reversed-phase and ion-exchange options for DNA and RNA oligos, and with particle pore sizes to cover the full range of oligonucleotide sizes, from small synthetic oligos to large plasmids.

Amino acids—the AdvanceBio Amino Acid Analysis columns provide a high-efficiency solution for analysis of 24 amino acids. Typical analysis times range from 14 minutes, with a 75 mm column, to 24 minutes with a 150 mm column.

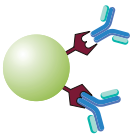


Monoclonal antibody

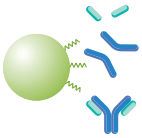
What is a biocolumn?

Biochromatography columns, or biocolumns, are liquid chromatography columns used for the separation of biological compounds, such as peptides and proteins, oligonucleotides and polynucleotides, and other biomolecules and complexes. Biocolumns are specifically designed for biomolecule analysis with larger pore sizes to accommodate the larger molecule sizes. Media are designed to minimize nonspecific binding of analytes for improved recovery. Separation mechanisms are chosen to either retain biological function so bioactivity is not lost during analysis, or to deliberately denature for primary structure characterization.

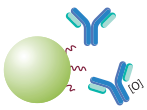
Agilent's biocolumn offering provides solutions for all the major characterization techniques required for your biomolecule analysis. These include:



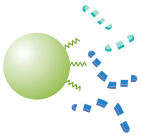
Titer determination
Use unique technology, such as AdvanceBio Bio-Monolith Protein A, to perform titer determination and cell line optimization.



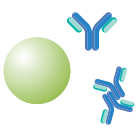
Intact and subunit purity using reversed-phase
Use key technologies such as AdvanceBio RP-mAb, ZORBAX RRHD 300 Å, and PLRP-S, for confidence in results from primary structural characterization through analysis of intact or fragmented proteins.



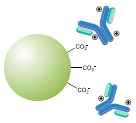
Intact analysis using hydrophobic interaction
Agilent AdvanceBio HIC columns will resolve various protein variants (PTMs) including oxidation in mAbs and drug-antibody species observed in ADCs.



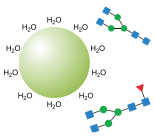
Peptide mapping
Detect and identify key post-translational modifications in digested protein samples using AdvanceBio Peptide Mapping.



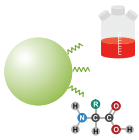
Aggregate and fragment analysis
AdvanceBio SEC accurately measures aggregates (such as dimers, trimers, and tetramers, and so on) and separates low molecular excipients and impurities from larger molecular weight proteins.



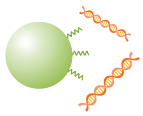
Charge variant analysis
Agilent ion-exchange columns include optimized chemistries for monoclonal antibody analysis, such as Bio MAb and Bio IEX for accurate isoform analysis.



Glycan analysis
Agilent hydrophilic interaction chromatography (HILIC) columns deliver accurate and reproducible glycan and glycopeptide analysis.



Amino acid and cell culture analysis
Analyze critical cell culture media components with either an LC/UV-based workflow with AdvanceBio AAA or an LC/MS-based workflow with AdvanceBio MS Spent Media.



Oligonucleotide analysis
Robust, high-efficiency solutions for DNA/RNA analysis.

Biocolumn Selection Guidelines

Agilent AdvanceBio columns are designed to advance accuracy and speed for your characterization of monoclonal antibodies and other intact proteins, aggregation with SEC, charge variants with IEX, intact mass, primary structure, and post-translational modifications (PTMs) by reversed-phase, and cleaved glycan analysis by hydrophilic interaction chromatography.

This guide provides more details on the complete Agilent biocolumn portfolio, along with information on choices within the AdvanceBio family to accurately characterize biotherapeutics.

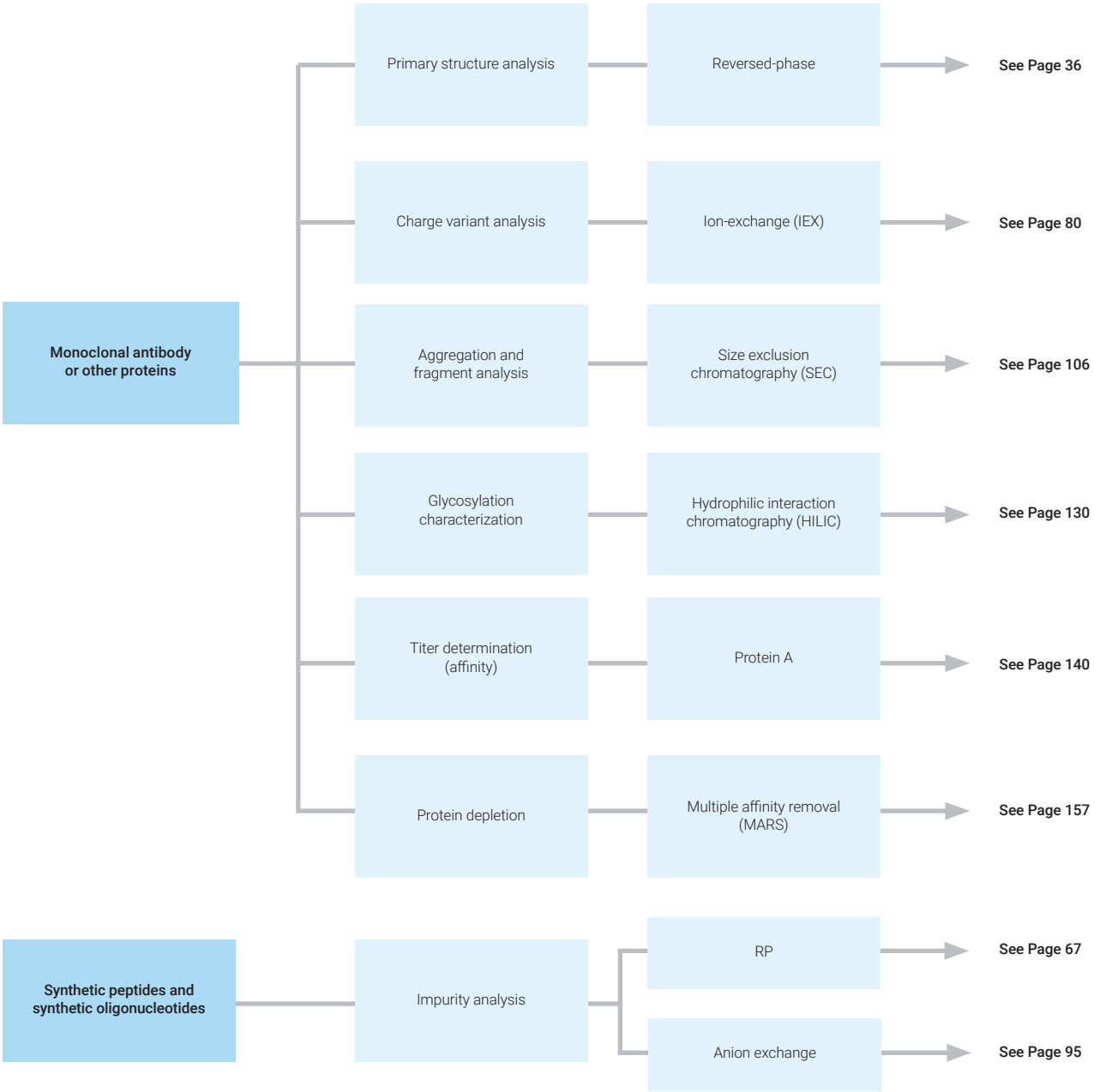
Tips and tools

To learn more about our AdvanceBio family of columns and various tools to advance your characterization needs, visit:
www.agilent.com/chem/advancebio

Column selection flowchart

The flowchart below indicates the page numbers that will take you to the selection guides in the individual chapters to help you choose the best column for your biomolecule application.

There are various guidelines that can be followed to help with the selection of the optimum column for a biomolecule separation. The starting point is the size of the molecule, as this determines the pore size of the HPLC method used for the separation. Secondly, consider the solubility of the molecule. Thirdly, note the separation mechanism, size, hydrophobicity, and charge.



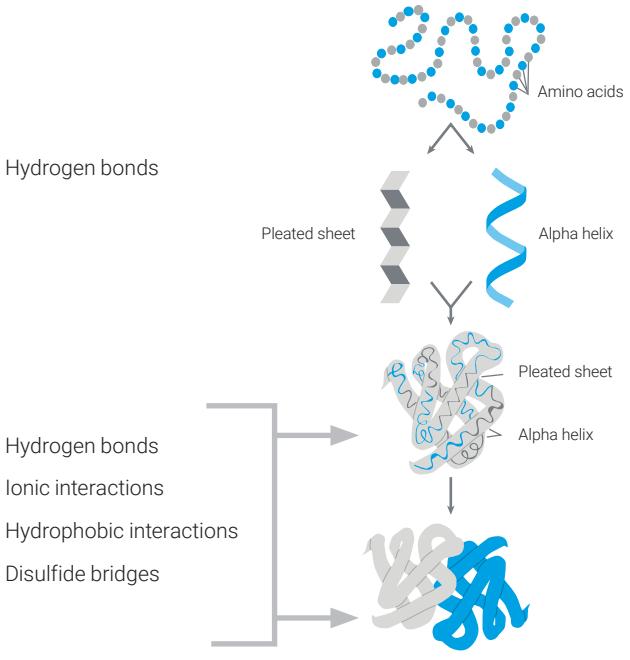
Biomolecule Separations

Protein separations

Proteins are complex molecules that require multiple techniques to provide full characterization. They exist as three-dimensional structures and these structures confer their biological activity.

The sequence of the amino acid chains defines the primary structure of the protein. Hydrogen bonding between amino acids of the primary structure then confers a secondary structure, typically in the form of alpha helices and pleated sheets. A further series of interactions, hydrogen bonding, ionic, hydrophobic, and disulfide bridges, between regions of the secondary structure, then provides the tertiary protein structure, or three-dimensional conformation. If the protein is composed of a number of amino acid chains, the interaction between these chains gives the quaternary structure.

When looking at methods for protein characterization, it is therefore clear from Figure 1 that techniques will be required that characterize the protein in its native state, without disrupting the tertiary and quaternary structures. We also need techniques for assessing the primary amino acid sequence, in the fully denatured state, with the three-dimensional structure stripped away.



Primary protein structure

A sequence of amino acids.

Secondary protein structure

Occurs when the sequence of amino acids are linked by hydrogen bonds.

Tertiary protein structure

Occurs when certain attractions are present between alpha helices and pleated sheet.

Quaternary protein structure

A protein consisting of more than one amino acid chain.

Figure 1. Schematic showing the various levels of protein structure.

The environment of the protein can influence, stabilize, or disrupt its structure. Factors to consider include pH, temperature, salt concentrations, aqueous or organic solvent content, and for some proteins, the presence of a stabilizing small molecule or metal ions. Protein structure can also be disrupted by the use of sulfhydryl reducing agents to break -S-S- bonds or chaotropic agents, such as urea or guanidine HCl. With the complexity of proteins and the intramolecular interactions that determine the three-dimensional structure, you can also expect that there will be intermolecular associations between protein molecules and other molecular entities and the surfaces with which they come into contact. This can result in protein complexes, aggregation (with possible precipitation), and deposition on surfaces, including those of the HPLC column and system. Therefore, you should consider the handling and environment in which the protein is maintained.

Protein Column Selection Guide

Application	Technique	Agilent Columns	Notes
Primary structure analysis	UHPLC/HPLC reversed-phase separations	AdvanceBio RP-mAb PLRP-S ZORBAX RRHD 300 Å Poroshell 300 Å ZORBAX 300 Å AdvanceBio Peptide Mapping	Reversed-phase separations require (or cause) denaturing of the protein to obtain detailed information about the amino acid sequence and amino acid modifications (including post-translational modifications).
Charge variant analysis	Ion-exchange separations	Agilent Bio IEX Agilent Bio MAb PL-SAX PL-SCX	The ratio of individual amino acids determines the net charge of the protein molecule. The pH at which the net charge is zero is called the isoelectric point (pI). When the solution pH is less than the pI, the protein will be positively charged (acidic), and when the solution pH is greater than the pI, the protein is negatively charged (basic). For ion-exchange analysis, we recommend the eluent pH be at least one pH unit away from its pI. Protein analysis using ionexchange columns requires buffered mobile phase and either salt gradients or pH gradients for elution.
Aggregation and fragment analysis	Size exclusion separations	AdvanceBio SEC Bio SEC-3 Bio SEC-5	Aggregates in protein biopharmaceuticals are of major concern, as they can induce an immunogenic response and can influence the composition of the final formulation.
Glycosylation characterization	Hydrophilic interaction chromatography	AdvanceBio Glycan Mapping ZORBAX RRHD 300 HILIC	Understanding glycosylation and glycan structures of proteins and mAbs is growing in importance due to the effect of immunogenicity and safety of the biotherapeutic. HILIC chromatography provides orthogonal information to reversed-phase columns as it retains the hydrophilic portion of the sample.
Titer determination	Affinity separation	Bio-Monolith Protein A Bio-Monolith Protein G	To monitor monoclonal antibody titer and yield from cell-culture supernatants before expensive preparative and large amounts of protein A are employed, a small (analytical) scale procedure is necessary to determine the titer of monoclonal antibody for the optimal time for harvest of the monoclonal antibody products.
Protein depletion	Affinity purification	MARS Human-14 MARS Human-7 MARS Human-6 MARS Human-6 High Capacity MARS Human-2 MARS Human-1 MARS Mouse-3	Remove the high-abundance proteins from biological samples. Removal of these abundant proteins improves the subsequent LC/MS and electrophoretic analysis of the sample by effectively expanding the dynamic range.

High speed, high resolution separation of Trastuzumab Variant IgG1

Column: **AdvanceBio RP-mAb C4**
795775-904
2.1 x 100 mm, 3.5 µm

Mobile phase: A: 0.1% TFA in water:IPA (98.2)
B: IPA:ACN:Mobile phase A (70:20:10)

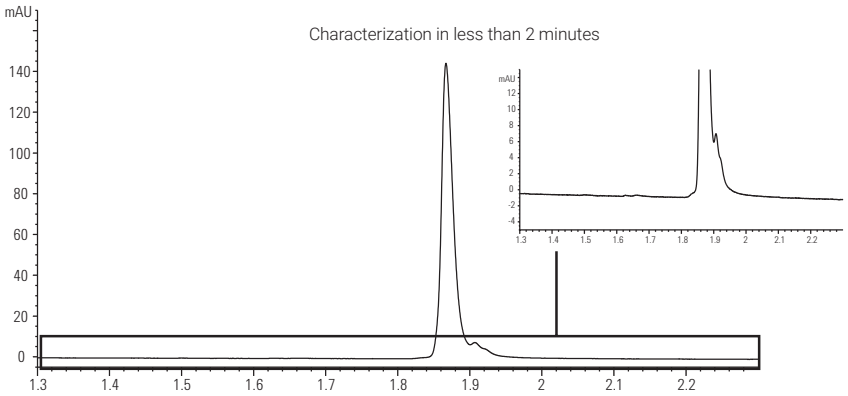
Flow rate: 1.0 mL/min

Gradient: 10–58% B in 4 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B

Temperature: 80 °C

Detector: UV, 254 nm

Sample: 5 µL injection of humanized recombinant Trastuzumab Variant IgG1 intact from Creative Biolabs (1 mg/mL)



AdvanceBio RP-mAb C4 provides a sharp peak and resolves fine detail in less than two minutes.

Higher resolution of oxidation

Column: **ZORBAX RRHD 300SB-C18**
857750-902
2.1 x 50 mm, 1.8 µm

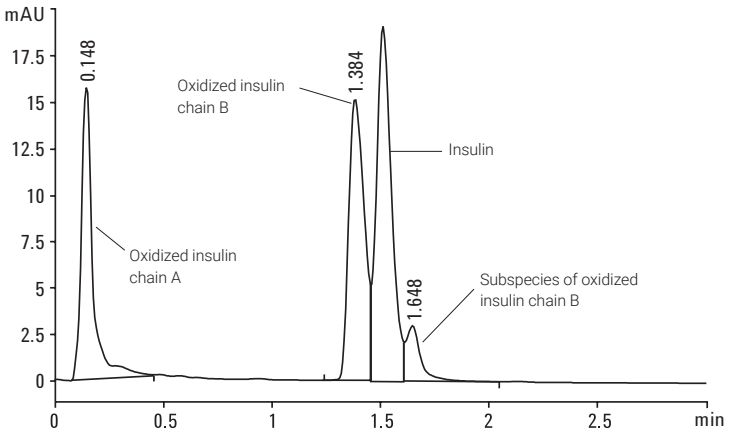
Mobile phase: A: 0.1% TFA
B: 0.01% TFA + 80% ACN

Flow rate: 1.0 mL/min

Gradient: 33–50% B, 0–4 min

Detector: 1290 Infinity LC with diode array detector at 280 nm

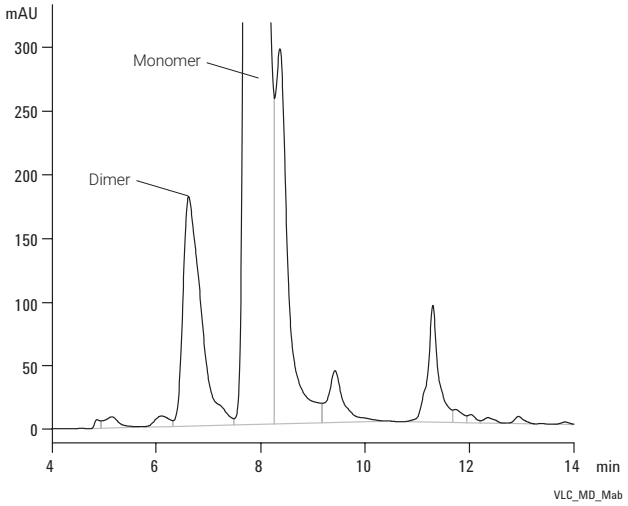
Sample: Insulin, insulin chain A and chain B, oxidized (BSA, Sigma-Aldrich, Corp., 1 mg/mL)



Oxidized insulin chains are resolved from insulin in under two minutes using the ZORBAX RRHD 300SB-C18 2.1 x 50 mm, 1.8 µm column.

Intact mAb monomer and dimer separation

Column:	**Bio SEC-3, 300 Å 5190-2511 7.8 x 300 mm, 3 µm**
Flow rate:	1.0 mL/min
Injection:	5 µL
Temperature:	Ambient
Detector:	UV, 220 nm
Buffer:	Sodium phosphate buffer 150 mM, pH 7.0
Isocratic:	0–100% buffer from 0–30 min



Tips and tools

Agilent recognizes the extraordinarily complicated and labor-intensive work that you do. We can help. Further information can be found in *BioPharma Workflow Solutions: How Agilent Helps Resolve Complex Analytical Challenges* (publication 5991-5235EN).
www.agilent.com/search

Separation of charge variants of human IgG1 with salt gradient

Column: **Bio MAb, PEEK**
5190-2407
4.6 x 250 mm, 5 µm

Mobile phase: A: 10 mM Na_2HPO_4, pH 5.5
B: A + 0.5 M NaCl

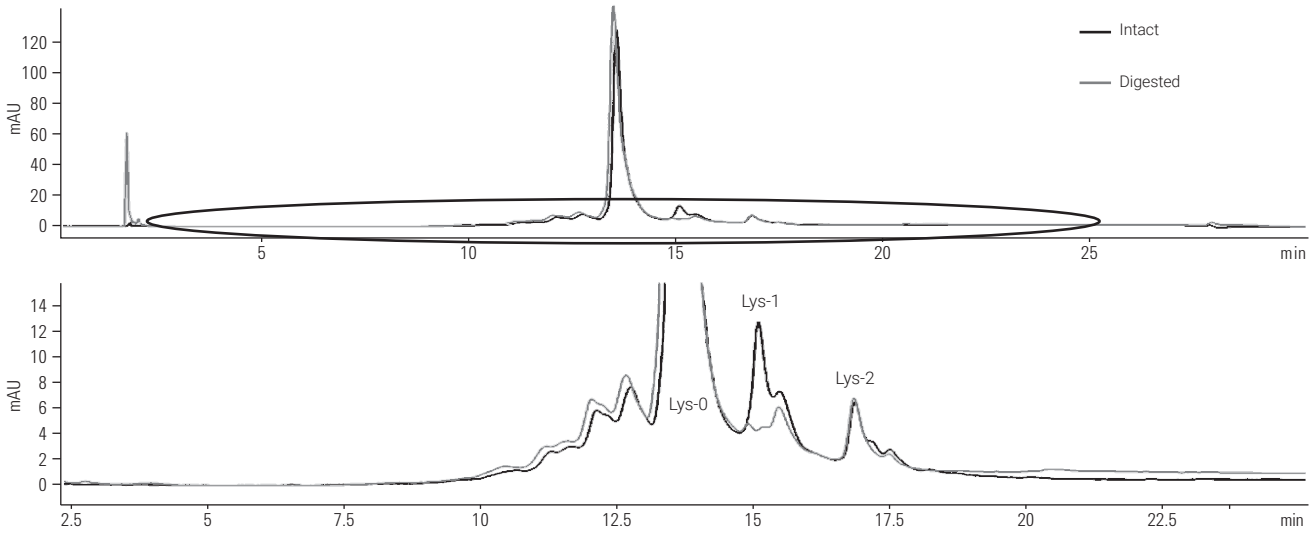
Flow rate: 0.85 mL/min

Gradient: 10 to 35% B from 0 to 25 min

Detector: UV, 225 nm

Instrument: 1260 Infinity Bio-inert Quaternary LC or
1100 series LC

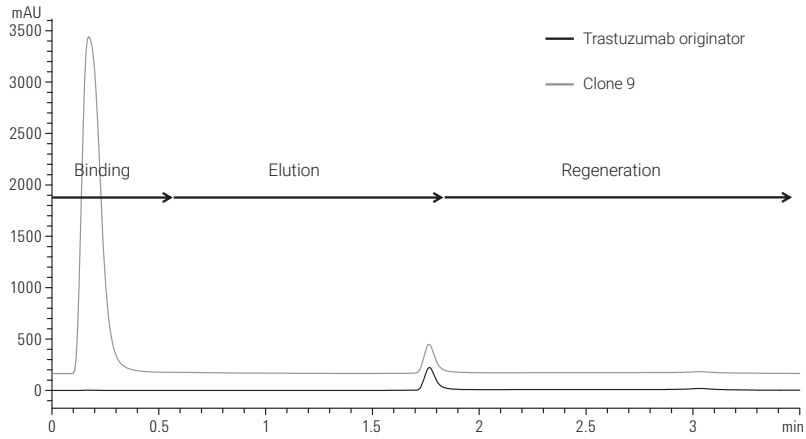
Sample: 5 µL of 1 mg/mL of intact or C-terminal digested IgG1



Separation of intact and C-terminal digested IgG1 using an Agilent Bio MAb 5 µm column.

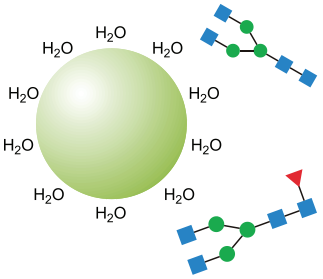
Titer determination of IgG1 from supernatant of CHO-cell

Column:	**Bio-monolith Protein A** **5069-3639** **5.2 x 4.95 mm**
Mobile phase:	A: 50 mM phosphate, pH 7.4 B: 100 mM citric acid, pH 2.8
Flow rate:	1 mL/min
Gradient:	**Time (min) % B** 0 to 0.5 0 (binding) 0.6 to 1.7 100 (elution) 1.8 to 3.5 0 (regeneration)
Injection volume:	50 µL
Detector:	UV, 280 nm
Fraction collection:	Time-based



AdvanceBio Bio-Monolith Protein A chromatogram of a Trastuzumab-producing CHO clone, clone 9, and of a Trastuzumab originator diluted in 50 mM Na-phosphate pH 7.4 to 0.2 mg/mL. Note that the supernatant was diluted 1:1 in phosphate buffer.

Glycan separations



Glycan profiling

Glycan analysis is required for the characterization of biotherapeutics as the glycosylation pattern can affect the safety and efficacy of the final product.

The intact glycoprotein is treated with an enzyme such as PNGase F to cleave the glycans from the protein. The glycans are then labeled with a fluorescent dye, as they are not inherently visible by UV or fluorescence. Following labeling, a cleanup step is performed to remove excess reagent and deglycosylated protein from the sample mixture. The purified, released glycan sample is then most commonly analyzed with hydrophilic interaction chromatography (HILIC) with either fluorescence or mass spectrometry detection.

The chromatographic profile is characteristic of the starting glycoprotein samples, and can vary widely in complexity. The AdvanceBio Glycan Mapping columns are well suited to delivering high resolution separation in a short amount of time.

Tips and tools

Agilent bio-inert supplies provide a metal-free sample flow path, which minimizes interactions with your biomolecules.

Visit: **www.agilent.com/chem/bio-inert-uhplc**

Super-fast glycan analysis: less than ten minutes with 1.8 µm particles

Column: **Agilent AdvanceBio Glycan Mapping 859700-913 2.1 x 150 mm, 1.8 µm**

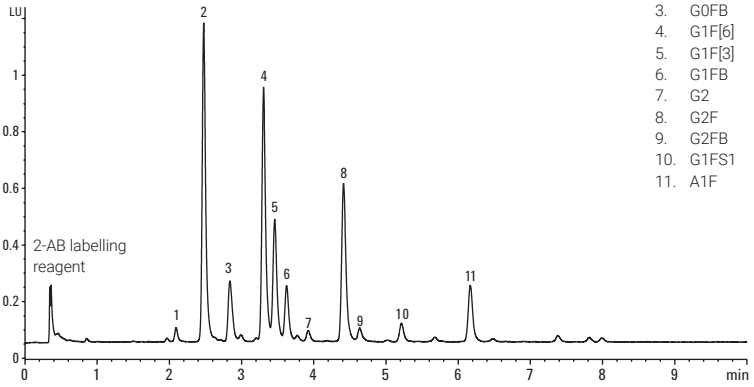
Mobile phase: A: 100 mM NH_4Formate, pH 4.5
B: ACN

Injection volume: 2 µL in 70:30 ACN:100 mM NH_4Formate

Fluorescence detection: Excitation = 260
Emission = 430

Instrument: Agilent 1290 Infinity LC with Agilent 1260 Infinity FLD

Sample: 2-AB labeled N-linked Human IgG glycan library (p/n 5190-6996)



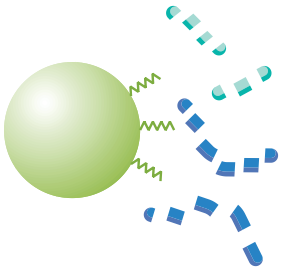
Fast, high resolution glycan mapping (1.8 µm column). This standard is used to test all AdvanceBio Glycan Mapping columns.

Time	%A	%B	Flow Rate (mL/min)
0	25	75	1.0
12	40	60	1.0
12.15	60	40	0.5
12.5	60	40	0.5
12.9	25	75	0.5
13.05	25	75	1.0
15	25	75	1.0

Peak	Glycan	Structure
1	G0	
2	G0F	
3	G0FB	
4	G1F	
5	G1F'	
6	G1FB	
7	G1FB Man6	
8	G2	
9	G2F	
10	G2FB	
11	G1FS1	
12	A1	
13	A1F	
14	A1FB	
15	A2	
16	A2F	
17	A2FB	

- Fucose
- Galactose
- Mannose
- N-acetylglucosamine
- N-acetylneuramic acid

Peptide separations



Peptide mapping

Peptide mapping is required for the characterization of proteins. It is used to confirm the identity of a protein and to identify and quantify post-translational modifications.

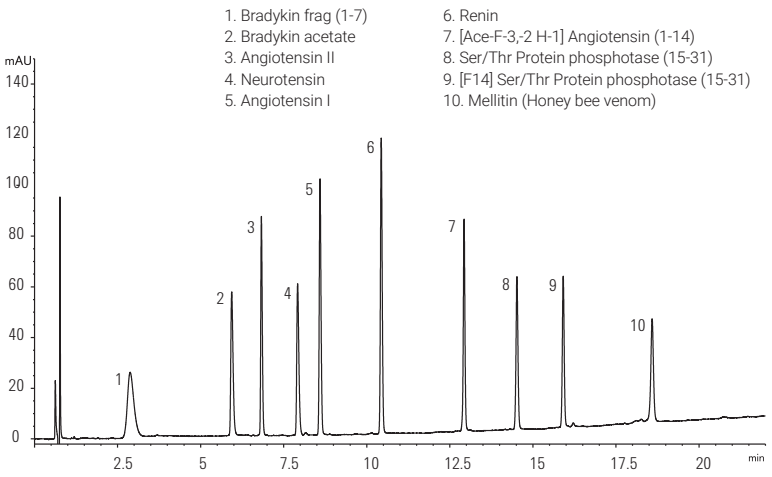
The purified protein is first digested using an enzyme, such as trypsin, yielding a range of peptide fragments. The specificity of the enzyme cleavage produces a fingerprint of peptides which is characteristic of that protein. Identification of the peptide fragments confirms the identity of the protein, and changes in the profile of the peptide digest can be used to identify post-translational modifications to the protein that may have occurred during the manufacturing or purification processes.

Reversed-phase UHPLC/HPLC is the preferred technique for the analysis of peptide digests with either MS or UV detection. LC/MS is used for the identification of the peptide fragments and determination of sequence coverage whereas LC/UV is more commonly used for peptide map comparisons in the monitoring/QC segments.

Peptide digests are complex mixtures, and for complete coverage, that is, resolution of the individual peptides, a high efficiency/high resolution column is required. AdvanceBio Peptide Mapping columns are designed to provide high-resolution peptide maps for protein identification and determination of post-translation modifications. These columns let you quickly resolve and identify amino acid substitutions/modifications in a protein primary sequence.

Quality assurance testing with Agilent peptide mix

Column:	**AdvanceBio Peptide Mapping 653750-902 2.1 x 150 mm, 2.7 µm**
Flow rate:	0.5 mL/min
Injection:	3 µL
Gradient:	A, water (0.1% TFA), B, ACN (0.1% TFA), 0-25 min, 15–65% B; 25–26 min, 65–95% B
Temperature:	55 °C
Detector:	220 nm
Sample:	Peptide Mapping Standards Mix (0.5-1.0 µg/µL per peptide) p/n 5190-0583



Test mix used for every batch of AdvanceBio Peptide Mapping media. The mixture contains 10 hydrophilic, hydrophobic, and basic peptides, ranging in molecular weight from 757 to 2845 Da. Every column is also tested with a small-molecule probe to ensure efficiency.

Tips and tools

Agilent InfinityLab well plates and sealing mats are the ideal sample containers for your high-throughput LC/MS applications.

Visit: **www.agilent.com/chem/well-plates**

AdvanceBio columns: for faster, more consistent biopharmaceutical analysis

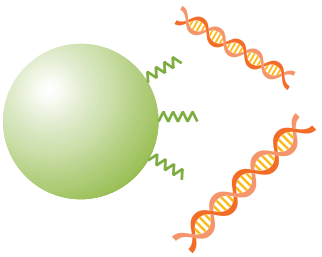
AdvanceBio Peptide Mapping columns are part of Agilent's growing state-of-the-art family of biocolumns. They are designed to deliver consistent, exceptional performance for the separation and characterization of peptides and proteins, antibodies, conjugates, new biological entities, and biopharmaceuticals. The science behind AdvanceBio columns helps to advance accuracy and productivity that support faster analysis and efficiency in your lab.

For ordering information on the Agilent Peptide Mapping solution, turn to **Page 62**.

Tips and tools

For more information on Agilent biocolumns, visit: **www.agilent.com/chem/bioHPLC**

DNA and RNA oligonucleotide separations



There is a renewed interest in oligonucleotides (oligos) as they are used in more and more applications, including potential therapeutics. The synthesis workflow is similar to that used for the more established synthetic peptide production, that is, an activated solid phase synthesis resin is used with sequential addition of specific nucleotides to build the desired sequence.

The nucleotide building blocks are protected at the 5' hydroxyl end with a dimethoxytrityl (DMT) group and the cleaved target oligo will have this protected group still attached. As DMT is hydrophobic, it is a useful handle that can be used for the first stage. To increase the stability of the oligonucleotide, particularly to enzyme degradation, it may be chemically modified, for example by replacing oxygen with sulfur to produce phosphorothioates. When using chemical synthesis to produce biomolecules, the coupling efficiency of each additional cycle is never 100%. The sample, after cleavage from the solid phase synthesis support, will contain deletion sequences, oligos where one or more residues are missing, and a certain amount of larger oligos produced by double coupling or branching. The sample mixture is complex and high-efficiency techniques are required for its analysis.

There are three UHPLC/HPLC techniques that are routinely used for oligonucleotide separations:

Trityl-on—This procedure is relatively simple to perform and separates the full-length target oligo, which still has the DMT group attached, from the deprotected failure sequences. The analytical information obtained is limited and this is considered to be a purification method.

Ion-exchange separations of the trityl-off, deprotected oligos—This method uses the negative charge on the backbone of the oligo to facilitate the separation. Resolution is good for the shorter oligos, but decreases with increasing chain length. Aqueous eluents are used, but oligos are highly charged and high concentrations of salt are needed to achieve elution from the column.

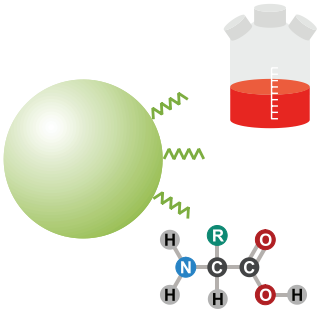
Ion-pair reversed-phase separation of the trityl-off, deprotected oligos—This technique uses organic solvents and volatile ion-pairing agents, and is suitable for LC/MS. The technique is best performed with high-efficiency particles. Conditions that fully denature the oligos and prevent association with complementary sequences are required. Thus, the separation is best performed at elevated temperatures.

DNA and RNA Oligonucleotide Column Selection

Application	Technique	Agilent Columns	Notes
Trityl-on/trityl-off oligonucleotides	Trityl-on	PLRP-S 50 µm media	Separates due to differences in hydrophobicity. Ideal for the separation of trityl-on from trityl-off oligos and is also used for ion-pair reversed-phase separations of deprotected oligos.
Deprotected oligonucleotides	Ion-pair reversed-phase separation of the trityl-off, deprotected oligos	PLRP-S 3 µm to 50 µm AdvanceBio Oligonucleotide	
Aggregation and fragment analysis	Ion-exchange separations of the trityl-off, deprotected oligos	PL-SAX 1000 Å	Separates deprotected oligos under denaturing high pH conditions. The quaternary amine functionality on the polymeric particles enables ion-exchange separations at high pH, improving chromatography for self-complementary sequences.

Amino acid analysis

The AdvanceBio AAA high efficiency column separates amino acids following an updated and improved protocol. During the production of proteins the cell culture medium is monitored to ensure that the correct nutrient balance and levels are maintained for the expression of the product protein. Amino acids are critical components of the feedstock and so must be monitored and adjusted during the production process. Reversed-phase chromatography is the primary technique used for amino acid analysis. Total analysis from injection to injection can be achieved in 14 minutes (9 minute analysis time) on shorter, 75 mm columns and 24 minutes (18 minute analysis time) on the 150 mm column. Sensitivity (5 to 50 pmol with diode array or fluorescence detectors) and reliability are achieved using both OPA- and FMOC-derivatization chemistries in one fully automated procedure using any Agilent LC.



AdvanceBio Amino Acid Analysis

Column: **AdvanceBio Amino Acid Analysis 655950-802 4.6 x 100 mm, 2.7 µm**

Mobile phase: A = 10mM Na_2HPO_4 and 10mM $Na_2B_4O_7$, pH 8.2
B = Acetonitrile:methanol:water (45:45:10, v:v:v:)

Flow rate: 1.5 mL/min

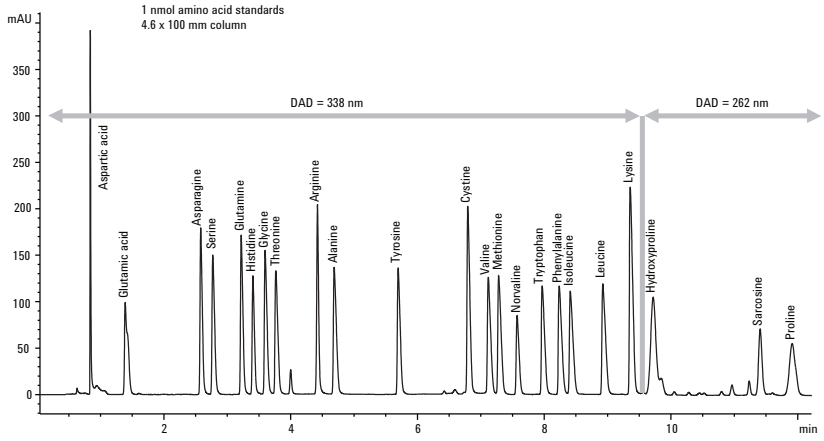
Injection: 3 µL

Gradient:

Time	%B
0	2
0.35	2
13.4	57
13.5	100
15.7	100
15.8	2
18	stop

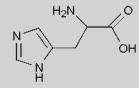
Column temperature: 40 °C

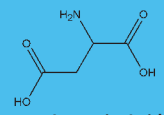
Detector: UV, 338 and 262 nm

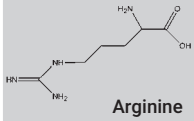


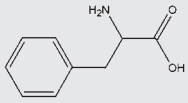
Separation of 1 nmol amino acid standards using the AdvanceBio Amino Acid Analysis column

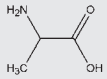
Amino acids

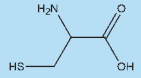
H
155.16
137.14
$C_6H_9N_3O_2$
His

Histidine

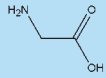
D
133.10
115.09
$C_4H_7NO_4$
Asp

Aspartic Acid

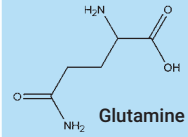
R
174.20
156.19
$C_6H_{14}N_4O_2$
Arg

Arginine

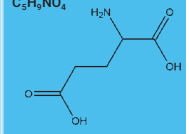
F
165.19
147.18
$C_9H_{11}NO_2$
Phe

Phenylalanine

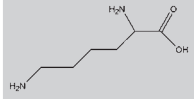
A
89.09
71.08
$C_3H_7NO_2$
Ala

Alanine

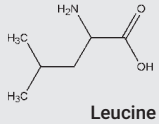
C
121.16
103.14
$C_3H_7NO_2S$
Cys

Cysteine

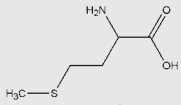
G
75.07
57.05
$C_2H_5NO_2$
Gly

Glycine

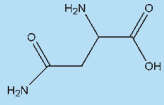
Q
146.15
128.13
$C_5H_{10}N_2O_3$
Gln

Glutamine

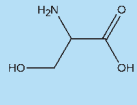
E
147.13
129.11
$C_5H_9NO_4$
Glu

Glutamic Acid

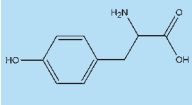
K
146.19
128.17
$C_6H_{14}N_2O_2$
Lys

Lysine

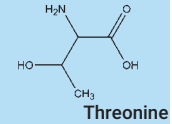
L
131.17
113.16
$C_6H_{13}NO_2$
Leu

Leucine

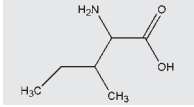
M
149.21
131.20
$C_5H_{11}NO_2S$
Met

Methionine

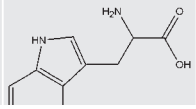
N
132.12
114.10
$C_4H_8N_2O_3$
Asn

Asparagine

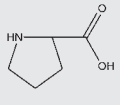
S
105.09
87.08
$C_3H_7NO_3$
Ser

Serine

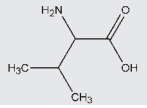
Y
181.19
163.17
$C_9H_{11}NO_3$
Tyr

Tyrosine

T
119.12
101.10
$C_4H_9NO_3$
Thr

Threonine

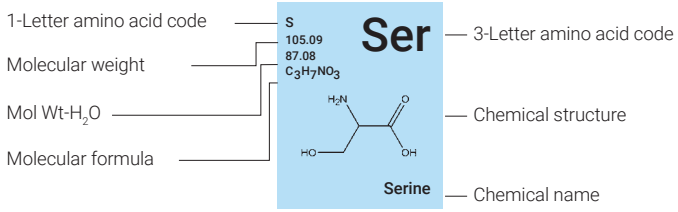
I
131.18
113.16
$C_6H_{13}NO_2$
Ile


W
204.23
186.21
$C_{11}H_{12}N_2O_2$
Trp


P
115.13
97.12
$C_5H_9NO_2$
Pro


V
117.15
99.13
$C_5H_{11}NO_2$
Val


- Basic
- Nonpolar (hydrophobic)
- Polar, uncharged
- Acidic



Method Development Guidelines

Primary structure analysis methods

This section on column selection strategy for primary structure analysis provides some critical details on method development for mAb, proteins, and peptides.

Peptides and polypeptides Mol Wt <10 kDa	Proteins Mol Wt >10 kDa	Reduced/fragmented Mol Wt <50 kDa	Intact Mol Wt >150 kDa
Initial bonded phase			
AdvanceBio Peptide Mapping Also consider: ZORBAX 300 Å StableBond C18	**ZORBAX 300 Å StableBond C8** Also consider: PLRP-S ZORBAX 300 Å StableBond C18 ZORBAX 300 Å StableBond C3 Poroshell 300 StableBond C18 Poroshell 300 StableBond C8 Poroshell 300 StableBond C3	**AdvanceBio RP-mAb StableBond C8** Also consider: PLRP-S AdvanceBio RP-mAb C4 AdvanceBio RP-mAb diphenyl ZORBAX 300 Å diphenyl ZORBAX 300 Å StableBond C8 ZORBAX 300 Å StableBond C3 ZORBAX 300 Å StableBond C18	**AdvanceBio RP-mAb C4** Also consider: PLRP-S AdvanceBio RP-mAb StableBond C8 AdvanceBio RP-mAb diphenyl Poroshell 300 StableBond C3 Poroshell 300 StableBond C8
Initial separation conditions			
Mobile phase: A: 95% H_2O:5% ACN with 0.1% TFA B: 5% H_2O:95% ACN with 0.085% TFA **Gradient:** 3 to 60% in 30 min **Temperature:** 40 °C	**Mobile phase:** A: 95% H_2O:5% ACN with 0.1% TFA B: 5% H_2O:95% ACN with 0.085% TFA **Gradient:** 5 to 70% in 20 min **Temperature:** 80 °C	**Mobile phase:** A: 100% H_2O with 0.1% TFA B: 10% H_2O:10% ACN:80% n-PA with 0.08% TFA **Gradient:** 5 to 40% in 10 min **Temperature:** 60 °C	**Mobile phase:** A: 98% H_2O:2% IPA with 0.1% TFA B: 10% H_2O:0% ACN:70% IPA with 0.08% TFA **Gradient:** 10 to 60% B in 5 min **Temperature:** 80 °C
2.1 mm id columns **Particle size** 2.7 µm SPP **Flow rate** 0.5 mL/min	**4.6 mm id columns** **Particle size** 2.7 µm SPP **Flow rate** 2.0 mL/min	**2.1 mm id columns** **Particle size** 1.8 µm TPP 3.5 µm TPP 5 µm TPP 5 µm SPP **Flow rate** 0.5 mL/min 0.2 mL/min 0.2 mL/min 1.0 mL/min	**4.6 mm id columns** **Particle size** 3.5 µm TPP 5 µm TPP **Flow rate** 1.0 mL/min 1.0 mL/min	**2.1 mm id columns** **Particle size** 3.5 µm SPP 1.8 µm TPP 3.5 µm TPP 5 µm TPP **Flow rate** 0.8 mL/min 0.3 mL/min 0.2 mL/min 0.2 mL/min	**4.6 mm id columns** **Particle size** 3.5 µm SPP 3.5 µm TPP 5 µm TPP **Flow rate** 4.0 mL/min 1.0 mL/min 1.0 mL/min	**2.1 mm id columns** **Particle size** 3.5 µm SPP 5 µm SPP **Flow rate** 1.0 mL/min	**4.6 mm id columns** **Particle size** 3.5 µm SPP **Flow rate** 5.0 mL/min

SPP = superficially porous particle, TPP = totally porous particle

Start at low pH with simple aqueous/organic gradient

Typically, a water:acetonitrile with 0.1% trifluoroacetic acid (TFA) gradient is used to elute all components of interest. A typical high resolution gradient on a 300 Å pore size column requires 30 to 50 min. An AdvanceBio RP-mAb column requires a shorter analysis time and a higher flow rate, and still provides exceptional resolution. To improve resolution, increase the gradient time, decrease column length, or increase flow rate. For LC/MS methods, TFA can reduce detector sensitivity and is often replaced with ammonium formate/formic acid.

Optimize sample solubility

For best peak shape and recovery at any pH, it is important to completely solubilize a sample. Highly acidic or neutral solvents can be used with AdvanceBio RP-mAb, ZORBAX 300 Å StableBond, Poroshell 300 StableBond, and AdvanceBio Peptide Mapping, while neutral solvents and dilute bases can be used with ZORBAX 300Extend-C18 and Poroshell 300Extend-C18.

Solvent choices to solubilize proteins and peptides

Weakest

- Water/phosphate buffer
- Dilute acid (TFA, acetic acid or HCl)
- Neutral pH, 6–8 M guanidine-HCl or isthiocyanate
- Acetic acid 5%/6 M urea
- Dilute acid + aqueous/organic solvents (ACE, MeOH, THF)
- Dilute base (ammonium hydroxide)
- DMSO or 0.1%–1% in DMSO
- Formamide

Strongest

Increase the temperature

Separations of proteins and peptides are influenced by temperature and higher column temperature can dramatically improve both resolution and recovery of proteins and hydrophobic and aggregating peptides.

AdvanceBio RP-mAb: Up to 90 °C
ZORBAX 300 StableBond, Poroshell 300 StableBond: Up to 80 °C
AdvanceBio Peptide Mapping: Up to 60 °C

Optimize mobile phase pH

Try mid and high pH if low pH does not work
If an optimized, low pH method does not provide an ideal separation, then mid or high pH mobile phase can be used. At high pH, selectivity is often very different because acidic amino acids become negatively charged and some basic amino acids may lose their charge. ZORBAX 300Extend-C18 is an excellent choice for mid to high pH separation.

Column:	**ZORBAX 300Extend-C18 4.6 x 150 mm, 5 µm 773995-902**	Gradient:	5–60% B in 30 min
Mobile phase:	A: 20 mM nH_4OH in H_2O B: 20 mM nH_4OH in 80% ACN	Temperature:	25–30 °C (<60 °C)
		Flow rate:	1 mL/min

Reversed-phase LC/MS methods

LC/MS of proteins and peptides is used to provide information for protein characterization, to accurately identify post-translational modifications of proteins, and to determine the molecular weight of synthetic and natural peptides. LC/MS is also used to provide protein identification in 2D separations for proteomics applications. Therefore, LC/MS of proteins and peptides is a critical separation area, which requires some special column and mobile phase recommendations. Smaller column sizes are often used for LC/MS and TFA is generally not used in mobile phase because of reduced sensitivity in the MS with this mobile phase additive.

Analytical LC/MS applications

2.1 mm id columns provide good sensitivity when sample size is not limited. With Poroshell columns, smaller 1 mm column ids may be used.

High sensitivity/proteomics applications

Capillary columns are used for high sensitivity protein and peptide applications. The 0.5 mm id columns are used for protein and protein digest separations, while the 0.3 mm id columns are most often used for protein digests. These can be analyzed at high pH with an ammonium hydroxide mobile phase. Nano columns (0.1 and 0.075 mm id) are often used in 2D LC/MS systems for proteomics and the initial choice is C18 bonded phase.

Charge variant analysis methods

This section on column selection strategy for charge variant analysis provides some critical details on method development for mAb, proteins, and peptides.

Bio MAb columns and monoclonal antibodies	Bio IEX columns, cation-exchange (SCX, WCX), monoclonal antibodies, proteins, peptides, and glycoproteins	Bio IEX columns, anion-exchange (SAX, WAX), oligonucleotides, and proteins

Recommended initial conditions

Bio MAb columns and monoclonal antibodies	Bio IEX columns, cation-exchange (SCX, WCX), monoclonal antibodies, proteins, peptides, and glycoproteins	Bio IEX columns, anion-exchange (SAX, WAX), oligonucleotides, and proteins
Mobile phase: A: 20 mM sodium phosphate, pH 5.5 B: Buffer A + 200 mM NaCl	**Mobile phase:** A: 20 mM sodium phosphate, pH 5.0 for WCX or pH 6.0 for SCX B: Buffer A + 500 mM NaCl	**Mobile phase:** A: 20 mM Tris-HCl, pH 8.5 B: A + 500 mM NaCl
Gradient: 10–35% B in 50 min	**Gradient:** 1–100% B in 30 min for 50 mm columns, 60 min for 250 mm columns	**Gradient:** 1–100% B in 30 min for 50 mm columns, 60 min for 250 mm columns
Sample size: 2 mg/mL, 5 µL injection	**Sample size:** 2 mg/mL, 5 µL injection	**Sample size:** 2 mg/mL, 5 µL injection
Temperature: Ambient	**Temperature:** Ambient	**Temperature:** Ambient
Detection: UV, 220 nm	**Detection:** UV, 220/280 nm	**Detection:** UV, 220/280 nm

Select flow rate based on column diameter and particle size

2.1 mm id columns

Particle size, µm	Flow rate, mL/min
5	0.1–0.5
1.7	0.1–0.8

4.6 mm id columns

Particle size, µm	Flow rate, mL/min
1.7	0.1–0.3
3	0.1–0.5
3.5	0.1–0.8
10	0.1–1.0

Optimize conditions

Some separations may require a specific buffer, ionic strength, pH, and temperature.

Ionic strength

Certain ionic strength is required to sustain the function of columns. Usually, a minimal concentration of 10 to 20 mM salt is required. However, greater than 20 mM strength may prevent the adsorption of biomolecules onto the column. Commonly used salts are sodium and potassium chloride and acetate. For elution, a typical salt concentration is 400 to 500 mM.

Note: Never use water alone for washing columns as it causes a significant increase in backpressure.

Selection of buffers and pH

Buffers play a key role in the optimization of separations. Phosphate buffers are typically used for antibodies and many biomolecules. The following are also recommended: MES, tris, and ACES buffers. Use buffers of pH 5.0 to 6.5. pH can be adjusted usually by +/- 0.2 units. For some specific proteins, buffers with higher pH (>pH 6.5) may be needed. Phosphoric acid, acetic acid, HCl, and NaOH can be used to adjust pH.

pH gradients can also be used for elution.

Additives

Organic solvents
Acetonitrile, ethanol, methanol, and other similar solvents can be used up to 50%.

Detergents
Nonionic, anionic, and zwitterionic detergents can be used. Cationic detergents are not recommended.

Selection of buffers and pH

For anion-exchange, acetate and phosphate buffers of pH 8.0 to 9.0 are recommended. pH can be adjusted usually by +/- 0.2 units. For some specific proteins, buffers with higher or lower pH may be needed. Phosphoric acid, acetic acid, HCl, and NaOH can be used to adjust pH.

pH gradients can also be used for elution.

Additives

Organic solvents
Acetonitrile, ethanol, methanol, and other similar solvents can be used up to 50%.

Detergents
Nonionic, cationic, and zwitterionic detergents can be used. Anionic detergents are not recommended.

Temperature

Agilent Bio MAb and IEX columns are stable up to 80 °C. However, many proteins and biomolecules are heat labile. Be sure to establish the temperature stability of your sample before routinely using high temperature for separation.

Charge variant analysis methods with Agilent Buffer Advisor software

Agilent Buffer Advisor is a software tool that enables more reproducible and precise ion exchange methods. For example, the software can generate methods using quaternary mixing for a salt gradient with constant pH, or for a pH gradient with a salt gradient for cleanup. These methods can be directly imported into Agilent LC acquisition software.

Recommended initial conditions

Salt gradient (see application note: 5991-0656EN)

Columns:	Bio WCX, 4.6 x 250 mm, 10 µm Bio WCX, 4.6 x 250 mm, 5 µm
Mobile phase:	A: water B: 1.6 M NaCl C. 40.0 mM NaH_2PO_4 D. 40.0 mM Na_2HPO_4 By combining predetermined proportions of C and D, 20 mM buffer solutions at the desired pH range are produced.
Gradient:	0 to 50% B, 0 to 20 min (constant pH, for example, pH 6.0) 50% B, 20 to 25 min 0% B, 25 to 35 min
Temperature:	Ambient
Injection volume:	10 µL
Detection:	UV, 220 nm
Instrument:	1260 Infinity bio-inert LC
Sample:	Ovalbumin, ribonuclease A, cytochrome c, lysozyme
Sample conc:	2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)

pH gradient (see application note: 5990-9629EN)

Column:	Bio MAb, 4.6 x 250 mm, 5 µm
Mobile phase:	A: water B: 1.6 M naCl C. 40.0 mM NaH_2PO_4 D. 40.0 mM Na_2HPO_4 By combining predetermined proportions of C and D, buffer solutions at the desired pH range are produced at the selected buffer strengths.
Gradient:	pH 6.0 to 8.0, 0 to 20 min 0 to 800 mM NaCl, 20 to 25 min 800 mM NaCl, 25 to 30 min
Temperature:	Ambient
Injection volume:	10 µL
Detection:	UV, 220 nm
Instrument:	1260 Infinity II bio-inert LC
Sample:	IgG monoclonal antibody
Sample conc:	2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)

Note: Similarly, the above approaches can be applied for Agilent WAX and SCX columns with modifications.
To access the Critical Quality Attributes Application Compendium, visit: **www.agilent.com/chem/cqa-applications**

Select flow rate based on column diameter and particle size

2.1 mm id columns

Particle size, µm	Flow rate, mL/min
1.7	0.1–0.3
3	0.1–0.5
5	0.1–0.8
10	0.1–1.0

4.6 mm id columns

Particle size, µm	Flow rate, mL/min
1.7	0.1–0.3
3	0.1–0.5
5	0.1–0.8
10	0.1–1.0

Note: Always start with a low flow rate and default to the recommended operating limit of the column.

Aggregation and fragment analysis methods

This section on column selection strategy for aggregation analysis provides some critical details on method development for mAb, proteins, and peptides.

Choose initial columns and conditions for size-based separation of biomolecules, aggregation analysis—peptides, polypeptides, and proteins

Peptides, polypeptides, proteins
Mol Wt >0.1–1,250 kda

Peptides, polypeptides, proteins
Mol Wt >0.1–10,000 kda

Select column based on molecular weight range and pore size

AdvanceBio SEC (2.7 µm)

Pore size	Mol Wt range, kDa
130 Å	0.1–120
300 Å	5–1,250

Bio SEC-3 (3 µm)

Pore size	Mol Wt range, kDa
100 Å	0.1–100
150 Å	0.5–150
300 Å	5–1,250

Bio SEC-5 (5 µm)

Particle size, µm	Flow rate, mL/min
100 Å	0.1–100
150 Å	0.5–150
300 Å	5–1,250
500 Å	15–5,000
1000 Å	50–7,500
2000 Å	>10,000

Recommended initial separation conditions

Columns: AdvanceBio SEC
Bio SEC (3 µm and 5 µm)

Mobile phase: Phosphate buffer 150 mM, pH 7.0*

Gradient: Isocratic in 15 to 60 min range

Temperature: Recommended 10 to 30 °C, maximum 80 °C

Flow rate: 0.1 to 0.4 mL/min for 4.6 mm id columns
0.1 to 1.25 mL/min for 7.8 mm id columns
1.0 to 10.0 mL/min for 21.2 mm id columns

Sample size: ≤5% of total column volume

* Other aqueous buffers with high and low salt can be used

For additional information, see:
Resolve Protein Aggregates and Degradants With Speed and Confidence (publication 5991-2898EN)
www.agilent.com/search

After the initial chromatogram, additional changes may be needed to improve the separation, maintain protein solubility, or to decrease sample interaction with the chromatographic media. The ionic strength of the mobile phase can be adjusted up or down to attain an optimized separation. pH can also be adjusted usually +/- 0.2 units. If further optimization is necessary, the upward or downward range should be expanded. A change of temperature or addition of an organic solvent can also be used.

For protocols requiring additional salt, these buffers are typical:

100 to 150 mM sodium chloride in 50 mM sodium phosphate, pH 7.0

100 to 150 mM sodium sulfate in 50 mM sodium phosphate, pH 7.0

50 to 100 mM urea in 50 mM sodium phosphate, pH 7.0

Other similar salts (for example, KCl) and guanidine hydrochloride can also be used.

pH range:

2.0 to 8.5

Potential organic solvent additions include:

5 to 10% ethanol (or other similar solvents) in 50 mM sodium phosphate, pH 7.0

5% DMSO in 50 mM sodium phosphate, pH 7.0

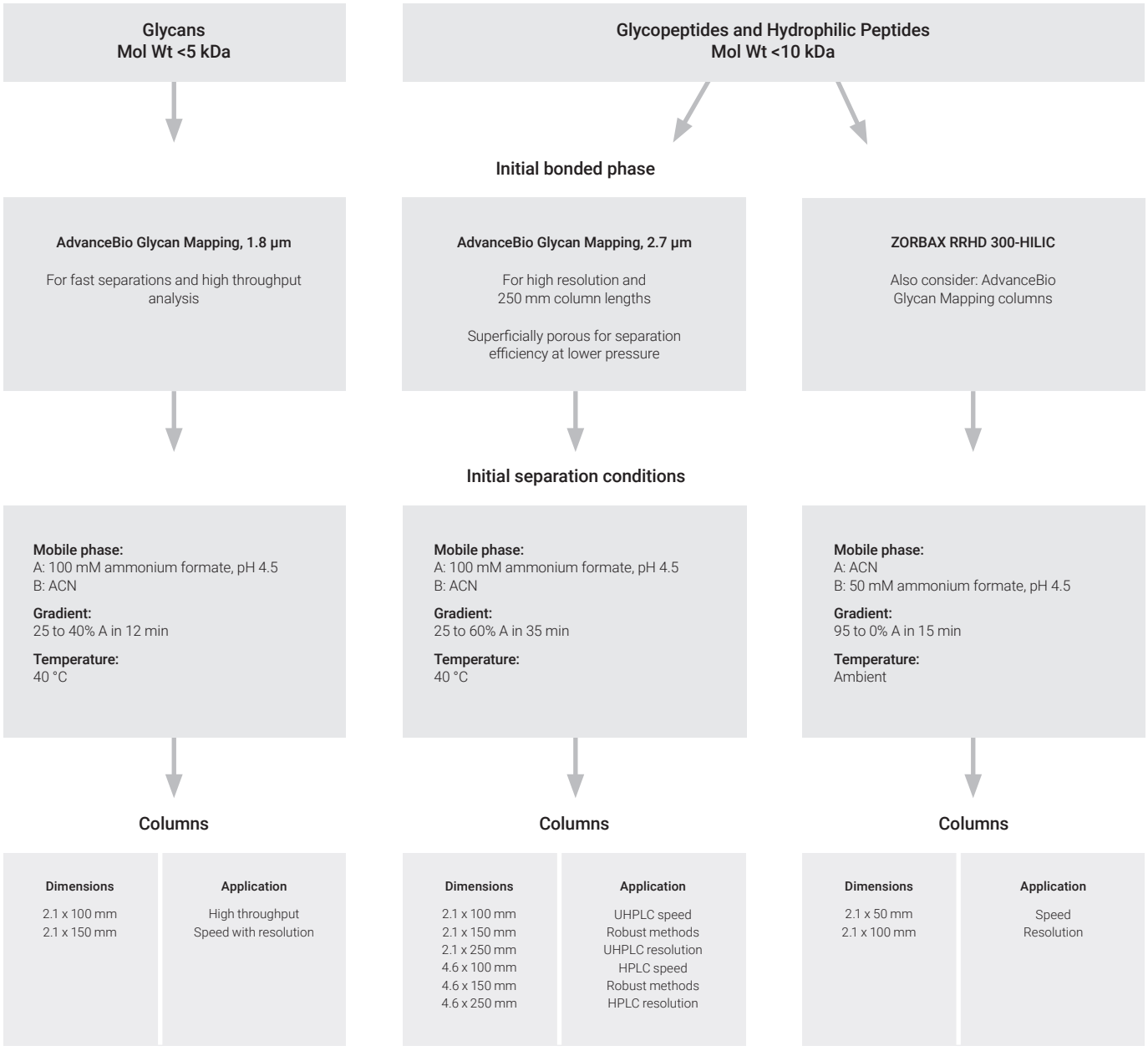
Particular care must be taken to avoid excessive pressure changes due to the high viscosity of some aqueous/organic solvent mixtures. Use reduced flow rate or increased temperature to help alleviate potential problems.

Temperature:

Typically, SEC separations are run at 20 to 30 °C. Separation of proteins and peptides may require higher temperatures to improve both resolution and recovery of proteins and hydrophobic peptides.

Maximum temperature of Bio SEC columns is 80 °C.

Glycan and hydrophilic/glycopeptide analysis



Titer determination and cell culture optimization methods

Agilent Bio-Monolith Protein A recommended conditions

Columns: Bio-Monolith Protein A (p/n 5069-3639)

Mobile phase: A: 50 mM phosphate, pH 7.4;
B: 100 mM citric acid, pH 2.8 mM, or 500 mM acetic acid, pH 2.6

Gradient:

Time (mins)	%A	%B	
0 to 0.5	100	0	Binding
0.6 to 1.7	0	100	Eluting
1.8 to 3.5	100	0	Re-equilibrating

Temperature: Ambient

Flow rate: 1 mL/min

Injection volume: Variable (50 µL, optimized for CHO cell culture supernatant contains IgG1)

Detection: UV, 280 nm

Sample: IgG1 (1 to 20 mg/mL) and CHO cell supernatant contains IgG1 (up to 20 mg/mL total protein)

Note: Additional salts such as sodium chloride can be added to mobile phases, up 150 mM. Higher salts should be determined experimentally.

Tips and tools

Agilent recognizes that there are many different factors that affect the quality of mAb and protein separations. To enable you to gain the best results, we have developed a series of 'how to' guides. For more information, see:

Keys for Enabling Optimum Peptide Characterizations: A Peptide Mapping "How to" Guide (publication 5991-2348EN)

Ion-exchange Chromatography for Biomolecule Analysis: A "How to" Guide (publication 5991-3775EN)

Size Exclusion Chromatography for Biomolecule Analysis: A "How to" Guide (publication 5991-3651EN)

For more on the above, and other guides that will help in your characterization, go to: **www.agilent.com/chem/getbioguides**

High sensitivity capillary column methods

Mobile phase considerations for reversed-phase methods

For LC/MS methods where the column eluent passes directly from the column to the MS detector the mobile phases must contain only volatile salts, and additives. And for maximum sensitivity there must be no ion suppression or adduct formation.

Low pH

TFA is generally not used for LC/MS separations of proteins and peptides as it suppresses ionization and increases the limit of detection. The first step is normally to replace TFA with 0.1 to 1% formic acid. Acetic acid up to 1% can also be used as an alternative mobile phase modifier. At low pH, the best separation may still be obtained with TFA in the mobile phase but with a reduction in sensitivity. In some cases, the TFA can be displaced postcolumn with an alternative acid, such as propionic acid by the use of a simple online desalting/counter ion-exchange.

Mid and high pH

High pH is less often used for protein characterization but LC/MS can also be done at high pH with 10 to 20 mM NH_4OH as a mobile phase additive.

Agilent Instruments for Protein Identification and Impurity Profiling

1260 Infinity II bio-inert LC

True bio-inertness for efficient biomolecule analysis

The only UHPLC that provides a metal-free sample flow path. Other advantages include:

100% bio-inertness

- No stainless steel: for utmost sample integrity
- pH 1 to pH 13 (pH 14 short-term) for highest instrument uptime
- Handles 2 M salt and 8 M urea
- Easy-to-use capillary technology

UHPLC capability

- 600 bar

Robust and easy to use

- Low surface activity, corrosion resistance, active seal wash, and quaternary buffer mixing

Ideal for protein identification

For best results, use with AdvanceBio Peptide Mapping, Bio MAb, and AdvanceBio SEC columns.

1260 Infinity II bio-inert LC

1290 Infinity II LC with high speed pump

Unmatched performance for ultimate confidence in your results

Best-in-class resolution per time, dispersion, sensitivity, accuracy, and precision in LC/UV and LC/MS. Combines innovative active damping, microfluidic mixing, and optofluidic waveguides detection technology to achieve:

- UHPLC power range with up to 1300 bar and 5 mL/min
- The fastest and easiest method transfer using ISET, Agilent's unique Intelligent System Emulation Technology
- UHPLC productivity with HPLC ownership costs

Use for impurity profiling, peptide mapping or ultra-fast gradients

For best results, use with ZORBAX RRHD 300 Å, 1.8 µm column.

1290 Infinity II LC with high speed pump

Achieve optimal performance and efficiency using Agilent LC supplies

Agilent InfinityLab supplies work seamlessly with InfinityLab LC series instruments to achieve the highest performance, operational efficiency, and lab safety. Smart consumables track usage for added confidence in your analysis.

Visit: **www.agilent.com/chem/infinitylab** or discover the InfinityLab LC Supplies catalog at **www.agilent.com/chem/catalog**

1260 Infinity II LC with binary pump

Raising the standard in analytical HPLC with 600 bar

100% HPLC compatibility plus UHPLC capability

- UHPLC performance with HPLC ownership costs
- Supports LC and LC/MS applications, with any narrow and standard bore analytical column (2.1 to 4.6 mm id)
- Superior gradient accuracy by high-pressure mixing

Use for any standard UHPLC application

1260 Infinity II LC with binary pump

1260 Infinity II Prime LC

Highest confidence and accuracy in your everyday analysis

Highest quaternary UHPLC performance facilitating seamless method transfer and automated buffer blending

- UHPLC power range with up to 800 bar and 5 mL/min
- BlendAssist, the easiest tool for accurate buffer and additive blending
- UHPLC productivity with HPLC ownership costs

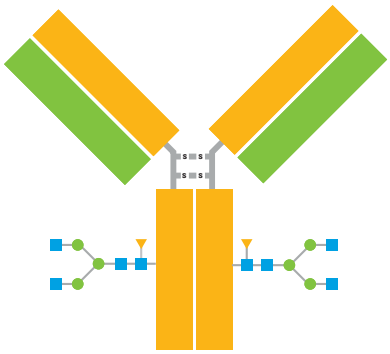
Use for method development or walk-up systems with accurate buffer blending

1260 Infinity II Prime LC

Tips and tools

For a closer look at these advanced systems, visit: **www.agilent.com/chem/BioHPLC**

Primary Structure Analysis



Accurately determine amino acid sequence and post-translational modifications, and analyze impurities of peptides and oligonucleotides

For full characterization of a protein, such as a monoclonal antibody, it is necessary to look at the primary amino acid sequence and any post-translational modifications to the sequence that may have occurred during the purification or formulation steps of manufacture. To perform this type of analysis, denaturing conditions are required, so reversed-phase LC is normally the technique of choice.

Agilent offers the most comprehensive range of wide-pore, 300 Å, 450 Å, and larger, reversed-phase BioHPLC columns, all backed by technical support experts and application chemists around the globe. The family includes 1.8, 3.5, and 5 µm porous particles for pressures from 400 to 1200 bar, three different superficially porous particles for UHPLC separations at lower pressure, and polymeric columns for analysis under a wide variety of conditions, including formic acid mobile phases for MS analysis.

For impurity analysis of either peptides or oligonucleotides, Agilent offers both silica-based and polymeric column options with high pH tolerance. Polymeric column options have the added benefit of being scalable from analytical to preparative separations.

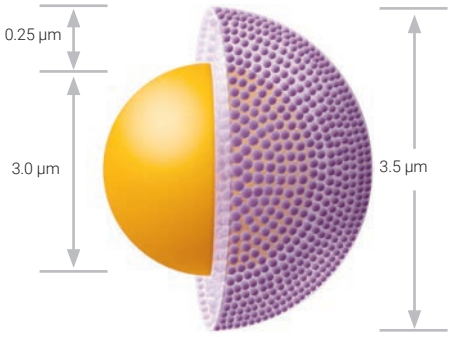
- **AdvanceBio RP-mAb columns** are the only reversed-phase columns designed especially for mAb characterizations. 450 Å pore size Poroshell technology and the right bonded phase selectivity provide fast, high resolution characterization of intact mAbs and mAb fragments.
- **PLRP-S columns** include macroporous polymer particles that deliver HPLC separations over the widest pH range. With three wide-pore sizes and eight particle sizes, the PLRP-S columns provide optimum solutions for analytical prep separations of peptides, proteins, and protein complexes.
- **ZORBAX RRHD 300 Å 1.8 µm columns** deliver UHPLC performance for reversed-phase separations of intact proteins, protein fragments, and digests with 1200 bar stability.
- **ZORBAX 300 Å 3.5, and 5 µm columns** are made from fully porous materials for HPLC and prep separations; many of the bonded phases scalable from the 1.8 µm particle.
- **Poroshell 300 columns** are the industry's first superficially porous small particle columns for fast polypeptide and protein separations.
- **AdvanceBio Peptide Mapping columns** quickly resolve and identify amino acid modifications in primary structure. With their 2.7 µm particles, and C18 functionality, AdvanceBio Peptide Mapping columns deliver excellent retention, resolution, and peak shape for basic hydrophobic peptides.
- **AdvanceBio Oligonucleotide columns** feature high efficiency, 2.7 µm superficially porous particles for high resolution analysis of oligonucleotides and their impurities. Using exclusive technology, the particles are chemically modified to make them resistant to the high pH mobile phase conditions necessary for oligonucleotide separations.

Reversed-phase column selection

Application	Agilent Columns	Notes	
Monoclonal antibodies and mAb fragments	AdvanceBio RP-mAb	Based on Poroshell technology featuring superficially porous particles that reduce diffusion distances and allow higher flow rates and steeper gradients to be used thus reducing run times—even on 600 bar systems. 450 Å pore size provides full access to the bonded phase by large molecules ensuring the best possible chromatography. Robust bonded phases designed for monoclonal antibody separations provide a range of selectivities that allow resolution to be optimized.	AB
	C4 SB-C8 Dipheny		
	PLRP-S 1000 Å	Macroporous polymeric PLRP-S delivers excellent mAb separations, especially with formic acid mobile phases for MS detection.	
Intact proteins, monoclonal antibodies, mAb fragments and polypeptides	ZORBAX 300 Å, 1.8 µm	Optimized packing processes achieve stability up to 1200 bar for use with the 1290 Infinity II LC. RRHD 1.8 µm columns are available in 50 and 100 mm lengths for fast or high resolution separations of the most complex samples. StableBond C18 is ideal for complex protein and protein digest separations.	AB
	RRHD 300SB-C18 RRHD 300SB-C8 RRHD 300SB-C3 RRHD 300-Diphenyl		
	ZORBAX 300 Å, 3.5, and 5 µm	Ideal for use with HPLC systems. StableBond C3 and CN are useful for larger, more hydrophobic compounds.	
	300SB-C18 300SB-C8 300SB-C3 300SB-CN		
	PLRP-S	Polymeric PLRP-S offers alternate selectivity to silica-based columns, along with excellent peak shape in formic acid mobile phases.	
	100 Å 300 Å 1000 Å 4000 Å		
	Poroshell 300	5 µm Poroshell particles with 300 Å pores enable rapid HPLC separations of intact proteins.	AB
	300SB-C18 300SB-C8 300SB-C3 300Extend-C18		
Proteins in protein digests	AdvanceBio Peptide Mapping	An ideal 120 Å pore size for identifying a wide molecular weight range of peptides. Tested with a challenging peptide mix to ensure performance. The unique Agilent Poroshell technology enables shorter run times and better resolution of the full peptide sequence.	AB
Synthetic peptide impurity analysis	PLRP-S 100 Å, 300 Å	Scalable from analytical to preparative separations. Superior pH stability enables extreme pH use, including for sanitization.	
Oligonucleotide analysis	PLRP-S 100 Å, 300 Å, 1000 Å, 4000 Å PL-SAX 1000 Å, 4000 Å	Multiple pore size options depending on oligonucleotide length. High temperature stability. Scalable from analytical to preparative separations. For more information on PL-SAX, please see **Page 95**.	
	AdvanceBio Oligonucleotide	Ideal for high-resolution analytical scale separations.	AB

AB Part of the AdvanceBio family

AdvanceBio RP-mAb



- **Improved accuracy:** superficially porous particles (3.5 µm) with wide pores (450 Å) increase mAb resolution while maintaining compatibility with all LC instruments.
- **Speed:** shorter analysis times compared to columns packed with fully porous particles.
- **Flexible method development:** range of chemistries—SB-C8, C4, and diphenyl.
- **Lower costs:** robust Poroshell packed bed and 2 µm inlet frit extend column lifetime by helping prevent inlet blockage.

Reversed-phase columns developed for the unique challenges of monoclonal antibody characterization

Analysis of intact and reduced monoclonal antibodies are critical measurements for characterizing therapeutic proteins and understanding their efficacy and stability. Poor chromatographic separations can result in rework and even compromise the accuracy of the characterization.

Long analysis times negatively impact the throughput of a laboratory and lead to delays in making decisions based on the results of characterization. To eliminate these problems, Agilent has developed a new reversed-phase column to optimize the performance of intact and reduced mAb analysis. The AdvanceBio RP-mAb column is based on Poroshell technology with unique engineering for pore size and bonded phases.

Tips and tools

For more information on the characterization of monoclonal antibody primary structure, see:
Better Characterization of Biomolecules using Agilent AdvanceBio Reversed-Phase Columns (publication **5991-2032EN**)

www.agilent.com/search

Large biomolecules, such as monoclonal antibodies, are typically separated slowly to reduce the potential peak broadening of these slow diffusing analytes. However, the Poroshell technology used in AdvanceBio RP-mAb columns features superficially porous particles made with a thin layer of porous silica, 0.25 µm thick, on a 3.0 µm solid silica core. This morphology reduces the diffusion distance, allowing higher flow rates and steeper gradients to be used—even on 600 bar systems. The wide 450 Å diameter of the pores in the thin layer provides full access to the bonded phase by the large monoclonal antibody molecules ensuring the best possible chromatography. A choice of robust bonded phases designed for monoclonal antibody separations, C4, SB-C8, and a unique diphenyl, provide a range of selectivities that allow resolution to be optimized.

AdvanceBio RP-mAb columns deliver higher resolution and shorter run times to provide fast, accurate, and reproducible results when analyzing monoclonal antibodies for biopharma discovery, development, and QA/QC applications.

Column Specifications

Bonded Phase	Pore Size	Temperature Limits*	pH Range*	Endcapped
AdvanceBio RP-mAb C4	450 Å	90 °C	1.0–8.0	Yes
AdvanceBio RP-mAb SB-C8	450 Å	90 °C	1.0–8.0	No
AdvanceBio RP-mAb Diphenyl	450 Å	90 °C	1.0–8.0	Yes

Specifications represent typical values only.

* Columns are designed for optimal use at low pH. At pH 6–8, highest column stability for all silica-based columns is obtained by operating at temperatures <40 °C and using low buffer concentrations in the range of 0.01–0.02 m.

High speed, high resolution separation of Trastuzumab Variant IgG1

Column: **AdvanceBio RP-mAb C4 795775-904 2.1 x 100 mm, 3.5 µm**

Mobile phase: A: 0.1% TFA in water:IPA (98.2)
B: IPA:ACN:mobile phase A (70:20:10)

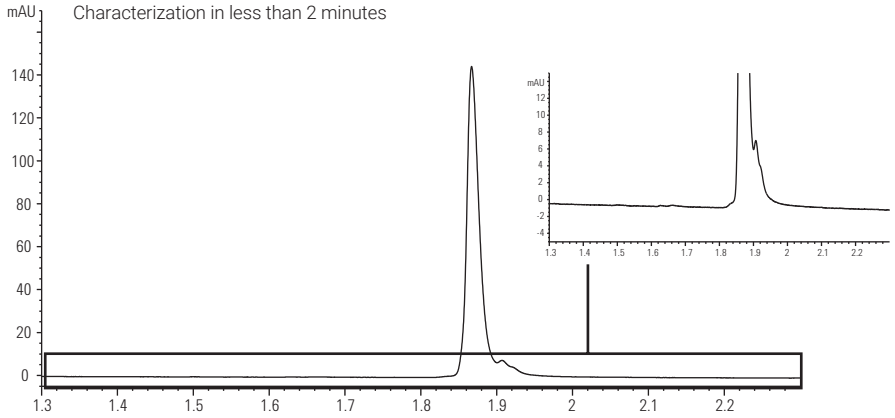
Flow rate: 1.0 mL/min

Gradient: 10–58% B in 4 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B

Temperature: 80 °C

Detector: UV, 254 nm

Sample: 5 µL injection of humanized recombinant Trastuzumab Variant IgG1 intact from Creative Biolabs (1 mg/mL)



AdvanceBio RP-mAb C4 provides a sharp peak and resolves fine detail in less than 2 minutes.

Separation of intact humanized recombinant Trastuzumab IgG1

Column: **AdvanceBio RP-mAb C4**
795775-904
2.1 x 100 mm, 3.5 µm

Mobile phase: A: 0.1% TFA in water:IPA (98:2)
B: IPA:ACN:mobile phase A (70:20:10)

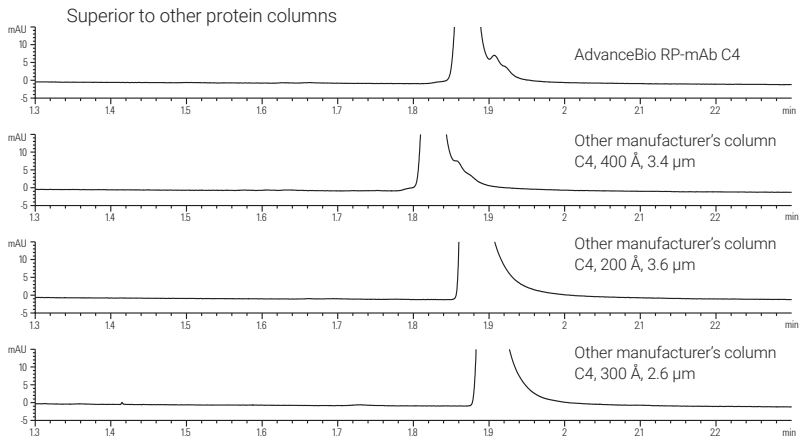
Flow rate: 1.0 mL/min

Gradient: 10–58% B in 4 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B

Temperature: 80 °C

Detector: UV, 254 nm

Sample: 5 µL injection of humanized recombinant Trastuzumab Variant IgG1 intact from Creative Biolabs (1 mg/mL)



Specifically designed for mAb separations, AdvanceBio RP-mAb provides superior peak shape and resolution when compared to other columns used for intact protein separations.

Selective diphenyl phase

Column: **AdvanceBio RP-mAb Diphenyl**
795775-944
2.1 x 100 mm, 3.5 µm

Mobile phase: A: 0.1% TFA in water: IPA (98:2)
B: IPA:ACN:mobile phase A (70:20:10)

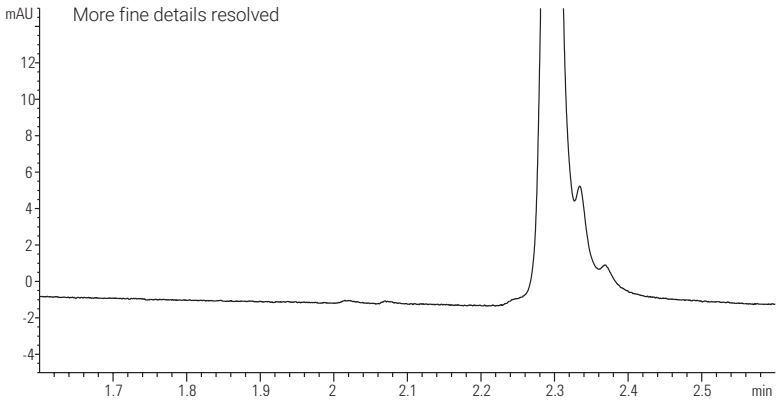
Flow rate: 1.0 mL/min

Gradient: 10–58% B in 4 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B

Temperature: 80 °C

Detector: UV, 254 nm

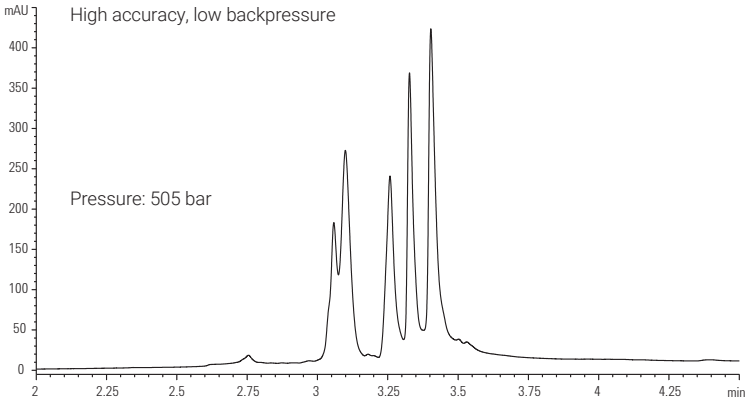
Sample: 5 µL injection of humanized recombinant Trastuzumab Variant IgG1 intact from Creative Biolabs (1 mg/mL)



The unique selectivity of AdvanceBio RP-mAb Diphenyl resolves even more fine detail.

The Poroshell advantage

Column:	**AdvanceBio RP-mAb SB-C8 785775-906 2.1 x 100 mm, 3.5 µm**
Mobile phase:	A: 0.1% TFA in water B: n-Propanol:ACN:mobile phase A (80:10:10)
Flow rate:	0.8 mL/min
Gradient:	5-40% B in 5 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B
Temperature:	60 °C
Detector:	UV, 220 nm
Sample:	1 µL injection of Fc/Fab, papain-digested humanized recombinant Trastuzumab Variant IgG1 from Creative Biolabs (2 mg/mL)



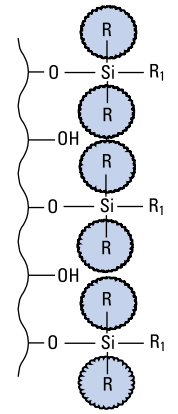
The wide-pore Poroshell technology of the AdvanceBio RP-mAb column delivers high efficiency, a short analysis time, and low pressure, at temperatures below 80 °C—the typical temperature of many reversed-phase methods.

AdvanceBio RP-mAb

Size (mm)	Particle Size (µm)	AdvanceBio RP-mAb C4 USP L26	AdvanceBio RP-mAb SB-C8 USP L7	AdvanceBio RP-mAb Diphenyl USP L11
4.6 x 150	3.5	793975-904	783975-906	793975-944
4.6 x 100	3.5	795975-904	785975-906	795975-944
4.6 x 50	3.5	799975-904	799975-906	799975-944
2.1 x 150	3.5	793775-904	783775-906	793775-944
2.1 x 100	3.5	795775-904	785775-906	795775-944
2.1 x 75	3.5	797775-904	787775-906	797775-944
2.1 x 50	3.5	799775-904	789775-906	799775-944

ZORBAX 300 Å StableBond

Agilent ZORBAX 300 Å StableBond columns are an ideal choice for the reproducible separations of proteins and peptides for two key reasons. First, wide-pore, 300 Å columns are necessary for an efficient separation of proteins and peptides, or other large molecules, to allow these analytes to completely access the bonded phase. Second, 300StableBond columns are unmatched in their durability at low pH, such as with TFA-containing mobile phases typically used for protein and peptide separations. For LC/MS separations at low pH, 300StableBond columns can also be used with formic acid and acetic acid mobile phase modifiers. These columns are available in five different bonded phases (C18, C8, C3, CN, and diphenyl (DP)) for selectivity and recovery optimization of proteins and polypeptides. To further increase sample recovery and improve efficiency for difficult proteins, 300StableBond columns can be used up to 80 °C. StableBond 300SB-C18 and 300SB-C8 columns are an ideal choice for complex protein and protein digest separations. These columns are also available in capillary (0.3 and 0.5 mm id) and nano (0.075 and 0.10 mm id) dimensions for reversed-phase LC/MS separations of protein digests. Capillary and nano columns can be used for either 1D or 2D proteomics separations.



Sterically Protected 300StableBond bonded phase

UHPLC Column Specifications

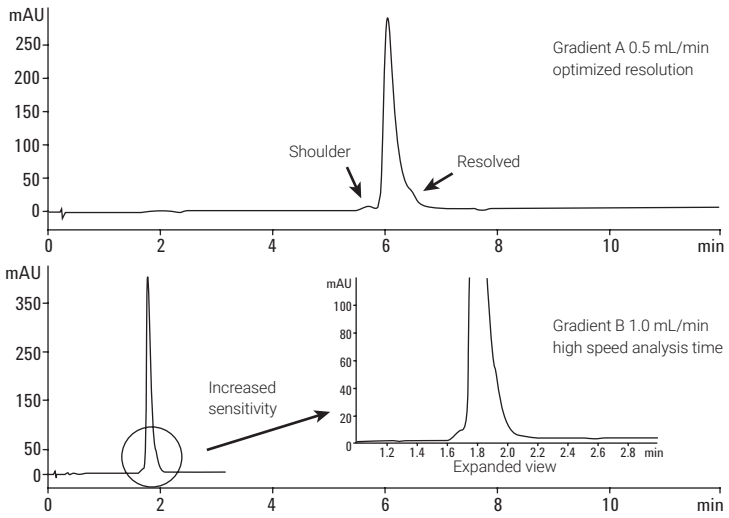
Bonded Phase	Pore Size	Temp Limits*	pH Range*	Endcapped
ZORBAX RRHD 300SB-C18	300 Å	90 °C	1.0–8.0	No
ZORBAX RRHD 300SB-C8	300 Å	80 °C	1.0–8.0	No
ZORBAX RRHD 300SB-C3	300 Å	80 °C	1.0–8.0	No
ZORBAX RRHD 300-Diphenyl	300 Å	80 °C	1.0–8.0	Yes
ZORBAX 300SB-C18	300 Å	80 °C	1.0–8.0	No
ZORBAX 300SB-C8	300 Å	80 °C	1.0–8.0	No
ZORBAX 300SB-C3	300 Å	80 °C	1.0–8.0	No
ZORBAX 300SB-CN	300 Å	80 °C	1.0–8.0	No

Specifications represent typical values only.

* 300StableBond columns are designed for optimal use at low pH. At pH 6–8, the highest column stability for all silica-based columns is obtained by operating at temperatures <40 °C and using low buffer concentrations in the range of 0.01–0.02 M. At mid or high pH, 300Extend-C18 is recommended.

Higher resolution of intact monoclonal antibody

Column:	**ZORBAX RRHD 300SB-C8 857750-906 2.1 x 50 mm, 1.8 µm**
Mobile phase:	A: H_2O:IPA (98:2) + 0.1% TFA (v/v) B: IPA:ACN:H_2O (70:20:10) + 0.1% TFA (v/v)
Flow rate:	Between 0.5 mL/min and 1.0 mL/min
Gradient:	Multisegmented and linear elution
Temperature:	80 °C
Detector:	1290 Infinity LC with autosampler, binary pump and thermostatted column compartment, and diode array detector (DAD)
Sample:	UV, 225 nm



Tips and tools

For more information on better characterization of biomolecules using AdvanceBio Reversed-Phase columns, see the white paper on this topic (publication **5991-2032EN**).

www.agilent.com/search

Reduced and alkylated mAb—separation of light chain and heavy chain variants

Column: **ZORBAX RRHD 300SB-C8**
858750-906
2.1 x 150 mm, 1.8 µm

Mobile phase: A: H_2O + 0.1% TFA (v/v)
B: n-propanol:ACN:H2O (80:10:10) + 0.1% TFA (v/v)

Flow rate: 0.5 mL/min

Injection: 3 µL (from 2.5 mg/mL sample)

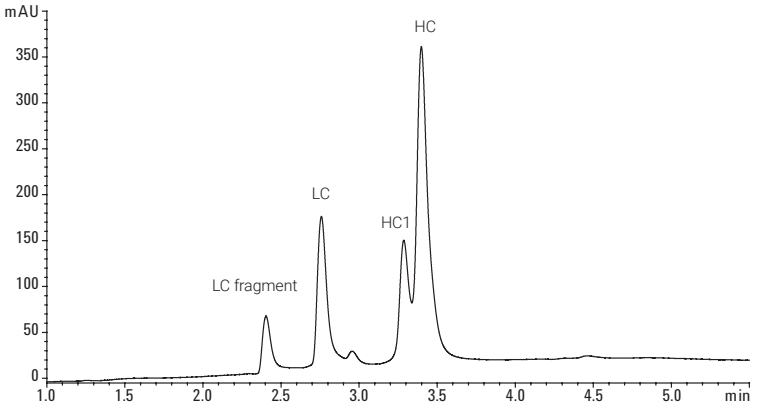
% Solvent B	Time (min)
20	0
35	3
40	4
40	5
90	5.1
90	5.5
25	6

Gradient: Multisegmented

Temperature: 75 °C

Detector: UV, 225 nm

Instrument: 1290 Infinity LC with autosampler, binary pump, thermostatted column compartment, and diode array detector (DAD)



For consecutive chromatographic runs, a 2-min postrun was added to re-equilibrate the column.

Tips and tools

Typical mobile phases for protein and peptide separations combine a very low pH with TFA (or other acids) to solubilize proteins. StableBond columns have extremely long lifetimes under these conditions. They are available in 300 Å pore size for proteins up to 100–500 kDa.

Improved reproducibility of monoclonal antibodies

Column: **ZORBAX RRHD 300SB-C8**
857750-906
2.1 x 50 mm, 1.8 µm

Mobile phase: A: H_2O:IPA (98:2) + 0.1% TFA (v/v)
B: IPA:ACN:H_2O (70:20:10) + 0.1% TFA

Flow rate: 1.0 mL/min

Temperature: 80 °C

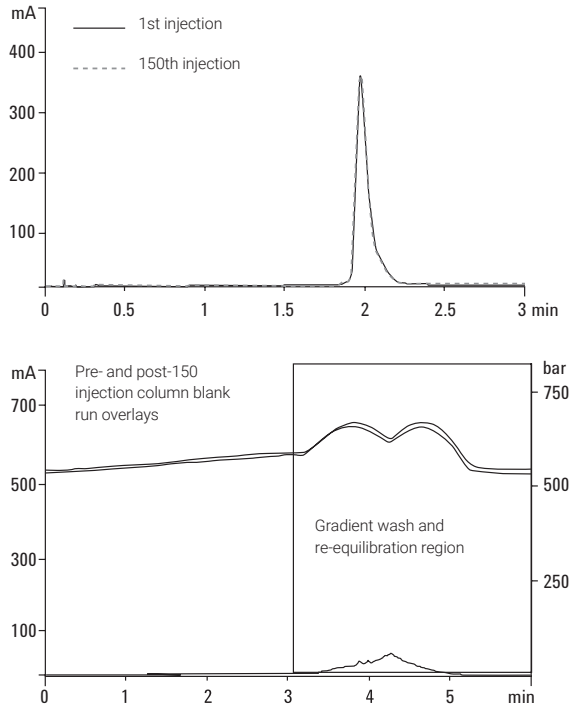
Detector: 1290 Infinity LC with diode array detector at 225 nm

Sample: mAb

Gradient

Particle size, µm	Flow rate, mL/min
0.00	25
3.00	35
4.00	90
5.00	25

Excellent column reproducibility and protein recovery using ZORBAX 300SB-C8.



Unique selectivity choices for mAb characterization

Columns:

ZORBAX RRHD 300SB-C18
858750-902
2.1 x 100 mm, 1.8 µm

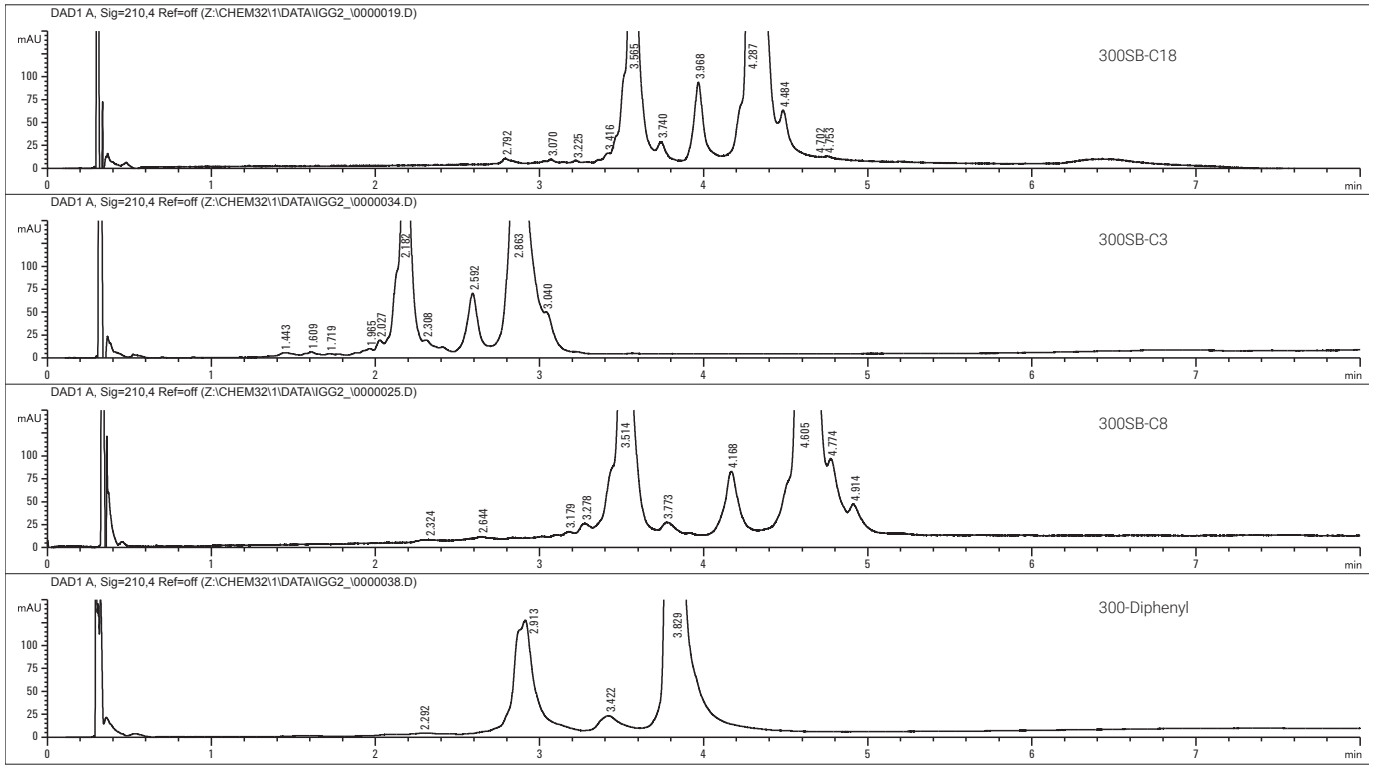
ZORBAX RRHD 300SB-C3
858750-909
2.1 x 100 mm, 1.8 µm

ZORBAX RRHD 300SB-C8
858750-906
2.1 x 100 mm, 1.8 µm

ZORBAX RRHD 300-Diphenyl
858750-944
2.1 x 100 mm, 1.8 µm

Mobile phase:	A: H_2O (0.1% TFA) (v/v) B: 80% nPA:10% ACN:10% H_2O (0.08% TFA) (v/v)
Injection volume:	3 µL (from 2.5 mg/mL sample)
Flow rate:	1.0 mL/min (3.5 µm*), 1.0 mL/min (1.8 µm)
Gradient:	25-35% B, 90% wash
Temperature:	80 °C
Detector:	UV, 215 nm

* Broad peaks at lower flow rates



Peptides/proteins: effect of elevated temperature

Column: **ZORBAX RRHD 300SB-C3**
883995-909
4.6 x 150 mm, 5 µm

Mobile phase: A: 5:95 ACN:water with 0.10% TFA (v/v%)
B: 95:5 ACN:water with 0.085% TFA (v/v%)

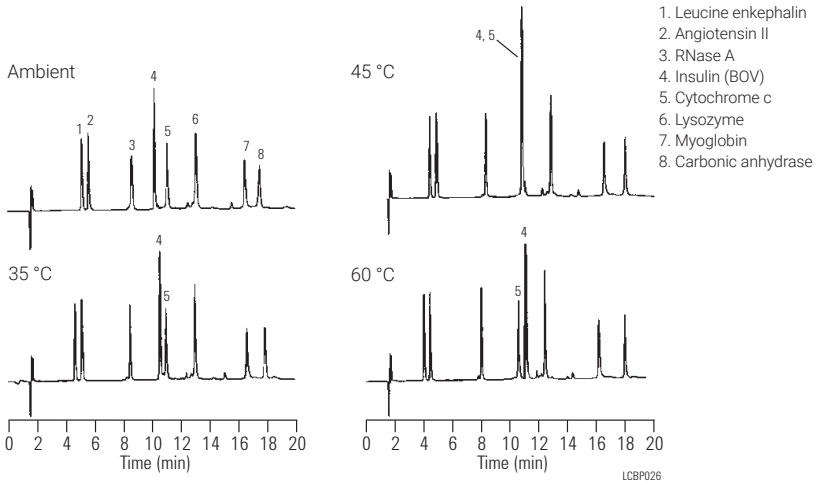
Flow rate: 1.0 mL/min

Gradient: 15–53% in 20 min, posttime 12 min

Temperature: Ambient–60 °C

Detector: UV, 215 nm

Sample: Polypeptides



1. Leucine enkephalin
2. Angiotensin II
3. RNase A
4. Insulin (BOV)
5. Cytochrome c
6. Lysozyme
7. Myoglobin
8. Carbonic anhydrase

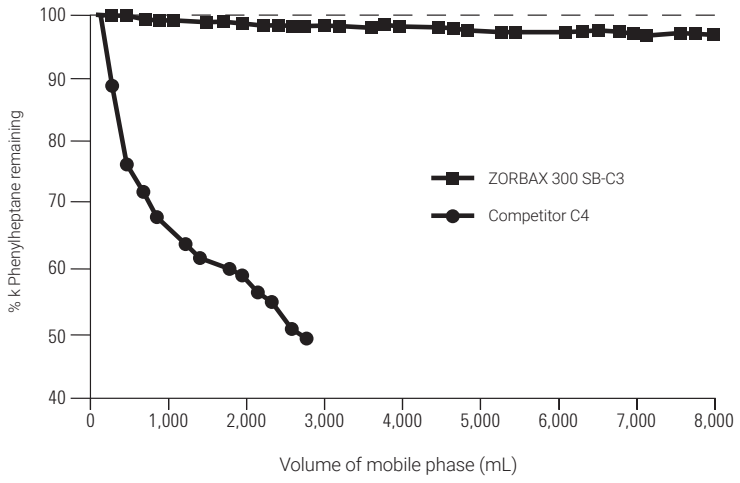
Short-chain ZORBAX 300SB-C3 is stable at low pH, high temperature

Column: **ZORBAX 300SB-C3**
883995-909
4.6 x 150 mm, 5 µm

Mobile phase: Gradient 0-100% B in 80 min
A: 0.5% TFA in water
B: 0.5% TFA in acetonitrile
Isocratic retention test conditions:
1-phenylheptane 50% A, 50% B

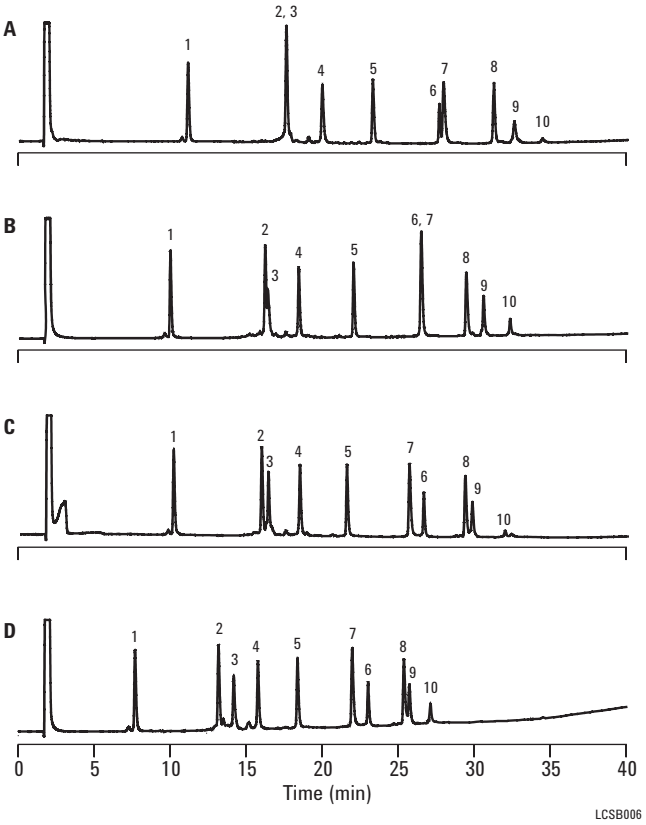
Flow rate: 1.0 mL/min

Temperature: 60 °C



Four different 300SB bonded phases optimize separation of large polypeptides

Column A:	**ZORBAX RRHD 300SB-C18 883995-902 4.6 x 150 mm, 5 µm**
Column B:	**ZORBAX 300SB-C8 883995-906 4.6 x 150 mm, 5 µm**
Column C:	**ZORBAX 300SB-C3 858750-909 4.6 x 150 mm, 5 µm**
Column D:	**ZORBAX 300SB-CN 858750-905 4.6 x 150 mm, 5 µm**
Mobile phase:	Linear gradient, 25–70% B in 40 min A: 0.1% TFA in water B: 0.09% TFA in 80% acetonitrile:20% water
Flow rate:	1.0 mL/min
Temperature:	60 °C
Sample:	3 µg each protein



1. RNase
2. Insulin
3. Cytochrome c
4. Lysozyme
5. Parvalbumin
6. CDR
7. Myoglobin
8. Carbonic anhydrase
9. S-100β
10. S-100α

The 300SB-C18, C8, C3, and CN bonded phases all provide a different separation of this group of polypeptides. This adds an important parameter for quickly optimizing protein separations. The 300SB-CN column offers unique selectivity for more hydrophilic polypeptides.

ZORBAX 300 Å StableBond

Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56	300-Diphenyl USP L11
Standard columns (no special hardware required)							
Semipreparative	9.4 x 250	5	880995-202	880995-206	880995-205	880995-209	
Analytical	4.6 x 250	5	880995-902	880995-906	880995-905	880995-909	
Analytical	4.6 x 150	5	883995-902	883995-906	883995-905	883995-909	
Analytical	4.6 x 50	5	860950-902	860950-906	860950-905	860950-909	
Rapid Resolution	4.6 x 150	3.5	863973-902	863973-906	863973-905	863973-909	
Rapid Resolution	4.6 x 100	3.5	861973-902	861973-906			
Rapid Resolution	4.6 x 50	3.5	865973-902	865973-906	865973-905	865973-909	
Solvent Saver Plus	3.0 x 150	3.5	863974-302	863974-306		863974-309	
Solvent Saver Plus	3.0 x 100	3.5		861973-306			
Narrow Bore	2.1 x 250	5	881750-902				
Narrow Bore	2.1 x 150	5	883750-902	883750-906	883750-905	883750-909	
Narrow Bore RR	2.1 x 150	3.5		863750-906			
Narrow Bore RR	2.1 x 100	3.5	861775-902	861775-906			
Narrow Bore RR	2.1 x 50	3.5	865750-902	865750-906			
Narrow Bore RRHD	2.1 x 100	1.8	858750-902	858750-906		858750-909	858750-944
Narrow Bore RRHD	2.1 x 50	1.8	857750-902	857750-906		857750-909	857750-944
MicroBore	1.0 x 250	5	861630-902				
MicroBore RR	1.0 x 150	3.5	863630-902	863630-906			
MicroBore RR	1.0 x 50	3.5	865630-902	865630-906			
MicroBore guard, 3/pk	1.0 x 17	5	5185-5920	5185-5920			
Guard cartridge, 2/pk	9.4 x 15	7	820675-124	820675-124	820675-124	820675-124	
Guard cartridge, 4/pk	4.6 x 12.5	5	820950-921	820950-918	820950-923	820950-924	

AB

(Continued)

AB Part of the AdvanceBio family

Tips and tools

To get the most out of your Agilent instruments, visit: **www.agilent.com/chem/getbioguides**

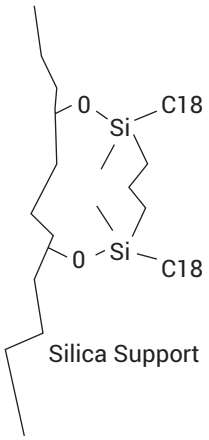
ZORBAX 300 Å StableBond

Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56	300-Diphenyl USP L11
Guard cartridge, 4/pk	2.1 x 12.5	5	821125-918	821125-918	821125-924	821125-924	
Guard hardware kit			840140-901	840140-901	840140-901	840140-901	
Guard hardware kit			820999-901	820999-901	820999-901	820999-901	
PrepHT cartridge columns (require endfittings kit 820400-901)							
PrepHT cartridge	21.2 x 250	7		897250-102	897250-106	897250-105	897250-109
PrepHT cartridge	21.2 x 150	7		897150-102	897150-106		897150-109
PrepHT cartridge	21.2 x 150	5		895150-902	895150-906		895150-909
PrepHT cartridge	21.2 x 100	5		895100-902	895100-906		895100-909
PrepHT cartridge	21.2 x 50	5		895050-902	895050-906		895050-909
PrepHT endfittings, 2/pk				820400-901	820400-901	820400-901	820400-901
PrepHT guard	17.0 x 7.5	5		820212-921	820212-918	820212-924	820212-924
Guard cartridge hardware				820444-901	820444-901	820444-901	820444-901
Capillary glass-lined columns							
Capillary	0.5 x 250	5	5064-8266				
Capillary	0.5 x 150	5	5064-8264				
Capillary	0.5 x 35	5	5064-8294				
Capillary RR	0.5 x 150	3.5	5064-8268				
Capillary RR	0.5 x 35	3.5	5065-4459				
Capillary	0.3 x 250	5	5064-8265				
Capillary	0.3 x 150	5	5064-8263				
Capillary	0.3 x 35	5	5064-8295				
Capillary RR	0.3 x 150	3.5	5064-8267	5065-4460			
Capillary RR	0.3 x 100	3.5	5064-8259	5065-4461			
Capillary RR	0.3 x 35	3.5	5064-8270	5065-4462			
Capillary RR	0.3 x 50	3.5	5064-8300	5065-4463			
Nano columns (PEEK fused silica)							
Nano RR	0.1 x 150	3.5	5065-9910				
Nano RR	0.075 x 150	3.5	5065-9911				
Nano RR	0.075 x 50	3.5	5065-9924	5065-9923			
Trap/guard, 5/pk	0.3 x 5	5	5065-9913	5065-9914			
Trap/guard hardware kit			5065-9915	5065-9915			

ZORBAX 300 Å Extend-C18

- Rugged, high and low pH separations of polypeptides and peptides from pH 2-11.5
- Different selectivity possible at high and low pH
- High efficiency and good recovery of hydrophobic peptides at high pH
- Ideal for LC/MS with ammonium-hydroxide-modified mobile phase

ZORBAX 300 Å Extend-C18 is a wide-pore HPLC column for high-efficiency separations of peptides from pH 2-11.5. The unique, bidentate bonded phase provides excellent lifetime and reproducibility at high and low pH. At high pH, retention and selectivity of peptides and polypeptides can change dramatically as a result of changes in charge on molecules. Excellent recoveries of hydrophobic polypeptides have been achieved at room temperature and high pH. LC/MS sensitivity of peptides and polypeptides can also be improved at high pH using a simple ammonium-hydroxide-containing mobile phase.



Novel bidentate C18-C18 bonding for Extend-C18 bonded phase

UHPLC Column Specifications

Bonded Phase	Pore Size	Temp Limits*	pH Range*	Endcapped
ZORBAX 300 Å Extend-C18 60 °C	300 Å	60 °C	2.0-11.5	Double

Specifications represent typical values only

* Temperature limits are 60 °C up to pH 8, 40 °C from pH 8-11.5.

Tips and tools

Selecting the right column is only part of the total solution. Don't forget key supplies such as our wide range of LC lamps. Visit: **www.agilent.com/chem/lamps** for more.

LC/MS analysis of angiotensin

Column: **ZORBAX Extend-C18**
773700-902
2.1 x 150 mm, 5 µm

Mobile phase: Acidic conditions:
A: 0.1% TFA in water
B: 0.085% TFA in 80% acetonitrile (ACN)
Basic conditions:
A: 10 mM NH_4OH in water
B: 10 mM NH_4OH in 80% ACN

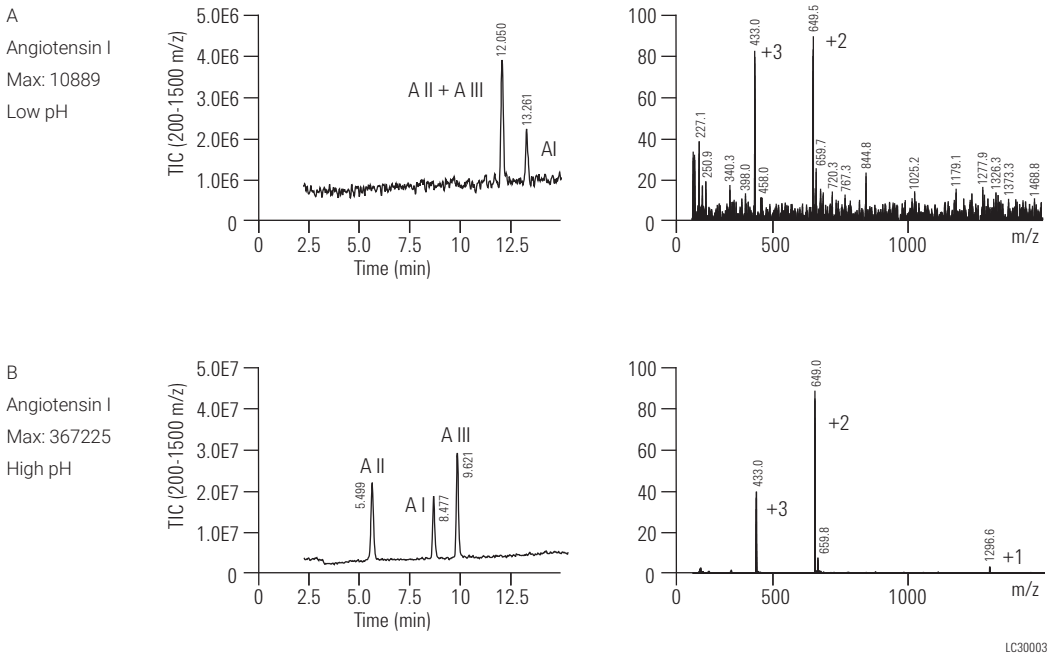
Flow rate: 0.2 mL/min

Gradient: 15–50% B in 15 min

Temperature: 35 °C

MS conditions: Pos. ion ESI; Vf 70 V, Vcap 4.5 kV, N_2, 35 psi, 12 L/min, 325 °C

Sample: Angiotensin I, II, III, 2.5 µL sample (50 pmol each)



Both small and large peptides demonstrate selectivity changes at high and low pH. At high pH, due to a change in charge, all three angiotensins can be resolved. In addition, the spectral clarity of angiotensin I is dramatically improved at high pH with the ammonium hydroxide mobile phase. The Extend-C18 column can be used for the analysis of small peptides at high pH as well.

Reference: B.E. Boyes. Separation and Analysis of Peptides at High pH Using RP-HPLC/ESI-MS, 4th WCBP, San Francisco, CA, Jan. 2000.

Long life at high pH

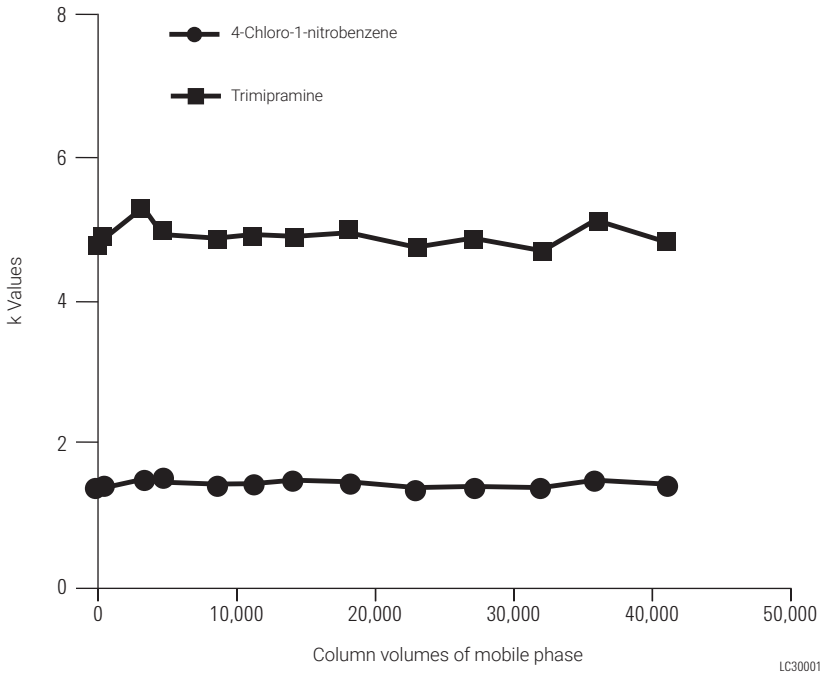
Column: **ZORBAX Extend-C18**
773450-902
4.6 x 150 mm, 5 µm

Mobile phase: 20% 20 mM NH_4OH, pH 10.5
80% methanol

Flow rate: 1.5 mL/min

Temperature: Aging 24 °C
Tests 40 °C

Each 10,000 column volume is approximately one working month.



Use ZORBAX Extend-C18 for alternate selectivity at high pH

Column: **ZORBAX Extend-C18**
773700-902
2.1 x 150 mm, 5 µm

Mobile phase: A: 0.1% TFA in water
B: 0.085% TFA in 80% ACN

A: 20 mM NH_4OH in water
B: 20 mM NH_4OH in 80% ACN

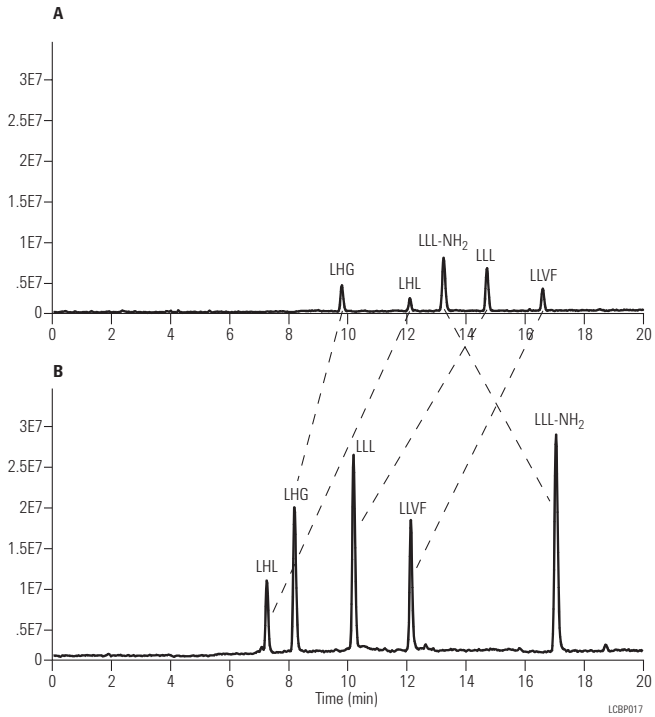
Flow rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

MS conditions: Pos. ion ESI; Vf 70 V, Vcap 4.5 kV,
N_2, 35 psi, 12 L/min, 300 °C
4 µL (50 ng each peptide)

The Extend column can be used for high pH separations of peptides. At high and low pH, very different selectivity can occur. Just by changing pH, a complementary method can be developed and it is possible to determine if all peaks are resolved. The Extend column can be used at high and low pH, and so the complementary separation can be investigated with one column. Better MS sensitivity for this sample is also achieved at high pH.



ZORBAX 300 Å Extend-C18

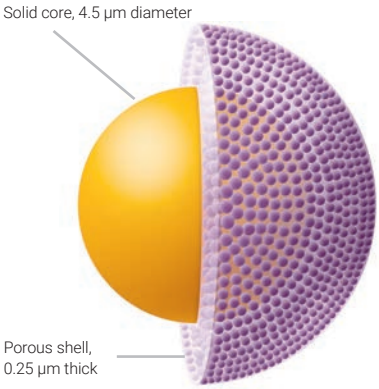
Description	Size (mm)	Particle Size (µm)	Part No.
Analytical	4.6 x 250	5	770995-902
Analytical	4.6 x 150	5	773995-902
Rapid Resolution	4.6 x 150	3.5	763973-902
Rapid Resolution	4.6 x 100	3.5	761973-902
Rapid Resolution	4.6 x 50	3.5	765973-902
Narrow Bore RR	2.1 x 150	3.5	763750-902
Narrow Bore RR	2.1 x 100	3.5	761775-902
Narrow Bore RR	2.1 x 50	3.5	765750-902
Guard cartridge, 4/pk	4.6 x 12.5	5	820950-932
Guard cartridge, 4/pk	2.1 x 12.5	5	821125-932
Guard hardware kit			820999-901
Capillary glass-lined columns			
Capillary RR	0.3 x 150	3.5	5065-4464
Capillary RR	0.3 x 100	3.5	5065-4465
Capillary RR	0.3 x 75	3.5	5065-4466
Capillary RR	0.3 x 50	3.5	5065-4467

Tips and tools

Guard columns and filters help protect your column and instruments from particulates that can cause blockages, which increase system pressure and negatively impact performance—interrupting your daily workflow. Agilent's new Fast guards for UHPLC and Bio LC columns help protect the column, leading to longer column lifetimes, minimizing interruptions in your workflow. For more information, visit: **www.agilent.com/chem/fastguards**

Poroshell 300 AB

- High speed separations of biomolecules with superficially porous particles
- 300 Å pores provide high efficiency and recovery with proteins (up to 1,000 kDa)
- Achieve long lifetime at low pH with Poroshell 300SB and at high pH with 300Extend-C18
- Optimize recovery and selectivity with four different bonded phases—300SB-C18, 300SB-C8, 300SB-C3, and 300Extend-C18



Poroshell 300 columns are ideal for fast separations of proteins and peptides because the 5 µm diameter superficially porous particle allows for fast flow rates to be used while maintaining sharp, efficient peaks. Poroshell columns with StableBond bonded phases provide excellent stability and selectivity choices with TFA and formic acid mobile phases. The Poroshell 300Extend-C18 column can be used from pH 2-11 for unique separations. These columns can also be used for analytical protein separations and LC/MS separations.

Peptides and proteins are typically separated slowly to reduce the potential peak broadening of these slow diffusing analytes. However, Poroshell columns use a superficially porous particle made with a thin layer of porous silica, 0.25 µm thick, on a solid core of silica. This reduces the diffusion distance for proteins, making rapid HPLC separations of peptides and proteins up to 500-1,000 kDa possible with 400/600 bar HPLC systems, including the 1260 Infinity II bio-inert LC.

UHPLC Column Specifications

Bonded Phase	Pore Size	Temp Limits*	pH Range*	Endcapped
Poroshell 300SB-C18, C8, C 3	300 Å	90 °C	1.0–8.0	No
Poroshell 300Extend-C18	300 Å	40 °C above pH 8 60 °C above pH 8	2.0–11.0	Yes

Specifications represent typical values only

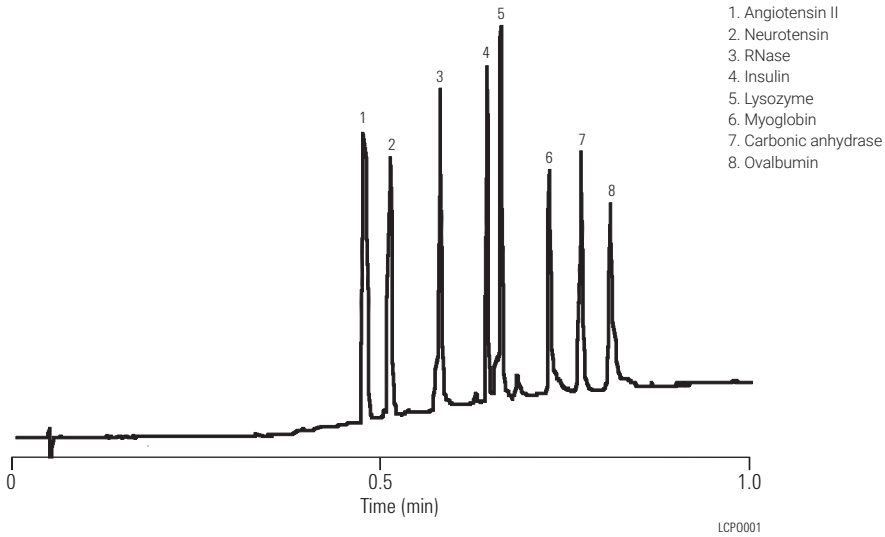
* 300StableBond columns are designed for optimal use at low pH. At pH 6–8, the highest column stability for all silica-based columns is obtained by operating at temperatures < 40 °C and using low buffer concentrations in the range of 0.01–0.02 M. At mid or high pH, 300Extend-C18 is recommended.

Poroshell 300 columns

AB Part of the AdvanceBio family

Poroshell 300 columns separate proteins and peptides in seconds

Column:	**Poroshell 300SB-C18** **660750-902** **2.1 x 75 mm, 5 µm**
Mobile phase:	A: 0.1% TFA in H_2O B: 0.07% TFA in ACN
Flow rate:	0.3 mL/min
Gradient:	5–100% B in 1.0 min
Temperature:	70 °C, 260 bar
Detector:	UV, 215 nm
Sample:	Proteins and peptides



This separation of eight polypeptides and proteins is completed in less than 60 seconds. Each peak is sharp and efficient.

Tips and tools

Further information can be found in:

Characterization of Glycosylation in the Fc Region of Therapeutic Recombinant Monoclonal Antibody (publication **5991-2323EN**)

Using the High-pH Stability of ZORBAX Poroshell 300Extend-C18 to Increase Signal-to-Noise in LC/MS (publication **5989-0683EN**)

www.agilent.com/search

Rapid high resolution analysis of fragmented IgG

Column: **Poroshell 300SB-C3**
660750-909
2.1 x 75 mm, 5 µm

Mobile phase: A: Water (5% AcOH, 1.0% FA, 0.05% TFA)
B: 70/20/10 IPA:ACN:water (5% AcOH, 1.0% FA, 0.05% TFA)

Flow rate: 1.0 mL/min

Injection volume: 2 µL

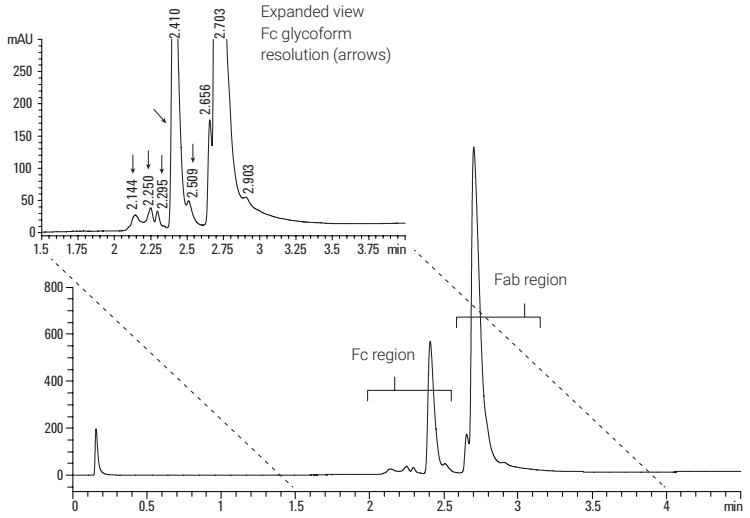
Gradient: Segmented

Time (min)	% B
0	20
4	45
8	45
9	90
10	20

Temperature: 80 °C

Detector: UV, 280 nm

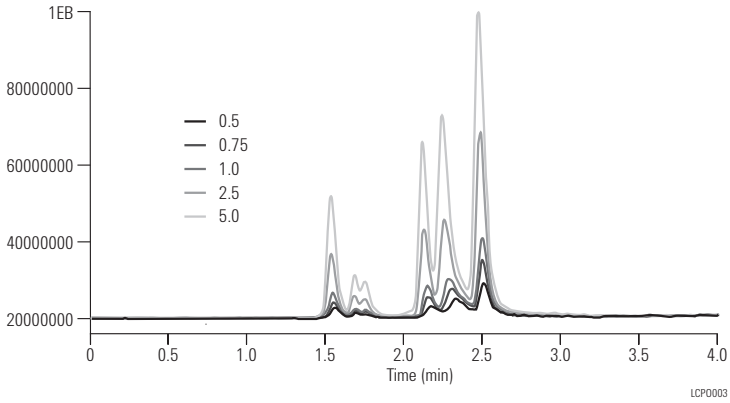
Instrument: 1200 Infinity series with high performance autosampler, binary pump, thermostatted column compartment (TCC), and diode array detector (DAD) coupled to a 6224 Accurate-Mass TOF LC/MS



Reversed-phase separation of IgG1 after papain digestion showing two primary peaks of the Fc and Fab fragments. The inset details partially resolved peaks representing variants of the Fc and Fab fragments (arrows).

MicroBore Poroshell 300 columns provide maximum sensitivity for LC/MS

Column:	**Poroshell 300SB-C18 661750-902 1.0 x 75 mm, 5 µm**	
Mobile phase:	A: Water + 0.1% formic acid B: ACN + 0.1% formic acid	
Flow rate:	600 µL/min	
Gradient:	20–100% B in 5.5 min	
Temperature:	80 °C	
MS conditions:	LC/MS:	Pos. ion ESI; Vcap 6,000 V
	Drying gas flow:	12 L/min
	Drying gas temperature:	350 °C
	Nebulizer:	45 psi
	Fragmentor voltage:	140 V
	Scan:	600-2,500
	Stepsize:	0.15 amu
	Peak width:	0.06 min
Sample:	1 µL	



With narrow bore diameters of 2.1 mm, 1.0 mm, and 0.5 mm, Poroshell columns make an ideal LC/MS partner. When the sample is very limited, the 1.0 mm or 0.5 mm id Poroshell columns are an excellent choice for high sensitivity LC/MS analyses. Sensitive MS molecular weight determinations are possible with as little as 0.5 to 5 pmole of protein on Poroshell columns. The columns have also been used for rapid MS identification of intact proteins, even in the presence of stabilizers and tissue culture media.

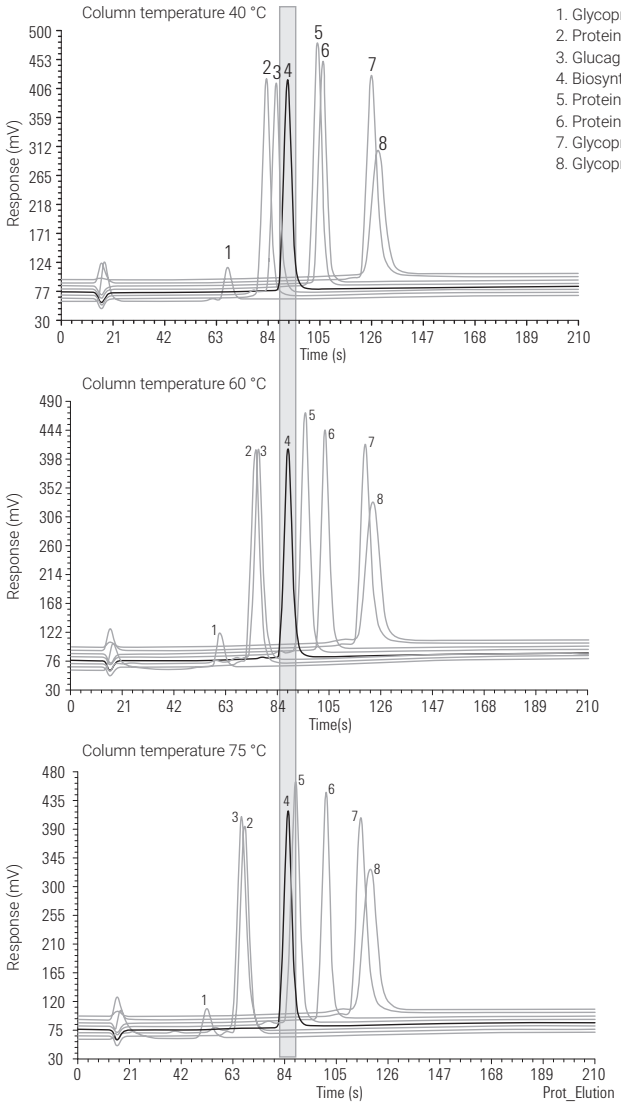
Tips and tools

Agilent offers an extensive selection of vials and sample containment solutions including polypropylene and deactivated and siliconized glass. To see the full range, see publication **5990-9022EN**.

www.agilent.com/chem/vials-productivity

Protein elution patterns

Column:	**Poroshell 300SB-C8 660750-906 2.1 x 75 mm, 5 µm**
Mobile phase:	A: 0.1% TFA in H_2O B: 0.1% TFA in ACN
Flow rate:	1.0 µL/min
Gradient:	20 to 70% B in 3 min
Detector:	UV, 214 nm



1. Glycoprotein X, mol wt ~22 kDa
2. Protein I, mol wt ~4 kDa
3. Glucagon, mol wt ~3.5 kDa
4. Biosynthetic human insulin, mol wt ~6 kDa
5. Protein J, mol wt ~3 kDa
6. Protein K, mol wt ~6 kDa
7. Glycoprotein Y, mol wt ~45 kDa
8. Glycoprotein Z, mol wt ~30 kDa

Poroshell 300

Description	Size (mm)	Partical Size (µm)	Poroshell 300SB-C18	Poroshell 300SB-C8	Poroshell 300SB-C3	Poroshell 300Extend-C18
Narrow Bore	2.1 x 75	5	660750-902	660750-906	660750-909	670750-902
MicroBore	1.0 x 75	5	661750-902	661750-906	661750-909	671750-902
Capillary	0.5 x 75	5		5065-4468		
Guard cartridge, 4/pk	2.1 x 12.5	5	821075-920	821075-918	821075-924	
Guard hardware kit			820999-901	820999-901	820999-901	
MicroBore guard, 3/pk	1.0 x 17	5	5185-5968	5185-5968	5185-5968	5185-5968

AdvanceBio Peptide Mapping

- **Greater analytical confidence:** each batch of AdvanceBio Peptide Mapping media is tested with a rigorous peptide mix to ensure suitability and reproducibility, and to enable the identification of key peptides in complex peptide maps.
- **Save time:** two to three times faster than fully porous HPLC columns.
- **Every instrument works harder:** 4.6, 3.0, and 2.1 mm id columns are stable to 600 bar, enabling you to get the most from your UHPLC instruments. They can also deliver excellent performance for your legacy 400 bar instruments, too.
- **Increased flexibility:** achieve increased MS sensitivity with formic acid mobile phases on any HPLC.

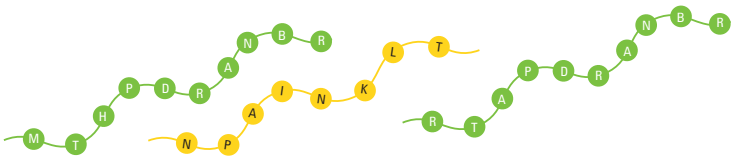
These advanced biocolumns feature a 120 Å pore size with superficially porous 2.7 µm particles. They are specially tested with a challenging peptides mix to ensure reliable peptide mapping performance. In addition, AdvanceBio Peptide Mapping columns deliver exceptional resolution and speed for UHPLC, and excellent results for conventional HPLC.

Column Specifications

Bonded phase	Pore Size	Temp Limits	pH Range	Endcapped
EC-C18	120 Å	60 °C	2.0–8.0	Double

Specifications represent typical values only

Tips and tools



Do you want to know how scientists are using the AdvanceBio Peptide Mapping column? See:

Amano, M. *et al.* Detection of Histidine Oxidation in a Monoclonal Immunoglobulin gamma (IgG) 1 Antibody. *Analytical Chemistry*, 2014, 86 (15): 7536 -7543

Leah G. Luna and Katherine Coady, Identification of *X. laevis* Vitellogenin Peptide Biomarkers for Quantification by Liquid Chromatography Tandem Mass Spectrometry. J. *Anal Bioanal Tech*, 2014, 5:3

High resolution peptide map of erythropoietin digest

Column: **AdvanceBio Peptide Mapping 651750-902 2.1 x 250 mm, 2.7 µm**

Mobile phase: A: H_2O + 0.1% formic acid (v/v)
B: Acetonitrile + 0.1% formic acid (v/v)

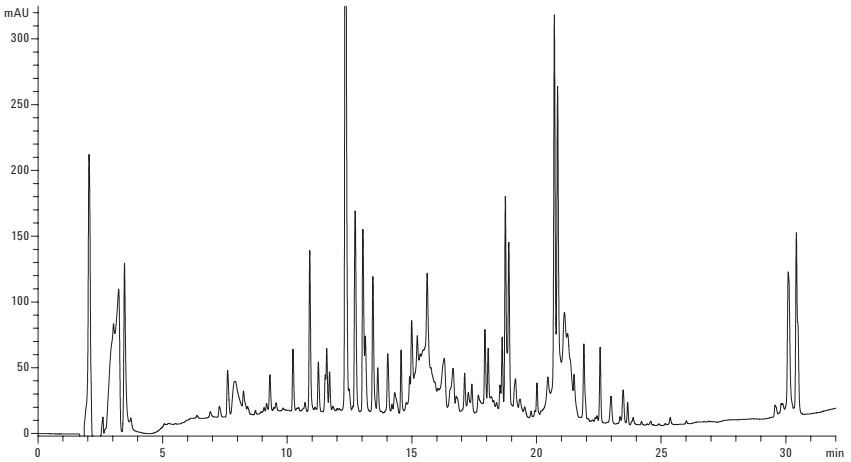
Flow rate: 0.4 mL/min

Gradient:

Time (min)	% B
0	3
28	45
33	60
34	95

Temperature: 55 °C

Sample: 5 µL (2 µg/µL)



Fast and efficient peptide mapping of IgG

Column: **AdvanceBio Peptide Mapping 655750-902 2.1 x 100 mm, 2.7 µm**

AdvanceBio Peptide Mapping 653750-902 2.1 x 150 mm, 2.7 µm

Mobile phase: A: H_2O + 0.1% FA (v/v)
B: 90% ACN + 0.1% FA (v/v)

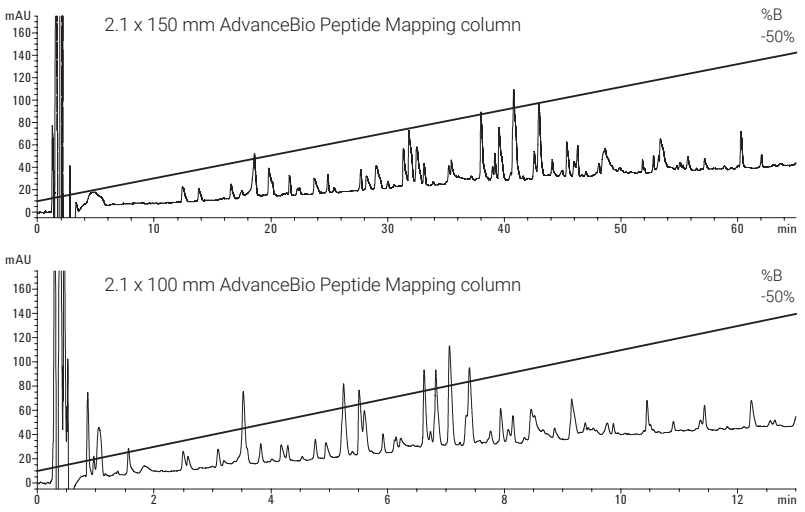
Flow rate: Various

Injection volume: 15 µL

Temperature: 40 °C

Detector: UV, 215/220 nm

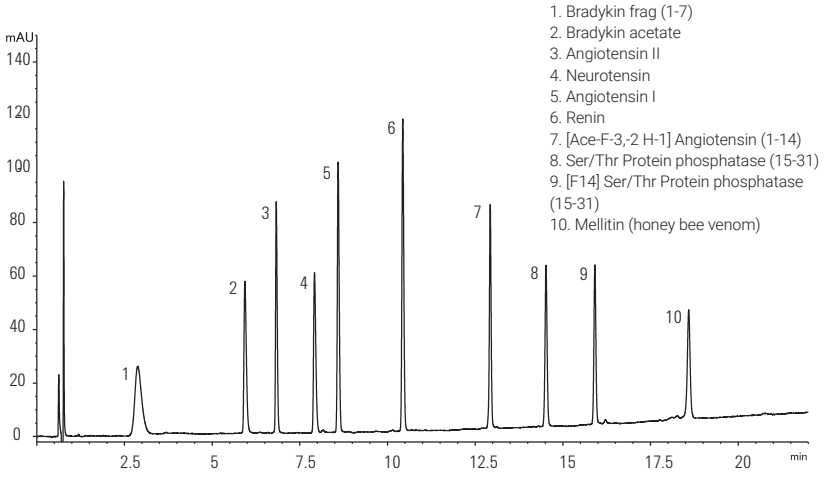
Sample: 1290 Infinity LC and 6530 accurate mass quadrupole time-of-flight LC/MS



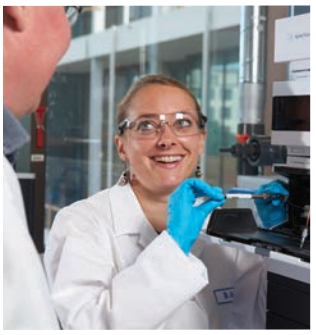
AdvanceBio Peptide Mapping column optimization for achieving a faster peptide mapping analysis. Gradient 10–40% B, DAD: 215 nm, 40 °C. Top panel, 75 min separation on a 2.1 x 150 mm column generated 59 peptide peaks (flow rate 0.2 mL/min, 211 bar). Bottom panel, optimized 14 min separation on a 2.1 x 100 mm column generated 57 peptide peaks (flow rate 0.6 mL/min, 433 bar).

Quality assurance testing with Agilent peptide mix

Column:	**AdvanceBio Peptide Mapping 653750-902 2.1 x 150 mm, 2.7 µm**
Flow rate:	3 µL
Gradient:	A, H_2O (0.1% TFA), B, ACN (0.1% TFA), 0–25 min, 15–65% B; 25–26 min, 65–95% B
Temperature:	55 °C
Detector:	220 nm
Sample:	Peptide Mapping Standards mix (0.5-1.0 µg/µL per peptide) p/n 5190-0583



Test mix used for every batch of AdvanceBio Peptide Mapping media. The mixture contains 10 hydrophilic, hydrophobic, and basic peptides, ranging in molecular weight from 757 to 2845 Da. Every column is also tested with a small-molecule probe to ensure efficiency.



Real stories from the lab

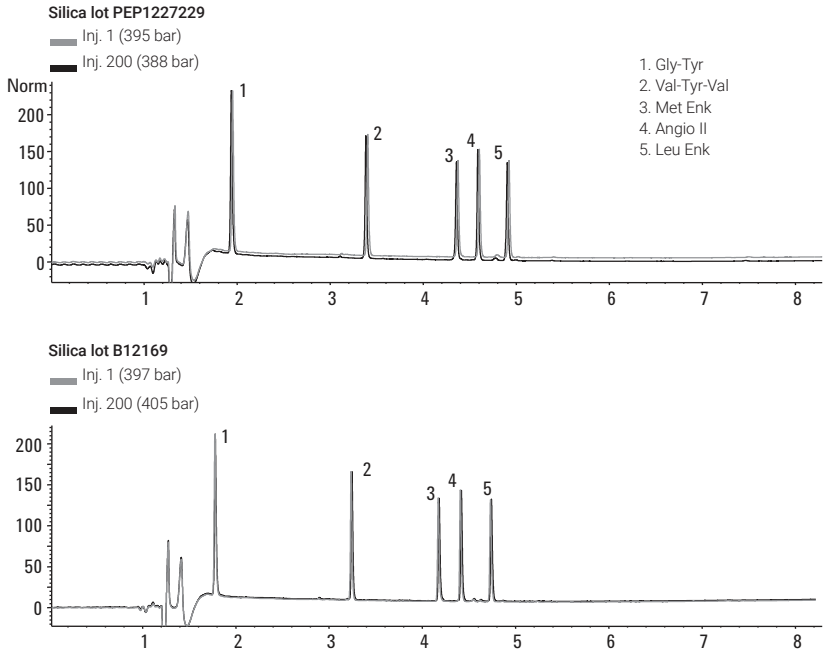
Bio bright spot

Learn how this lab manager was able to dramatically reduce downtime and improve user confidence.

www.agilent.com/chem/story25

Lot-to-lot reproducibility after 200 injections

Column:	**AdvanceBio Peptide Mapping 651750-902 2.1 x 250 mm, 2.7 µm**
Flow rate:	0.5 mL/min
Injection:	1 µL
Gradient:	A, H_2O (0.1% TFA), B, ACN (0.08% TFA), 0–8 min, 10–60% B; 8.1–9 min, hold 95% B
Temperature:	55 °C
Detector:	220 nm
Sample:	Sigma HPLC peptide standards



Injection	RT2 (min)	RT3 (min)	RT4 (min)	RT5 (min)
1	3.39	4.36	4.59	4.90
200	3.52	4.48	4.70	5.02
Injection	**PW2**	**PW3**	**PW4**	**PW5**
1	0.020	0.021	0.020	0.022
200	0.020	0.021	0.019	0.021

Injection	RT2 (min)	RT3 (min)	RT4 (min)	RT5 (min)
1	3.36	4.29	4.52	4.85
200	3.24	4.18	4.41	4.74
Injection	**PW2**	**PW3**	**PW4**	**PW5**
1	0.019	0.020	0.019	0.020
200	0.019	0.020	0.019	0.020

Superior reproducibility, lot-to-lot and run-to-run. A 2.1 x 250 mm AdvanceBio Peptide Mapping column was used for maximum resolution.

AdvanceBio Peptide Mapping

Description	Part No.
4.6 x 150 mm, 2.7 µm	653950-902
3.0 x 150 mm, 2.7 µm	653950-302
2.1 x 250 mm, 2.7 µm	651750-902
2.1 x 150 mm, 2.7 µm	653750-902
2.1 x 100 mm, 2.7 µm	655750-902
4.6 x 5 mm, Fast guard*	850750-911
3.0 x 5 mm, Fast guard*	853750-911
2.1 x 5 mm, Fast guard*	851725-911

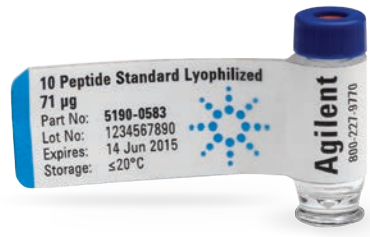
* Fast guards extend column lifetime without slowing down the separation or affecting resolution.

Agilent peptide quality control standard

Use Agilent's ten-peptide quality control standard, the same standard Agilent uses to QC its columns, to evaluate your column performance over its lifetime. It can be used for HPLC or LC/MS. Approximately 20 injections per vial.

Agilent Peptide Quality Control Standard

Description	Part No.
Peptide quality control standard, 71 µg in 2 mL vial	5190-0583



Tips and tools

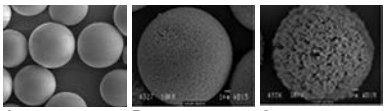
Peptide mapping is a powerful technique and the most widely used identity test for proteins, particularly those produced by recombinant means. There are several considerations to be made in addition to column selection for reproducible and accurate peptide maps, including protein digestion, sample preparation, method optimization, and so on. For fundamental techniques used in peptide mapping procedures and considerations when optimizing your peptide mapping separations, see *Keys for Enabling Optimum Peptide Characterizations: A Peptide Mapping "How to" Guide* (publication **5991-2348EN**).

www.agilent.com/search

PLRP-S

- Contain durable and resilient polymer particles that deliver reproducible results over longer lifetimes
- Thermally and chemically stable
- Comply with USP L21 designation
- Used in bioscience, chemical, clinical research, energy, environmental, food and agriculture, material science, and pharmaceutical industries
- Pore sizes (100 Å to 4000 Å) for separations of small molecules to large complexes and polynucleotides

The PLRP-S family of columns consists of a range of pore sizes and particle sizes, all with identical chemistry and fundamental adsorptive characteristics. The particles are inherently hydrophobic. Therefore, no bonded phase or alkyl ligand is required for reversed-phase separations. This gives a highly reproducible material that is free from silanols and heavy metal ions. Columns within the extensive product range are suitable for micro separations, including bottom-up and top-down proteomics, analytical separations, and preparative purifications. In addition, process columns can be packed with bulk media.



Scanning electron micrographs (SEM) of PLRP-S 10 µm particles.

The difference in pore size is clearly seen.
A is the small pore 100 Å
B the larger pore 300 Å
C the gigaporous 4000 Å

Column Specifications

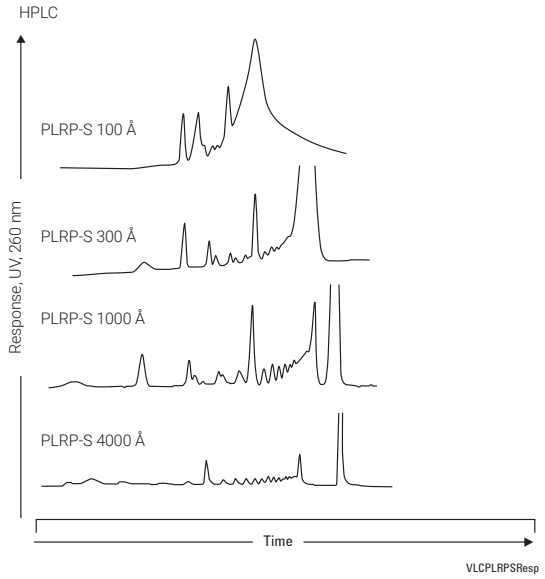
pH range	1-14
Buffer content	Unlimited
Organic modifier	1-100%
Temperature limits	200 °C
Maximum pressure	3 µm: 275 bar/4000 psi
	5 µm, 8 µm, and 10 µm: 207 bar/3000 psi
	10–15 µm, 15–20 µm, and 30 µm: 103 bar/1500 psi

PLRP-S Applications

Pore size	Application
100 Å	Small molecules/synthesis
300 Å	Recombinant peptides/proteins
1000 Å	Large proteins
4000 Å	DNA/high speed

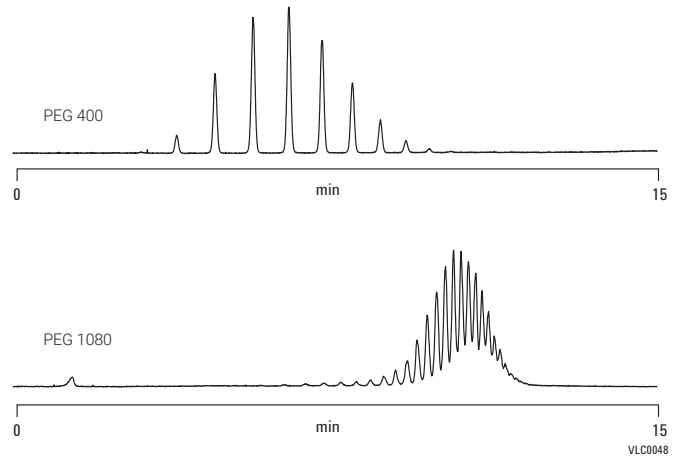
HPLC of 25 bp DNA ladder

Column:	**PLRP-S, 2.1 x 150 mm**
Mobile phase:	A: 100 mM TEAA B: 100 mM TEAA in 50% water:50% ACN
Flow rate:	200 µL/min
Gradient:	12.5–50% B in 150 min



Polyethylene glycols

Column:	**PLRP-S 100 Å** **PL1111-3500** **4.6 x 150 mm, 5 µm**
Mobile phase:	A: H_20 B: ACN
Flow rate:	1 µL/min
Injection volume:	10 µL
Gradient:	10–30% B in 12 min, held at 30% B for 3 minutes
Detector:	ELS (neb=50 °C, evap=70 °C, gas=1.6 SLM)
Sample conc:	1 mg/mL



Exploiting chemical stability—TFA concentration

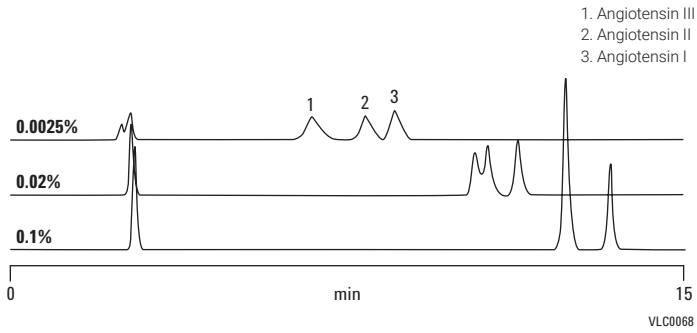
Column: **PLRP-S 100 Å**
PL1512-5500
4.6 x 250 mm, 5 µm

Mobile phase: A: TFA (various %) in water
B: TFA (various %) in ACN

Flow rate: 1.0 mL/min

Gradient: Linear 12–40% B in 15 min

Detector: ELS (neb=75 °C, evap=85 °C, gas=1.0 SLM)



Exploiting chemical stability—NH_4OH concentration

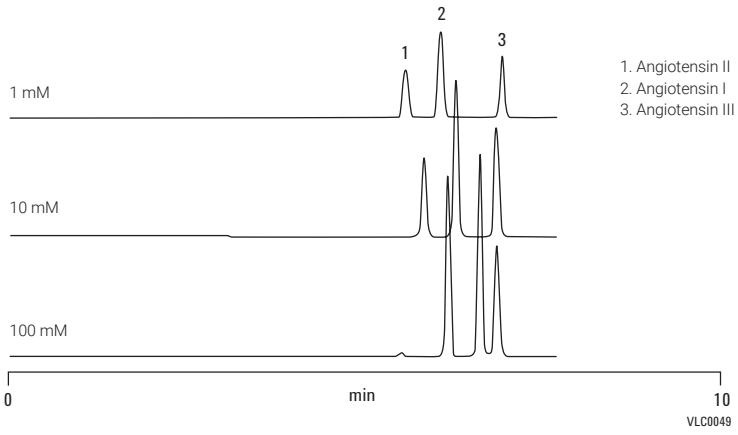
Column: **PLRP-S 100 Å**
PL1512-5500
4.6 x 250 mm, 5 µm

Mobile phase: A: NH_4OH (various mM) in water
B: NH_4OH (various mM) in ACN

Flow rate: 1.0 mL/min

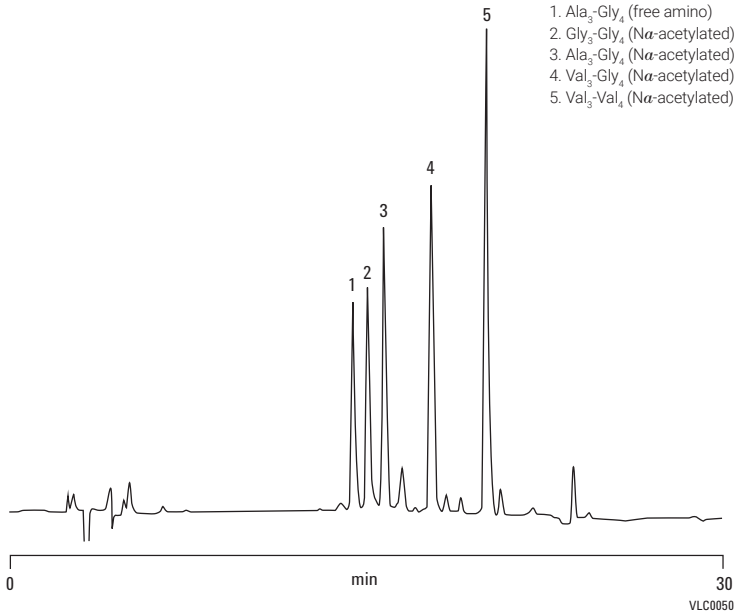
Gradient: Linear 10–100% B in 15 min

Detector: ELS (neb=80 °C, evap=85 °C, gas=1.0 SLM)



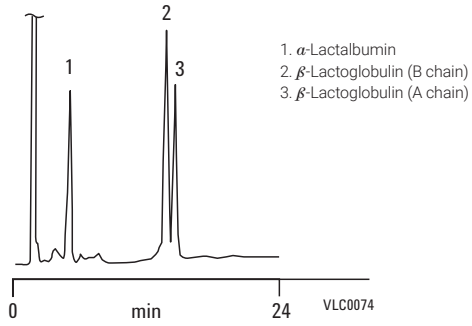
Alberta Peptide Institute test mix

Column:	**PLRP-S 100 Å** **PL1512-5500** **4.6 x 250 mm, 5 µm**
Mobile phase:	A: 0.1% TFA in 99% water:1% ACN B: 0.1% TFA in 70% water:30% ACN
Flow rate:	1 µL/min
Gradient:	0–100% B in 30 min
Detector:	UV, 220 nm



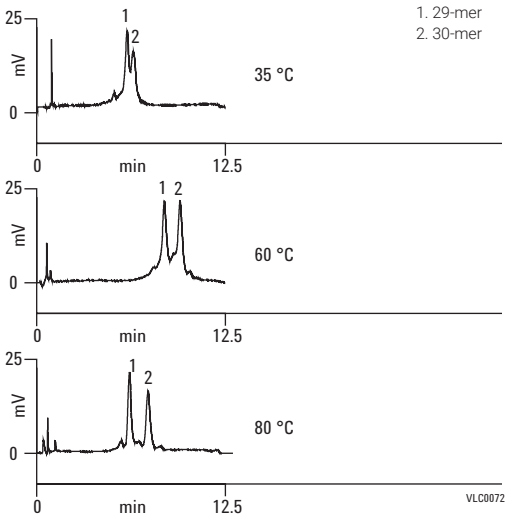
Whey proteins in dairy samples—milk

Column:	**PLRP-S 300 Å** **PL1512-3801** **4.6 x 150 mm, 8 µm**
Mobile phase:	A: 0.1% TFA in 99% water: 1% ACN B: 0.1% TFA in 1% water: 99% ACN
Flow rate:	1.0 mL/min
Injection volume:	10 µL
Gradient:	36–48% B, 0–24 min, 48–100% B, 24–30 min 100% B, 30–35 min, 100-36% B, 35–40 min
Detector:	UV, 220 nm



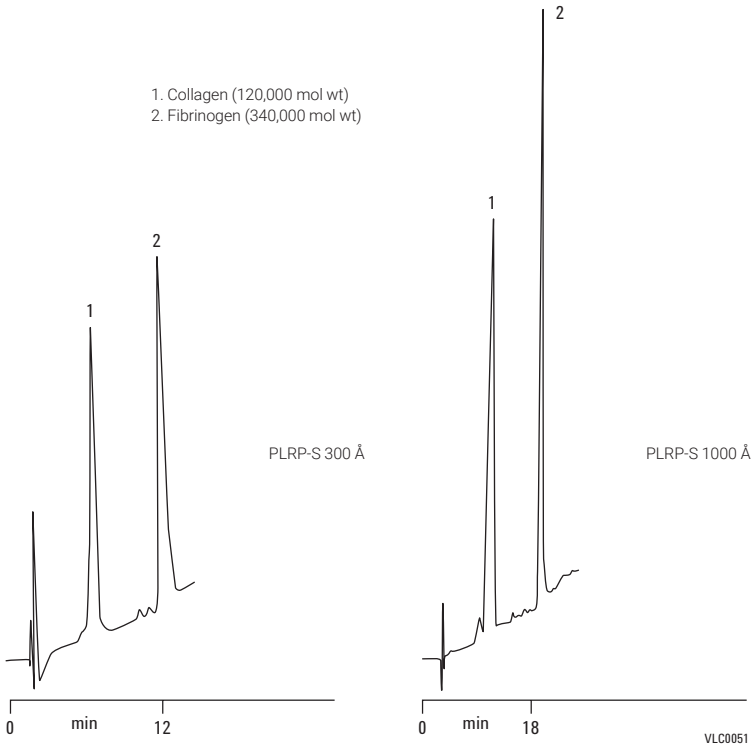
Temperature as a tool to enhance mass transfer and improve resolution of oligonucleotides in ion-pair reversed-phase HPLC

Column:	**PLRP-S 100 Å** **PL1512-1300** **4.6 x 50 mm, 3 µm**
Mobile Phase:	A: 100 mM TEAA B: 100 mM TEAA in 25% ACN
Flow Rate:	1.0 mL/min
Gradient:	5% change in buffer B over 5 min
Temperature:	35 °C, 60 °C, or 80 °C
Detector:	UV, 254 nm



Large fibrous proteins

Column:	**PLRP-S 300 Å** **PL1512-3801** **4.6 x 150 mm, 8 µm**
Mobile phase:	A: 0.25% TFA in water B: 0.25% TFA in 5% water:95% ACN
Flow rate:	1.0 mL/min
Injection volume:	10 µL
Gradient:	20–60% B in 15 min
Detector:	UV, 220 nm



PLRP-S HPLC Columns

Size (mm)	Particle Size (µm)	PLRP-S 100 Å USP L21	PLRP-S 300 Å USP L21	PLRP-S 1000 Å USP L21	PLRP-S 4000 Å USP L21
4.6 x 250	8	PL1512-5800	PL1512-5801	PL1512-5802	
4.6 x 150	8	PL1512-3800	PL1512-3801	PL1512-3802	PL1512-3803
4.6 x 50	8		PL1512-1801	PL1512-1802	PL1512-1803
4.6 x 250	5	PL1512-5500	PL1512-5501		
4.6 x 150	5	PL1111-3500	PL1512-3501		
4.6 x 50	5	PL1512-1500	PL1512-1501	PL1512-1502	PL1512-1503
4.6 x 150	3	PL1512-3300	PL1512-3301		
4.6 x 50	3	PL1512-1300	PL1512-1301		
2.1 x 250	8		PL1912-5801		
2.1 x 150	8		PL1912-3801	PL1912-3802	PL1912-3803
2.1 x 50	8		PL1912-1801	PL1912-1802	PL1912-1803
2.1 x 250	5	PL1912-5500	PL1912-5501		
2.1 x 150	5	PL1912-3500	PL1912-3501		
2.1 x 50	5	PL1912-1500	PL1912-1501	PL1912-1502	PL1912-1503
2.1 x 150	3	PL1912-3300	PL1912-3301		
2.1 x 50	3	PL1912-1300	PL1912-1301		
1.0 x 50	8			PL1312-1802	
1.0 x 50	5	PL1312-1500		PL1312-1502	
1.0 x 10	5			PL1C12-2502	
1.0 x 150	3	PL1312-3300			
1.0 x 50	3	PL1312-1300			
PLRP-S guard cartridges for 3.0 x 5.0 mm, 2/pk		PL1612-1801	PL1612-1801	PL1612-1801	PL1612-1801
Guard cartridge holder for 3.0 x 5.0 mm cartridges		PL1310-0016	PL1310-0016	PL1310-0016	PL1310-0016

Tips and tools

For microbore columns ordering information, turn to **Page 169.**

For PLRP-S prep to process columns and media ordering information, turn to **Page 182**.

AdvanceBio Desalting-RP

AdvanceBio Desalting-RP, p/n PL1612-1102 and cartridge holder, p/n 820999-901

Reversed-phase desalting for on-line removal of salt ions before MS detection

Affinity, ion exchange, and size exclusion chromatography are commonly used techniques in the analysis of proteins such as monoclonal antibodies. However, these techniques require aqueous mobile phases that contain nonvolatile salts. These nonvolatile salts present a problem when using MS detection as they cause signal suppression and can contaminate the MS detector through salt deposition leading to increased maintenance and instrument downtime. AdvanceBio Desalting-RP cartridges solve this problem, enabling fast and efficient on-line removal of salt ions before MS detection. These cartridge-style columns can be used on their own on any LC system to desalt collected fractions. Or on 1290 Infinity II 2D-LC system as the second dimension to desalt after the first dimension separation.

AdvanceBio Desalting-RP

Description	Part No.
AdvanceBio Desalting-RP, 2.1 x 12.5 mm, 2/pk	PL1612-1102
Cartridge holder	820999-901

AdvanceBio Oligonucleotide

To successfully separate trityl-off, deprotected oligonucleotides, you need columns that have high resolving power and are robust enough to withstand challenging conditions.

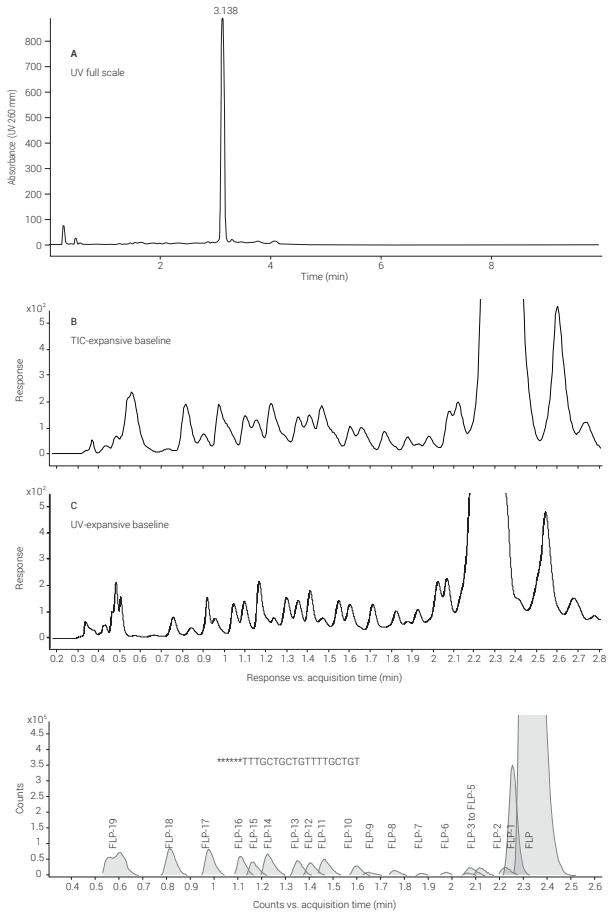
Agilent AdvanceBio oligonucleotide columns feature high efficiency, 2.7 µm superficially porous Poroshell particles. Using technology exclusive to Agilent, we chemically modify the particles to make them exceedingly resistant to high pH mobile phases. We also bond them with an end-capped C18 phase that delivers excellent selectivity for oligonucleotides. What's more, we test every batch of AdvanceBio oligonucleotide media with a resolution standard to ensure consistent performance.

The family of AdvanceBio products are designed to deliver consistent, exceptional performance for the complete characterization of proteins, antibodies, conjugates, new biological entities and biopharmaceuticals.

Column Specifications

Bonded Phase	Particle Size	Pore Size	Temp Limit	pH Range	Endcapped	Pressure Limit
C18	2.7 µm	100 Å	65 °C	3.0–11.0	Double	600 bar

Column:	**AdvanceBio Oligonucleotide 2.1 x 50 mm (p/n 659750-702)**
Mobile phase:	A: HFIP:TEA (400 mM:15mM) in water B: MeOH:mobile phase A (50:50)
Flow rate:	0.4 mL/min
Gradient:	30–40% B in 0.5 min; 40–70% B in 5 min
Sample:	25 mer DNA
Temperature:	65 °C
Detection:	UV at 260 nm
Detection:	MS
Min range:	400 m/z
Max range:	1,700 m/z
Scan rate:	3.00 spectra/s
Ion polarity:	-ve
VCap:	3,500
Nozzle voltage:	1,000 V
Fragmentor:	200



Deconvoluted data from TIC of a 25-mer DNA oligonucleotide separated by the AdvanceBio Oligonucleotide column

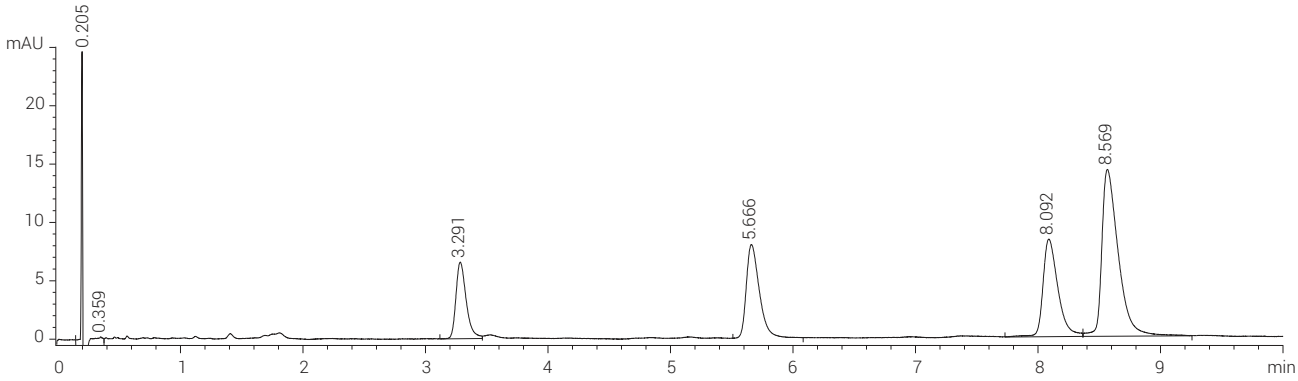
AdvanceBio Oligonucleotide

Description	id (mm)	Length (mm)	Particle Size (µm)	Part No.
Conventional	2.1	150	2.7	653750-702
Conventional	2.1	100	2.7	655750-702
Conventional	2.1	50	2.7	659750-702
UG Guard, 600 bar	2.1	5	2.7	821725-921
Conventional	4.6	150	2.7	653950-702
Conventional	4.6	100	2.7	655950-702
Conventional	4.6	50	2.7	659950-702
UG Guard, 600 bar	4.6	5	2.7	820750-921

AdvanceBio Oligonucleotide standards

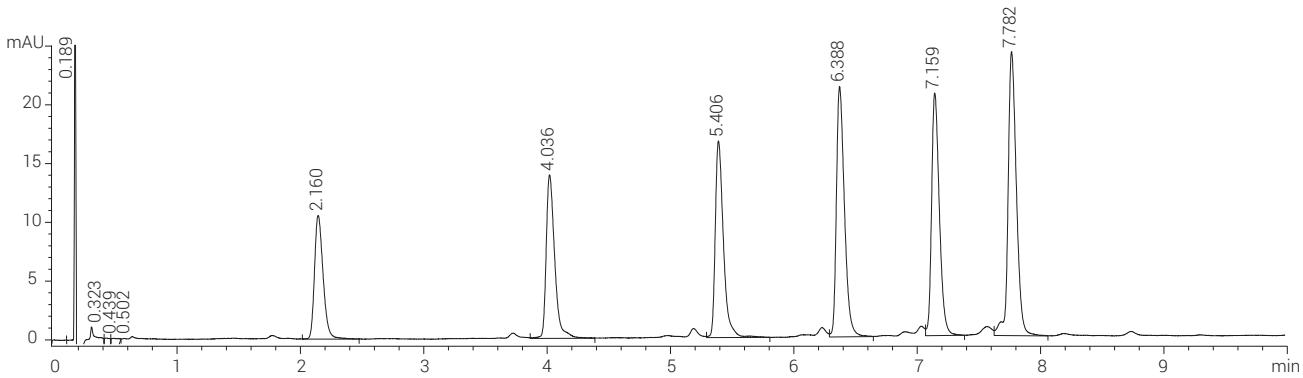
To ensure performance for your separations, every batch of AdvanceBio Oligonucleotide media is tested with the Agilent Oligonucleotide Resolution standard. The Oligonucleotide Resolution standard containing 14, 17, 20, and 21 mer synthetic oligonucleotides is designed to demonstrate N / N-1 resolution.

Column:	**AdvanceBio Oligonucleotide 2.1 x 50 mm (p/n 659750-702)**
Mobile phase:	A: 100 mM TEAA in water B: 100 mM TEAA in acetonitrile
Gradient:	6 to 8% B in 12 min
Stop time:	13 min
Post run:	5 min
Flow rate:	0.6 mL/min
Sample:	Agilent Oligonucleotide Resolution Standard (p/n 5190-9028)
Temperature:	65 °C
Injection:	0.5 µL
Detection:	UV at 260 nm



Agilent also offers an Oligonucleotide Ladder standard containing 15, 20, 25, 30, 35, and 40 mer synthetic oligodeoxythymidines, an excellent tool for demonstrating column selectivity and reproducibility.

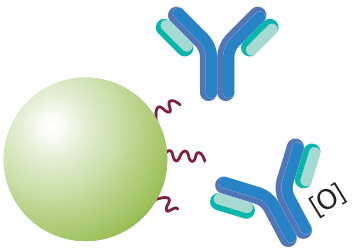
Column:	**AdvanceBio Oligonucleotide 2.1 x 50 mm (p/n 659750-702)**
Mobile phase:	A: 100 mM TEAA in water
	B: 100 mM TEAA in acetonitrile
Gradient:	10 to 14% B in 10 min
Stop time:	11 min
Post run:	5 min
Flow rate:	0.6 mL/min
Column temperature:	65 °C
Sample:	Agilent Oligonucleotide Ladder standard (p/n 5190-9029)
Injection:	10 µL
Detection:	UV at 260 nm



AdvanceBio Oligonucleotide Standards

Description	Part No.
Oligonucleotide Resolution Standard	5190-9028
Oligonucleotide Ladder Standard	5190-9029

Intact Analysis Using Hydrophobic Interaction Chromatography



AdvanceBio HIC

AdvanceBio HIC columns deliver high resolution, robust, and reproducible separations of native proteins at the intact level.

Built using the capabilities of the ZORBAX fully porous particles and proprietary bonding technology, these columns provide new levels of hydrophobicity and versatile single chemistry to address particularly challenging molecules such as monoclonal antibodies (mAbs), antibody drug conjugates (ADCs), and other recombinant proteins.

Together with the 1260 Infinity II bio-inert LC system, the AdvanceBio HIC provides uncompromised performance and data consistency during characterization and validation.

- Optimized selectivity: ideal for mAb oxidation and ADC DAR ratios.
- Single chemistry: reduces the need for multiple-column screening for different CQAs.
- Enhanced robustness: improved column lifetime for ultimate confidence in your data.
- Proven performance: every batch of media is tested with NIST mAb.
- High quality: each column is individually tested to ensure packing efficiency.
- Greater productivity: shorter columns reduce analysis time while maintaining separation performance.

Column Specifications

Pore Size	Particle Size	Temperature Limit	pH Range	Pressure Limit	Flow Rate*
450 Å	3.5 µm	60 °C (at pH 7)	2.0–8.0 (at 35 °C)	400 bar (typical operating pressure <200 bar)	0.5–1.0 mL/min (4.6 mm id)

*In some cases, lowering the flow rate to 0.3 mL/min and extending gradient time may further improve resolution.

Column: **AdvanceBio HIC 4.6 x 100 mm, 3.5 µm**

Eluent A: 2 M Ammonium Sulfate, 50 mM Sodium Phosphate, pH 7.0

Eluent B: 50 mM Sodium Phosphate, pH 7.0

Gradient:

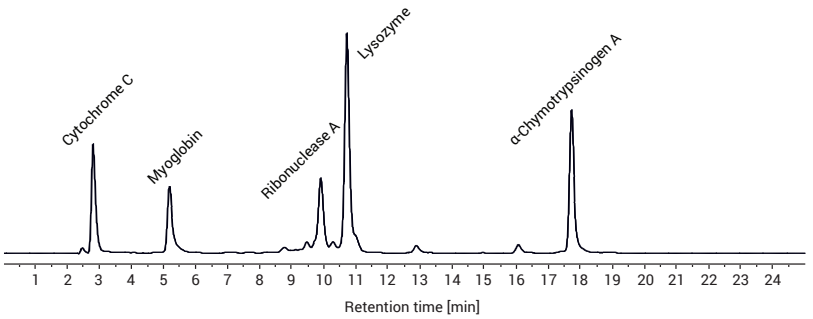
Time (min)	%A	%B
0	100	0
20	0	100
25	0	100
30	100	0
40	100	0

Flow rate: 0.5 mL/min

Temperature: 30 °C

Injection volume: 5 µL

Detection: UV, 220 nm



Column: **AdvanceBio HIC 4.6 x 100 mm, 3.5 µm**

Eluent A: 2 M Ammonium Sulfate, 50 mM Sodium Phosphate, pH 7.0

Eluent B: 50 mM Sodium Phosphate, pH 7.0

Gradient:

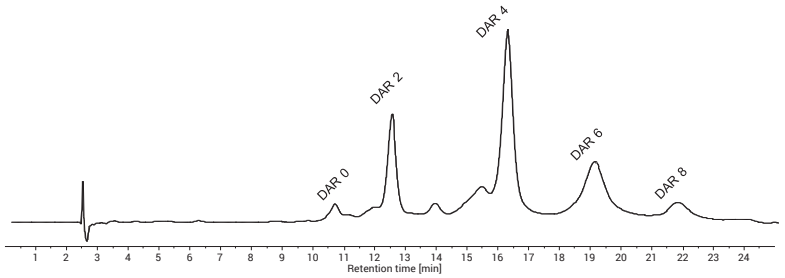
Time (min)	%A	%B	%C
0	50	45	5
20	0	75	25
25	0	75	25
30	50	45	5
40	50	45	5

Flow rate: 0.5 mL/min

Temperature: 30 °C

Injection volume: 5 µL

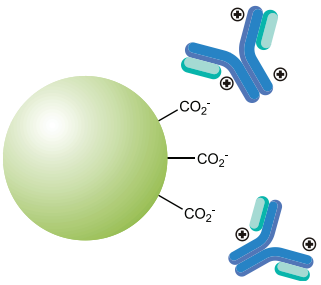
Detection: UV, 220 nm



Agilent AdvanceBio HIC Columns

Description	Part No.
AdvanceBio HIC, 4.6 x 100 mm, 450 Å, 3.5 µm	685975-908
AdvanceBio HIC, 4.6 x 30 mm, 450 Å, 3.5 µm	681975-908

Charge Variant Analysis



Purify proteins and other charged molecules

Ion-exchange chromatography (IEX) is a highly sensitive technique that allows you to separate ions and polar molecules based on their charge. Like SEC, IEX can be used to separate proteins in their native state.

Applying IEX to charge variant analysis

During production and purification, antibodies can exhibit changes in charge heterogeneity as a result of amino acid substitutions, glycosylation, phosphorylation, and other post-translational or chemical modifications. Because these changes can impact stability and activity—or cause immunologically adverse reactions—the analysis of charge heterogeneity in monoclonal antibody (mAb) preparations is critical to biopharmaceutical manufacture.

In protein analysis, charge 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 charge variants), and can be resolved by IEX chromatography. IEX is also useful as a preparative technique.

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

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.

The pages that follow describe Agilent's family of weak and strong ion-exchangers—both anionic and cationic.

- Nonporous Bio IEX columns are designed for high resolution, high efficiency, and high recovery separations.
- Bio MAb columns are optimized for separating charge isoforms of monoclonal antibodies.
- Porous IEX columns (PL-SAX and PL-SCX) are chemically stable, and are available in two pore sizes, allowing you to separate peptides, oligonucleotides, and very large proteins.
- Bio-Monolith IEX columns are uniquely suited for separating antibodies, viruses, and DNA.
- Buffer Advisor software is an ideal solution for automated protein separation by ionic strength gradients.

Tips and tools

For more information about the Agilent Buffer Advisor software, see publication **5991-1408EN**

www.agilent.com/search

Ion-exchange column selection

Application	Agilent Columns	Notes
Monoclonal antibodies	Bio MAb	Thorough characterization of monoclonal antibodies includes the identification and monitoring of acidic and basic isoforms. Bio MAb HPLC columns feature a unique resin specifically designed for high resolution charge-based separations of monoclonal antibodies.
Peptides and proteins	Bio IEX	Bio IEX columns are packed with polymeric, nonporous, ion-exchange particles. The Bio IEX columns are designed for high resolution, high recovery, and highly efficient separations.
Proteins, peptides, and deprotected synthetic oligonucleotides	PL-SAX • 1000 Å • 4000 Å	The strong anion-exchange functionality, covalently linked to a fully porous chemically stable polymer, extends the operating pH range. In addition, the anion-exchange capacity is independent of pH. For synthetic oligonucleotides, separations using denaturing conditions of temperature, organic solvent, and high pH are all possible. The 5 µm media deliver separations at high resolution while the 30 µm media are used for medium pressure liquid chromatography
Globular proteins and peptides	PL-SAX 1000 Å	
Very large biomolecules/high speed	PL-SAX 4000 Å	
Small peptides to large proteins and very large biomolecules	PL-SCX • 1000 Å • 4000 Å	PL-SCX is a macroporous PS/DVB matrix with a very hydrophilic coating and strong cation-exchange functionality. This process is controlled to provide the optimum density of strong cation-exchange moieties for the analysis, separation, and purification of a wide range of biomolecules. The 5 µm media deliver separations at higher resolution while the 30 µm media are used for medium pressure liquid chromatography.
Antibodies (IgG, IgM), plasmid DNA, viruses, phages, and other macro biomolecules	Bio-Monolith • Bio-Monolith QA • Bio-Monolith DEAE • Bio-Monolith SO_3	Strong cation-exchange, strong and weak anion-exchange phases. Bio-Monolith HPLC columns are compatible with InfinityLab LC series.
Viruses, DNA, large proteins	Bio-Monolith QA	
Plasmid DNS, bacteriophages	Bio-Monolith DEAE	
Proteins, antibodies	Bio-Monolith SO_3	

Bio MAb HPLC columns

- A packing support composed of a rigid, spherical, highly cross-linked polystyrene divinylbenzene (PS/DVB) nonporous bead
- Particles grafted with a hydrophilic polymeric layer, virtually eliminating nonspecific binding of antibody proteins
- A different process is used to layer the weak cation-exchange phase to the particle giving it a higher density than the Bio WCX column particles
- Specifically designed for the separation of charge isoforms of monoclonal antibodies

Thorough characterization of monoclonal antibodies includes the identification and monitoring of acidic and basic isoforms. Bio MAb HPLC columns feature a unique resin specifically designed for high resolution, charge-based separations of monoclonal antibodies. These columns are compatible with aqueous solution buffers, acetonitrile/acetone/methanol, and water mixtures. Commonly used buffers are phosphate, tris, MES, and acetate.

Bio MAb columns are available in 1.7, 3, 5, and 10 µm sizes, providing higher resolution with smaller particles.

Column Specifications

Bonded Phase	id	Particle Size	pH Stability	Operating Temperature Limit	Flow Rate
Weak cation-exchange (carboxylate)	2.1 and 4.6 mm	1.7, 3, 5, and 10 µm	2–12	80 °C	0.1–1.0 mL/min

Tips and tools

Are you looking to increase your throughput for charge variant analysis of monoclonal antibodies? If so, see:

Reducing Cycle Time for Charge Variant Analysis of Monoclonal Antibodies (publication **5991-4722EN**)

www.agilent.com/search

Consistent ion-exchange mAb separation

Column: **Bio MAb, PEEK**
5190-2407
4.6 x 250 mm, 5 µm

Mobile phase: A: Sodium phosphate 10 mM, pH 5.5
B: A + sodium chloride 0.5 M

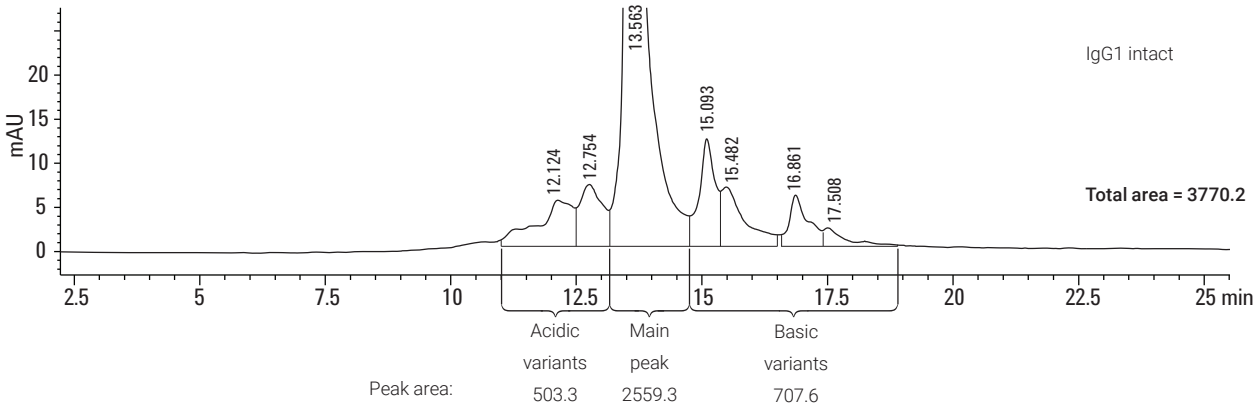
Flow rate: 0.85 mL/min

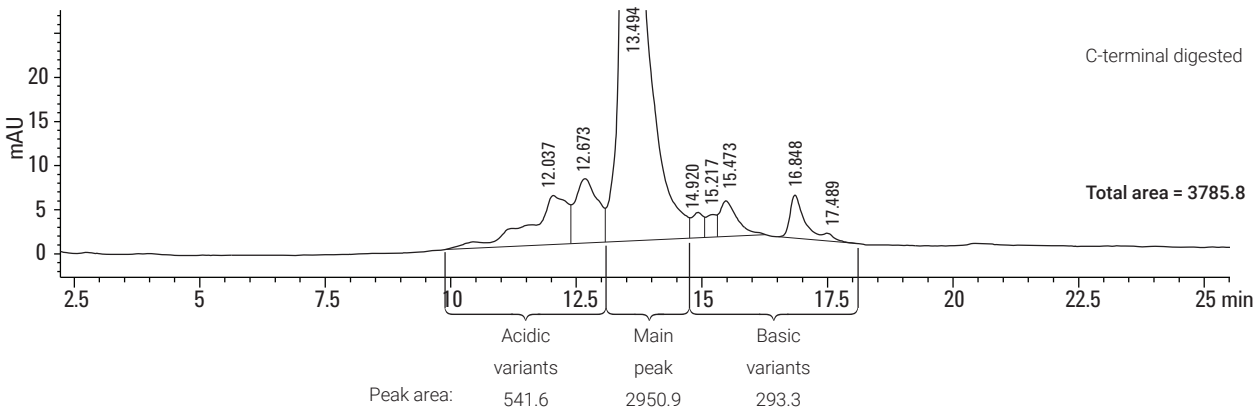
Gradient: 10 to 35% B from 0 to 25 min
(unless otherwise stated)

Detector: UV, 225 nm

Sample: 5 µg of 1 mg/mL of intact or C-terminal digested IgG1

Instrument: 1260 Infinity bio-inert quaternary LC or
1100 Series LC





Calculation of C-terminal digested IgG1 using a Bio MAb 5 µm column on the 1260 Infinity bio-inert quaternary LC.

Reproducibility and precision—Bio MAb columns enable precise quantitation, robust methods

Column: **Bio MAb, PEEK**
5190-2407
4.6 x 50 mm, 5 µm

Mobile phase: A: 10 mM Sodium phosphate buffer, pH 6.0
B: 10 mM Sodium bicarbonate buffer, pH 9.5

Flow rate: 1.0 mL/min

Injection volume: 10 µL (needle with wash, flush port active for 7 s)

Gradient:

Time (min)	% B
0	0
25	100
27	100
30	0

Data acquisition: 214, and 280 nm

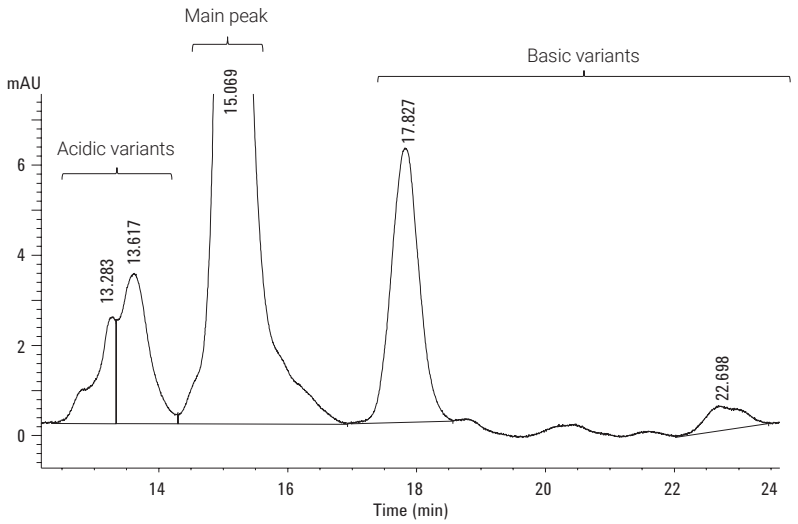
Acquisition rate: 20 Hz

Flow cell: 60 mm path

Column temperature: 30 °C

Sample thermostat: 50 °C

Posttime: 5 min



WCX separation of Fab and Fc fragments of Trastuzumab

Column: **Bio MAb, PEEK**
5190-2411
2.1 x 250 mm, 5 µm

Mobile phase: A: 20 mM MES, pH 5.6
B: 20 mM MES, pH 5.6 + 300 mM NaCl

Flow rate: 170 µL/min

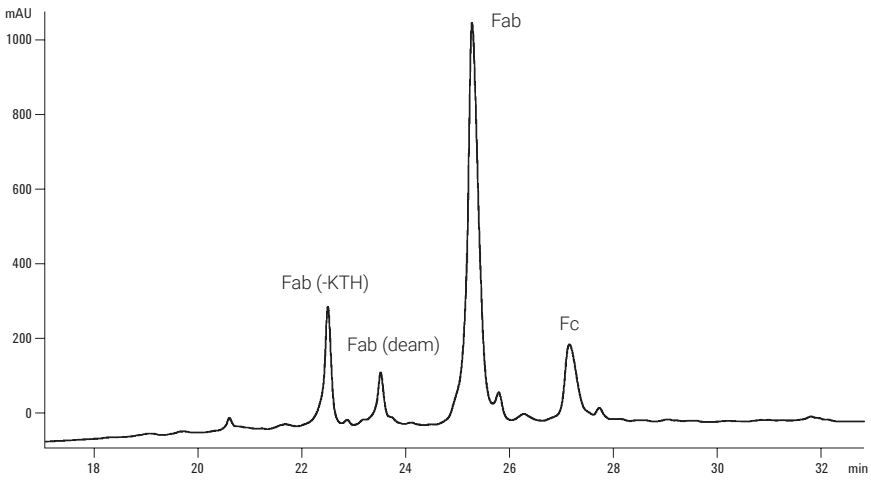
Injection volume: 16 µL

Gradient:

Time (min)	% B
0	0
39.5	80
40	100
50	100
50.5	2
60	2

Temperature: 30 °C

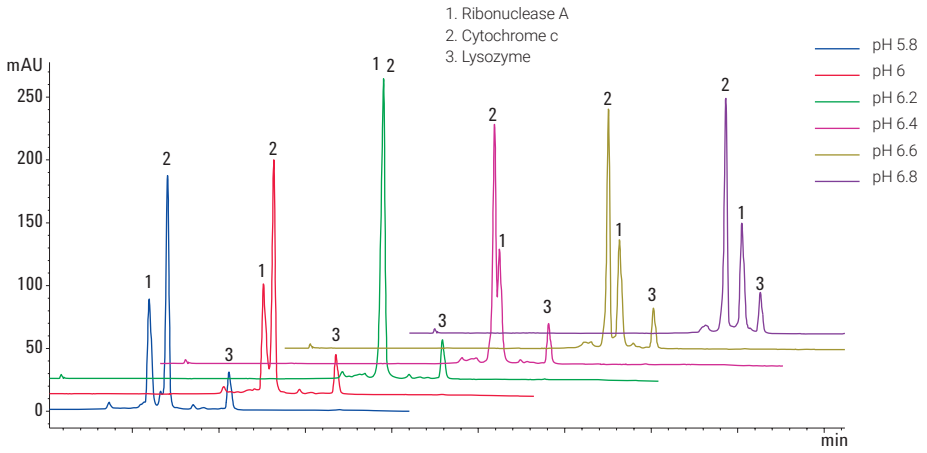
Instrument: 1100 series



Method development using Buffer Advisor software—determination of optimum pH

Column: **Bio MAb, PEEK**
5190-2407
4.6 x 250 mm, 5 µm

Instrument:	1260 Infinity Bio-inert Quaternary LC
Buffer:	A: H_2O B: NaCl 3 M C: MES (2-N-morpholino) ethanesulfonic acid monohydrate) 60 mM D: MES-Na (2-(N-morpholino) ethanesulfonic acid sodium salt) 35 mM
Sample:	Mix of three proteins, dissolved in PBS (phosphate buffered saline), pH 7.4 Ribonuclease A: 13,700 Da, pI 9.6 Cytochrome c: 12,384 Da, pI 10-10.5 Lysozyme: 14,307 Da, pI 11.35
Flow rate:	1 mL/min
Gradient:	0 min–20 mM NaCl 5 min–20 mM NaCl 30 min–500 mM NaCl 35 min–1,000 mM NaCl 36 min–20 mM NaCl
Injection volume:	10 µL
Thermostat:	4 °C
Temperature TCC:	25 °C
DAD:	280 nm/4 nm Reference: OFF
Peak width:	>0.05 min (1.0 s respose time)(5 Hz)



pH scouting for the separation of a three-protein mix using dynamically mixed quaternary gradients

Virtually eliminate retention time variations

Column: **Bio MAb, stainless steel**
5190-2413
4.6 x 250 mm, 10 µm

Mobile phase: A: 10 mM Sodium phosphate, pH 6.0
B: A + 1.0 M Sodium chloride

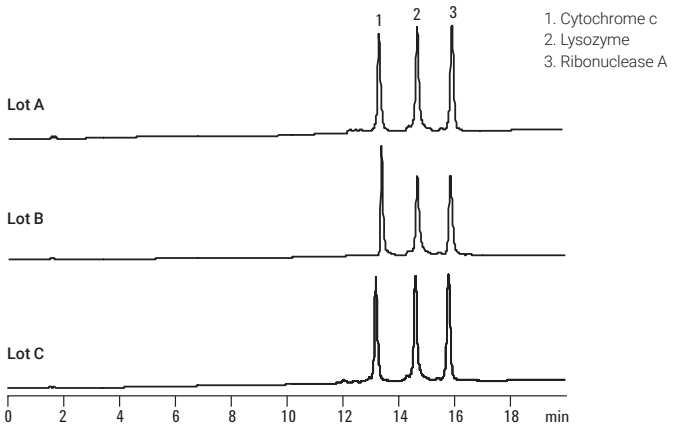
Flow rate: 1.0 mL/min

Gradient: 0–100% B in 42 min

Temperature: 25 °C

Detector: UV, 214 nm

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 MAb HPLC columns

Size (mm)	Particle Size (µm)	Bio MAb PEEK	Pressure Limit	Bio MAb Stainless Steel	Pressure Limit
21.2 x 250	5			5190-6885	275 bar, 4000 psi
10 x 250	5			5190-6884	275 bar, 4000 psi
4.6 x 250	10	5190-2415	275 bar, 4000 psi	5190-2413	275 bar, 4000 psi
4.6 x 50	10	5190-2416	275 bar, 4000 psi		
4.6 x 250	5	5190-2407	400 bar, 5800 psi	5190-2405	400 bar, 5800 psi
4.6 x 50	5	5190-2408	400 bar, 5800 psi		
4.6 x 50	3			5190-2403	551 bar, 8000 psi
4.6 x 50	1.7			5190-2401	600 bar, 8700 psi
4.0 X 10, guard	10			5190-2414	275 bar, 4000 psi
4.0 X 10, guard	5			5190-2406	413 bar, 6000 psi
4.0 X 10, guard	3			5190-2404	551 bar, 8000 psi
4.0 X 10, guard	1.7			5190-2402	600 bar, 8700 psi
2.1 x 250	10	5190-2419	275 bar, 4000 psi		
2.1 x 50	10	5190-2420	275 bar, 4000 psi		
2.1 x 250	5	5190-2411	400 bar, 5800 psi		
2.1 x 50	5	5190-2412	400 bar, 5800 psi		

Bio IEX HPLC columns

- Highly cross-linked and rigid nonporous poly(styrene divinylbenzene) (PS/DVB) particles are grafted with a hydrophilic polymeric layer, eliminating nonspecific binding
- Uniform, densely packed ion-exchange functional groups are chemically bonded to the hydrophilic layer (multiple ion-exchange groups per anchor) to increase column capacity
- Particles, coating, and bonding are resistant to high pressures, promoting higher resolution and faster separations
- Multiple ion-exchange groups are captured on one anchor to increase cap

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 peptides, oligonucleotides, and proteins.

The Bio IEX family includes strong cation-exchange (SCX), weak cation-exchange (WCX), strong anion-exchange (SAX), and weak anion-exchange (WAX) phases. All phases are available in 1.7, 3, 5, and 10 µm nonporous particles.

Column Specifications

Bonded Phase	id	Particle Size	pH Stability	Operating Temperature Limit	Flow Rate
SCX (strong cation-exchange)–SO_3H	2.1 and 4.6 mm	1.7, 3, 5, and 10 µm	2–12	80 °C	0.1–1.0 mL/min
WCX (weak cation-exchange)–COOH					
SAX (strong anion-exchange)–$N(CH_3)_3$					
WAX (weak cation-exchange)–$N(C_2H_5)_2$					

Tips and tools

For further information on optimizing your charged variant analysis, refer to: *Ion-exchange chromatography for biomolecule analysis: a "how-to" guide* (publication **5991-3775EN**), and *Agilent ion-exchange BioHPLC columns, characterize charged variants of proteins with speed and confidence* (publication **5991-2449EN**)

www.agilent.com/search

Shortened and simplified charge variant workflow

Column: **Bio WCX, stainless steel**
5190-2443
4.6 x 50 mm, 3 µm

Bio SCX, stainless steel
5190-2423
4.6 x 50 mm, 3 µm

Mobile phase: A: Water
B: Sodium chloride 1.5 M
C: Monosodium phosphate 40 mM
D: Disodium phosphate 40 mM
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.

Flow rate: 1.0 mL/min

Injection volume: 10 µL

Gradient: Conditions for chromatograms shown:
pH 5.0 to 7.0, 10 to 25 mM buffer strength
sodium chloride (NaCl) 0 to 500 mM, 0 to 15 min
sodium chloride (NaCl) 500 mM, 15 to 20 min
DOE experiments
pH 5.0 to 7.0
0 to 200 mM, 0 to 250 mM, and 0 to 300 mM NaCl

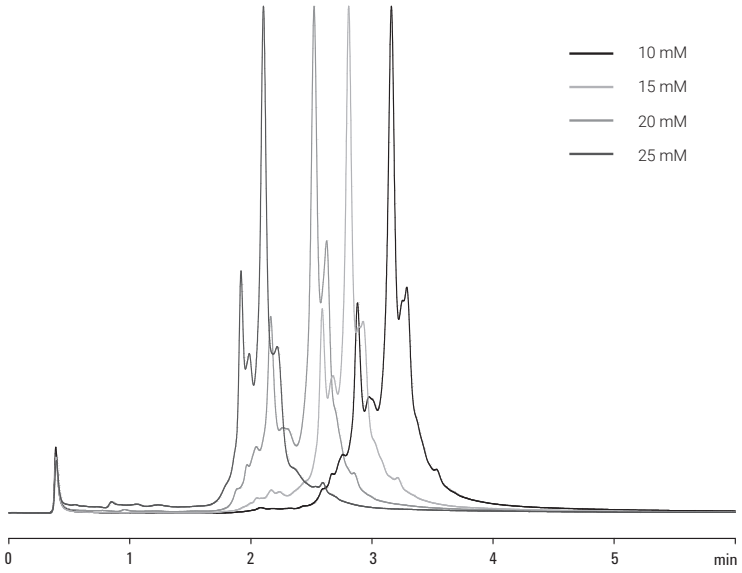
Temperature: Ambient

Detector: UV, 220 nm

Sample: IgG monoclonal antibody

Sample conc: 2 mg/mL (in sodium phosphate buffer 20 mM, pH 6.0)

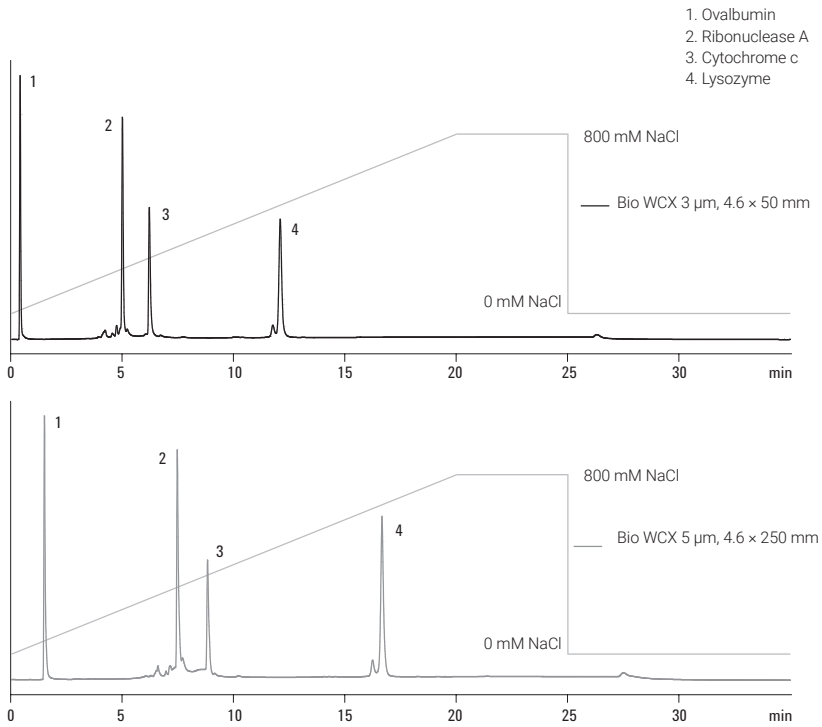
Instrument: 1260 Infinity bio-inert quaternary LC



Automated method development for optimized charge variant separations. Optimizing buffer strength at pH 6.5 from the screening chromatograms of a monoclonal IgG separation.

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

Column:	**Bio WCX, stainless steel** **5190-2445** **4.6 x 250 mm, 5 µm** **Bio WCX, stainless steel** **5190-2443** **4.6 x 50 mm, 3 µm**
Mobile phase:	A: Sodium phosphate 20 mM, pH 6.5 B: A + sodium chloride 1.6 M
Flow rate:	1.0 mL/min
Injection volume:	10 µL
Gradient:	0 to 50% B
Temperature:	Ambient
Detector:	UV, 220 nm
Sample conc:	0.5 mg/mL
Instrument:	1260 Infinity bio-inert quaternary LC

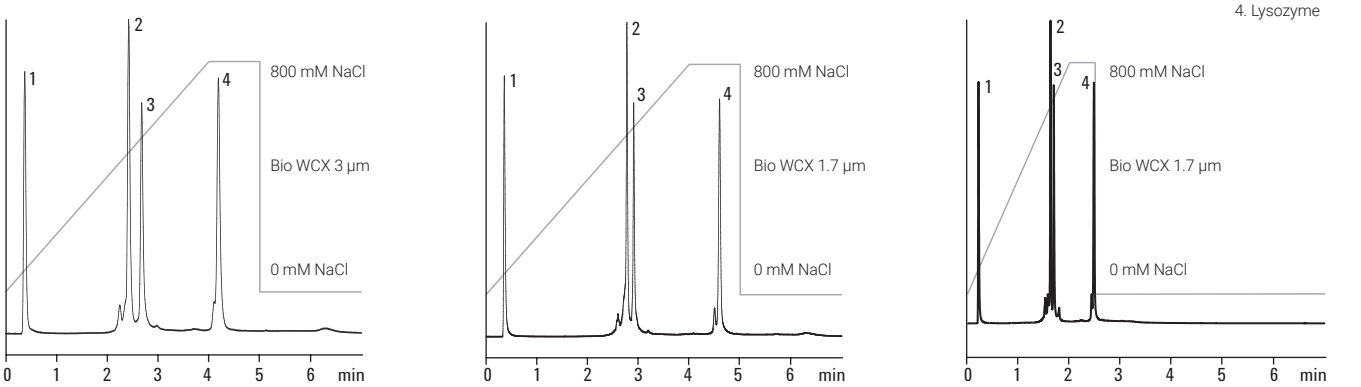


Protein separation on a Bio WCX 4.6 x 50 mm, 3 µm column versus a Bio WCX 4.6 x 250 mm, 5 µm column (flow rate 1.0 mL/min). Faster analysis times were achieved through smaller particle size and shorter column length—samples eluted from the longer column in 17 min, and in just 12 min from the shorter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

Smaller particle sizes provide increased resolution

Column:	**Bio WCX, stainless steel** **5190-2443** **4.6 x 50 mm, 3 µm**
	Bio WCX, stainless steel **5190-2441** **4.6 x 50 mm, 1.7 µm**
Mobile phase:	A: Sodium phosphate 20 mM, pH 6.5 B: A + sodium chloride 1.6 M
Injection volume:	10 µL
Gradient:	0 to 50% B
Temperature:	Ambient
Detector:	UV, 220 nm
Sample conc:	0.5 mg/mL
Instrument:	1260 Infinity bio-inert quaternary LC

1. Ovabumin
2. Ribonuclease A
3. Cytochrome c
4. Lysozyme



Left and middle: Protein separation on a Bio WCX 3 µm column versus a Bio WCX 1.7 µm column (flow rate 1.0 mL/min).

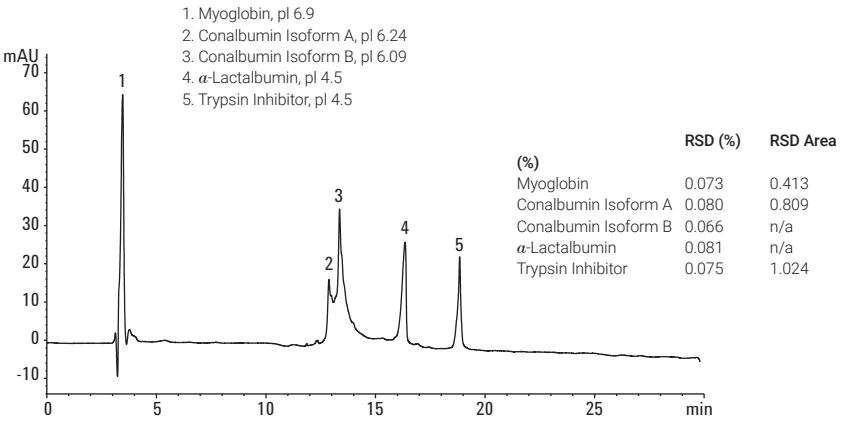
Right: By increasing the flow rate to 1.7 mL/min, the separation time was reduced to less than 3 minutes. (A Bio WCX column was used.)

Reduce analysis time—without sacrificing peak shape and resolution—by increasing flow rate.

Analysis of proteins by anion-exchange columns using the Agilent 1260 Infinity bio-inert quaternary LC system

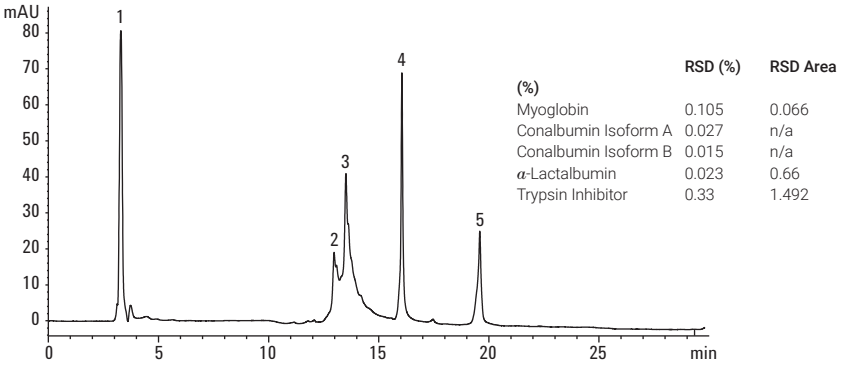
Column:	**Bio WAX, PEEK** **5190-2487** **4.6 x 250 mm, 5 µm**
Buffer:	A: 20 mM tris, pH 7.6 B: 20 mM tris, pH 7.6 + 2 M NaCl, 1 M KCl, 1 M CH_3COONa, 1 M $[(CH_3)4N]Cl$
Gradient 1 M:	5 min–100% A 20 min–70% B 25 min–100% B
Gradient 2 M:	5 min–100% A 20 min–35% B 25 min–50% B 25.01 min–100% B
Stop time:	30 min
Posttime:	20 min
Temperature:	25 °C
Flow rate:	0.5 mL/min
Injection volume:	5 µL
DAD:	280 nm
Peak width:	0.025 min (0.5 s response time) (10 Hz)

For further information see application note
5990-9614EN (**www.agilent.com/search**)



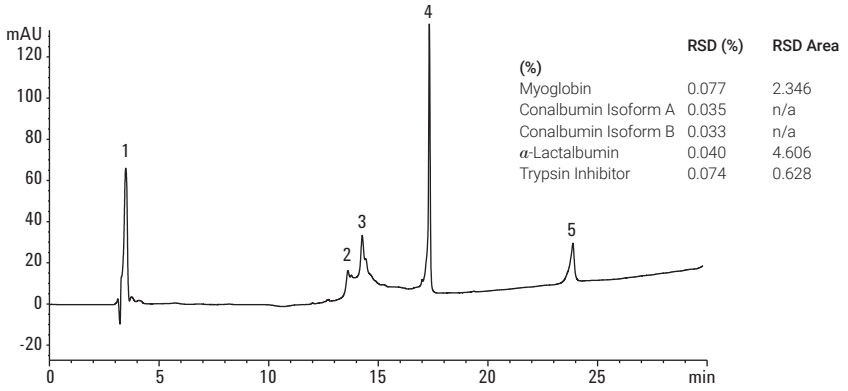
(%)	RSD (%)	RSD Area
Myoglobin	0.073	0.413
Conalbumin Isoform A	0.080	0.809
Conalbumin Isoform B	0.066	n/a
α-Lactalbumin	0.081	n/a
Trypsin Inhibitor	0.075	1.024

Figure 1. Protein separation by AEX by a linear gradient using 2 M NaCl as eluting salt.



(%)	RSD (%)	RSD Area
Myoglobin	0.105	0.066
Conalbumin Isoform A	0.027	n/a
Conalbumin Isoform B	0.015	n/a
α-Lactalbumin	0.023	0.66
Trypsin Inhibitor	0.33	1.492

Figure 2. Protein separation by AEX by a linear gradient using 1 M KCl as eluting salt.



(%)	RSD (%)	RSD Area
Myoglobin	0.077	2.346
Conalbumin Isoform A	0.035	n/a
Conalbumin Isoform B	0.033	n/a
α-Lactalbumin	0.040	4.606
Trypsin Inhibitor	0.074	0.628

Figure 3. Protein separation by AEX by a linear gradient using 1 M $[(CH_3)4N]Cl$) as eluting salt.

Bio IEX HPLC Columns, PEEK

Size (mm)	Particle Size (µm)	Pressure Limit	Bio SCX Part No.	Bio WCX Part No.	Bio SAX Part No.	Bio WAX Part No.
4.6 x 250	10	275 bar, 4000 psi	5190-2435	5190-2455	5190-2475	5190-2495
4.6 x 50	10	275 bar, 4000 psi	5190-2436	5190-2456	5190-2476	5190-2496
4.6 x 250	5	400 bar, 5800 psi	5190-2427	5190-2447	5190-2467	5190-2487
4.6 x 50	5	400 bar, 5800 psi	5190-2428	5190-2448	5190-2468	5190-2488
2.1 x 250	10	275 bar, 4000 psi	5190-2439	5190-2459	5190-2479	5190-2499
2.1 x 50	10	275 bar, 4000 psi	5190-2440	5190-2460	5190-2480	5190-2500
2.1 x 250	5	400 bar, 5800 psi	5190-2431	5190-2451	5190-2471	5190-2491
2.1 x 50	5	400 bar, 5800 psi	5190-2432	5190-2442	5190-2462	5190-2492

Tips and tools

Use Agilent solvent filters to remove particles from self-prepared buffers and mobile phases.

Visit: **www.agilent.com/chem/solvent-filters-degassers**

Bio IEX HPLC Columns, Stainless Steel

Size (mm)	Particle Size (µm)	Pressure Limit	Bio SCX Part No.	Bio WCX Part No.	Bio SAX Part No.	Bio WAX Part No.
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	275 bar, 4000 psi	5190-2433	5190-2453	5190-2473	5190-2493
4.6 x 250	5	413 bar, 6000 psi	5190-2425	5190-2445	5190-2465	5190-2485
4.6 x 150	3					5190-6875
4.6 x 50	3	551 bar, 8000 psi	5190-2423	5190-2443	5190-2463	5190-2483
4.6 x 50	1.7	600 bar, 8700 psi	5190-2421	5190-2441	5190-2461	5190-2481
4.0 x 10, guard	10	275 bar, 4000 psi	5190-2434	5190-2454	5190-2474	5190-2494
4.0 x 10, guard	5	413 bar, 6000 psi	5190-2426	5190-2446	5190-2466	5190-2486
4.0 x 10, guard	3	551 bar, 8000 psi	5190-2424	5190-2444	5190-2464	5190-2484
4.0 x 10, guard	1.7	600 bar, 8700 psi	5190-2422	5190-2442	5190-2462	5190-2482

Tips and tools

For further information see:

Optimizing protein separations with Agilent weak cation-exchange columns (publication **5990-9628EN**)
Faster separations using Agilent weak cation-exchange columns (publication **5990-9931EN**)
pH Gradient elution for improved separation of monoclonal antibody variants (publication **5990-9629EN**)
Optimizing protein separations with cation-exchange chromatography using Agilent Buffer Advisor (publication **5991-0565EN**)

www.agilent.com/search

PL-SAX Strong Anion-Exchange columns

- Small particles deliver excellent chromatographic performance
- Wide range of particle sizes and two pore sizes for flexible analysis to scale up purification
- Exceptional stability for long column lifetime

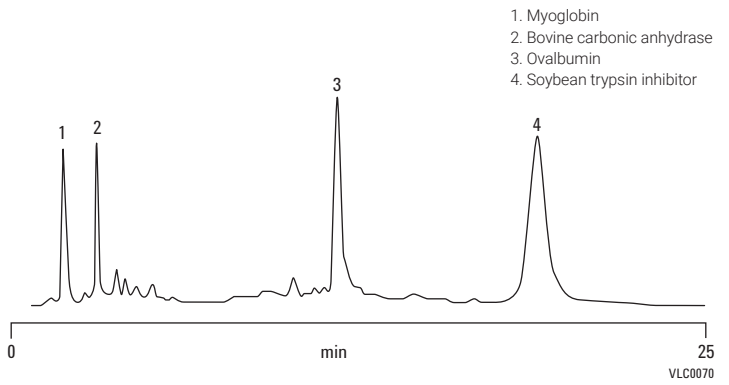
PL-SAX -$N(CH_3)_3^+$ is ideal for the anion-exchange HPLC separations of proteins, peptides, and deprotected synthetic oligonucleotides under denaturing conditions. The strong anion-exchange functionality, covalently linked to a chemically stable fully porous polymer, extends the operating pH range. In addition, the anion-exchange capacity is independent of pH. For synthetic oligonucleotides, separations using denaturing conditions of temperature, organic solvent, and high pH are all possible. PL-SAX delivers improved chromatography for self-complementary or G-rich sequences that may associate to form aggregates or hairpin structures. The 5 µm material provides high-efficiency separations of n and n-1 sequences. A wide range of particle sizes and column geometries permits analysis scale-up to purification. The strong anion-exchange functionality provides a material with exceptional chemical and thermal stability, even with sodium hydroxide eluents, leading to long column lifetime.

Column Specifications

Bonded Phase	id	Particle Size (mm)	Pore Size (µm)	pH Stability	Operating Temperature Limit
Strong anion-exchange	2.1, 4.6, 7.5, 25, 50, and 100	5, 8, 10, and 30	1000 Å and 4000 Å	1–14	80 °C

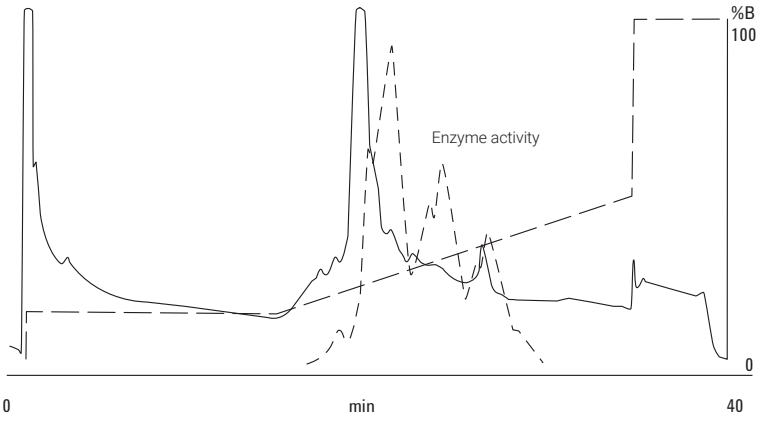
Standard ion-exchange protein separation

Column:	**PL-SAX 1000 Å** **PL1551-1502** **4.6 x 50 mm, 5 µm**
Mobile phase:	A: 10 mM tris HCl, pH 8 B: A + 350 mM sodium chloride, pH 8
Gradient:	0–100% B in 20 min
Flow rate:	1.0 mL/min
Detector:	UV, 220 nm



Analysis of choline kinase

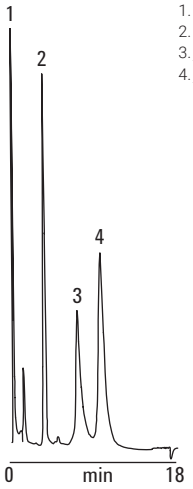
Column:	**PL-SAX 4000 Å** **PL1551-1803** **4.6 x 50 mm, 8 µm**
Mobile phase:	A: 20 mM tris 5% ethylene glycol, pH 7.5 (The following are required to retain enzyme activity) 1.0 mM ethylene glycol tetraacetic acid 2.0 mM ß-mercaptoethanol 0.2 mM phenylmethylsulfonyl fluoride B: A + 1 M potassium chloride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



Separation courtesy of T Porter, Purdue University, USA

Analysis of representative whey proteins

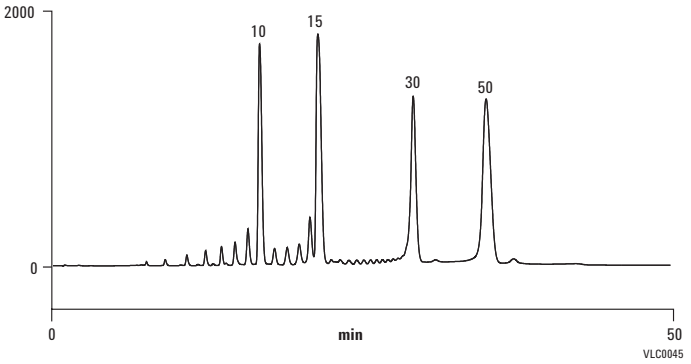
Column:	**PL-SAX 1000 Å** **PL1551-1802** **4.6 x 50 mm, 8 µm**
Mobile phase:	A: 20 mM tris HCl, pH 7 B: A + 500 mM sodium acetate, pH 7
Flow rate:	1.0 mL/min
Gradient:	Linear 0-50% B in 10 min
Detector:	UV, 280 nm



1. Carbonic anhydrase
2. α-Lactalbumin
3. β-Lactoglobulin B
4. β-Lactoglobulin A

Reliable separations of synthetic oligonucelotides—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 1000 Å** **PL1551-1802** **4.6 x 50 mm, 8 µm**
Mobile phase:	A: 7:93 v/v ACN: 100 mM TEAA, pH 8.5 B: 7:93 v/v ACN: 100 mM TEAA, 1 M ammonium chloride, pH 8.5
Gradient:	0–40% B in 10 min, followed by 40–70% B in 14 min and 70–100% B in 25 min
Flow rate:	1.0 mL/min
Temperature:	60 °C
Detector:	UV, 220 nm



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.

PL-SAX Strong Anion-Exchange Columns

Size (mm)	Particle Size (µm)	Pressure Limit	PL-SAX 1000 Å	PL-SAX 4000 Å
100 x 300	10	207 bar, 3000 psi	PL1851-2102	PL1851-2103
50 x 150	30	207 bar, 3000 psi	PL1751-3702	PL1751-3703
50 x 150	10	207 bar, 3000 psi	PL1751-3102	PL1751-3103
25 x 150	30	207 bar, 3000 psi	PL1251-3702	PL1251-3703
25 x 150	10	275 bar, 4000 psi	PL1251-3102	PL1251-3103
25 x 50	10	207 bar, 3000 psi	PL1251-1102	PL1251-3103
4.6 x 250	30	207 bar, 3000 psi	PL1551-5702	PL1551-5703
4.6 x 150	30	207 bar, 3000 psi	PL1551-3702	PL1551-3703
4.6 x 250	10	207 bar, 3000 psi	PL1551-5102	PL1551-5103
4.6 x 150	10	207 bar, 3000 psi	PL1551-3102	PL1551-3103
4.6 x 150	8	207 bar, 3000 psi	PL1551-3802	PL1551-3803
4.6 x 50	8	207 bar, 3000 psi	PL1551-1802	PL1551-1803
4.6 x 50	5	207 bar, 3000 psi	PL1551-1502	PL1551-1503
2.1 x 150	8	207 bar, 3000 psi	PL1951-3802	PL1951-3803
2.1 x 50	8	207 bar, 3000 psi	PL1951-1802	PL1951-1803
2.1 x 50	5	207 bar, 3000 psi	PL1951-1502	PL1951-1503
1.0 x 50	5	207 bar, 3000 psi	PL1351-1502	PL1351-1503

PL-SAX Strong Anion-Exchange Bulk Media

Size (mm)	Particle Size (µm)	Pressure Limit	PL-SAX 1000 Å	PL-SAX 4000 Å
100 g	30	207 bar, 3000 psi	PL1451-4702	PL1451-4703
100 g	10	207 bar, 3000 psi	PL1451-4102	PL1451-4103

PL-SCX Strong Cation-Exchange columns

- Optimal design for effective separation of biomolecules
- Pore sizes allow use of a range of solute sizes
- Exceptional stability for long column lifetime

PL-SCX -SO_3 is a macroporous PS/DVB matrix with a very hydrophilic coating and strong cation-exchange functionality. This process is controlled to provide the optimum density of strong cation-exchange moieties for the analysis, separation, and purification of a wide range of biomolecules, from small peptides to large proteins. Two pore sizes are available, 1000 Å and 4000 Å, to provide good mass-transfer characteristics for a range of solute sizes. The 5 µm media deliver separations at higher resolution with the 30 µm media used for medium pressure liquid chromatography.

Column Specifications

Bonded Phase	id (mm)	Particle Size (µm)	Pore Size	pH Stability	Operating Temperature Limit
Strong cation-exchange	2.1, 4.6, 7.5, 25, 50, and 100	5, 8, 10, and 30	1000 Å and 4000 Å	1–14	80 °C

Standard protein separation

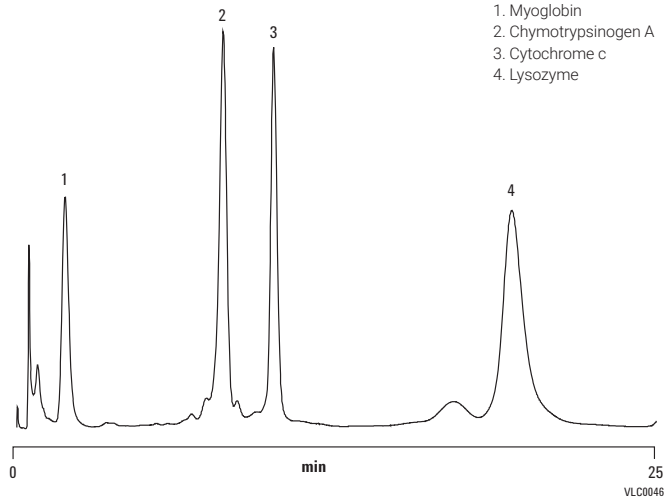
Column: **PL-SCX 1000 Å**
PL1545-1502
4.6 x 50 mm, 5 µm

Mobile phase: A: 20 mM potassium dihydrogen phosphate, pH 6.0
B: A + 1 M sodium chloride

Gradient: 0–100% B in 20 min

Flow rate: 1.0 mL/min

Detector: UV, 280 nm



PL-SCX Strong Cation-Exchange Columns

Size (mm)	Particle Size (µm)	Pressure Limit	PL-SCX 1000 Å	PL-SCX 4000 Å
100 x 300	10	207 bar, 3000 psi	PL1845-2102	PL1845-2103
50 x 150	30	207 bar, 3000 psi	PL1745-3703	PL1745-3703
50 x 150	10	207 bar, 3000 psi	PL1745-3102	PL1745-3103
25 x 150	30	207 bar, 3000 psi	PL1245-3702	PL1245-3703
25 x 150	10	207 bar, 3000 psi	PL1245-3102	PL1245-3103
25 x 50	10	207 bar, 3000 psi	PL1245-1102	PL1245-1103
4.6 x 250	30	207 bar, 3000 psi	PL1545-5703	PL1545-5703
4.6 x 150	30	207 bar, 3000 psi	PL1545-3702	PL1545-3703
4.6 x 250	10	207 bar, 3000 psi	PL1545-5102	PL1545-5103
4.6 x 150	10	207 bar, 3000 psi	PL1545-3102	PL1545-3103
4.6 x 150	8	207 bar, 3000 psi	PL1545-3802	PL1545-3803
4.6 x 50	8	207 bar, 3000 psi	PL1545-1802	PL1545-1803
4.6 x 50	5	207 bar, 3000 psi	PL1545-1502	PL1545-1503
2.1 x 150	8	207 bar, 3000 psi	PL1945-3802	PL1945-3803
2.1 x 50	8	207 bar, 3000 psi	PL1945-1802	PL1945-1803
2.1 x 50	5	207 bar, 3000 psi	PL1945-1502	PL1945-1503
1.0 x 50	5	207 bar, 3000 psi	PL1345-1502	PL1345-1503

PL-SCX Strong Cation-Exchange Bulk Media

Size (mm)	Particle Size (µm)	PL-SCX 1000 Å	PL-SCX 4000 Å
100 g	30	PL1445-4702	PL1445-4703
100 g	10	PL1445-4102	PL1445-4102

Tips and tools

PL-SAX and PL-SCX columns and bulk media are also available for prep to process.

Turn to **Pages 190–195**.

Bio-Monolith Ion-Exchange HPLC columns

- Polymer-based, monolith HPLC columns designed for macro biomolecule separations
- Flow-rate independent separations; no diffusion, no pores, and no void volume make transport between mobile and stationary phase very rapid
- Monolith disk is 5.2 x 4.95 mm (100 µL column volume) with continuous channels, eliminating diffusion mass transfer
- Extremely fast separations speed up method development time and decrease costs; locking in method parameters takes significantly less time and buffer

Bio-Monolith Ion-Exchange HPLC columns provide high resolution and rapid separations of antibodies (IgG, IgM), plasmid DNA, viruses, phages, and other macro biomolecules. The product family offers strong cation-exchange, strong and weak anion-exchange, and Protein A phases. Bio-Monolith HPLC columns are compatible with InfinityLab LC series.

Bio-Monolith Ion-Exchange HPLC column

Bio-Monolith HPLC Column Selection Guide

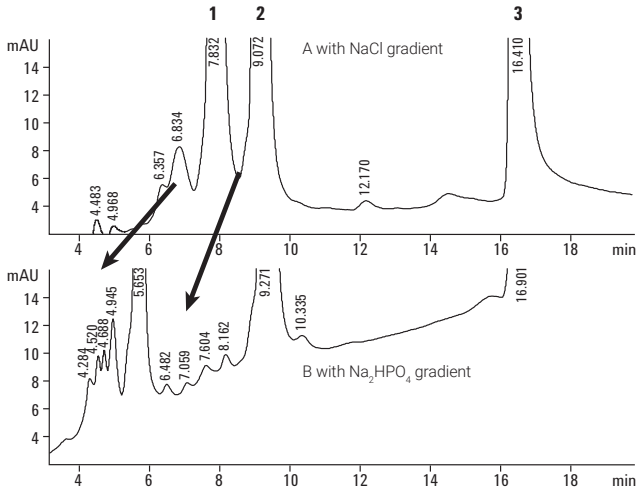
Column	Description	Key Applications	Part No.
Bio-Monolith QA	The quaternary amine bonded phase (strong anion-exchange) is fully charged over a working pH range of 2–13, binding negatively charged biomolecules.	– Adenovirus process monitoring and quality control – IgM purification monitoring and quality control – Monitoring DNA impurity removal – Monitoring endotoxin removal – HSA purity	5069-3635
Bio-Monolith DEAE	The diethylaminoethyl bonded phase (weak anion-exchange) offers increased selectivity of biomolecules with negative charge over a working pH range of 3–9.	– Process monitoring and quality control of bacteriophage manufacturing and purification – Process monitoring and quality control of plasmid DNA purification	5069-3636
Bio-Monolith SO_3	The sulfonyl bonded phase (strong cation-exchange) is fully charged over a working pH range of 2–13, binding positively charged biomolecules.	– Fast and high resolution analytical separations of large molecules such as proteins and antibodies – Hemoglobin A1c fast analytics	5069-3637

Column Specifications

Dimensions	5.2 mm x 4.95 mm
Column volume	100 µL
Maximum pressure	150 bar (15 MPa, 2,200 psi)
Temperature min/max	Operating: 2–40 °C
	Storage: 2–8 °C
Recommended pH	Operating range: 2–13
	Cleaning-in-place: 1–14
Materials of construction	Hardware: stainless steel
	Packing: poly(glycidyl methacrylate-co-ethylene dimethacrylate) highly porous monolith
Color ring identifier	Bio-Monolith QA: blue
	Bio-Monolith DEAE: green
	Bio-Monolith SO_3: red
Shelf life/expiration date	SO_3, QA, DEAE: 24–36 months

Baseline expansion of a separation of protein standards

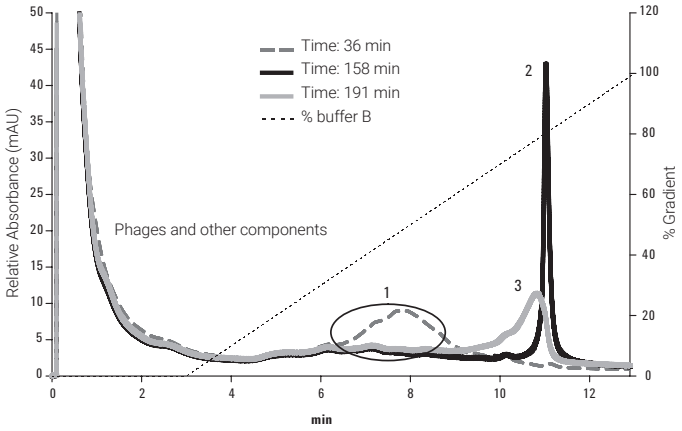
Column:	**Bio-Monolith CM15, 5.5 x 15 mm**
Mobile phase:	A: 10 mM sodium hydrogen phosphate, pH 6.0 B: A + 500 mM sodium chloride or just 500 mM sodium hydrogen phosphate, pH 6.0
Flow rate:	2 mL/min
Gradient:	0.5 min hold with mobile phase A followed by a linear gradient to 45% B in 15 min (elapsed time 15.5 min), then 60% B at 15.6 min continued to 20 min. Column flushed with 100% B for 15 min before re-equilibration for the next run. pH gradient: A: 5 mM sodium hydrogen phosphate, buffer, pH 5.5 and B: 40 mM sodium hydrogen phosphate (not buffered, pH 8.9). 2% B/min at 1 mL/min for 15 min, followed by a column wash with 90% B for 5 min.
Detector:	UV, 220 nm
Sample:	One mg each/mL in mobile phase A 1. RNAse from bovine pancreas (pI 9.6) 2. Cytochrome c from bovine heart (pI 10.37-10.8) 3. Lysozyme from chicken egg (pI 11.35) (0.5 mg)
Instrument:	1200 Infinity series with diode array detector



B shows a better resolution of protein contaminants.

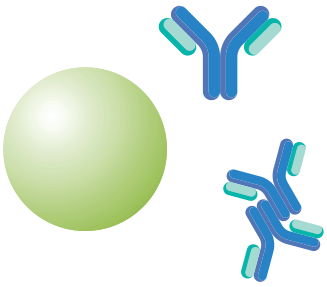
Monitor phage production during fermentation

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



As phage proliferation progresses, the genomic DNA (gDNA) concentration increases as the host cells are lysed. In the late stages of fermentation, gDNA begins to degrade into fragments. These gDNA fragments cannot be easily removed by purification media, and so it is critical to stop the fermentation cycle before the degradation of the gDNA. 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.

Aggregation and Fragment Analysis



Accurately determine biomolecule aggregation, fragmentation, and chemical ligation/modification

Size exclusion chromatography (SEC) is a technique for separating proteins, oligonucleotides, and other complex biopolymers by size, using aqueous eluents. In particular, it is an essential tool for quantification of aggregates present in protein biotherapeutics. Manufacture of a biopharmaceutical, such as a monoclonal antibody, is a complex process and aggregation of the protein is an issue that can arise during cell culture, isolation, purification, and formulation. The presence of dimers and higher aggregates can affect both efficacy and safety of the final product; quantification of aggregate content must be carried out during process development to establish the product's critical quality attributes (CQA) as well as during final product characterization to ensure the extent of aggregation is minimized and controlled at safe levels.

Applying SEC to aggregation studies

The size, type, and content of aggregates present in protein biopharmaceuticals can affect both efficacy and formulation—or worse, induce an immunogenic response. Aggregate formation occurs through a variety of mechanisms, including disulfide bond formation and noncovalent interactions.

Because the size of protein aggregates, including dimers, is sufficiently different from the protein monomer, you can separate the various forms using SEC. In fact, SEC with UV or light scattering is a standard technique for quantifying protein aggregation.

Applying SEC to quantitation and molecular weight determination

For proteins and other molecules of discrete molecular weight, SEC can be used to detect and quantitate monomers, dimers, aggregates, and fragments. SEC can also separate oligonucleotide mixtures.

For biopolymers containing varying chain lengths, such as starches and other polysaccharides, SEC can provide data on molecular weight distribution and branching (with the proper detectors)

As a leading manufacturer of SEC columns and instruments for over 30 years, Agilent is continually developing new SEC products that will provide even higher resolution and quicker separations. This section highlights Agilent's broad family of SEC columns for protein biopolymer analysis:

- AdvanceBio SEC is available in two particle sizes for SEC-UV or SEC-LS measurements. The 2.7 µm particles are available in 130 Å or 300 Å pore sizes to cover a wide variety of sample sizes. The 1.9 µm particles have a 200 Å pore and offer the highest resolution. Both particles have a hydrophilic polymer coating, resulting in minimal secondary interactions. This makes them robust for challenging samples like ADCs, as well as for routine analysis.
- Bio SEC-3 and Bio SEC-5 columns are available in a variety of pore sizes, and are well suited for protein analysis with UV or MS detection—especially when determining the presence of dimers and aggregates in therapeutic biologicals. Note that 3 µm Bio SEC-3 columns provide higher resolution than our industry-standard 5 µm Bio SEC-5 columns.
- ProSEC 300S columns work well with globular proteins under high salt conditions.
- ZORBAX GF-250 and GF-450 columns are legacy products that should be employed where protocols still require use of USP designation L35. Alternatively, we recommend using Bio SEC columns for improved performance.
- PL aquagel-OH columns can be used to analyze biopolymers of broad molecular weights, such as PEGs, oligo—and polysaccharides, starches, and gums. Please see aqueous and polar GPC/SEC columns product guide (publication 5990-7995EN).

Size Exclusion Chromatography (SEC)

Application	Agilent Columns	Notes	USP Designation
Peptides, proteins, aggregate analysis	AdvanceBio SEC	Robust hydrophilic polymer coating yielding minimal secondary interactions; 2.7 µm particles with 130 Å or 300 Å, or 1.9 µm particles with 200 Å pore size for highest resolution.	L59
	Bio SEC-3	Higher resolution and faster separation from 3 µm particles, with 100 Å, 150 Å, and 300 Å pore sizes.	L59
Large biomolecules and samples with multiple molecular weight components	Bio SEC-5	More pore size options (100 Å, 150 Å, 300 Å, 500 Å, 1000 Å, and 2000 Å) to cover a wider range of analytes.	L59
Globular proteins, antibodies	ProSEC 300S	Single column option for protein analysis in high salt conditions.	L33
Proteins, globular proteins	ZORBAX GF-250/450	Legacy products that should be employed where protocols still require use of USP designation L35.	L35

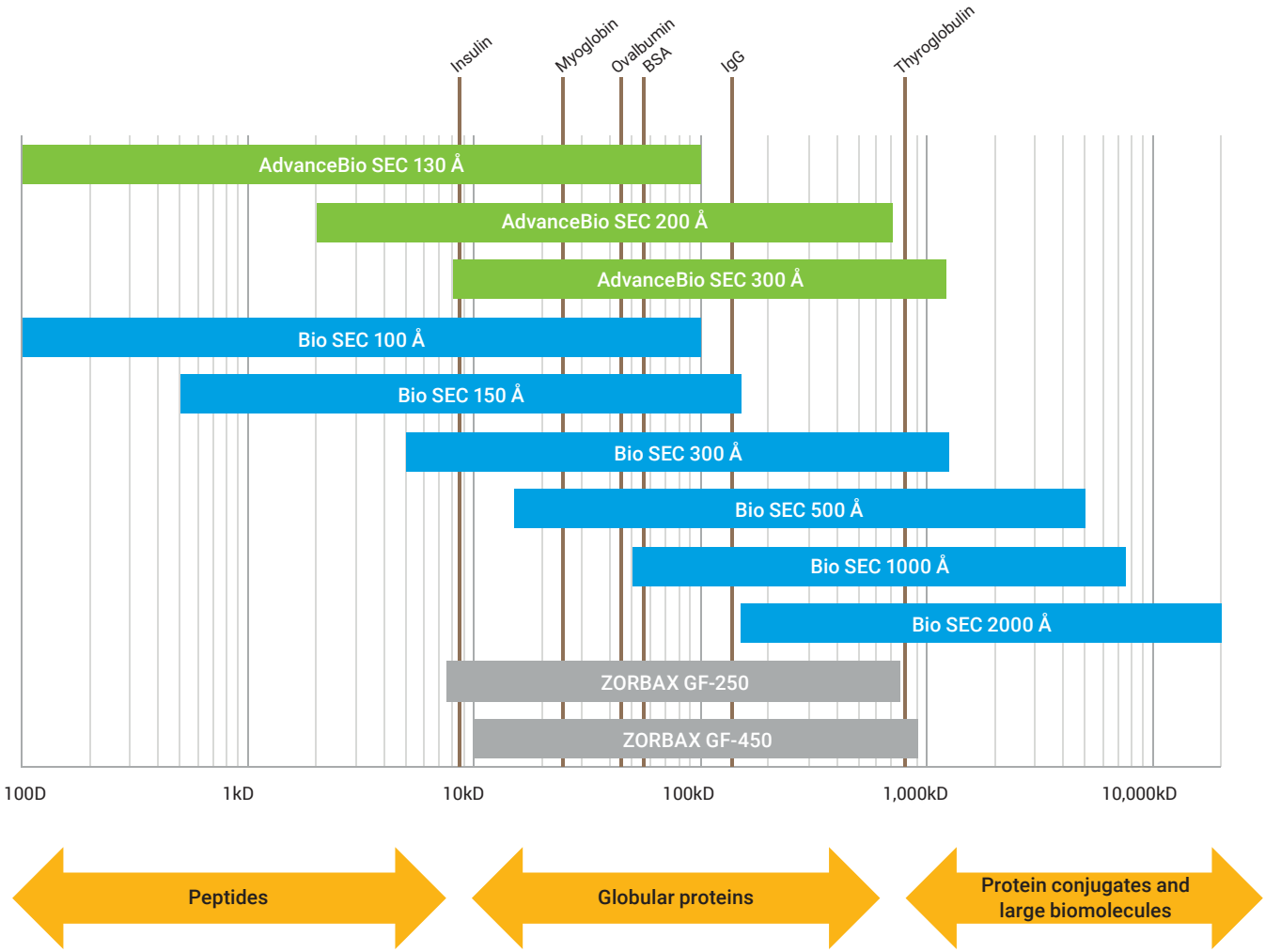
Tips and tools

Use Agilent solvent filters to remove particles from self-prepared buffers and mobile phases.

Visit: **www.agilent.com/chem/solvent-filters-degassers**

Which SEC column is right for your application?

Agilent has a wide selection of SEC columns that give you the choices you need to perfect separations based on your analytes and method parameters. This chart offers an overview of the pore size ranges that yield the best results for common molecule types. We recommend that you begin your method development with AdvanceBio SEC columns.



AdvanceBio SEC

AdvanceBio SEC columns deliver accurate, precise quantitation for mAb aggregation and protein analysis. These innovative size exclusion chromatography columns were designed and engineered by Agilent to improve lab productivity by providing robust, reliable methods that eliminate sample reanalysis. Consistent results are achieved from column to column, batch to batch, and lab to lab, ensuring that methods can be transferred across departments and locations to put an end to uncertainty.

- Increased analytical speed to help you meet vital deadlines
- Increased resolution for more accurate quantitation
- Increased sensitivity to quantitate aggregates, even at low levels
- Increased reproducibility to eliminate rework
- Uniquely designed standards for AdvanceBio SEC columns, providing optimal calibration and performance verification

Column Specifications

Pore Size	Particle Size	Mol Wt Range	pH Range	Max Pressure	Flow Rate
130 Å	2.7 µm	100–120,000	2–8.5	400 bar (typical operating pressure < 200 bar)	0.1–2.0 mL/min (7.8 mm id)
					0.1–0.7 mL/min (4.6 mm id)
300 Å	2.7 µm	5,000–1,250,000	2–8.5	400 bar (typical operating pressure < 200 bar)	0.1–2.0 mL/min (7.8 mm id)
					0.1–0.7 mL/min (4.6 mm id)
200 Å	1.9 µm	2,000–700,000	2–8	620 bar	0.1– 0.7 mL/min (4.6 x 150 mm)
					0.1– 0.5 mL/min (4.6 x 300 mm)

AdvanceBio SEC, 2.7 µm

Description	130 Å	300 Å
Analytical Columns		
4.6 x 300 mm, 2.7 µm	PL1580-5350	PL1580-5301
4.6 x 150 mm, 2.7 µm	PL1580-3350	PL1580-3301
7.8 x 300 mm, 2.7 µm	PL1180-5350	PL1180-5301
7.8 x 150 mm, 2.7 µm	PL1180-3350	PL1180-3350
Analytical Guards		
4.6 x 50 mm, 2.7 µm	PL1580-1350	PL1580-1301
7.8 x 50 mm, 2.7 µm	PL1180-1350	PL1180-1301

AdvanceBio SEC, 1.9 µm

Description	200 Å
Analytical Columns	
4.6 x 300 mm, 1.9 µm	PL1580-5201
4.6 x 150 mm, 1.9 µm	PL1580-3201
Guard Column	
4.6 x 30 mm, 1.9 µm	PL1580-1201

Real stories from the lab

Reducing complexity drives better lab economics

Learn how the CrossLab team helped a large pharma lab manage different types of instruments, from different manufacturers.

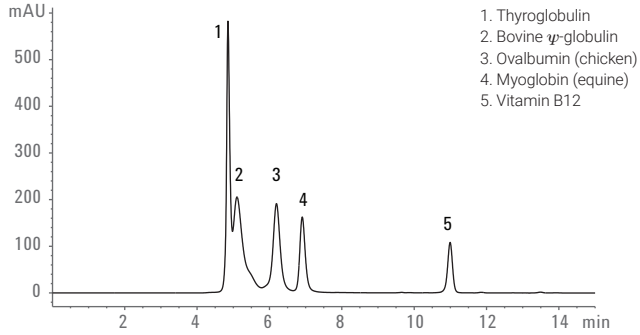
www.agilent.com/chem/story92

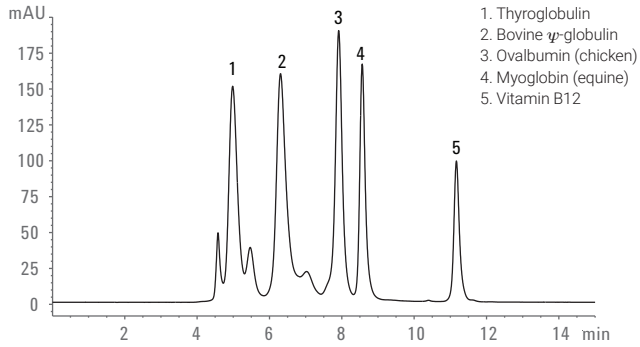
SEC molecular weight standard separations

Column: **AdvanceBio SEC 7.8 x 300 mm**

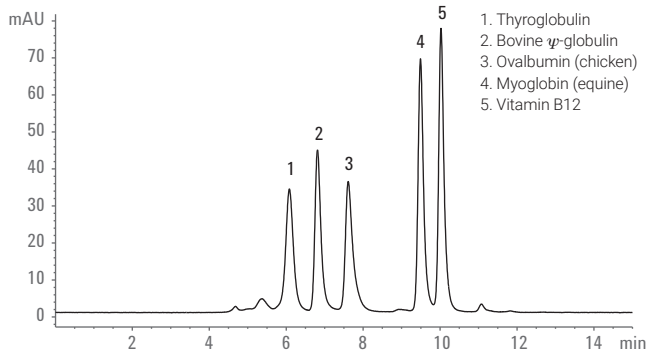
Sample: BioRad Gel Filtration standard #1511901

Mobile phase: A: 150mM sodium phosphate, pH 7.0





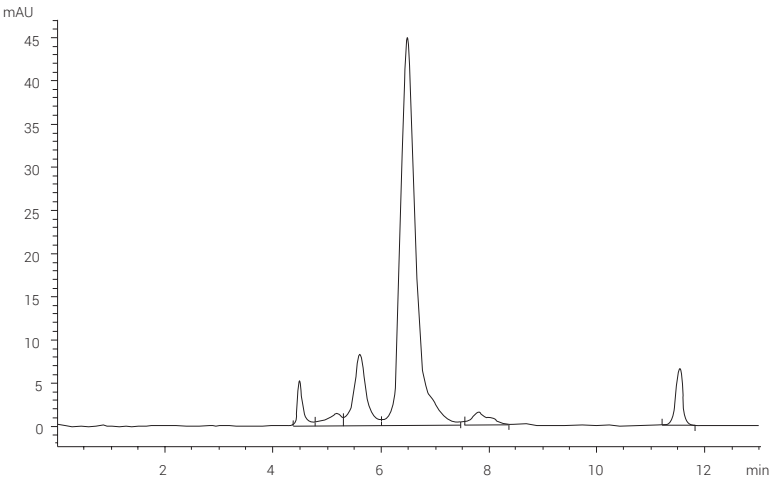
AdvanceBio SEC 300 Å and 130 Å separation of the BioRad Gel Filtration mix. The 300 Å provides increase resolution.



AdvanceBio SEC 130 Å separation of protein and peptide mix showing the resolution of small peptides and proteins.

AdvanceBio SEC recommended starting conditions

Column:	**AdvanceBio SEC 300 Å, 2.7 µm 7.8 x 300 mm (p/n PL1180-5301)**
Flow rate:	1 mL/min
Mobile phase:	150mM phosphate buffer, pH 7.0
Wavelength:	220 nm
Temperature:	ambient
Injection volume:	5 µL
Sample:	IgG



High-resolution separation of an IgG sample, showing the monomer, aggregates, and degradation products.

AdvanceBio SEC 2.7 µm Operating Parameters

Mobile phase compatibility	150 mM phosphate buffer, pH 7.0 (recommended starting conditions) Other aqueous buffers with high and low salt can be used. Mixtures of water and acetonitrile can be used. (Check solubility of buffer components and system pressure.)
pH stability	2 to 8.5
Operating temperature	20–30 °C (recommended) 80 °C (maximum)
Typical operating pressure	< 200 bar (2,900 psi) (single column)
Maximum pressure	400 bar (5,800 psi)
Working flow rate	0.1 to 2.0 mL/min for 7.8 mm i.d. columns (recommended) 0.1 to 0.7 mL/min for 4.6 mm i.d. columns (recommended) For two columns in series, lower flow rates may be necessary to ensure maximum pressure does not exceed 400 bar (5,800 psi).

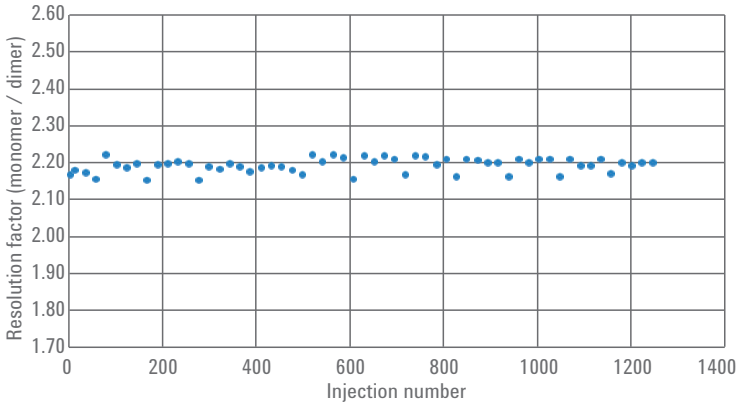
Note: Working at extremes of the operating parameters is likely to reduce column lifetime.

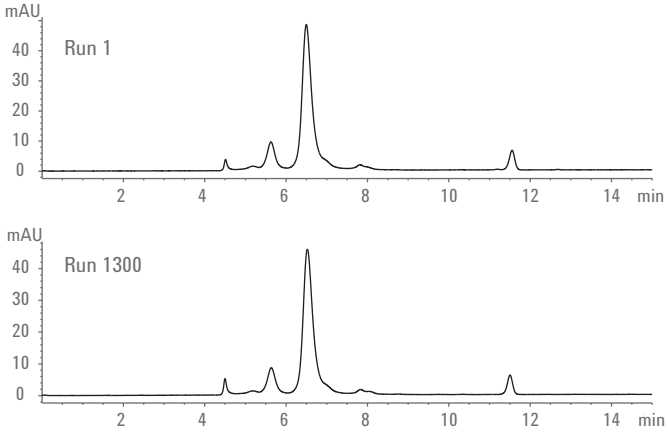
Column: **AdvanceBio SEC 300 Å 7.8 x 300 mm**

Mobile phase: 150 mM sodium phosphate, pH 7.0

Sample: IgG

Plot showing the resolution between IgG monomer and dimer over a 1300 injection sequence.





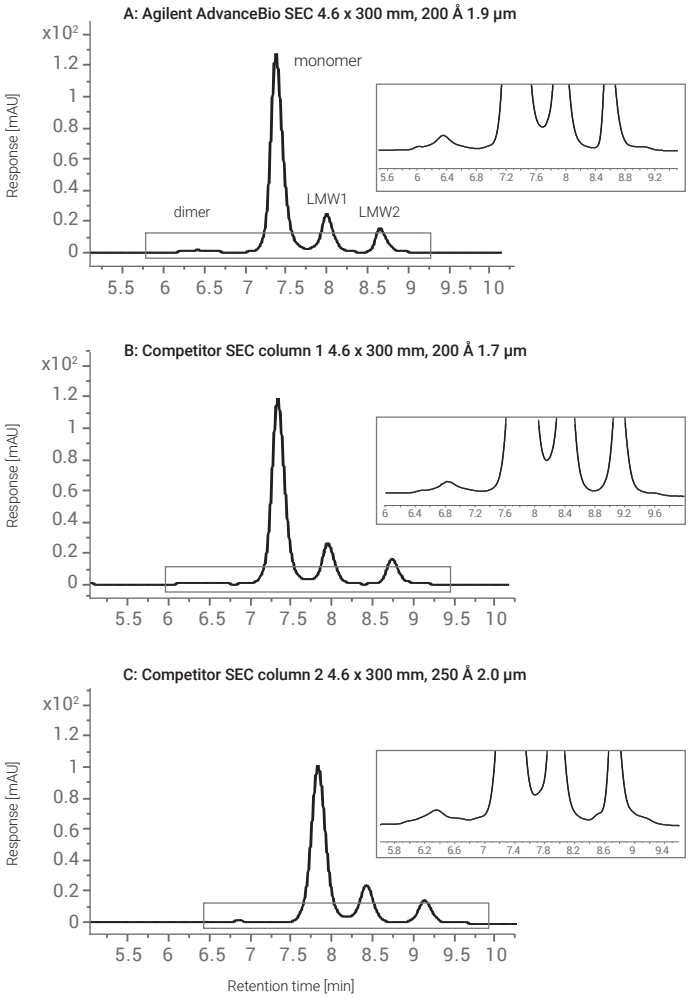
The profile of an IgG sample did not change—even after 1300 injections. Resolution factors and quantitation of the IgG monomer and dimer also remained within working range throughout the column lifetime.

AdvanceBio SEC, 1.9 µm

Instrument:	1260 Infinity II bio-inert LC system
Software:	Agilent OpenLab CDS
Flow rate:	0.35 mL/min
Eluent:	50 mM sodium phosphate, 200 mM NaCl, pH 7.0
Temperature:	25 °C
Injection volume:	1 µL
Detection:	UV, 220 nm

The 1.9 µm silica particles modified by unique Agilent hydrophilic bonding chemistry ensure high resolution and separation efficiency. In addition, they minimize secondary interactions with mAbs, ADCs, and other proteins.

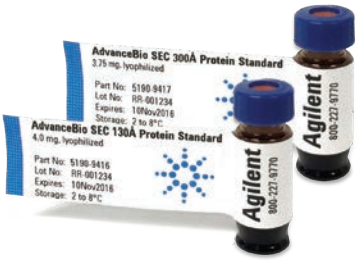
AdvanceBio SEC columns demonstrate better resolution and separation efficiency, compared to competitive columns.



Size exclusion chromatograms of SigmaMAb mixed with LMW1 and LMW2 fragments.

	Peak Width 50%			Resolution		
	Monomer	LMW1	LMW2	Dimer/Monomer	Monomer/LMW1	Back Pressure (bar)
Agilent Advance Bio SEC 1.9 µm	0.159	0.154	0.148	2.79	2.28	340
Competitor SEC column 1	0.172	0.166	0.160	2.46	2.09	354
Competitor SEC column 2	0.194	0.182	0.169	2.49	1.83	260

AdvanceBio SEC standards



AdvanceBio SEC protein standards, p/n 5190-9416 and p/n 5190-9417

130 Å AdvanceBio SEC protein standards

A protein mix consisting of 5 carefully selected proteins (Ovalbumin, Myoglobin, Aprotinin, Neurotensin, Angiotensin II) designed to calibrate Agilent's 130 Å AdvanceBio size exclusion columns. This standard can be used regularly to calibrate the column and ensure ideal system performance in various applications involving protein purification and analysis.

300 Å AdvanceBio SEC protein standards

A protein mix consisting of 5 carefully selected proteins (Thyroglobulin, g-globulin, Ovalbumin, Myoglobin, Angiotensin II) designed to calibrate Agilent's 300 Å AdvanceBio size exclusion columns. This standard can be used regularly to calibrate the column and ensure ideal system performance in various applications involving protein purification and analysis.

AdvanceBio SEC Standards

Description	Size	Part No.
130 Å	1.5 mL vial	5190-9416
300 Å	1.5 mL vial	5190-9417

Bio SEC-3

Higher resolution for faster peptide and protein separations

Bio SEC-3 columns offer speed and resolution advantages over other SEC columns, thanks to their small, efficient particles.

- Faster separations than large-particle SEC columns
- High resolution: sharper peaks and better protein recovery
- Exceptional loading capacity and recovery due to proprietary hydrophilic layer
- Flexible method development: compatible with most aqueous buffers
- Excellent stability under both high- and low-salt conditions
- Reliable, consistent performance: narrowly dispersed particles; proprietary hydrophilic layer provides for minimal secondary interactions
- Robust particles compatible with multi-detectors including light scattering
- MS-compatible

Bio SEC-3 columns help you achieve more consistent SEC separations. Each column is packed with spherical, narrowly dispersed 3 µm silica particles coated with a proprietary hydrophilic layer for high recovery and minimal secondary interactions, which provides more consistent separations. This thin polymeric layer is chemically bonded to pure, mechanically stable silica under controlled conditions, ensuring a highly efficient and stable size exclusion particle.

Tips and tools

Deactivated/silanized vials have inert surfaces that will not interact with metals, biologicals or proteins, and will not cause pH shifts. Avoid standard polypropylene vials for biological or light-sensitive compounds.

Column Specifications

Pore Size	Particle Size	Mol Wt Range	pH Range	Max Pressure	Flow Rate
100 Å	3 µm	100–100,000	2–8.5	137 bar, 2000 psi	1.0–10.0 mL/min (21.2 mm id)
					0.2–1.2 mL/min (7.8 mm id)
					0.1–0.4 mL/min (4.6 mm id)
150 Å	3 µm	500–150,000	2–8.5	137 bar, 2000 psi	1.0–10.0 mL/min (21.2 mm id)
					0.2–1.2 mL/min (7.8 mm id)
					0.1–0.4 mL/min (4.6 mm id)
300 Å	3 µm	5,000–1,250,000	2–8.5	137 bar, 2000 psi	1.0–10.0 mL/min (21.2 mm id)
					0.2–1.2 mL/min (7.8 mm id)
					0.1–0.4 mL/min (4.6 mm id)

Calibration curves—Bio SEC-3

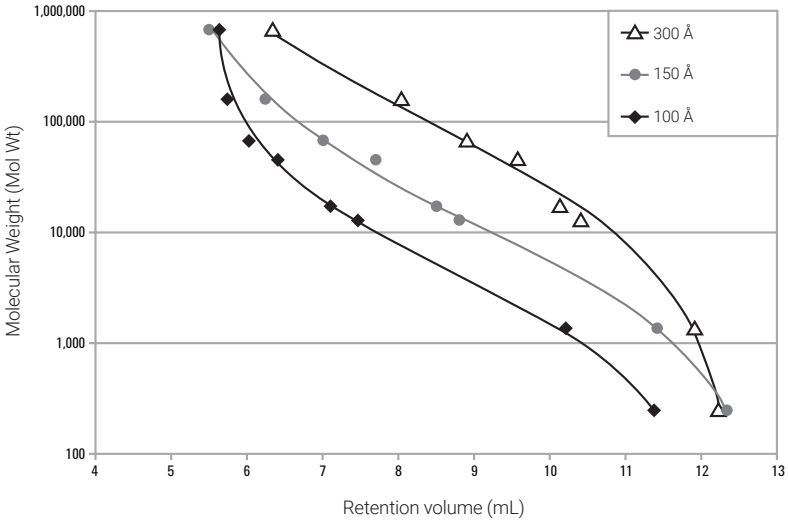
Column: **Bio SEC-3**
7.8 x 300 mm, 3 µm

Mobile phase: Sodium phosphate 150 mM, pH 7.0

Flow rate: 1.0 mL/min

Detector: UV

		Pore Size		
Proteins	**Mol Wt**	**300 Å**	**150 Å**	**100 Å**
Thyroglobulin	670,000	6.34	5.50	5.63
γ-Globulin	150,000	8.03	6.24	5.74
BSA	67,000	8.90	7.00	6.03
Ovalbumin	45,000	9.57	7.70	6.41
Myoglobin	17,000	10.12	8.50	7.10
Ribonuclease A	12,700	10.40	8.80	7.46
Vitamin B12	1,350	11.90	11.40	10.20



Pore size choice

The choice of media pore size will influence the resolution in SEC. As the separation is based on differences in molecular size in solution, the sample must be able to permeate the porous structure of the particles. If the pore size is too small, the samples will be excluded from the pores and elute in the void volume of the column, and if too large, then all will be able to fully permeate the particles and so there will be very little separation.

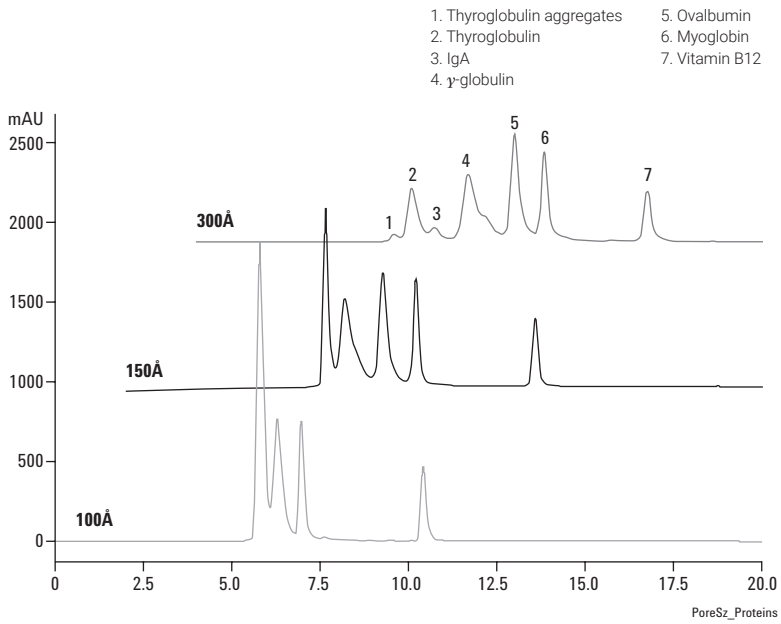
Pore size choice: proteins

Column A: **Bio SEC-3, 100 Å**
5190-2503
4.6 x 300 mm, 3 µm

Column B: **Bio SEC-3, 100 Å**
5190-2508
4.6 x 300 mm, 3 µm

Column C: **Bio SEC-3, 100 Å**
5190-2513
4.6 x 300 mm, 3 µm

Mobile phase:	Sodium phosphate 100 mM + sodium chloride 150 mM, pH 6.8
Flow rate:	0.35 mL/min
Gradient:	10–58% B in 4 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B
Detector:	UV, 220 nm
Sample:	Bio-Rad gel filtration standards mix



Pore size choice: mouse IgG

Column A: **Bio SEC-3, 100 Å**
5190-2503
4.6 x 300 mm, 3 µm

Column B: **Bio SEC-3, 100 Å**
5190-2508
4.6 x 300 mm, 3 µm

Column C: **Bio SEC-3, 100 Å**
5190-2513
4.6 x 300 mm, 3 µm

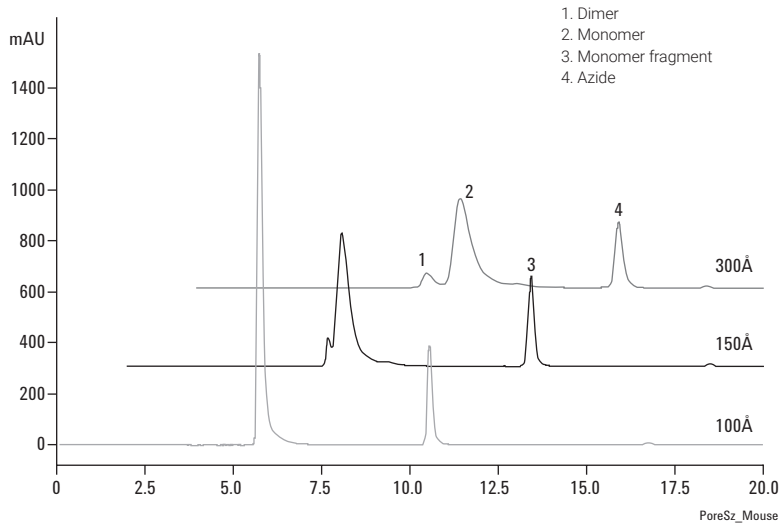
Mobile phase: Sodium phosphate 100 mM + sodium chloride 150 mM, pH 6.8

Flow rate: 0.35 mL/min

Gradient: 10-58% B in 4 min, 1 min wash at 95% B, 1 min re-equilibration at 10% B

Detector: UV, 220 nm

Sample: Mouse IgG



Bio SEC-3

Size (mm)	Particle Size (µm)	Bio SEC-3 100 Å USP L59	Bio SEC-3 150 Å USP L59	Bio SEC-3 300 Å USP L59
21.2 x 300	3	5190-6850	5190-6851	5190-6852
21.2 x 50, guard	3	5190-6854	5190-6855	5190-6856
7.8 x 300	3	5190-2501	5190-2506	5190-2511
7.8 x 150	3	5190-2502	5190-2507	5190-2512
7.8 x 50, guard	3	5190-2505	5190-2510	5190-2515
4.6 x 300	3	5190-2503	5190-2508	5190-2513
4.6 x 150	3	5190-2504	5190-2509	5190-2514
4.6 x 50, guard	3	5190-6846	5190-6847	5190-6848

Tips and tools

To further understand molecular weight determination and aggregation analysis using the 1260 Infinity Multi-detector Bio-SEC Solution along with BioSEC-3 columns, see:

Detailed Aggregation Characterization of Monoclonal Antibodies Using the Agilent 1260 Infinity Multi-Detector Bio-SEC Solution with Advanced Light Scattering Detection (publication 5991-3954EN), and *Determination of Protein Molecular Weight and Size Using the Agilent 1260 Infinity Multi-Detector Bio-SEC Solution with Advanced Light Scattering Detection* (publication **5991-3955EN**)

www.agilent.com/search

Bio SEC-5

- Exceptional resolution for large molecules
- High stability and efficiency due to a proprietary neutral hydrophilic layer
- Improved peak capacity and resolution due to specially designed packing that increases pore volume
- Rugged performance: outstanding reproducibility and column lifetime
- Excellent stability, even under high-pH, high-salt, and low-salt conditions
- Flexible method development: compatible with most aqueous buffers
- Broad applicability: up to 2000 Å pore size for vaccines and high molecular weight biomolecules
- MS-compatible

For large biomolecules and samples with components of multiple molecular weights, Bio SEC-5 columns are an ideal choice. They are packed with 5 µm silica particles coated with a proprietary, neutral, hydrophilic layer for maximum efficiency and stability, with six different pore sizes to provide optimum resolution over the molecular weight range.

Column Specifications

Pore Size	Particle Size	Mol Wt Range	pH Range	Max Pressure	Flow Rate
100 Å	5 µm	100–100,000	2–8.5	137 bar, 2000 psi	1.0–10.0 mL/min (21.2 mm id)
					0.2–1.2 mL/min (7.8 mm id)
					0.1–0.4 mL/min (4.6 mm id)
150 Å	5 µm	500–150,000	2–8.5	137 bar, 2000 psi	1.0–10.0 mL/min (21.2 mm id)
					0.2–1.2 mL/min (7.8 mm id)
					0.1–0.4 mL/min (4.6 mm id)
300 Å	5 µm	5,000–1,250,000	2–8.5	137 bar, 2000 psi	1.0–10.0 mL/min (21.2 mm id)
					0.2–1.2 mL/min (7.8 mm id)
					0.1–0.4 mL/min (4.6 mm id)
500 Å	5 µm	15,000–5,000,000	2–8.5	137 bar, 2000 psi	1.0–10.0 mL/min (21.2 mm id)
					0.2–1.2 mL/min (7.8 mm id)
					0.1–0.4 mL/min (4.6 mm id)
1000 Å	5 µm	50,000–7,500,000	2–8.5	137 bar, 2000 psi	1.0–10.0 mL/min (21.2 mm id)
					0.2–1.2 mL/min (7.8 mm id)
					0.1–0.4 mL/min (4.6 mm id)
2000 Å	5 µm	>10,000,000	2–8.5	137 bar, 2000 psi	1.0–10.0 mL/min (21.2 mm id)
					0.2–1.2 mL/min (7.8 mm id)
					0.1–0.4 mL/min (4.6 mm id)

Comparing Bio SEC-3 and Bio SEC-5

Analysis of monoclonal antibody

Column: **Bio SEC-3, 300 Å**
5190-2511
7.8 x 300 mm, 3 µm

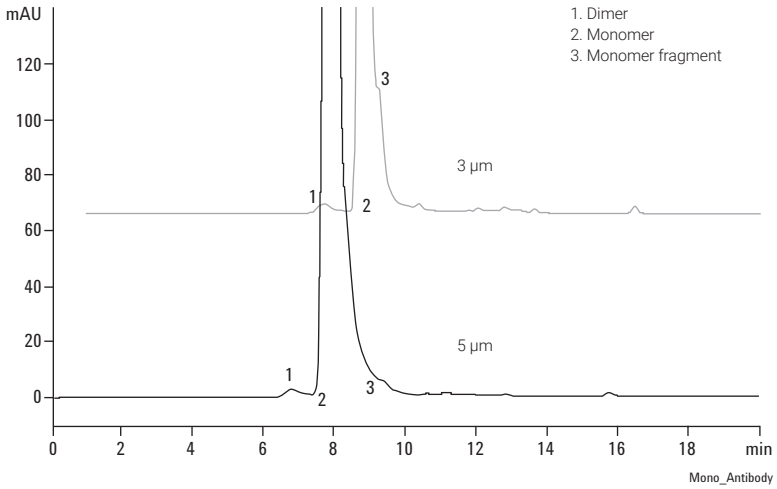
Column: **Bio SEC-5, 300 Å**
5190-2526
7.8 x 300 mm, 5 µm

Mobile phase: Sodium phosphate 150 mM, pH 7.0

Flow rate: 1 mL/min

Detector: UV, 220 nm

Sample: Humanized monoclonal antibody



The 3 µm column gives higher definition of the fragmentation plan.

Tips and tools

There are many things to consider when developing aggregation analysis for your proteins; the effect of solute size and molecular weight, column selection choices, important mobile phase considerations, and more. For a guide on all of the above, see:

Size exclusion chromatography for biomolecule analysis: A "How to" guide (publication **5991-3651EN**)

www.agilent.com/search

Calibration curves—Bio SEC-5

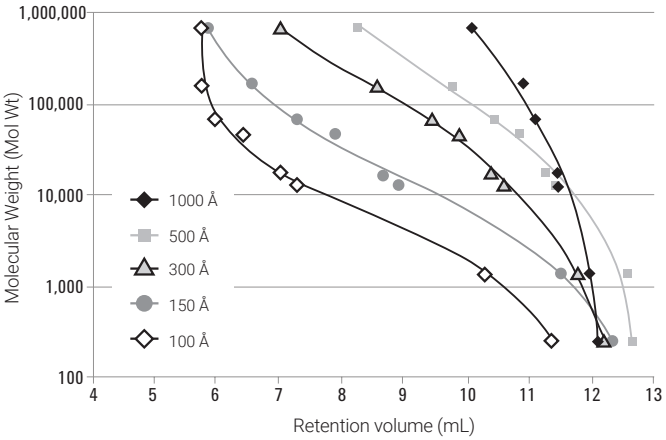
Column: **Bio SEC-5**
7.8 x 300 mm, 5 µm

Mobile phase: Sodium phosphate 150 mM, pH 7.0

Flow rate: 1.0 mL/min

Detector: UV, 214 nm

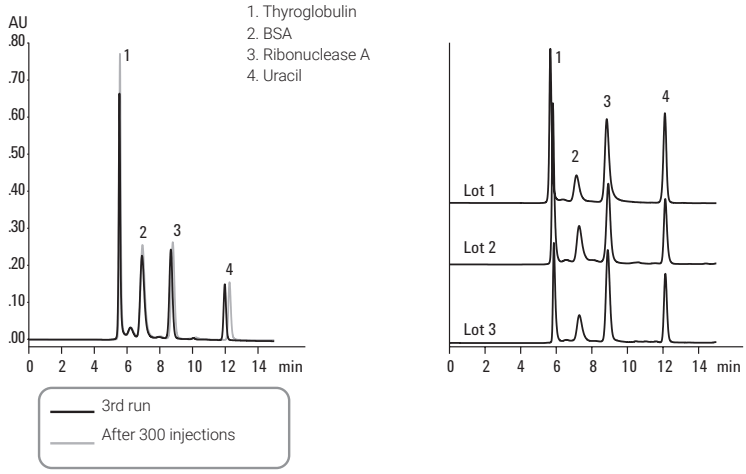
		Retention Volume				
Proteins	**Mol Wt**	**1000 Å**	**500 Å**	**300 Å**	**150 Å**	**100 Å**
Thyroglobulin	670,000	10.07	8.23	7.03	5.82	5.77
γ-Globulin	150,000	10.88	9.80	8.57	6.55	5.79
BSA	67,000	11.13	10.44	9.44	7.29	6.00
Ovalbumin	45,000	11.28	10.83	9.89	7.90	6.40
Myoglobin	17,00 0	11.44	11.28	10.42	8.66	7.05
Ribonuclease A	12,700	11.52	11.41	10.58	8.93	7.32
Vitamin B12	1,350	12.00	12.59	11.78	11.49	10.30
Uracil (total permeation marker)	112	12.08	12.68	12.21	12.13	11.41



Exceptional lot-to-lot reproducibility

Column: **Bio SEC-5, 150 Å**
5190-2521
7.8 x 300 mm, 5 µm

Mobile phase: Sodium phosphate 150 mM, pH 7.0



The four-protein mixture shows excellent retention time reproducibility over 300 injections and on three columns from different manufacturing lots.

Bio SEC-5

Size (mm)	Particle Size (µm)	Bio SEC-5 100 Å USP L59	Bio SEC-5 150 Å USP L59	Bio SEC-5 300 Å USP L59	Bio SEC-5 500 Å USP L59	Bio SEC-5 1000 Å USP L59	Bio SEC-5 2000 Å USP L59
21.2 x 300	5	5190-6863	5190-6864	5190-6865	5190-6866	5190-6867	5190-6868
21.2 x 50, guard	5	5190-6869	5190-6870	5190-6871	5190-6872	5190-6873	5190-6874
7.8 x 300	5	5190-2516	5190-2521	5190-2526	5190-2531	5190-2536	5190-2541
7.8 x 150	5	5190-2517	5190-2522	5190-2527	5190-2532	5190-2537	5190-2542
7.8 x 50, guard	5	5190-2520	5190-2525	5190-2530	5190-2535	5190-2540	5190-2545
4.6 x 300	5	5190-2518	5190-2523	5190-2528	5190-2533	5190-2538	5190-2543
4.6 x 150	5	5190-2519	5190-2524	5190-2529	5190-2534	5190-2539	5190-2544
4.6 x 50, guard	5	5190-6857	5190-6858	5190-6859	5190-6860	5190-6861	5190-6862

Tips and tools

Update your SEC technology to AdvanceBio SEC 300 Å for higher resolution and fewer secondary interactions.

See **Page 110** for more information.

ProSEC 300S

- Stable performance: mechanically robust silica particles that do not bleed during use
- Easy method development: extended linear resolving range eliminates the need for pore size selection—a single column to analyze most globular proteins
- Choices to help you perfect your separation: two column ids to suit multi-detector SEC
- Increased sensitivity when used with light-scattering detectors, to identify dimers, trimers, and aggregates

The ProSEC 300S column is specifically designed as a single column solution for globular protein analysis. The pore size selection and optimization provides an extended linear resolving range so that this single column can be used for analysis across the full range of globular proteins.

The particles are extremely robust and do not fragment during use to leach particulates. This gives exceptionally stable baselines making this column an ideal choice for use with light scattering detectors.

Two column dimensions, 7.5 mm id and 4.6 mm id, to suit multi-detector size exclusion chromatography, provide an option for the analysis of small masses.

ProSEC 300S Column Specifications

Bonded Phase	Pore Size	Particle Size	Protein Mol Wt Range	pH Range	Flow Rate	Max Pressure
ProSEC 300S	300 Å	5 µm	1,500–800,000	2–7.5	<1.5 mL/min (7.5 mm id)	250 bar, 3700 psi
					<0.5 mL/min (4.6 mm id)	

ProSEC 300S

Dimensions	Particle Size (µm)	Part No.
7.5 x 600	5	PL1147-8501
7.5 x 300	5	PL1147-6501
4.6 x 250	5	PL1547-5501
Guard columns		
7.5 x 50	5	PL1147-1501
4.6 x 50	5	PL1547-1501

ZORBAX GF-250 and GF-450 gel filtration columns

GF-250 gel filtration columns

- Legacy products to be used where protocols state USP designation L35
- Semiprep and prep column dimensions
- Compatible with organic modifiers and denaturants
- Wide usable pH range (3 to 8)

ZORBAX GF-250 and GF-450 size exclusion (gel filtration) columns are ideal for size separations of proteins and other biomolecules. The separation range is 4,000-900,000 for globular proteins when using GF-250 and GF-450 columns in series. The GF-250/GF-450 size exclusion columns have a hydrophilic diol bonded phase for high recovery of proteins (typically >90%) and a unique zirconia modification of the silica for a pH operating range from 3 to 8. The GF-250 and GF-450 columns are packed with precisely sized porous silica microspheres with narrow pore size and particle size distributions. The result is an efficient, rugged, and reproducible size exclusion column that can be used for both analytical and preparative separations of proteins with flow rates of up to 3 mL/min. These columns are compatible with organic modifiers (<25%) and denaturants in the mobile phase to reduce protein aggregation. Some common applications include separations of protein monomers, dimers and aggregates, desalting, protein molecular weight estimation, and separations of modified proteins.

UHPLC Column Specifications

Bonded Phase	Pore Size	Particle Size	Mol Wt Range	Surface Area	pH Range	Flow Rate	Max Pressure
ZORBAX GF-250	150 Å	4 µm	4,000–400,000	140 m^2/g	3.0–8.0	<3.0 mL/min	350 bar
ZORBAX GF-450	300 Å	6 µm	10,000–900,000	50 m^2/g	3.0– 8.0	<3.0 mL/min	350 bar

Specifications represent typical values only

ZORBAX GF-250 (USP L35) and GF-450 (USP L35) Gel Filtration Columns

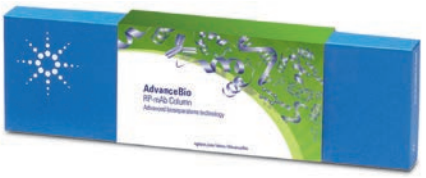
Description	Size (mm)	Particle Size (µm)	Part No.
GF-250, 150 Å	9.4 x 250	4	884973-901
GF-250, 150 Å	4.6 x 250	4	884973-701
GF-450, 300 Å	9.4 x 250	6	884973-902
Guard Columns (hardware required)			
GF-450 Diol, guard cartridge, 2/pk	9.4 x 15	6	820675-111
GF-250 Diol, guard cartridge, 4/pk	4.6 x 12.5	6	820950-911
GF-450 Diol, guard cartridge, 2/pk	9.4 x 15	6	820675-111
Prep guard hardware kit			840140-901
Guard hardware kit			820999-901
PrepHT Columns			
PrepHT GF-250, 150 Å	21.2 x 250	6	877974-901
PrepHT GF-450, 300 Å	21.2 x 250	6	877974-910
PrepHT endfittings, 2/pk			820400-901
PrepHT guard cartridge, 2/pk	17.0 x 7.5	5	820212-911
Guard cartridge hardware			820444-901

Tips and tools

Column user guides are excellent resources, with instructions for use and column care, as well as suggested starting methods:

www.agilent.com/chem/biolc-columns-user-guides

Glycosylation Characterization



Post-translational modifications to the primary amino acid sequence, including glycosylation, have functional consequences and can impact efficacy and immunogenicity of a biopharmaceutical. The structure of the glycan also contributes to the half-life of the protein in plasma and the ability of the monoclonal antibody to trigger the immune response required for efficacy. Regulatory authorities consider glycosylation to be one of the critical quality attributes and, therefore, it must be characterized and quantified, with acceptable ranges determined, as part of the development process for a glycoprotein innovator, biosimilar or biobetter pharmaceutical.

There are a number of analytical methods that are used to obtain information about the structure and form of protein glycosylation.

- For structural identification, including identification of glycosylation sites, mass spectrometry detection is used with reversed-phase and hydrophilic interaction chromatography (HILIC).
- The sialic acid containing glycans will also impart more charge to the protein and can be characterized by ion-exchange chromatography.

Having characterized the glycoprotein and glycopeptide fragments to obtain information about the number and position of the glycosylation sites, it is then necessary to identify and quantify the individual glycans. To do this, the glycans must be cleaved from the protein and analyzed using HILIC columns. As glycans have no chromaphore, derivatization with a fluorophore is carried out to enable FLD detection to map and quantify the glycans.

Hydrophilic Interaction Column Selection

Application	Agilent Columns	Notes	
Glycans cleaved from a glycoprotein including monoclonal antibodies	AdvanceBio Glycan Mapping	Amide bonded phase for rapid equilibration and enhanced selectivity for glycans.	AB
	1.8 µm	Based on a fully porous particle for high-speed separations and high throughput applications. Stability to 1200 bar for use with the 1290 Infinity II LC.	
	2.7 µm	Based on Poroshell technology to give a superficially porous particle with reduced diffusion distances. This gives high-resolution separations at lower pressures, and enables the use of longer column lengths for increased separation efficiency.	
Hydrophilic peptides and glycopeptides	ZORBAX RRHD 300 Å, 1.8 µm	A 300 Å silica particle to provide an orthogonal separation to the ZORBAX RRHD 300 Å, 1.8 µm reversed-phase columns.	AB
	AdvanceBio Glycan Mapping	The amide bonded phase provides an alternative HILIC functionality for small hydrophilic peptides and glycopeptides.	

AB Part of the AdvanceBio family

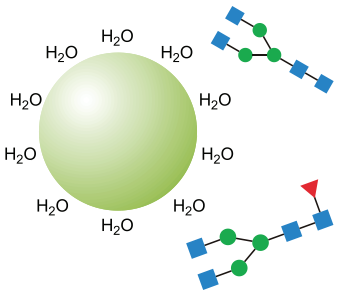
Benefit from a complete Agilent N-glycan workflow

Agilent provides a single source offering for instruments and consumables, from sample to trusted answer.

With the addition of ProZyme products and services, our expertise now covers the complete N-glycan analysis workflow. The Agilent portfolio now includes Gly-X N-glycan sample preparation technology with InstantPC, providing best-in-class throughput and analytical sensitivity. Standalone reagents for glycobiology research, such as glycan standards and glycoenzymes, and a wide range of analytical services are also available.

For more information, please visit: **www.agilent.com/chem/better-together**

AdvanceBio Glycan Mapping columns



AdvanceBio Glycan Mapping columns, standards, and sample preparation products for the selective removal of the N-glycans from a glycoprotein, including monoclonal antibodies.

Speed of analysis—1.8 µm columns provide high throughput N-glycan analysis where speed is the primary concern either due to the number of samples or to the immediate requirement for data.

Resolution—high resolution separations are achieved using the 2.7 µm particles packed in the 250 mm column. This increased resolution enables accurate quantitation of target glycans and changes to the protein glycosylation profile, which may have occurred during expression.

Comprehensive methods—for sample preparation, chromatographic analysis, and data interpretations to ensure reproducibility, and accuracy of identification and quantitation

Simplicity of ordering—a single part number to order the full sample preparation workflow for protein solubilization to purification of 2-AB labeled glycans, plus kits for each part of the sample preparation workflow for versatility.

Column Specifications

Bonded Phase	id (mm)	Particle	Endcapped	pH Stability	Operating Temperature	Pressure Limit
Amide HILIC	2.1 and 4.6	1.8 µm, fully porous	No	2–7	40 °C	1200 bar
Amide HILIC	2.1 and 4.6	2.7 µm, superficially porous	No	2–7	40 °C	600 bar

The mapping of the N-linked glycan component of a glycoprotein, including monoclonal antibodies, requires the N-glycans to be enzymatically cleaved, using PNGase F, from the protein amino acid backbone. The cleaved N-glycans can be analyzed by hydrophilic interaction chromatography with MS detection, or after derivatization with a fluorophore, 2-aminobenzamide (2-AB) analyzed using HPLC/UHPLC using either FLD or MS. The AdvanceBio Glycan columns provide both speed of analysis, 1.8 µm, and resolution, 2.7 µm, for the identification and quantitation of the cleaved glycans.

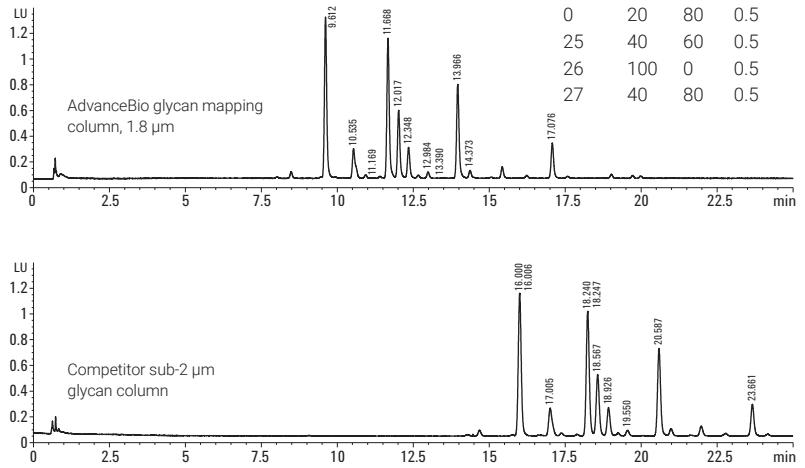
Speed of analysis

The AdvanceBio Glycan Mapping 1.8 µm columns are recommended for high throughput analysis where short run times are required.

Superior results—in 40% less time than the competition

Column:	**AdvanceBio Glycan Mapping 859700-913 2.1 x 150 mm, 1.8 µm**
Column B:	Competitor sub-2 µm glycan column
Mobile phase:	100 mM NH_4Formate, pH 4.5 B: ACN
Injection volume:	2 µl in 70:30 ACN: 100 mM NH_4Formate
Sample thermostat:	10 °C
FLD:	Excitation = 260 Emission = 430
Flow rate:	0.35 mL/min
Instrument:	1290 Infinity LC with 1260 Infinity fluorescence detector (FLD)
Sample:	2-AB labeled N-linked Human IgG glycan library (p/n 5190-6996)

Time	%A	%B	Flow Rate mL/min
0	20	80	0.5
25	40	60	0.5
26	100	0	0.5
27	40	80	0.5



The AdvanceBio Glycan Mapping column delivers better resolution, narrower bands, and higher peak capacity than the non-Agilent sub-2 µm column in a 2.1 x 150 mm configuration.

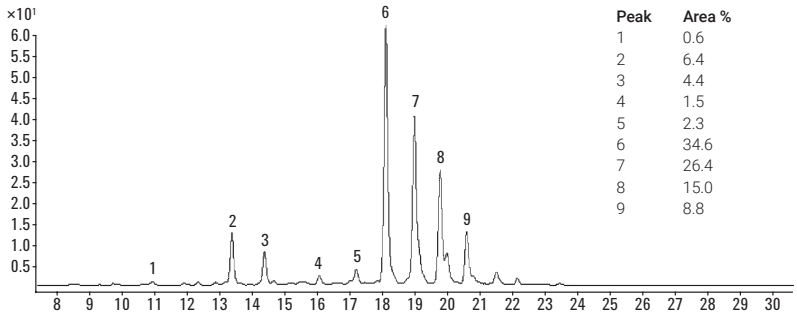
Resolution

The AdvanceBio Glycan Mapping 2.7 µm media, in longer column lengths, are recommended for high resolution separations.

Superior results—in 40% less time than the competition

Column: **AdvanceBio Glycan Mapping 859700-913 2.1 x 150 mm, 1.8 µm**

Instrument:	1290 Infinity binary LC	
Buffer:	A: 100 mM ammonium formate in water, pH 4.5	
	B: Acetonitrile	
MS conditions:	Gas temperature:	250 °C
	Sheath gas temperature:	250 °C
	Gas flow:	8 L/min
	Sheath gas flow:	8 L/min
	Nebulizer:	25 psi
	Vcap:	3,500 V
	Nozzle:	1,000 V
	Fragmentor:	200 V
	Skimmer:	45 V
	Oct 1 RF Vpp:	550
	Collision energies:	15 and 30 V
	Mode:	MS and targeted MS/MS



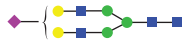
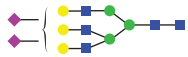
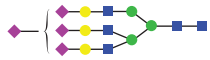
Peak	Area %
1	0.6
2	6.4
3	4.4
4	1.5
5	2.3
6	34.6
7	26.4
8	15.0
9	8.8

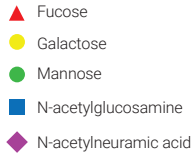
The N-glycans cleaved from fetuin using PNGase F were analyzed after 2-AB derivatization using UHPLC —FLD. The peak assignment by MS shows that the N-glycans cleaved from fetuin are complex biantennary and triantennary glycans containing N-acetylneuramic acid (NeuAc) but no fucose. Fetuin 2-AB N-glycans analyzed using HILIC-UHPLC with peak assignments determined by MS.

Instrument conditions

	Antibody Standard Gradient	Fetuin Gradient	Ovalbumin Gradient
Starting flow rate	0.5 mL/min	0.5 mL/min	0.5 mL/min
Gradient	0 min 85% B	0 min 75% B	0-6 min 85% B
	5 min 75% B	45 min 50% B	10 min 80% B
	35 min 64% B	47 min 40% B, flow 0.5 mL/min	60 min 70% B
	40 min 50% B	47.01 min, flow 0.25 mL/min	65 min 50% B, flow 0.5 mL/min
	42 min, flow 0.5 mL/min 42.01 min, flow 0.25 mL/min	49 min 0% B	65.01 min, flow 0.25 mL/min
	43 min, 0% B	51 min 0% B	68 min 0% B
	48 min 0% B	51.01 min 75% B, flow 0.25 mL/min	73 min 0% B
	50 min 85% B 50.01 min, flow 0.25 mL/min	52.00 min, flow 0.5 mL/min flow	74 min 85% B, 0.25 mL/min
	51 min, flow 0.5 mL/min		75.00 min, flow 0.5 mL/min
Stop time	51 min	52 min	75 min
Posttime	20 min	20 min	20 min
Injection volume	5 µL	1 µL	1 µL
Thermostat autosampler	5 °C		
FLD	Excitation = 260 nm Emission = 430 nm		
Peak width	>0.013 min (0.25 s resp. time) (37.04 Hz)		

Detailed information of N-glycan ovalbumins

Peak	Oxford	Structure
1	A2G2S1	
2, 3	A2G2S2	
4	A3GGS2	
5	A3G3S3, A3G3S2 (trace)	
6	A3G3S3, A3G3S2 (trace)	
7	A3G3S3, A3G3S4 (trace)	
8	A3G3S4, A3G3S3	
9	A3G3S4	

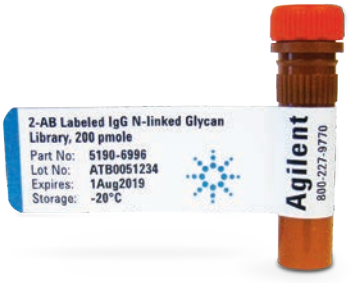


N-Glycan standards

Agilent provides the reference materials, IgG N-linked glycan standard, and dextran ladder standard, needed as part of the workflow to ensure optimum performance of both sample preparation and the LC system. The two standards are available with the 2-AB label attached and also without the 2-AB label for use as sample preparation reference materials.

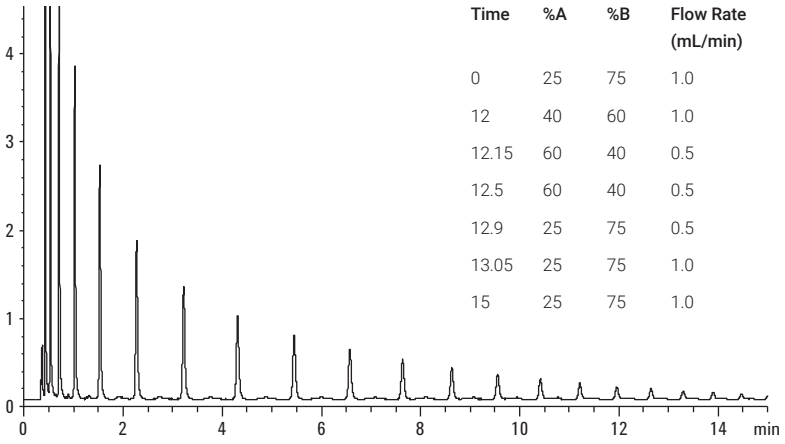
The IgG N-linked glycan standard is used as a QA test for every batch of the AdvanceBio Glycan mapping media to ensure each column meets the stringent reproducibility requirements for this demanding analysis.

The dextran ladder standard is used for calibrating the system based on elution times of the glucose units (GU) in the dextran homologous series and for reporting out GU relative retention data.



Separation of a 2-AB labeled dextran ladder

Column:	**AdvanceBio Glycan Mapping 859700-913 2.1 x 150 mm, 1.8 µm**
Mobile phase:	A: 100 mM NH4Fc, pH 4.5 B: ACN
FLD:	Excitation = 260 nm Emission = 430 nm
Injection volume:	2 µL (10 pmol total glycan/1 µL 75:25 ACN:water)
Sample:	2-AB (p/n 5190-6998) labeled dextran ladder



Time	%A	%B	Flow Rate (mL/min)
0	25	75	1.0
12	40	60	1.0
12.15	60	40	0.5
12.5	60	40	0.5
12.9	25	75	0.5
13.05	25	75	1.0
15	25	75	1.0

This analysis uses the Agilent dextran ladder standard, together with an AdvanceBio Glycan Mapping column to correlate retention times of unknown glycans.

AdvanceBio Glycan Mapping, 1.8 µm, Stable to 1200 Bar

Size (mm)	Part No.
2.1 x 150	859700-913
2.1 x 100	858700-913
2.1, 1.8 µm, Fast Guard	821725-905

AdvanceBio Glycan Mapping, 2.7 µm, Superficially Porous, Stable to 1200 Bar

Size (mm)	Part No.
4.6 x 250	680975-913
4.6 x 150	683975-913
4.6 x 100	685975-913
2.1 x 250	651750-913
2.1 x 150	683775-913
2.1 x 100	685775-913
2.1, 2.7 µm, Fast Guard	821725-906

N-Glycan Standards

Size (mm)	Part No.
Dextran ladder standard, 10 µg, 0.5 mL vial	5190-6997
2-AB labeled dextran ladder standard, 200 pmol	5190-6998
IgG N-linked glycan library, 20 µg, 0.5 mL	5190-6995
2-AB labeled IgG N-linked glycan library, 200 pmol	5190-6996

Hydrophilic and glycopeptide analysis

Peptide analysis demands high selectivity and run-to-run reproducibility as provided by reversed-phase chromatography. However, reversed-phase columns have limited retention and selectivity for hydrophilic peptides, including glycopeptides. The ZORBAX RRHD 300-HILIC, 1.8 µm columns provide increased retention of hydrophilic and glycopeptides compared to reversed-phase columns so that valuable information is not lost when doing peptide mapping experiments.

The two techniques are orthogonal and provide complementary information for protein primary structure analysis.

- A ZORBAX 300 Å particle for analysis across the range of peptide sizes
- The 1.8 µm particle delivers UHPLC performance with 1200 bar stability
- Provides UHPLC orthogonality when used with the ZORBAX RRHD 300 Å reversed-phase columns

Column Specifications

Bonded Phase	id (mm)	Particle Size	Endcapped	pH Stability	Operating Temperature	Pressure Limit
Silica	2.1	1.8, fully porous	No	1-8	40 °C	1200 bar

Peptide mapping is used for characterization and impurity profiling of protein biotherapeutics. Reversed-phase UHPLC/HPLC is routinely used but when the digest contains hydrophilic peptides, such as glycopeptides, valuable information may be missed. The ZORBAX RRHD 300-HILIC column retains the hydrophilic glycopeptides and, when coupled with mass spectrometry, provides identification of this important group of protein fragments.

Glycopeptide identification in a protein tryptic digest

Column: **ZORBAX RRHD 300-HILIC**
858750-901
2.1 x 100 mm, 1.8 µm

Mobile phase: A: 100% ACN
B: 50 mM ammonium formate, pH 4.5

Flow rate: 0.4 mL/min

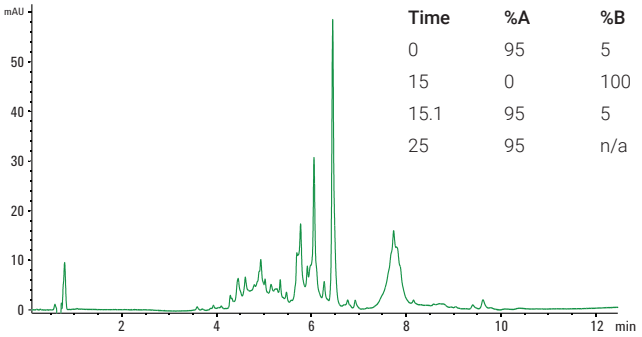
Injection: 5 µg

Detector: UV, 280 nm

Instrument: 1290 Infinity LC, 6224 accurate-mass time-of-flight, dual ESI source in positive ion mode

Sample: Glycopeptide from digested EPO protein (1 mg/mL)

UV



Gradient

Time	%A	%B
0	95	5
15	0	100
15.1	95	5
25	95	n/a

MS

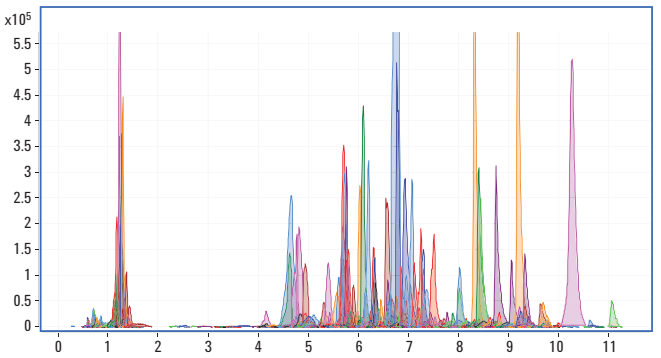
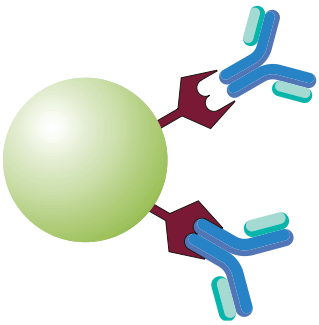


Figure UV shows the separation of an erythropoietin (EPO) peptide map using the ZORBAX RRHD 300-HILIC 2.1 x 100 mm column and Figure MS shows the extracted compound chromatograms of matched EPO. Seven peptides were identified from the HILIC-MS data that were not identified by RP-MS. HILIC is orthogonal to RP and provides additional resolution of the hydrophilic glycopeptides, in a protein enzyme digest.

ZORBAX RRHD 300-HILIC 1.8 µm Columns

Size (mm)	id (mm)	Particle Size (µm)	Part No.
ZORBAX RRHD 300-HILIC	2.1 x 100	1.8	858750-901
ZORBAX RRHD 300-HILIC	2.1 x 50	1.8	857750-901

Titer Determination



Affinity chromatography is a powerful technique that takes advantage of highly specific molecular interactions, frequently between specific proteins (for example, antigen/antibody). Agilent offers several specialty affinity products, monolithic Protein A and monolithic Protein G columns for the isolation and quantitation of IgG and a series of multiple affinity removal systems for the elimination of high abundance proteins in biological samples.

Bio-Monolith HPLC columns

- Designed for the analytical separation of all IgG (human and mouse)
- Flow rate independent separations; no diffusion, no pores, and no void volume make transport between mobile and stationary phase very rapid
- Extremely fast separations speed up method development time and decrease costs
- Locking in method parameters takes significantly less time and buffer

Bio-Monolith Protein A and Protein G HPLC columns are part of the Bio-Monolith column family. Protein A and Protein G Bio-Monolith columns are compatible with HPLC and preparative LC systems, including 1100, 1200, and 1260 bio-inert quaternary LC.

Bio-Monolith Protein A column, 5069-3639

Tips and tools

For more information on salt tolerance for mAb binding and acidic buffers compatibility for mAb elution on Bio-Monolith Protein A columns, see publication **5991-2990EN**.

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Column Specifications	
Dimensions	5.2 mm x 4.95 mm
Column volume	100 µL
Maximum pressure	150 bar (15 MPa, 2,200 psi)
Temperature min/max	Operating: 2-40 °C
	Storage: 2-8 °C
Recommended pH	Operating range: 2-13
	Cleaning-in-place: 1-14
Materials of construction	Hardware: stainless steel
	Packing: poly (glycidyl methacrylate-co-ethylene dimethacrylate) highly porous monolith
Color ring identifier	Bio-Monolith Protein A: white Bio-Monolith Protein G: orange
Shelf life/expiration date	12 months

Bio-Monolith Protein A and Protein G

Description	Part No.
Bio-Monolith Protein A, 4.95 x 5.2 mm	5069-3639
Bio-Monolith Protein G, 4.95 x 5.2 mm	5190-6900

Tips and tools

Further information can be found in:

mAb Titer Analysis with the Agilent Bio-Monolith Protein A Column (publication **5991-5135EN**)

Agilent Bio-Monolith Protein A Monitors Monoclonal Antibody Titer from Cell Cultures (publication **5991-2990EN**)

Cell Clone Selection Using the Agilent Bio-Monoltih Protein A Column and LC/MS (publication **5991-5124EN**)

Cell Culture Optimization Using an Agilent Bio-Monolith Protein A Column and LC/MS (publication **5991-5125EN**)

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Binding Affinity of Bio-Monolith Protein A and G for Different IgG Subclasses

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		
Human Fab	+	+
Human F(ab')2	+	+
Human scFv	+	+
Human Fc	+	+
Human κ	+	+
Human λ	+	+

++++ = Strong affinity
+++ = Moderate affinity
++ = Weak affinity
+ = Slight affinity
– = No affinity

Rapid humanized monoclonal antibody quantitation

Column: **Bio-Monolith Protein A**
5069-3639
5.2 x 4.95 mm

Mobile phase: A: 50 mM phosphate, pH 7.4
B: 100 mM citric acid, pH 2.8

Flow rate: 1 mL/min

Injection volume: Variable (50 µL, optimized for CHO cell culture supernatant contains IgG1)

Gradient:

Time (min)	%A	%B	
0 to 0.5	100	0	Binding
0.6 to 1.7	0	100	Eluting
1.8 to 3.5	100	0	Re-equilibrating

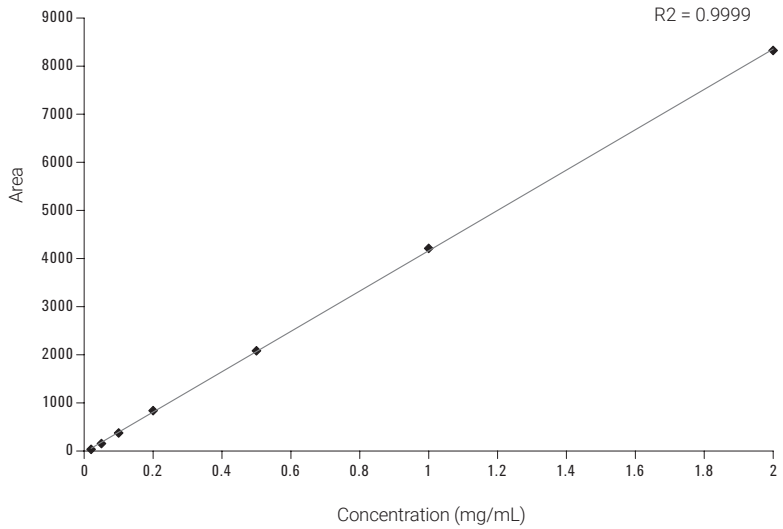
Temperature: Ambient

Detector: UV, 280 nm

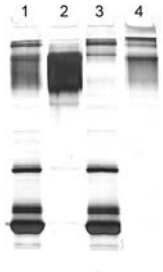
Fraction collection: Time-based

Sample: IgG1 (1-20 mg/mL) and CHO cell supernatant contains IgG1 (up to 20 mg/mL total protein)

	RT (min)	Peak Area
1	383	1.666
2	372	1.666
3	365	1.665
4	389	1.667
5	383	1.666
6	378	1.666
7	379	1.668
8	377	1.666
9	376	1.667
10	377	1.667
Mean	378	1.667
S	6.52	0.001
%RSD	1.73	0.060

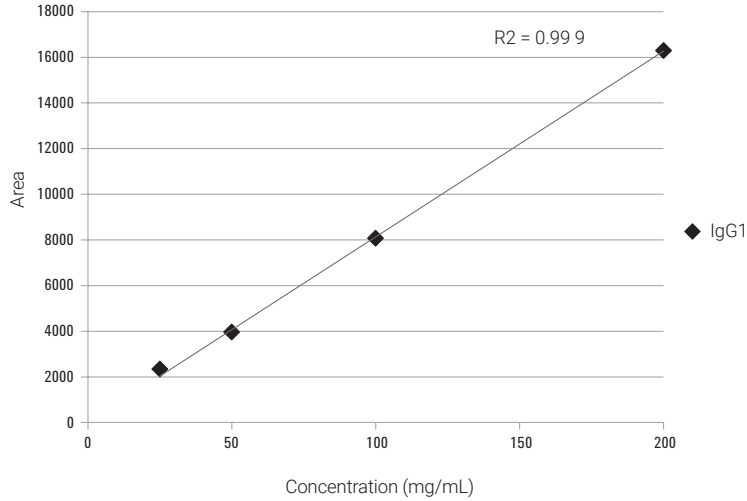


Calibration curves of modified humanized Trastuzumab (panel A: 0-2 mg/mL, and B: 25-200 mg/mL)



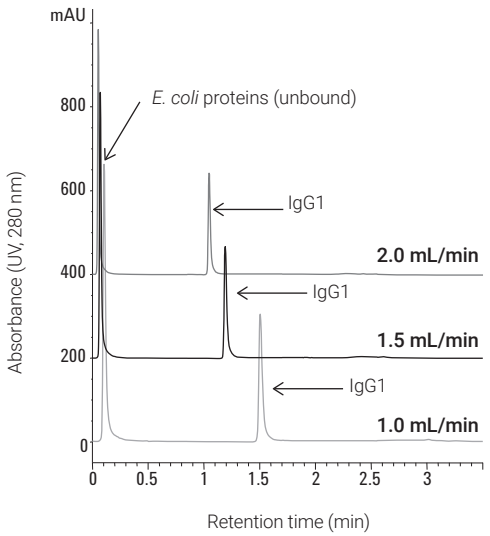
Key:
Lane 1: Whole serum before separation
Lane 2: IgG standard
Lane 3: Peak 1 (flow-through fraction)
Lane 4: Peak 2 (Protein A-bound fraction; for example, IgG1)

SDS PAGE analysis of fractions from the separation



No impact on binding efficiency with high flow rate

Column:	**Bio-Monolith Protein A** **5069-3639** **5.2 x 4.95 mm**
Mobile phase:	A: Sodium phosphate buffer, 20 mM, pH 7.4 B: Citric acid, 0.1 M, pH 2.8
Flow rate:	1.0, 1.5, and 2.0 mL/min
Injection:	4 µL (from 2.5 mg/mL IgG1 spiked with 20 mg/mL of E.coli supernatant) detector: UV, 280 nm
Gradient:	0% B for 0.5 min, 100% B from 0.6-1.7 min, 0% B from 1.8-3 min
Temperature:	25 °C
Sample:	Humanized IgG1 and *E.coli* lysate
Instrument:	1260 Infinity bio-inert LC

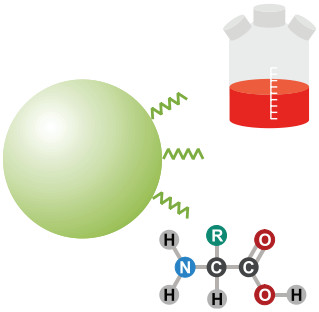


Binding of IgG1 with the Bio-Monolith Protein A column evaluated at several flow rates. More sample was loaded for this study to easily observe changes in chromatogram and signal integration.

Flow Rate Versus Peak Relative Area on Unbound Proteins and IgG1

Flow Rate (mL/min)	Unbound Area (mAu/S)	IgG1 Area (mAu/S)	Unbound Relative area (%)	IgG1 Relative area (%)	Pressure (bar)
1.0	1230	738	63	37	32
1.5	840	492	63	37	47
2.0	636	363	64	36	68

Cell Culture and Amino Acid Analysis



AdvanceBio columns from Agilent make it easier for your biotech lab to analyze amino acids and other small metabolites in spent cell culture media—with or without sample derivatization. Columns for both solutions are tested with amino acids to ensure quality and performance. Simply choose the workflow that suits your needs.

Choose the Agilent AdvanceBio Amino Acid Analysis kit for industry-standard LC/UV analysis

- Get automated online derivatization of amino acids with reverse-phase LC separation and UV detection.
- Use any Agilent LC system.
- Minimize your investment in instrumentation and expertise.

Choose Agilent AdvanceBio MS Spent Media columns for fast, underivatized LC/MS analysis

- Analyze amino acids and other cell culture metabolites with a single method: HILIC LC separation with MS detection.
- Eliminate the need to derivatize your sample.
- Use any LC/MS system.
- No need for baseline chromatographic resolution with MS detection.

Tips and tools

Agilent InfinityLab well plates and sealing mats are the ideal sample containers for your high-throughput LC/MS applications.

Visit: **www.agilent.com/chem/well-plates**

Agilent solutions for spent media analysis

Derivatization?

Yes

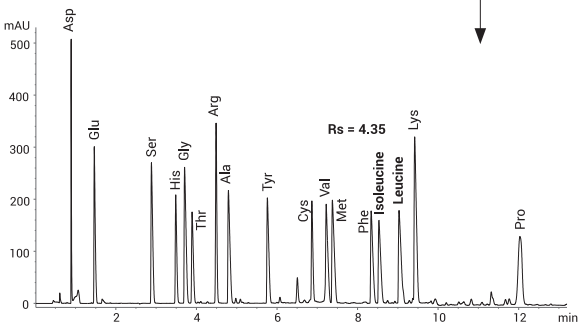
No

AdvanceBio
Amino Acid Analysis
Ready to use reagents

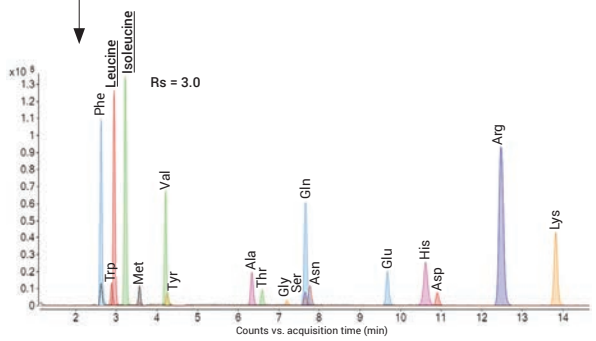
AdvanceBio Amino Acid Analysis
LC/UV

Fast, reliable results

AdvanceBio MS Spent Media
LC/MS



Amino acids



Amino acids and cell culture metabolites

AdvanceBio Amino Acid Analysis (AAA)

Agilent's AdvanceBio Amino Acid Analysis (AAA) columns deliver fast, sensitive, and reproducible separations of amino acids in protein hydrolysates and cell culture media.

AdvanceBio AAA includes proven reagents for amino acid derivatization, a ready-to-use amino acid standards kit, columns based on Agilent's innovative Poroshell technology, and expert support from Agilent. Alongside Agilent InfinityLab LC Series instruments, AdvanceBio AAA provides a complete solution for amino acid analysis.

These columns are part of the Agilent AdvanceBio family—designed as innovative solutions for biomolecule characterization.

- Reliable results: high resolution separations delivered by efficient Poroshell particle morphology.
- Reduced costs: long column lifetimes from robust, high-pH resistant, chemically modified silica.
- Increased flexibility: compatibility with both HPLC and UHPLC systems via 2.7 µm diameter particles.
- Quality control: AdvanceBio AAA columns are batch tested with amino acid standards to ensure quality.
- Easy ordering: standards and reagents available as kits.
- Automated online derivatization: with Agilent analytical injection systems.

Column Specifications

Bonded Phase	Particle Size	Pore Size	Temp Limit	pH Range	Endcapped	Pressure Limit
C18	2.7 µm	100 Å	60 °C	3.0–11.0	Double	600 bar

Tips and tools

To learn about Agilent's end-to-end solution for amino acid analysis, please visit: **www.agilent.com/chem/aaa-how-to-guide**

AdvanceBio Amino Acid Analysis (AAA) Columns

Description	Part No.
AdvanceBio Amino Acid Analysis 100 Å, 3.0 x 100 mm, 2.7 µm	695975-322
AdvanceBio Amino Acid Analysis 100 Å, 4.6 x 100 mm, 2.7 µm	655950-802
AdvanceBio Amino Acid Analysis 100 Å, 3.0 x 5 mm, 2.7 µm (3-pack guards)	823750-946
AdvanceBio Amino Acid Analysis 100 Å, 4.6 x 5 mm, 2.7 µm (3-pack guards)	820750-931

LC/UV

Column: **Agilent AdvanceBio Amino Acid Analysis 4.6 x 100 mm p/n 655950-802**

Column temperature: 30 °C

Mobile phase: Low pH, positive ion mode MS detection: A = 10 mM Na_2HPO_4, 10 mM Na2B4O7, pH 8.2 B = acetonitrile:methanol:water, 45:45:10 (v:v:v)

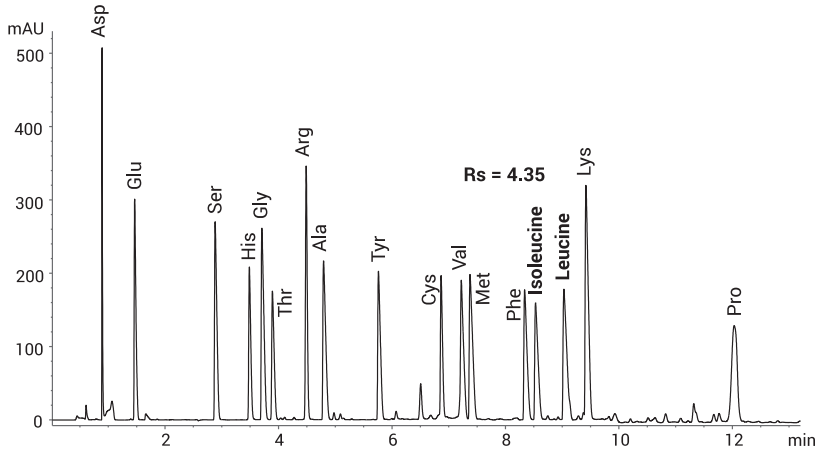
Flow rate: 1.5 mL/min

Gradient:

Time (min)	%B
0	2
0.35	2
13.4	57
13.5	100
15.7	100
15.7	2
18	end

Sample: Protein hydrolysate

Detection: Agilent 1260 Infinity II DAD WR



Ultraviolet chromatogram of amino acids from protein hydrolysate. The resolution between leucine and isoleucine is 4.35, which easily meets the European Pharamacopoeia requirement that resolution be greater than 1.5. [European Pharmacopoeia 9.0 (2.2.56) Amino Acid Analysis.]

AdvanceBio Amino Acid Analysis standards and kit

All necessary derivatization reagents and amino acid standards for quantitation are included in a single part number. Individual components may be reordered as needed.

AdvanceBio Amino Acid Analysis (AAA) Standards and Reagents

Description	Part No.
Standards and reagents kit	5190-9426
Kit contents (can be ordered separately)	
Buffer, borate, 100 mL	5061-3339
FMOC reagent, 10 ampoules, 1 mL each, for AAA	5061-3337
OPA reagent, 10 mg/mL, 6 ampoules, 1 mL each	5061-3335
Dithiodiproprionic acid (DTDPA), 5g	5062-2479
AA standard, 1 nmol, 10/pk	5061-3330
AA standard, 250 pmol, 10/pk	5061-3331
AA standard, 100 pmol, 10/pk	5061-3332
AA standard, 25 pmol, 10/pk	5061-3333
AA standard, 10 pmol, 10/pk	5061-3334
AA supplement, 1 g each	5062-2478

Buffer, borate, 100 mL,
5061-3339

AA supplement, 1 g each,
5062-2478

Each amino acid standard contains the following amino acids:

- Glycine
- L-cysteine
- L-histidine
- L-tyrosine
- L-leucine
- L-methionine
- L-serine
- L-alanine
- L-phenylalanine
- L-glutamic acid
- L-proline
- L-isoleucine
- L-arginine
- L-threonine
- L-valine
- L-lysine
- L-aspartic acid

AdvanceBio MS Spent Media

Agilent AdvanceBio MS Spent Media columns are HILIC columns that deliver fast, sensitive, and reproducible separations of underivatized amino acids and other polar metabolites found in bioprocessor cell culture media for mass spectrometric detection.

Alongside Agilent InfinityLab LC series instruments and Agilent MS instruments, AdvanceBio MS Spent Media provides a complete solution for spent media analysis.

AdvanceBio MS Spent Media analysis is a new addition to the Agilent AdvanceBio family, designed as innovative solutions for biomolecule production and characterization.

- Fast, MS-based workflow
- No sample derivatization needed, saving time and resources
- PEEK-lined stainless steel column hardware for an inert flow path to achieve excellent peak shape and recovery of challenging ionic metabolites
- Baseline chromatographic resolution of leucine and isoleucine isomers
- Columns are tested with amino acids to ensure quality and performance
- Excellent analytical sensitivity with MS-friendly mobile phases
- Compatibility with both HPLC and UHPLC systems via 2.7 µm Poroshell particle

Column Specifications

Bonded Phase	Pore Size	Particle Size	Temperature Limit	pH Range	Pressure Limit
HILIC-Z	100 Å	2.7 µm	80 °C (at pH 7)	3.0–11.0 (at 35 °C)	600 bar

AdvanceBio MS Spent Media

Description	Part No.
AdvanceBio MS Spent Media 100 Å, 2.1 x 50 mm, 2.7 µm	679775-901
AdvanceBio MS Spent Media 100 Å, 2.1 x 100 mm, 2.7 µm	675775-901
AdvanceBio MS Spent Media 100 Å, 2.1 x 150 mm, 2.7 µm	673775-901

LC/MS

Column: **Agilent AdvanceBio MS Spent Media 2.1 x 100 mm p/n 675775-901**

Column temperature: 30 °C

Mobile phase: Low pH, positive ion mode MS detection:
A = 10% 200 mM ammonium formate in water pH 3, 90% water
B = 10% 200 mM ammonium formate in water pH 3, 90% acetonitrile
Final salt concentration is 20 mM.
We recommend preparing mobile phases from a concentrated buffer stock to ensure robust and consistent mobile phases.

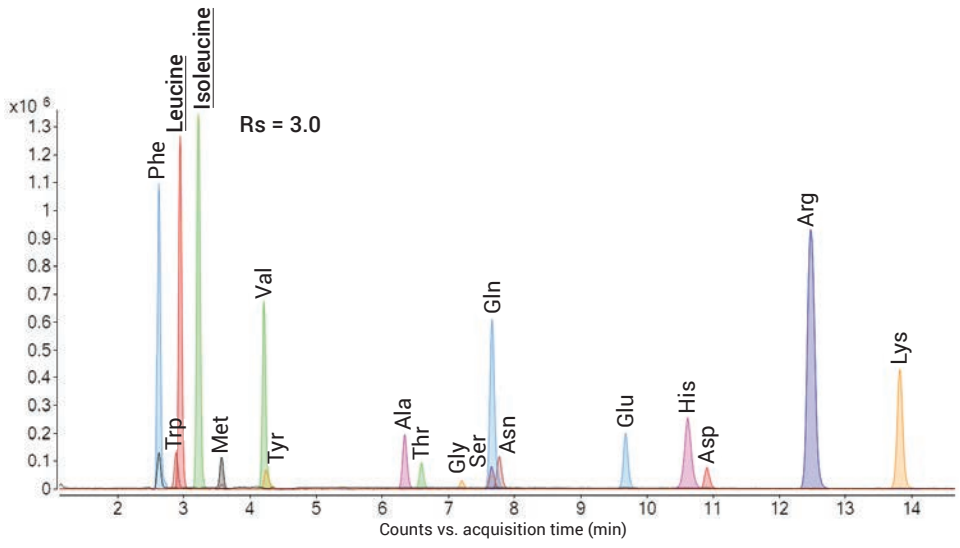
Flow rate: 0.5 mL/min

Gradient:

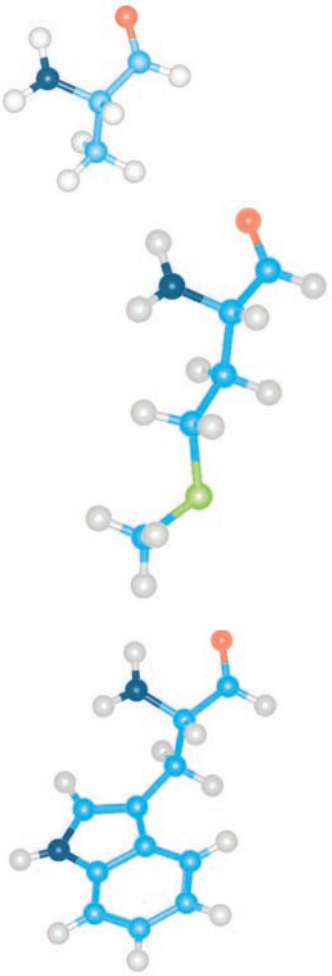
Time (min)	%B (Low pH, positive ion mode))	% B (High pH, negative ion mode)
0	1.00	100
15	80	80
15.5	100	100
20	100	100

Sample: Cell culture media, diluted five-fold with mobile phase B

Detection: Agilent 6230 time-of-flight LC/MS



ZORBAX Eclipse Amino Acid Analysis (AAA)



- Tested for amino acid analysis
- Uses well-known OPA and FMOC precolumn derivatization chemistry
- Easily automated using a detailed online, derivatization protocol available for use with the InfinityLab LC series

The ZORBAX Eclipse AAA column separates amino acids following an updated and improved protocol. Total analysis from injection-to-injection can be achieved in 14 min (9 min analysis time) on shorter, 75 mm length columns and 24 min (18 min analysis time) on the 150 mm column length. Sensitivity (5-50 pmol with DAD, FLD) and reliability are achieved using both OPA and FMOC derivatization chemistries in one fully automated procedure using the InfinityLab LC series.

For high speed amino acid analysis on UHPLC systems, ZORBAX Eclipse Plus C18 1.8 µm columns give excellent results.

ZORBAX Eclipse Amino Acid Analysis (AAA) Columns

Description	Size (mm)	Particle Size (µm)	Part No.
Analytical routine sensitivity	4.6 x 150	5	993400-902
Analytical routine sensitivity, high resolution using FLD	4.6 x 150	3.5	963400-902
Analytical routine sensitivity, high throughput	4.6 x 75	3.5	966400-902
Solvent Saver high sensitivity, high resolution	3.0 x 150	5	961400-302
Guard cartridges, 4/pk	4.6 x 12.5	5	820950-931
Guard hardware kit			820999-901

ZORBAX Eclipse Plus

Description	Size (mm)	Particle Size (µm)	Eclipse Plus C18 USP L1
Narrow bore RRHD, 1200 ba	2.1 x 50	1.8	959757-902
Narrow bore RRHT, 600 bar	2.1 x 50	1.8	959741-902

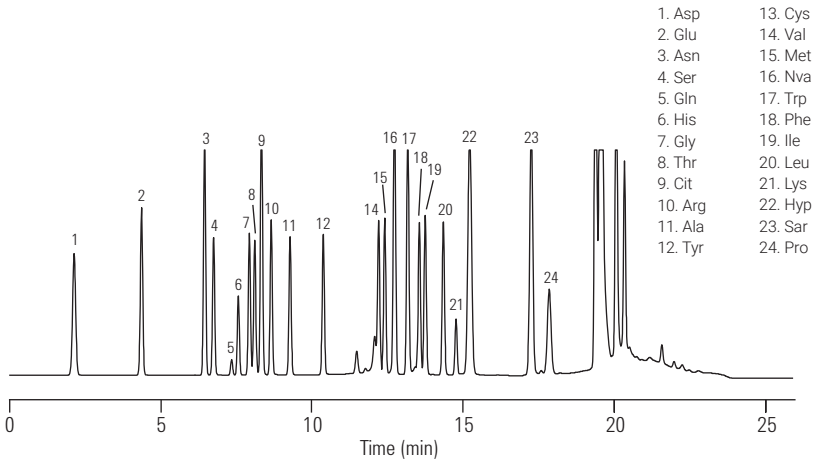
Tips and tools

Further information can be found in: *Automatic Precolumn Derivatization of Amino Acids and Analysis by Fast LC using the Agilent 1290 Infinity LC System* (publication **5990-5599EN**)

www.agilent.com/search

High resolution of 24 amino acids

Column:	**ZORBAX Eclipse AAA 963400-902 4.6 x 150 mm, 3.5 µm**
Mobile phase:	A: 40 mM Na_2HPO_4, pH 7.8
	B: ACN:MeOH:water, 45:45:10 v/v
Flow rate:	2 mL/min
Temperature:	40 °C
Detector:	Fluorescence
Sample:	24 amino acids



This high resolution separation of 24 amino acids is achieved in 18 minutes. If the Rapid Resolution 4.6 x 75 mm Eclipse AAA column is selected, these amino acids are resolved in 9 minutes.

Tips and tools

Quick reference guides list the common supplies you should have on hand to keep your Agilent InfinityLab LC series operating at peak efficiency. Download your free copy at **www.agilent.com/chem/getguides**

Protein Depletion

To more easily isolate and identify proteins in biological samples, such as serum, plasma, and cerebrospinal fluid (CSF), the multiple affinity removal system is designed to chromatographically eliminate interfering high-abundance proteins from biological samples. Removal of these abundant proteins improves the subsequent LC/MS and electrophoretic analysis of the sample by effectively expanding the dynamic range.

Agilent protein fractionation system and proteomics reagents

- LC/MS analysis of biological samples
- Preparation for electrophoretic analysis
- Sample preparation for biomarker discovery
- Instrument and workflow validation
- Cost-effective immunodepletion
- Sample desalting, concentration, and fractionation

For sample fractionation and desalting, the Agilent mRP-C18 high-recovery protein column is designed to simultaneously desalt, concentrate, and fractionate in one easy step with extremely high recovery of samples as compared to conventional RP-HPLC columns that are fully compatible with LC/MS analysis.

In addition, validated reagents for sample preparation in biomarker discovery and other proteomics applications are also available, including a complex standard, and proteomics grade trypsin. For your convenience, these reagents are fully compatible with Agilent LC/MS methods and require no additional sample pretreatments.

Large volume requirements and custom column dimensions can also be fulfilled with our custom configurations.

Multiple affinity removal system

The multiple affinity removal system enables the identification and characterization of high-value, low abundant proteins and biomarkers found in serum, plasma, and other biological fluids.

The multiple affinity removal system reproducibly and specifically removes up to fourteen high-abundant proteins found in human biological fluids and three high-abundant proteins found in mouse biological fluids.

The multiple affinity removal system is available in a variety of LC column dimensions and in spin cartridge format. When combined with Agilent optimized buffers, convenient spin filters, and concentrators, the multiple affinity removal system creates an automated, integrated depletion solution compatible with most LC instruments (columns), and bench-top centrifuges (spin cartridges).

Samples depleted using the multiple affinity removal system are ready for downstream analyses such as 2D gel electrophoresis, LC/MS, and other analytical techniques.

Multiple affinity removal system

Tips and tools

For more information on how to reduce your cycle time for affinity chromatography, see:

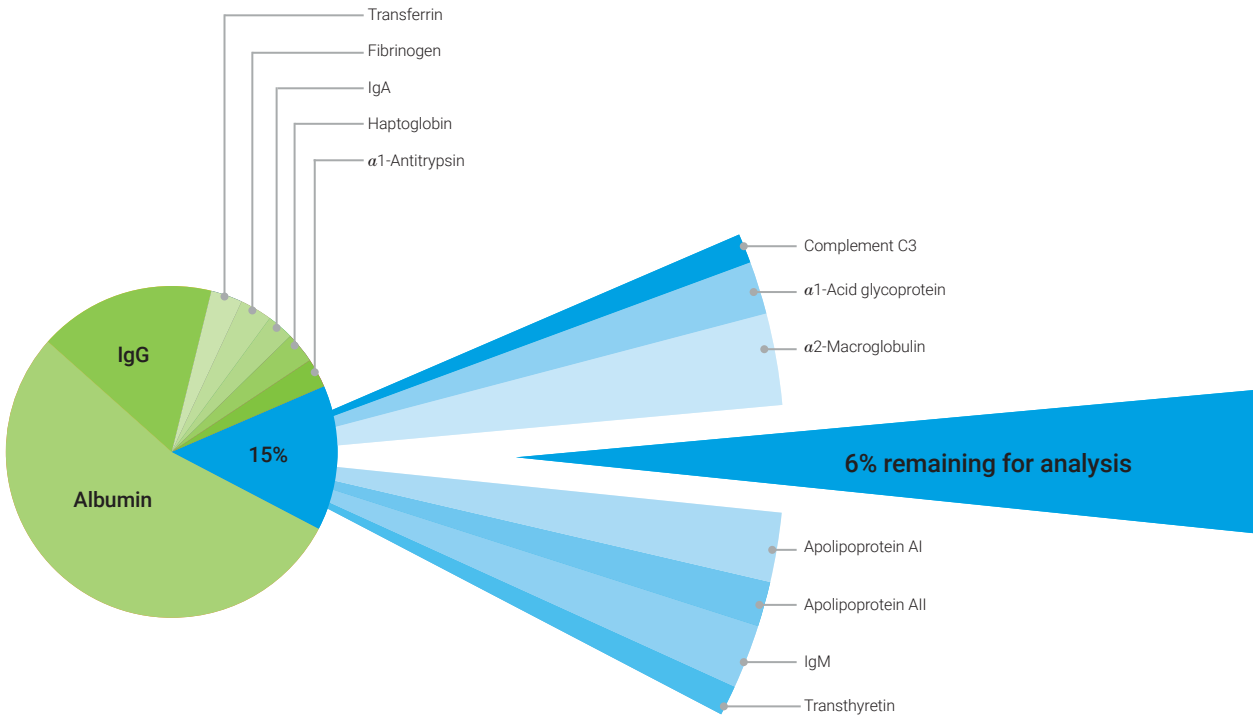
Reducing Cycle Time for Affinity Removal of High-Abundant Proteins in Human Plasma. Alternating Column Regeneration Using an Agilent 1200 Infinity Series Quick-Change Bio-inert 2-position/10-port Valve and an Agilent 1290 Infinity Flexible Cube (publication **5991-4721EN**)

www.agilent.com/search

Multiple Affinity Removal System Selection Guide

Product	Proteins Removed	Total Protein Removed	Dimensions	Load Capacity	Part No.
MARS Human-14	Albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein. AII, complement C3, transthyretin	94%	Spin cartridge	8–10 µL	5188-6560
			4.6 x 50 mm	20 µL	5188-6557
			4.6 x 100 mm	40 µL	5188-6558
			10.0 x 100 mm	250 µL	5188-6559
MARS Human-7	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, fibrinogen	88–92%	Spin cartridge	12–14 µL	5188-6408
			4.6 x 50 mm	30–35 µL	5188-6409
			4.6 x 100 mm	60–70 µL	5188-6410
			10.0 x 100 mm	250–300 µL	5188-6411
MARS Human-6	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin	85–90%	Spin cartridge	7–10 µL	5188-5230
			4.6 x 50 mm	15–20 µL	5185-5984
			4.6 x 100 mm	30–40 µL	5185-5985
MARS Human-High Capacity	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin	85–90%	Spin cartridge	14–16 µL	5188-5341
			4.6 x 50 mm	30–40 µL	5188-5332
			4.6 x 100 mm	60–80 µL	5188-5333
			10.0 x 100 mm	up to 340 µL	5188-5336
MARS Human-2	Albumin, IgG	69%	Spin cartridge	50 µL	5188-8825
			4.6 x 50 mm	100 µL	5188-8826
MARS Human-1	Albumin	50–55%	Spin cartridge	65 µL	5188-5334
			4.6 x 50 mm	130 µL	5188-6562
MARS Mouse-3	Albumin, IgG, transferrin	80%	Spin cartridge	25–30 µL	5190-2534
			4.6 x 50 mm	37–50 µL	5188-5217
			4.6 x 100 mm	75–100 µL	5188-5218

High abundance proteins removed by Agilent multiple affinity removal columns and spin cartridges



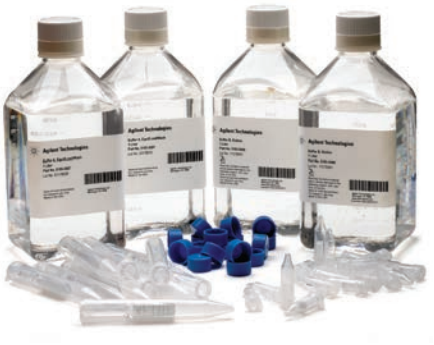
Tips and tools

Learn more about Agilent's complete services portfolio at **www.agilent.com/chem/services**

Multiple affinity removal system starter kits

The LC Column and Spin Cartridge reagent Starter Kits include all the required supplies to use with the multiple affinity removal system. These buffers provide optimal conditions for column longevity and sample reproducibility.

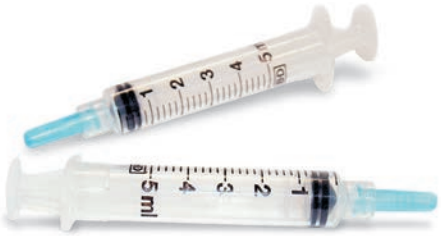
- The kits provide enough Buffer A and Buffer B for approximately 200 sample depletions using the 4.6 x 50 mm LC columns, approximately 100 sample depletions using the 4.6 x 100 mm LC columns and 200 spin cartridge uses.
- Buffer A, the loading buffer, minimizes protein-protein interactions, allowing low-abundant proteins often bound to high-abundant proteins to pass through the column, while the targeted high-abundant proteins bind to their associated antibodies.
- Buffer B, the elution buffer, then disrupts the antibody-protein interaction eluting the high-abundant proteins off the column.



LC column reagent starter kit, 5185-5986

Multiple Affinity Removal System Starter Kits

Description	Part No.
High concentration sample dilution buffer, 50 mL	5188-8283
LC column reagent starter kit includes:	5185-5986
Buffer A, for loading, washing, and equilibrating, 1 L	5185-5987
Buffer B, for eluting, 1 L	5185-5988
0.22 µm cellulose acetate, 25/pk, 1 L	5185-5990
Spin concentrators, 5K MWCO, 4 mL, 25/pk	5185-5991
Multiple affinity removal spin cartridge reagent kit includes:	5188-5254
Buffer A, for loading, washing, and equilibrating, 1 L	5185-5987
Buffer B, for eluting, 1 L	5185-5988
2 x spin filters, 0.22 µm cellulose acetate, 25/pk	5185-5990
Spin concentrators, 5K MWCO, 4 mL, 25/pk	5185-5991
Luer-Lok adapters, 2/pk	5188-5249
Plastic syringe, 5 mL, Luer-Lok, 2/pk	5188-5332
6 x microtube, 1.5 mL, screw top, 100/pk	5188-5251
Caps and plugs, 6/pk	5188-5252
PTFE needles, Luer-Lok, 10/pk	5188-5253



Luer-Lok syringe, 5188-5250

Luer-Lok adapters, 5188-5249

Luer-Lok needles, 5188-5253

Specialty Dimensions

Capillary and nano columns

- Highest sensitivity for your smallest sample sizes
- Compatible with all LC/MS interfaces
- Internal diameters of 0.5, 0.3, 0.1, and 0.075 mm
- 300 Å pore sizes for biomolecule analysis
- Ideal for 1D and 2D (proteomics) applications

Nano columns

ZORBAX capillary (0.5 and 0.3 mm id) and nano columns (0.1 and 0.075 mm id) are available in a wide variety of phases, and dimensions. These columns are ideal for very sample-limited applications because they provide enhanced sensitivity by reducing on-column sample dilution.

Tips and tools

Agilent offers various e-seminars and on-site training to help you learn how to be a more effective chromatographer.

For more information, visit: **www.agilent.com/chem/education**

High sensitivity protein digest analysis by LC/MS

Column: **ZORBAX 300SB-C18**
5065-9911
0.075 x 150 mm, 3.5 µm

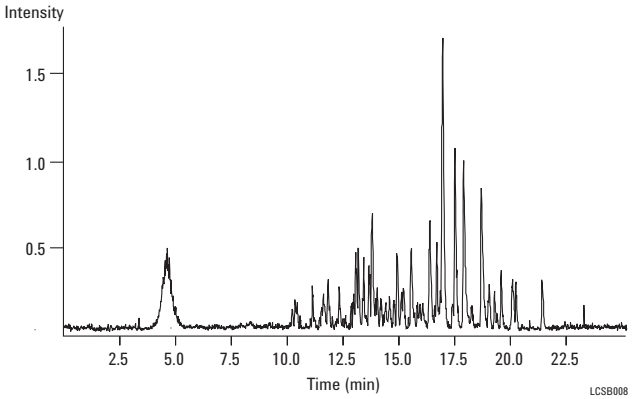
Mobile phase: A: Water + 0.1% formic acid
B: ACN + 0.1% formic acid

Flow rate: 600 nL/min

Gradient: 2% B to 52% B in 25 min

Detector: Positive ion nano Electrospray MS

Sample: Digest of eight proteins 100 fm (1 µL)



A ZORBAX nano HPLC column, 0.075 mm id, is used for high-sensitivity LC/MS analysis of a protein digest sample.

High sensitivity with capillary columns

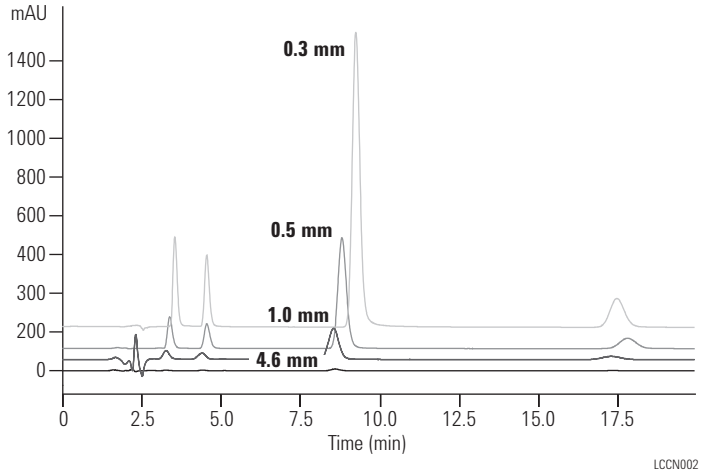
Column: **ZORBAX SB-C18**
5064-8255
0.3 x 150 mm, 5 µm

Column: **ZORBAX SB-C18**
5064-8256
0.5 x 150 mm, 5 µm

Column: **ZORBAX SB-C18**
863600-902
1.0 x 150 mm, 3.5 µm

Column: **ZORBAX SB-C18**
883975-902
4.6 x 150 mm, 5 µm

Sample: Biphenyl 200 ng



Sample-limited applications require capillary column dimensions to minimize on-column sample dilution and to enhance sensitivity. The 0.3 mm capillary in this example provides 100 times more sensitivity than the standard 4.6 mm column. Agilent nanobore (0.1 mm to 0.075 mm id) columns can provide up to 2,000 times more sensitivity for your most limited sample applications.

Human serum: low abundance protein isolation and identification from 1D gel band by LC/MS

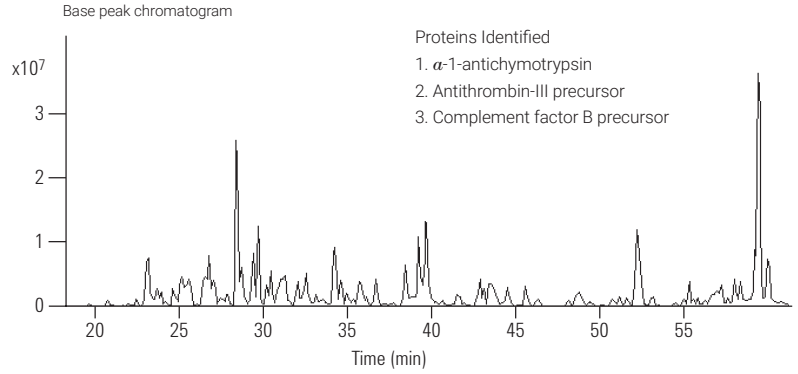
Column: **ZORBAX 300SB-C18**
Trap: 0.3 x 5 mm, 5 µm, 5065-9913
Analytical: 0.3 x 150 mm, 5 µm, 5064-8263

Mobile phase: A: Water + 0.1% formic acid
B: Acetonitrile + 0.1% formic acid

Flow rate: 6 µL/min

Gradient: 0 min 3% B
5 min 3% B (loading)
50 min 45% B
52 min 80% B
57 min 80% B
60 min 3% B

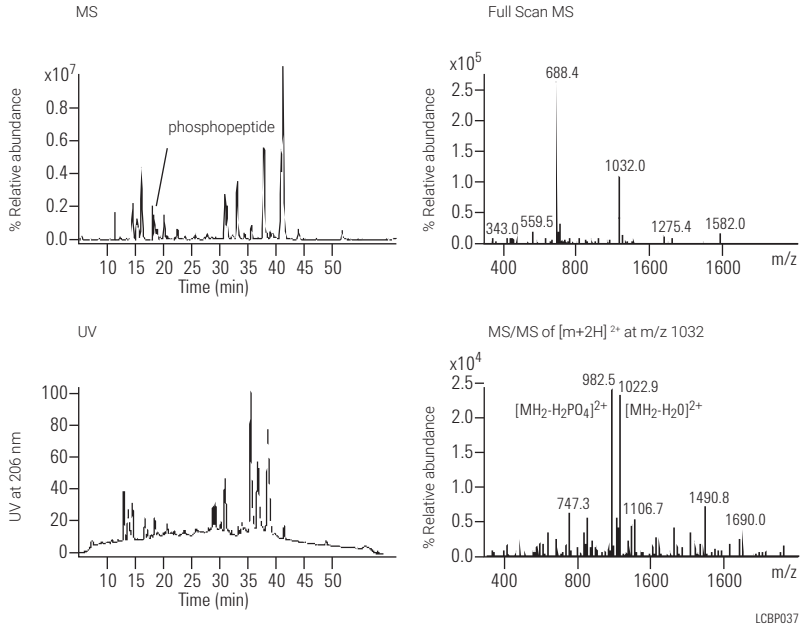
Sample: Band from 1D in gel digest



Sample preparation of human serum: Major serum proteins removed using multiple affinity removal column, 4.6 x 100 mm (p/n 5185-5985)
Followed by 1D gel digest

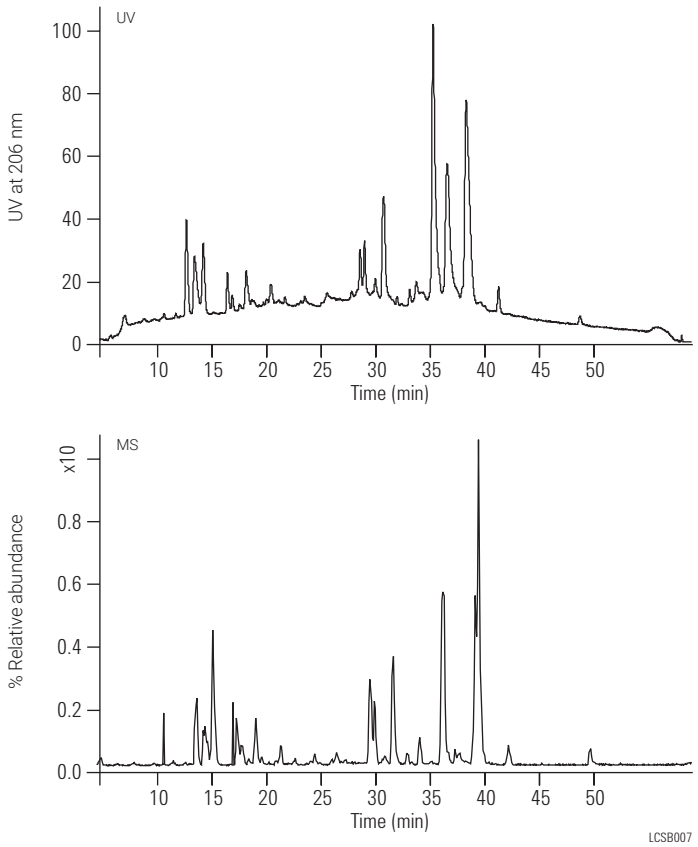
Peptide phosphorylation sites, LC and LC/MS using capillary LC columns

Column:	**ZORBAX 300SB-C18** **5064-8268** **0.5 x 150 mm, 3.5 µm**	
Mobile phase:	A: Water + 0.1% formic acid B: Acetonitrile + 0.1% formic acid	
Flow rate:	5.5 µL/min	
Gradient:	5-55% B in 50 min, to 85% B from 55-57 min	
Detector:	UV, 206 nm	
MS conditions:	LC/MS:	Pos. ion ESI with LC/MSD trap
	Vcap:	4,000 V
	Drying gas flow:	7 L/min
	Drying gas temperature:	250 °C
	Nebulizer:	15 psi
	Capillary exit volt:	50 V max
	Accum time:	300 ms
	Total averages:	3
	Isolation width:	3 m/z
	Frag amplitude:	1.0 V
Sample:	Beta case in digest 100 nL (4 pmol)	



Capillary columns for HPLC analyses with UV and MS detection

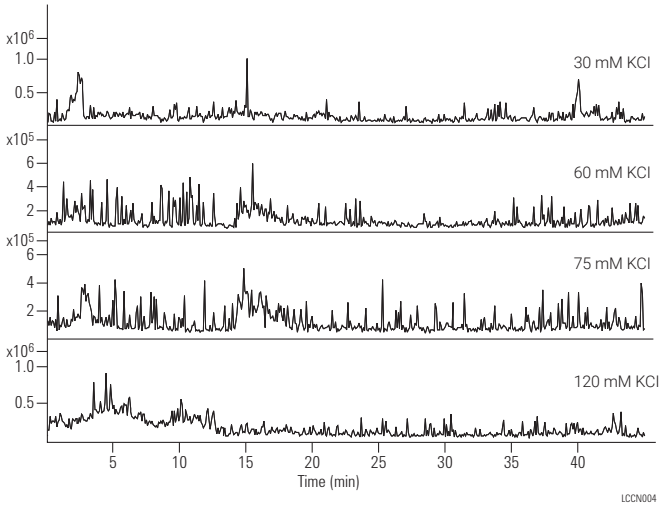
Column:	**ZORBAX 300SB-C18** **5064-8263** **0.3 x 150 mm, 5 µm**	
Mobile phase:	5-55% B in 50 min, to 85% B from 55-57 min A: Water + 0.1% formic acid B: Acetonitrile + 0.1% formic acid	
Flow rate:	5.5 µL/min	
Detector:	UV, 206 nm	
MS conditions:	LC/MS:	Pos. ion ESI with LC/MSD trap, Vcap 4,000 V
Drying gas flow:	7 L/min	
	Drying gas temperature:	250 °C
	Nebulizer:	15 psi
	Capillary exit volt:	50 V
	Max accum time:	300 ms
	Total averages:	3
	Isolation width:	3 m/z
	Frag amplitude:	1.0 V
Sample:	Beta casein digest 100 nL (4 pmol)	



A ZORBAX 300SB-C18 capillary column (0.3 mm id) is used for the separation of the protein digest. Detection is by both UV and Electrospray MS. MS detection can be used for identification of peptide fragments.

Proteins in a complex sample by 2D HPLC with nano HPLC columns

Column:	**ZORBAX 300SB-C18** **5065-9913** **0.3 x 5 mm, 5 µm**
Column:	**ZORBAX 300SB-C18** **5065-9911** **0.075 x 150 mm, 3.5 µm**
Mobile phase:	Quaternary pump: 3% acetonitrile:0.1% formic acid Nano pump: A; water, 0.1% formic acid, B; ACN, 0.1% formic acid
Flow rate:	Quaternary pump: 30 µL/min Nano pump: 300 nL/min
Gradient:	Quaternary pump: isocratic Nano pump: 6 min = 3% B, 120 min = 60% B, 125 min = 80% B, 130 min = 80% B, 131 min = 3% B, 140 min = 3% B
MS conditions:	Source: nano ESI, drying gas flow: 5 L/min, drying gas temp: 225 °C Ion trap: skim: 1:35 V, cap exit offset: 115 V, octupole 1:12 V, octupole 2:3.5 V, trap drive: 80 V. ICC: on, averages: 4, max accum time: 150 ms; target 60.000, ion mode positive, MS/MS mode
Sample:	Tryptic digest of bovine serum albumin Volume: 1 to 8 µL Salt step elution: 8 mL of 10 mM to 100 mM KCl (10 mM increments), 125 mM, 150 mM, 200 mM, 300 mM, 500 mM, 1 M



Tryptic digest of bovine serum albumin (BSA). The base peak chromatograms show a selection of fractions from a 2D HPLC separation. Single chromatograms represent peptides from BSA eluting at a given salt concentration followed by enrichment and reversed-phase chromatography.

ZORBAX Bio-SCX Series II

ZORBAX Bio-SCX Series II columns are designed for optimized 2D separations of peptides and proteins using LC/MS. This packing is based on ultrapure 3.5 µm ZORBAX silica particles, bonded with a bio-friendly polymer that is functionalized with sulfonic acid groups. This gives strong retention and good peak shape in the ion-exchange step of 2D analysis of peptides and proteins.

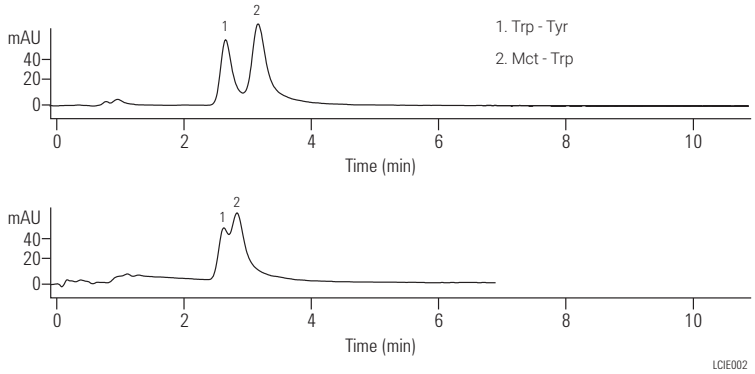
Nano columns

HPLC Column Specifications

Bonded Phase	Pore Size	Surface Area	pH Range	Functionality	Max Pressure
ZORBAX Bio-SCX Series II	300 Å	90 m^2/g	2.5-8.5	Sulfonic acid	350 bar

More retention of small peptides

Column:	**ZORBAX Bio-SCX Series II 5065-9912 0.3 x 35 mm, 3.5 µm**
Mobile phase:	95% 40 mM NaCl: 5% ACN, 0.3% formic acid
Flow rate:	5 µL/min
Detector:	UV, 230 nm
Sample:	Synthetic dipeptides



The ZORBAX Bio-SCX Series II column retains smaller peptides more strongly than some other SCX columns. The result is increased resolution of more hydrophilic peptide fragments and more accurate identification when these columns are used in 2D HPLC analysis.

Tips and tools

Agilent bio-inert supplies provide a metal-free sample flow path, which minimizes interactions with your biomolecules.

Visit: **www.agilent.com/chem/bio-inert-uhplc**

ZORBAX HPLC Capillary Columns (Glass-Lined Stainless Steel)

Description	Size (mm)	Particle Size (mm)	300SB-C18	300SB-C8	300Extend-C18
Capillary	0.5 x 250	5	5064-8266		
Capillary	0.5 x 150	5	5064-8264		
Capillary RR	0.5 x 150	3.5	5064-8268		
Capillary	0.5 x 35	5	5064-8294		
Capillary RR	0.5 x 35	3.5	5065-4459		
Capillary	0.3 x 250	5	5064-8265		
Capillary	0.3 x 150	5	5064-8263		
Capillary	0.3 x 35	5	5064-8295		
Capillary RR	0.3 x 150	3.5	5064-8267	5065-4460	5065-4464
Capillary RR	0.3 x 100	3.5	5064-8259	5065-4461	5065-4465
Capillary RR	0.3 x 75	3.5	5064-8270	5065-4462	5065-4466
Capillary RR	0.3 x 50	3.5	5064-8300	5065-4463	5065-4467
Replacement screens, 10/pk			5065-4427	5065-4427	5065-4427

ZORBAX Nano HPLC Columns (PEEK)

Description	Size (mm)	Particle Size (mm)	300SB-C18 USP L1	300SB-C8 USP L7
Nano RR	0.1 x 150	3.5	5065-9910	
Nano RR	0.075 x 150	3.5	5065-9911	
Nano RR	0.075 x 50	3.5	5065-9924	5065-9923
Trap/guard, 5/pk	0.3 x 5	5	5065-9913	5065-9914
Trap/guard hardware kit			5065-9915	5065-9915

ZORBAX 300SB-C18 trap/guard, 5065-9913

MicroBore (1.0 mm id) columns

- High sensitivity for small sample sizes
- Compatible with LC/MS interfaces
- Wide variety of bonded phases
- Silica and polymeric particles

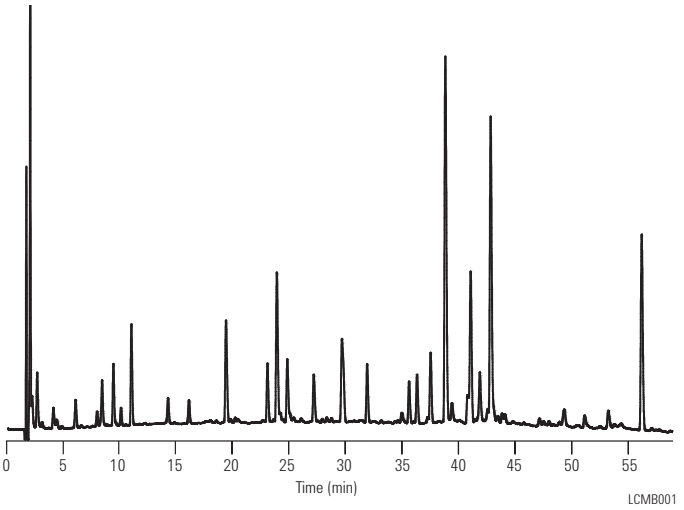
Sterically protected 300StableBond bonded phase

MicroBore (1.0 mm id) columns are a good choice when sample sizes are limited. They can improve detection limits by five times, compared to 2.1 mm id columns when the same sample mass is used. This increase in sensitivity can be critical. MicroBore columns use low flow rates (typically ~50 µL/min). Therefore, these columns are ideal for use with detectors requiring low flow rates such as some mass spectrometers and with capillary LC systems.

Optimum performance is achieved when MicroBore columns are used with UHPLC/HPLC Microbore systems. A wide variety of bonded phases is available for up to 400 bar including StableBond, 300SB-C18, 300SB-C8, and Poroshell columns. Polymeric reversed-phase, PLRP-S, and ion-exchange PL-SAX and PL-SCX are also available for applications requiring exceptionally stable wide-pore particles. Guard columns are also now available with an adjustable tube depth-stop to provide perfect zero dead volume connection every time.

Separation of a tryptic digest

Column:	**ZORBAX 300SB-C18** **863630-902** **1.0 x 150 mm, 3.5 µm**
Mobile phase:	Gradient: 2-60% B in 60 min A: 0.1% TFA B: 0.075% TFA:80% ACN
Flow rate:	50 µL/min
Temperature:	50 °C
Detector:	UV, 215 nm
Sample:	Tryptic digest of rhGH 2 µL



This example of a tryptic digest separated on a MicroBore column demonstrates the high sensitivity and resolution possible with 1.0 mm id columns.

Microbore HPLC for sensitive peptide analysis

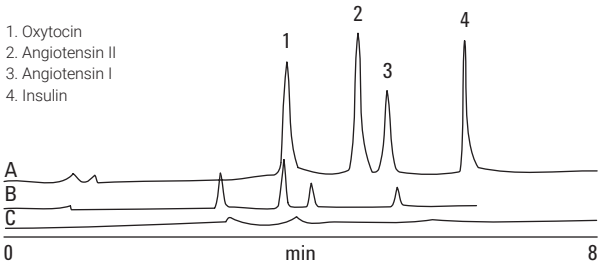
Column:	**PLRP-S 100 Å 5 µm 150 mm x various id**
Mobile phase:	A: 0.01 M tris HCl, pH 8 B: A + 0.35 M NaCl, pH 8
Flow rate:	1 mL/min
Gradient:	linear 20% ACN, 0.1% TFA to 50% ACN, 0.1% TFA over 15 min
Injection volume:	0.5 µL
Sample conc:	0.25 mg/mL
Detector:	UV, 220 nm

Peak identification

A. 1.0 mm id (flow rate 47 µL/min)

B. 2.1 mm id (flow rate 200 µL/min)

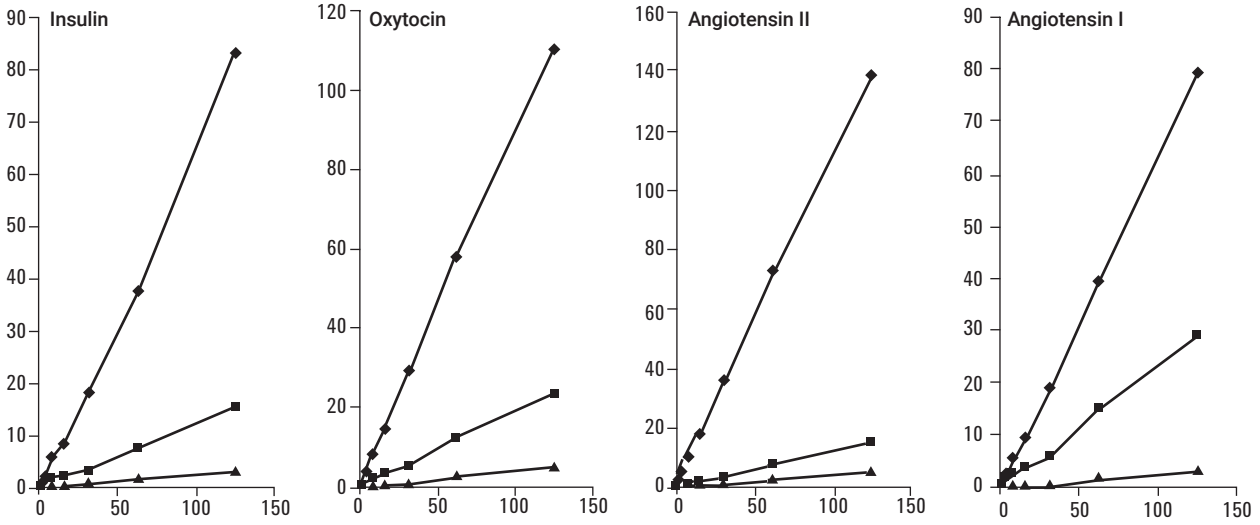
C. 4.6 mm id (flow rate 1 mL/min)



Peptide separation on PLRP-S 100 Å 5 µm columns

Peak identification

- **1.0 mm**
- **2.1 mm**
- **4.6 mm**



Standard curve data-point graphs on PLRP-S columns. Reduced column internal diameter reduces the limit of detection and enables quantitation of lower amounts of sample.

MicroBore (1.0 mm id)

Description	Size (mm)	Particle Size (mm)	300SB-C18 USP L1	300SB-C8 USP L7
MicroBore	1.0 x 250	5	861630-902	
MicroBore RR	1.0 x 150	3.5	863630-902	863630-906
MicroBore RR	1.0 x 50	3.5	865630-902	865630-906
MicroBore guard, 3/pk	1.0 x 17	5	5185-5920	5185-5920

Description	Size (mm)	Particle Size (mm)	Poroshell 300SB-C18	Poroshell 300SB-C8	Poroshell 300SB-C3	Poroshell 300Extend-C18
MicroBore	1.0 x 75	5	661750-902	661750-906	661750-909	671750-902
MicroBore guard, 3/pk	1.0 x 17	5	5185-5968	5185-5968	5185-5968	

Description	Size (mm)	Particle Size (µm)	PLRP-S 100 Å USP L21	PLRP-S 300 Å USP L21	PLRP-S 1000 Å USP L21	PLRP-S 4000 Å USP L21
MicroBore	1.0 x 150	3	PL1312-3300			
MicroBore	1.0 x 50	8			PL1312-1802	PL1312-1803
MicroBore	1.0 x 50	5	PL1312-1500	PL1312-1501	PL1312-1502	PL1312-1503
MicroBore	1.0 x 50	3	PL1312-1300	PL1312-1301		

Description	Size (mm)	Particle Size (µm)	PL-SAX 1000 Å	PL-SAX 4000 Å	PL-SCX 1000 Å	PL-SCX 4000 Å
MicroBore	1.0 x 50	5	PL1351-1502	PL1351-1503	PL1345-1502	PL1345-1503

2D-LC

- Combine two orthogonal LC techniques into a single analysis
- Provides greater peak capacity compared even with UHPLC methods
- Interfaces size exclusion and ion-exchange methods to MS

The heterogeneity and complexity of biomolecules necessitates the use of multiple LC techniques to identify and characterize a target biopharmaceutical. By combining two orthogonal techniques into one analysis, for example hydrophilic interaction and reversed-phase, or cation-exchange and reversed-phase, you can achieve unmatched separating power to enable identification and analysis of critical quality attributes.

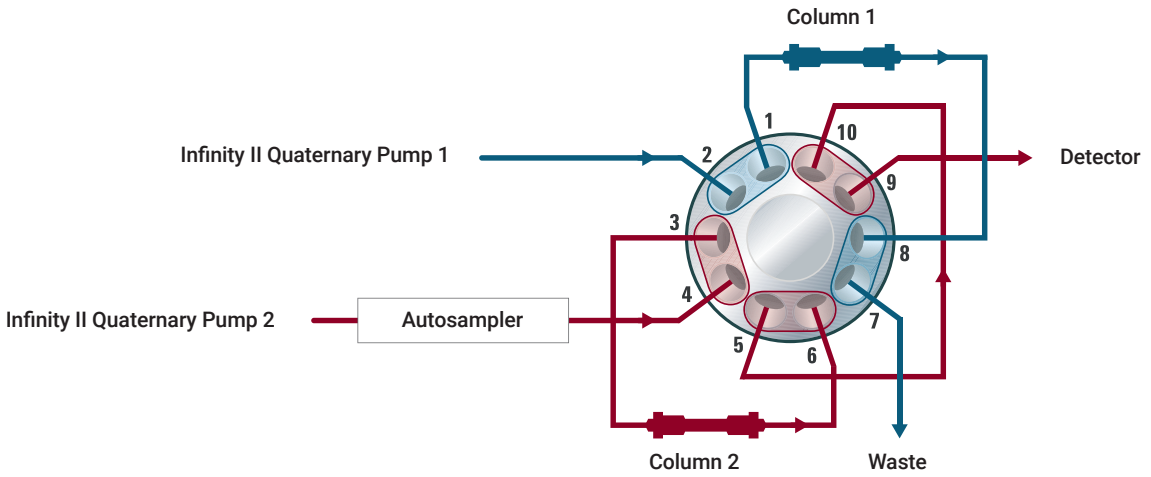
Applications for 2D-LC range from simple online desalting of a fraction obtained from a Protein A capture of a monoclonal antibody, to interfacing hydrophilic interaction and reversed-phase peptide mapping methods to characterize the full spectrum of peptide fragments—hydrophilic, glyco-, and hydrophobic peptide fragments.

Achieve increased productivity by using 2D-LC to shorten analysis times and maximize data generation/interpretation:

- Method scouting and application switching
- Offline column regeneration
- Online impurity analysis
- Heart-cutting 2D-LC
- Comprehensive 2D-LC

Offline column regeneration

For robust charge variant analysis using ion-exchange LC, a robust column cleanup and equilibration is required. This adds time to the total analysis and so ways of decreasing this time to increase sample throughput are needed. One way of doing this is to use offline column regeneration, which has been shown to reduce cycle time by as much as 40%.



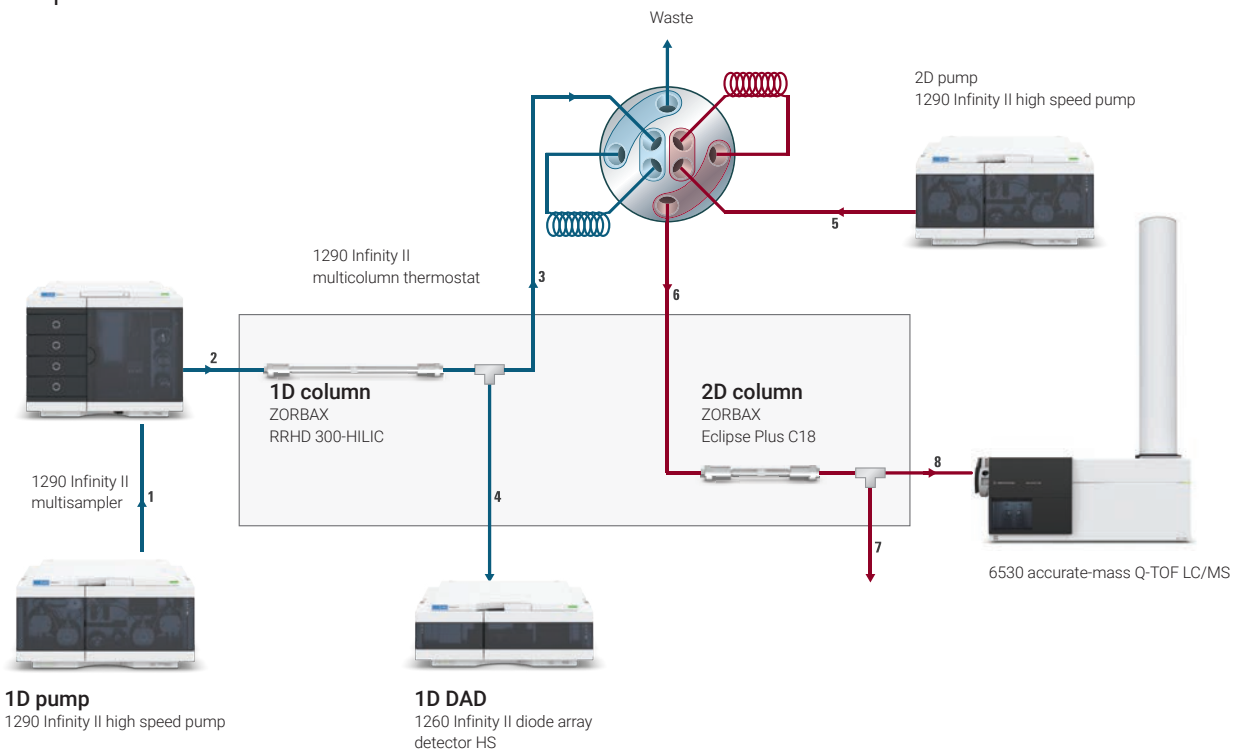
A schematic showing the valve arrangement required for offline column regeneration as would be used for mAb charge variant analysis using the 1260 Infinity II bio-inert LC, and two Bio MAb PEEK, 2.1 x 250 mm, 5 µm columns.

Intra- (n = 6) and Inter-column (n = 12) Precision of Retention Time and Area

	Intra-column % RSD RT	Intra-column % RSD Area	Inter-column % RSD RT	Inter-column % RSD Area
CV1	0.205	2..50	0.247	3.39
CV2	0.183	1.91	0.218	1.63
CV3	0.247	1.13	0277	2.56
CV4	0.302	6.73	0.286	6.67
CV5	0.301	1.63	0.255	1.41
CV6	0.252	2.78	0.213	2.93

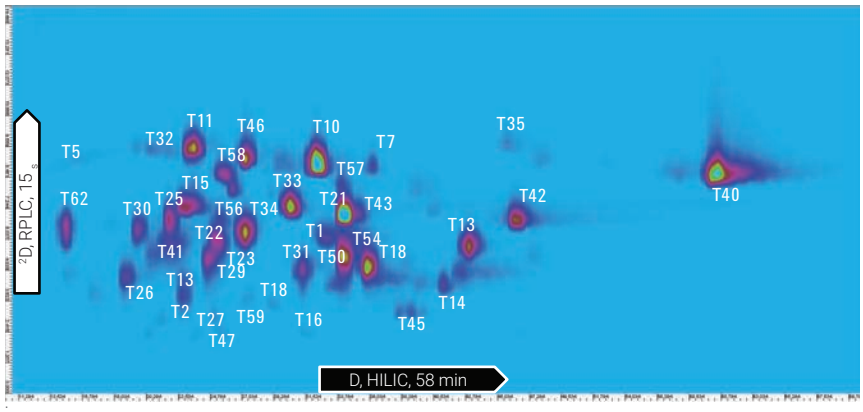
Good inter- and intra-column precision of retention time and area is achieved for the offline regeneration of the Bio MAb PEEK column.
Intra-column (n = 6) and inter-column (n = 12).

Comprehensive 2D-LC



1	1D pump to autosampler	Calibration capillary (G1312-67500)
2	Autosampler to 1D column (1.6 µL heat exchanger)	Stainless steel, 0.17 mm
3	Tee 1 to 2D-LC valve	Stainless steel, 0.12 x 200 mm
4	Tee 1 to 1D DAD	Stainless steel, 0.12 x 140 mm
5	2D pump to 2D-LC valve	Stainless steel, 0.17 mm
6	2D-LC valve to 2D column (1.6 µL heat exchanger)	Stainless steel, 0.12 x 270 mm
7	Tee 2 to waste	Stainless steel, 0.12 x 340 mm
8	Tee 2 to detector (AJS source or DAD 2D)	Stainless steel, 0.075 x 340 mm (5067-4783)

InfinityLab 2D-LC system configuration for comprehensive 2D analysis of monoclonal antibody digests, and peptide mapping, using HILIC and RPLC-MS.



LCxLC contour plot for the analysis of the tryptic digest of trastuzumab generated with MS total ion count data. The two dimensions, HILIC in the first and RP in the second, had good orthogonality for this analysis.

Agilent has a wide range of phases available in the column sizes required. If you cannot find the combination of pore size, particle size, phase, and column size you require, contact our custom columns team who have a wealth of expertise in producing columns to meet specific performance criteria.

Tips and tools

To find out more about the fundamentals of 2D-LC, see our 2D-LC primer: *Principles, Practical Implementation and Applicati ons of Two-Dimensional Liquid Chromatography* (publication **5991-2359EN**)

www.agilent.com/chem/2DLC-Primer

Purification—Prep HPLC

Agilent has a comprehensive range of silica and polymeric HPLC columns and media designed for biomolecule purification. There are high efficiency small particle prep columns optimized for the purification of µg and mg amounts of a biopharmaceutical drug candidate, and fully porous bulk media, to pack development and process columns to purify g, kg, and multi-kg of API.

Some columns are specifically designed to address the needs of high-efficiency purification, while other products provide easy scale-up from small particle analytical columns to full-scale API production. Table 1 shows prep column/media options and the quantity of product that can be purified.

Polymeric Prep HPLC columns

Biopharmaceutical Lifecycle		Discovery µ mg high efficiency	Development g	Production kg multi-kg high throughput
Reversed-phase	mrP-C18	→		
	ZORBAX Prep HT 300 Å StableBond	→	→	
	VariTide RPC	→	→	→
	PLRP-S 100 Å, 300 Å, 1000 Å, 4000 Å	→	→	→
Ion-exchange	Bio MAb	→	→	
	Bio IEX	→	→	
	PL-SAX	→	→	→
	PL-SCX	→	→	→
Size exclusion	Bio SEC-3 Bio SEC-5	→	→	

Agilent columns and media for biomolecule purification—chromatographic type, product family, and purification scale.

Purification Column Selection

Application	Technique	Notes	Agilent Columns
Proteomics	Reversed-phase	A specialist high recovery column for proteomics applications. It is designed for µg scale purification with maximum recovery.	mRP-C18
All biomolecules	Reversed-phase	High efficiency 300 Å silica-based particles.	ZORBAX PrepHT 300SB
Synthetic peptides	Reversed-phase	Polymeric material designed for the purification of synthetic peptides. It is a high efficiency single-column solution for the full range of synthetic peptides, acidic, basic, hydrophobic, and hydrophilic, and covers the size range of peptides produced by both solution and solid phase synthesis.	VariTide RPC
All biomolecules	Reversed-phase	The premium polymeric reversed-phase family with a range of pore sizes and particle sizes to enable high efficiency laboratory scale purification using small particle prep columns, and scale-up to high-yield production purification with larger particles at the process scale. Use PLRP-S when purification will be scaled up to produce APIs and will need regulatory documentation.	PLRP-S
		– 3 µm and 5 µm for high efficiency – 8 µm, 10 µm, 10-15 µm, 15-20 µm, 30 µm, and 50 µm particles for larger scale and low pressure purification	
Monoclonal antibodies	Ion-exchange	A nonporous weak cation-exchanger	Bio MAb
All biomolecules	Ion-exchange	Nonporous ion-exchangers	Bio IEX
		SAX, WAX, SCX, and WCX functionalities to provide options for purification of acidic and basic molecules Nonporous 5 µm particle for highest efficiency lab prep	
All biomolecules	Ion-exchange	A fully porous strong anion-exchanger	PL-SAX
		– 5 µm particle size for high-efficiency separations – 8 µm, 10 µm, and 30 µm particles for larger scale medium and low pressure purification	
		A fully porous strong cation-exchanger	PL-SCX
		– 5 µm particle size for high-efficiency separations – 8 µm, 10 µm, and 30 µm particles for larger scale medium and low pressure purification	
All biomolecules	Size exclusion	Silica based SEC materials with a range of pore sizes	Bio SEC-3 and 5
		– 3 µm, and 5 µm particle sizes for high efficency – Pore sizes for 100 Å to 2000 Å cover a wide range of sample molecular sizes	

Tips and tools

Further information can be found in:

Biomolecule Purification (publication **5990-8335EN**)

www.agilent.com/search

mRP-C18 high-recovery protein columns

The mRP (macroporous reversed-phase) C18 high-recovery protein column is designed for high recovery, high-resolution separation, fractionation, and simultaneous desalting of complex protein samples, such as immunodepleted serum or plasma proteins.

- Greater than 95 to 99% protein sample recovery has been observed with immunodepleted serum using the multiple affinity removal system—LC column
- Can load up to 380 µg of total protein mass without reducing chromatographic resolution of the proteins
- Column packed with macroporous C18-bonded ultrapure 5 µm particle silica designed to reduce or eliminate strong adsorption of proteins
- Maximum operating pressure of 250 bar (4,000 psi)
- Compatible with water and all common organic solvents

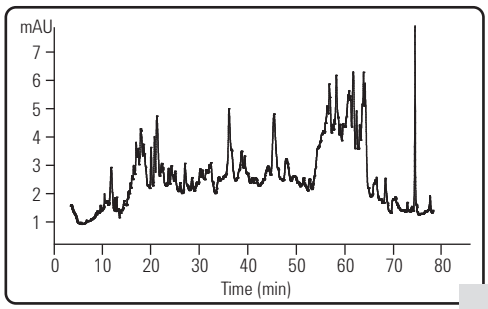
mRP-C18 high-recovery protein column, 4.6 x 50 mm, 5188-5231

mRP-C18 High-Recovery Protein Columns

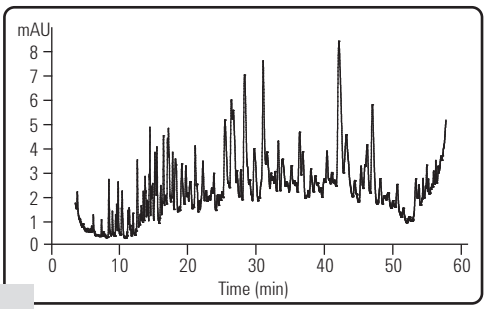
Description	Protein Load Capacity	Part No.
mRP-C18, 0.5 x 100 mm	10 ng-5 µg	5188-6510
mRP-C18, 2.1 x 75 mm	8-85 µg	5188-6511
mRP-C18, 4.6 x 50 mm	40-380 µg	5188-5231

Protein fractionation of complex samples on the mRP column

mRP-C18, 4.6 x 50 mm

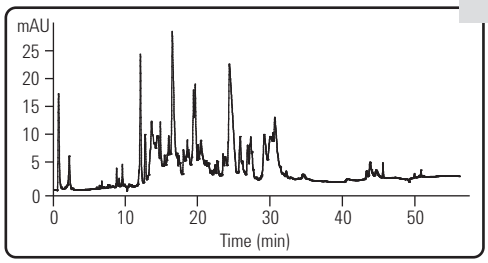


Hela membrane prep

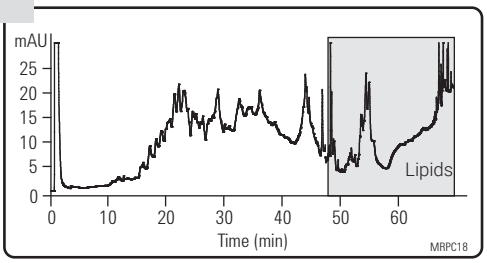


Hela cell lysate (352 µg)

Highest recovery



"Top-6" depleted human serum



Human brain membrane lipid raft prep (500 µg)

ZORBAX PrepHT

- Easy scale-up from analytical to preparative scale with ZORBAX phases
- Fast preparative separations, up to 2,000 mg
- 5 to 7 µm particles for high efficiency and high yield
- Easy to install finger-tight connections seal up to 5,000 psi/350 bar
- Use to maintain selectivity of the analytical phase in your prep separations

High purity, high recovery, and high throughput can be easily achieved with ZORBAX PrepHT columns. Available in a variety of bonded phases—StableBond 300 Å, C18, C8, C3, and CN—for optimized resolution and loadability under any conditions.

ZORBAX PrepHT columns are packed with 5 and 7 µm particle sizes for very high resolution. The high resolution allows high loadability, high yield, and high purity of compounds. The larger diameter columns and mechanically stronger ZORBAX particles allow for flow rates up to 100 mL/min, thus increasing throughput.

ZORBAX PrepHT columns are designed for rapid scale-up from analytical to preparative scale without losing resolution. For complex separations on larger columns (21.2 mm id, 150 mm length and longer), Agilent has carefully chosen the 7 µm particle size to achieve a balance between high efficiency and high loadability.

ZORBAX 300 Å StableBond Prep HT Cartridge columns

ZORBAX 300 Å StableBond

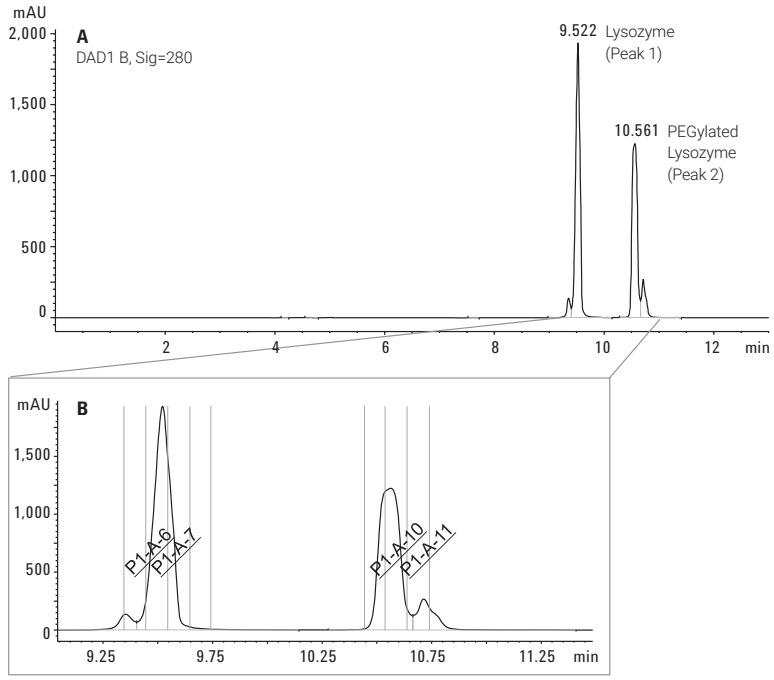
Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56
PrepHT Cartridge Columns (require endfittings kit 820400-901)						
PrepHT cartridge	21.2 x 250	7	897250-102	897250-106	897250-105	897250-109
PrepHT cartridge	21.2 x 150	7	897150-102	897150-106		897150-109
PrepHT cartridge	21.2 x 150	5	895150-902	895150-906		895150-909
PrepHT cartridge	21.2 x 100	5	895100-902	895100-906		895100-909
PrepHT cartridge	21.2 x 50	5	895050-902	895050-906		895050-909
PrepHT endfittings, 2/pk			820400-901	820400-901	820400-901	820400-901
PrepHT guard cartridge, 2/pk	17.0 x 7.5	5	820212-921	820212-918	820212-924	820212-924
Guard cartridge hardware			820444-901	820444-901	820444-901	820444-901

Semipreparative RP-HPLC of PEG lysozyme reaction mixture

Column: **ZORBAX SB-C18**
880975-202
9.4 x 250 mm, 5 µm

A: Semipreparative RP-HPLC of PEG lysozyme reaction mixture on a ZORBAX semipreparative 300 SB-C18 column

B: Zoom-in showing fraction collection



PLRP-S for prep to process

- Discovery stage to multi-kg CGMP production reduces method development time
- Chemical stability for separations, optimization, sanitation, and regeneration increases selectivity and column lifetime
- Single batch packing of multiple columns reduces system downtime and validation costs

The PLRP-S media, rigid poly(styrene/divinylbenzene) particles, are available in a range of pore sizes for small molecule, synthetic biomolecule, and macromolecule purification. Their thermal and chemical stability makes them ideal for purifications that require extreme conditions for sample preparation, compound elution, and column regeneration.

Capacity and resolution are two key parameters for maximizing the throughput of a purification. With a wide choice of pore sizes and extended range of operating conditions, PLRP-S provides more options to achieve the optimum process. Particle sizes range from 3 µm to 50 µm for scale-up from the µg/mg discovery stage to multi-kg CGMP production. Excellent chemical stability, up to 1 M NaoH, permits sanitation and regeneration that increase column lifetime. PLRP-S media batch sizes of up to 600 L are available, providing single batch packing of multiple columns.

As part of our commitment to quality and continuity of supply, all manufacturing is carried out under a fully documented process, and facility audits are routinely conducted.

PLRP-S Prep to Process Application Guide

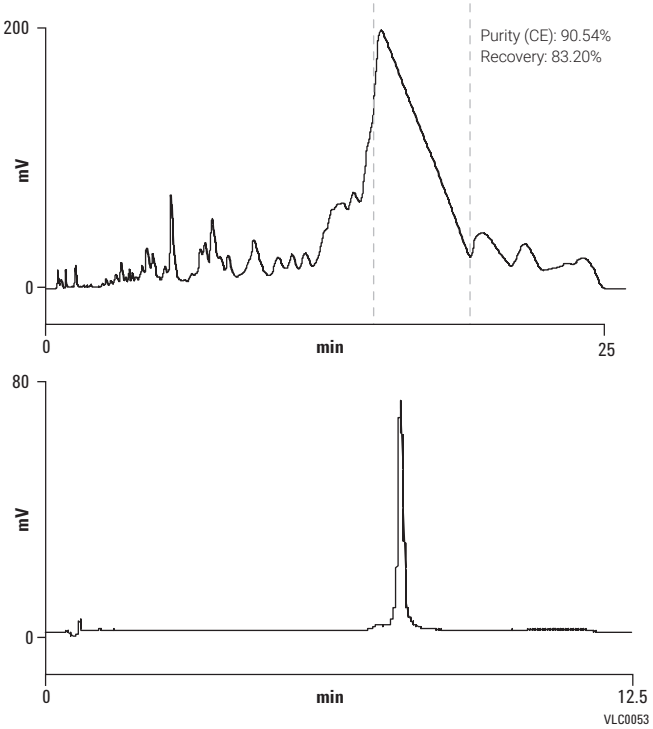
	PLRP-S Media Pore Size			
Application	**100 Å**	**300 Å**	**1000 Å**	**4000 Å**
Synthetic biomolecules, peptides, oligonucleotides	✓	✓		
Recombinant biomolecules, peptides, proteins	✓	✓		
Large biomolecules, antibodies, DNA fragments			✓	✓
Small molecules, unstable compounds including metal sensitivity	✓			

UHPLC Column Specifications

pH range	1–14
Buffer content	Unlimited
Organic modifier	1–100%
Temperature limits	200 °C
Maximum pressure	5–8 µm: 3,000 psi (210 bar)
	3 µm: 4,000 psi (300 bar)

Purification of a 25-mer trityl-off oligonucleotide and analytical quantitation of the fraction using PLRP-S 100 Å, 4.6 x 50 mm

Column:	**PLRP-S 100 Å PL1512-1300 4.6 x 50 mm, 3 µm**
Mobile phase:	A: 100 mM Triethylammonium acetate (TEAA) B: 100 mM TEAA in 25:75 Acetonitrile:water
Flow rate:	1 mL/min
Gradient:	25% B 0 min, 35% B 2 min, 45% B 22.5 min, 45% B 23 min, 25% B 23.05 min, 25% B 26 min
Temperature:	80 °C



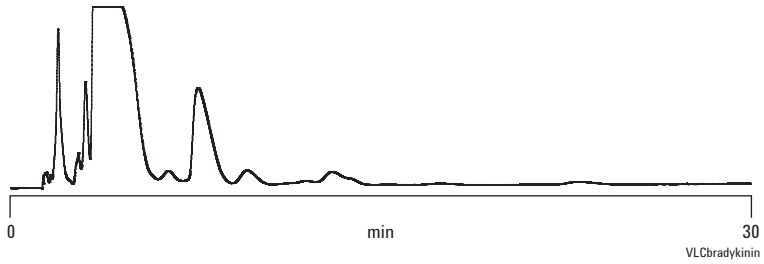
Crude bradykinin prep load

Column: **PLRP-S 100 Å**
PL1512-5100
4.6 x 250 mm, 10 µm

Mobile phase: 0.1% TFA in 21% ACN:79% water

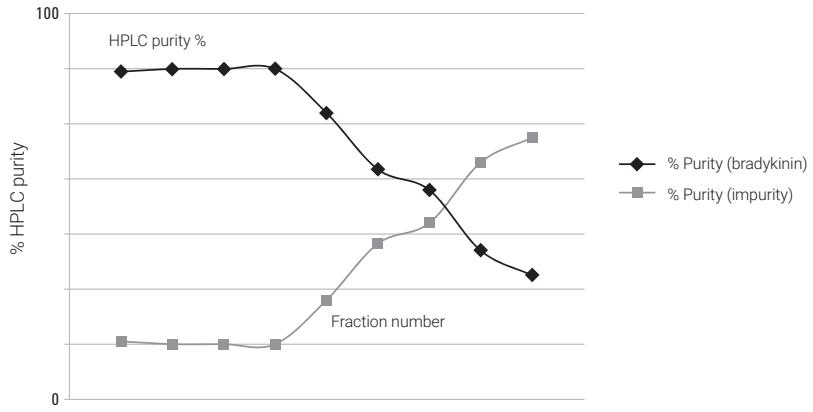
Flow rate: 1 mL/min (360 cm/h)

Sample: 30 µL containing 1.5 mg of crude peptide



Fraction analysis—the concentration overload purification

HPLC analysis of the fractions collected across the peak showed that fractions 1 to 4 contained only the peptide of interest and that the level of the critical impurity increased with increasing fraction number. Using the high efficiency PLRP-S column it was possible to obtain from the crude, 91.7% pure, a recovery of 97% with 100% purity. For more information, see application note 5990-7736EN.



Prep to Process PLRP-S

Size (mm)	Particle Size (µm)	PLRP-S 100 Å	PLRP-S 300 Å	PLRP-S 1000 Å	PLRP-S 4000 Å
100 x 300	30			PL1812-3102	PL1812-3103
100 x 300	15-20	PL1812-6200	PL1812-6201		
100 x 300	10-15	PL1812-6400	PL1812-6401		
100 x 300	10	PL1812-6100	PL1812-6101		
100 x 300	8	PL1812-6800	PL1812-6801		
50 x 300	8	PL1712-6800	PL1712-6801		
50 x 150	30			PL1712-3702	PL1712-3703
50 x 150	15-20	PL1712-3200	PL1712-3201		
50 x 150	10-15	PL1712-3400	PL1712-3401		
50 x 150	10	PL1712-3100	PL1712-3101	PL1712-3102	PL1712-3103
50 x 150	8	PL1712-3800	PL1712-3801		
25 x 300	15-20	PL1212-6200	PL1212-6201		
25 x 300	10-15	PL1212-6400	PL1212-6401		
25 x 300	10	PL1212-6100	PL1212-6101		
25 x 300	8	PL1212-6800	PL1212-6801		
25 x 150	30			PL1212-3702	PL1212-3703
25 x 150	10	PL1212-3100	PL1212-3101	PL1712-3102	PL1712-3103
25 x 150	8	PL1212-3800	PL1212-3801		
25 x 50	10			PL1212-1102	PL1212-1103
PLRP-S Method Development Columns					
4.6 x 250	30			PL1512-5702	PL1512-5703
4.6 x 250	15-20	PL1512-5200	PL1512-5201		
4.6 x 250	10-15	PL1512-5400	PL1512-5401		
4.6 x 250	10	PL1512-5100	PL1512-5101	PL1512-5102	PL1512-5103
4.6 x 250	8	PL1512-5800	PL1512-5801		
4.6 x 150	30			PL1512-3702	PL1512-3703
4.6 x 150	15-20	PL1512-3200	PL1512-3201		
4.6 x 150	10-15		PL1512-3401		
4.6 x 150	10	PL1512-3100	PL1512-3101	PL1512-3102	PL1512-3103
4.6 x 150	8	PL1512-3800	PL1512-3801		

PLRP-S Bulk Media

Particle Size (µm)	Unit	PLRP-S 100 Å	PLRP-S 300 Å	PLRP-S 1000 Å	PLRP-S 4000 Å
50	1 kg	PL1412-6K00	PL1412-6K01	PL1412-6K02	
	100 g	PL1412-4K00	PL1412-4K01	PL1412-4K02	
30	100 g			PL1412-4702	PL1412-4703
15-20	1 kg	PL1412-6200	PL1412-6201		
	100 g	PL1412-4200	PL1412-4201		
10-15	1 kg	PL1412-6400	PL1412-6401		
	100 g	PL1412-4400	PL1412-4401		
10	1 kg	PL1412-6100	PL1412-6101		
	100 g	PL1412-4100	PL1412-4101	PL1412-4102	PL1412-4103
8	1 kg	PL1412-6800	PL1412-6801		

Custom column and bulk media ordering.
If you do not see the combination of pore size/particle size and column dimension or the bulk media quantity you require in these tables, contact your local sales office for assistance with our custom ordering process.

Tips and tools

Column user guides are excellent resources, with instructions for use and column care, as well as suggested starting methods:

www.agilent.com/chem/biolc-columns-user-guides

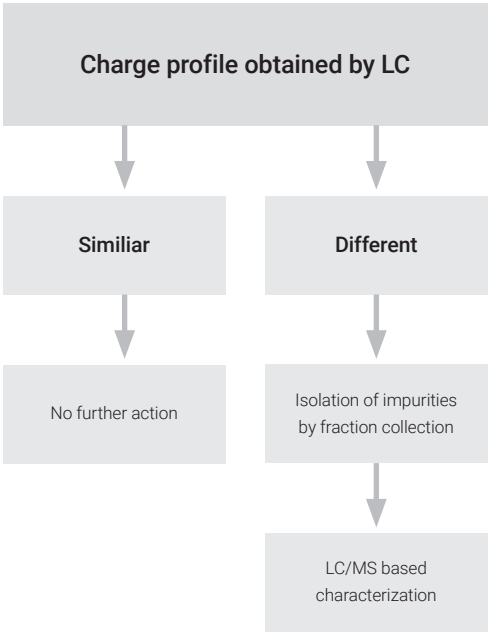
Bio MAb and Bio IEX

Analytical to high efficiency prep

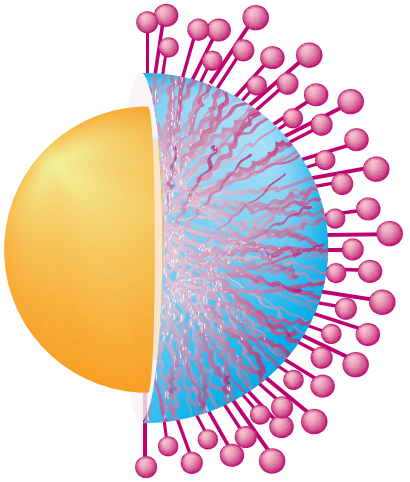
- Nonporous particle to eliminate mass transfer and provide highest efficiency to purify even closely related impurities
- Five functionalities: SAX, WAX, SCX, WCX, and a CX optimized specifically for mAbs to provide maximum resolution for increased sample load
- Scale-up from analytical to semiprep and prep with the same 5 µm particle

The outer hydrophilic layer on these particles reduces nonspecific interaction and delivers high sample recovery.

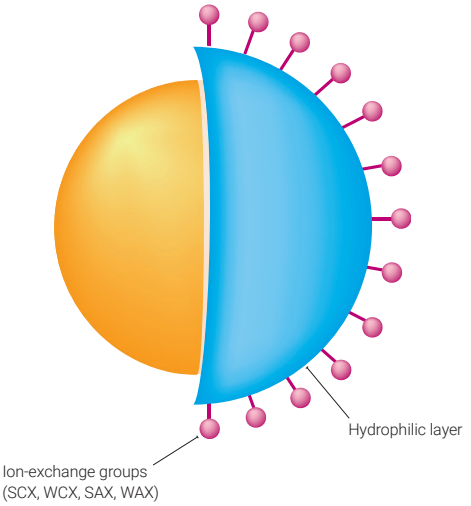
With analytical, semiprep, and prep columns packed with the same 5 µm particles, any unexpected charge variant seen during process development of a biopharmaceutical can quickly be purified for further characterization and identification.



Bio MAb particle



Bio IEX particle



Bio MAb HPLC Columns

Size (mm)	Particle Size (µm)	Part No.
21.2 x 250	5	5190-6885
10 x 250	5	5190-6884

Bio IEX HPLC Columns, Stainless Steel

Size (mm)	Particle Size (µm)	Bio SCX Part No.	Bio WCX Part No.	Bio SAX Part No.	Bio WAX Part No.
21.2 x 250	5	5190-6879	5190-6881	5190-6883	5190-6877
10 x 250	5	5190-6878	5190-6880	5190-6882	5190-6878

Tips and tools

You can connect your Agilent BioHPLC column with InfinityLab Quick Connect fittings and metal-free bio-inert capillaries.

Learn more: **www.agilent.com/chem/5991-7469EN**

PL-SAX and PL-SCX for prep to process

- Ion-exchange purifications over a wider pH range extend applications
- HPLC flow rates and rapid equilibration reduce purification cycle times
- Large pore size for improved mass transfer delivers high speed, high resolution purifications

These rigid, strong ion-exchange materials are extremely hydrophilic and are designed for purification of biomolecules. The PL-SAX and PL-SCX materials are totally polymeric and are chemically and thermally stable over a full range of HPLC conditions. The strong ion-exchange functionalities, covalently linked to a chemically stable polymer, facilitate ion-exchange purifications over a wider pH range. This stability can be exploited for column sanitation and cleanup. Thermal stability also enables the use of denaturing conditions and stabilizing/ solubilizing agents for the purification of target compounds, as encountered in the purification of synthetic oligonucleotides with self-complementary sequences.

Both the 1000 Å and 4000 Å wide-pore materials are mechanically stable and robust and can be operated over a wide range of linear velocities, with fast loading of dilute solutions and wash cycles. HPLC flow rates, and rapid equilibration reduce purification cycle times.

Packing in dynamic axial compression (DAC) column hardware is straightforward and high efficiency columns are achieved with excellent reproducibility and lifetimes. The 1000 Å pore size is for high-capacity purifications and the 4000 Å gigaporous particles with improved mass transfer are intended for large biomolecules and high speed, high resolution purifications.

UHPLC Column Specifications

	PL-SAX	PL-SCX
Matrix	Fully polymeric	Fully polymeric
Pore sizes	1000 Å, 4000 Å	1000 Å, 4000 Å
Particle sizes	10 µm, 30 µm	10 µm, 30 µm
Bead form	Rigid spherical	Rigid spherical
Functionality	Quaternary amine	Sulfonic acid
Pressure stability	3,000 psi	3,000 psi
Temperature stability	80 °C	80 °C
pH range	1–14	1–14
Eluent compatibility	All anion-exchange buffers	All cation-exchange buffers
Packed bed density	0.39 g/mL	0.39 g/mL

Purification of a large oligonucleotide

Column: **PL-SAX 1000 Å, 8 µm**

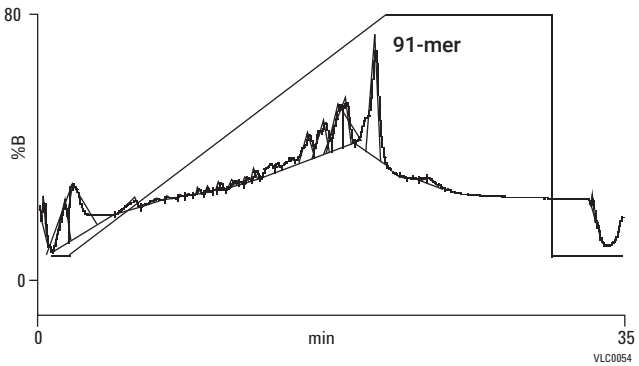
Mobile phase: A: 93% 100 mM TEAA, pH 7:7% Can
B: 93% 100 mM TEAA, 3.24 M ammonium acetate, pH 7:7% ACN

Flow rate: 1.5 mL/min

Gradient: 0–100% B in 20 min

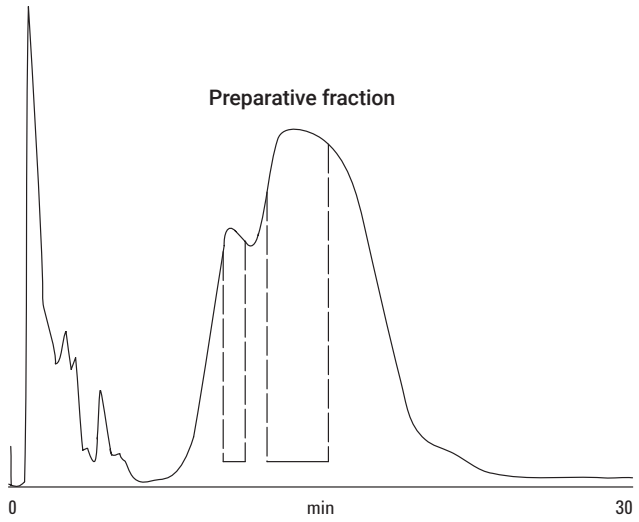
Temperature: 60 °C

Detector: UV, 290 nm



Preparative fractionation of a culture filtrate containing amyloglucosidases

Column:	**PL-SAX 4000 Å** **PL1551-1803** **4.6 x 50 mm, 8 µm**
Mobile phase:	A: 10 mM tris HCL, pH 8 B: A + 500 mM NaCl, pH 8
Flow rate:	4.0 mL/min
Gradient:	Linear 0–100% B in 2 min
Temperature:	60 °C
Detector:	UV, 280 nm



Prep to Process PL-SAX and PL-SCX

Dimensions	Particle Size (µm)	PL-SAX 1000 Å	PL-SAX 4000 Å	PL-SCX 1000 Å	PL-SCX 4000 Å
50 x 150	30	PL1751-3702	PL1751-3703	PL1745-3702	PL1745-3703
50 x 150	10	PL1751-3102	PL1751-3103	PL1745-3102	PL1745-3103
25 x 150	30	PL1251-3702	PL1251-3703	PL1245-3702	PL1245-3703
25 x 150	10	PL1251-3102	PL1251-3103	PL1245-3102	PL1245-3103
25 x 50	10	PL1251-1102	PL1251-1103	PL1245-1102	PL1245-1103
7.5 x 150	8	PL1151-3802	PL1151-3803		
7.5 x 50	8	PL1151-1802	PL1151-1803	PL1145-1802	PL1145-1803
PL-SAX and PL-SCX Method Development Columns					
4.6 x 250	30	PL1551-5702	PL1551-5703	PL1545-5702	PL1545-5703
4.6 x 250	10	PL1551-5102	PL1551-5103	PL1545-5102	PL1545-5103
4.6 x 150	30	PL1551-3702	PL1551-3703	PL1545-3702	PL1545-3703
4.6 x 150	10	PL1551-3102	PL1551-3103	PL1545-3102	PL1545-3103

Prep to Process PL-SAX and PL-SCX columns and bulk media

PL-SAX and PL-SCX Bulk Media

Particle Size (µm)	Unit	PL-SAX 1000 Å	PL-SAX 4000 Å	PL-SCX 1000 Å	PL-SCX 4000 Å
30	100 g	PL1451-4702	PL1451-4703	PL1445-4702	PL1445-4703
10	100 g	PL1451-4102	PL1451-4103	PL1445-4102	PL1445-4103

Custom column and bulk media ordering.
If you do not see the combination of pore size/particle size and column dimension or the bulk media quantity you require in these tables, contact your local sales office for assistance with our custom ordering process.

Peptide purification

VariTide is a cost-effective solution for the production of synthetic peptides. This column lets you manage the cost and efficiency of high-volume synthetic peptide purification, from µg to g scale. VariTide provides a solution for peptide houses that manufacture small quantities of hundreds or thousands of peptides where manufacturing time is the economic driving force.

VariTide RPC columns

VariTide RPC columns for synthetic peptides

- A single column to cover the full range of synthetic peptides
- Small particle size for maximum efficiency, even with 1 and 2 in prep columns
- Bulk media to pack 1 and 2 in prep columns for the purification of mg to g quantities

VariTide RPC columns and media are part of the VariPep peptide solution. This is the recommended option for cost-effective separation and purification of synthetic peptides using generic methods.

VariTide RPC Columns for Synthetic Peptides

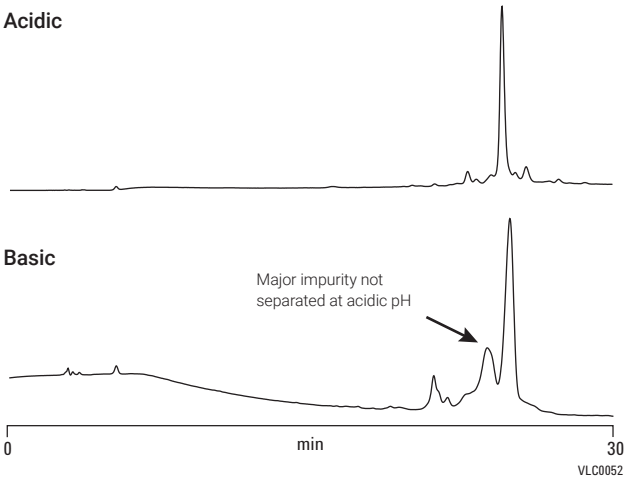
Size (mm)	Part No.
21.2 x 250	PL1E12-5A05
10.0 x 250	PL1012-5A05
4.6 x 250	PL1512-5A05

VariTide RPC Bulk Media

Description	Part No.
100 g	PL1412-4A05
1 kg	PL1412-6A05

Crude peptide screen

Column:	**VariTide RPC PL1512-5A05 4.6 x 250 mm**
Mobile phase:	Acidic A: 0.1% TFA in 95% water: 5% ACN B: 0.1% TFA in 50% water: 50% Can Basic A: 5% ACN, 95% 20 mM ammonium carbonate, pH 9.5 B: 50% ACN, 50% 20 mM ammonium carbonate, pH 9.5
Flow rate:	1.0 mL/min (360 cm/h)
Gradient:	0–100% B in 30 min
Detector:	UV, 220 nm



VariPure IPE

- Prepacked for convenience
- Removal of ion-pairing agents for improved productivity
- High performance and economy for excellent efficiency

VariPure IPE is a polymer-supported quaternary-amine resin with a bicarbonate counter ion, designed for removing acidic ion-pair reagents, such as trifluoroacetic acid (TFA), formic acid, or acetic acid. VariPure IPE is a high performance and economical acid-removal material conveniently supplied as prepacked SPE type devices. The particle size, capacity, and device geometry are matched to provide sufficient residence time to achieve effective ion-air extraction under gravity flow. For acid-labile peptides, removal of the ion-pairing agent prevents acid degradation of the peptide during post-HPLC work-up, and increases the yield of purified product

VariPure IPE

Loading	Counter-ion Removal Capacity	Unit	Part No.
100 mg per 3 mL tube	~5 mL 0.1% TFA	50/pk	PI3540-d603VP
500 mg per 6 mL tube	~25 mL 0.1% TFA	50/pk	PI3540-C603VP
1 g per 20 mL tube	~50 mL 0.1% TFA	25/pk	PI3540-P603VP
25 g			PI3549-3603VP

Load & Lock preparative HPLC columns

Agilent offers a complete range of laboratory scale Load & Lock columns and the Mobile Packing Station. Designed to let you easily and quickly pack your own preparative high-efficiency columns, this is the right solution for development applications of pharmaceutical compounds, peptides, and natural products. Our Load & Lock columns have a unique fluid/sample distribution system to maximize productivity. This improves column performance by diffusing the sample more efficiently over the complete bed surface.

- Highest performance: achieve superior results with a unique flow distribution system
- Maximum flexibility: all the 1 inch, 2 inch, and 3 inch Load & Lock columns conveniently use the same mobile packing station and perform both Dynamic Axial Compression (DAC) or Static Axial Compression (SAC)
- Greater convenience: pack or unpack your column in a few minutes
- Maximum mobility: column and packing station are combined in one easy-to-move skid, wherever it's needed

Dual mode packing formats ensure easy operation and the delivery of consistent, high-performance results by using the DAC or SAC mode.

Agilent laboratory scale Load & Lock columns combine excellent packed-bed stability with enhanced flow distribution to deliver the highest quality purification possible with maximum speed, flexibility, and ease of operation.

Requiring only compressed air, the Load & Lock packing station uses no power supply, making it safe to use with any type of solvent and the solution of choice for hazardous environments. The quick-release single-bolt clamp offers speedy and easy packing and unpacking within minutes.

Load & Lock Preparative HPLC Columns

Description	Water Jacket	Size (mm)	Part No.
Load & Lock column	No	27.0 x 500	PCG93LL500X25
	Yes	27.0 x 500	PCG93LL500X25WJ
	Spare parts kit		PCG931AAKIT
Mobile packing station (air-driven hydraulic)			PCG93LLSTAND123

Bio SEC

Purification based on size

- Six pore sizes to provide size separations across the range of biopharmaceuticals
- Scale-up from 3 µm, and 5 µm analytical columns to lab prep with the same particles
- The thin hydrophilic polymer layer minimizes nonspecific interactions and provides good peak shapes and increased sample capacity

These silica based SEC materials have optimized pore sizes and pore volume to provide high resolution separations under HPLC conditions of pressure and flow rate. The 3 µm particles with 100 Å, 150 Å, and 300 Å pore sizes provide highest efficiency for lab prep, and the 5 µm particles provide a wide range of pore sizes for fractionation of larger biomolecules and conjugates.

Tips and tools

Column user guides are excellent resources, with instructions for use and column care, as well as suggested starting methods:

www.agilent.com/chem/biolc-columns-user-guides

Bio SEC-3 HPLC Columns for Faster Peptide and Protein Separations

Size (mm)	Particle Size (µm)	Bio SEC-5 100 Å USP L33	Bio SEC-5 150 Å USP L33	Bio SEC-5 300 Å USP L33
21.2 x 300	3	5190-6850	5190-6851	5190-6852
Prep guards				
21.2 x 50	3	5190-6854	5190-6855	5190-6856

Bio SEC-5 HPLC Columns for Size-Based Biomolecules

Size (mm)	Particle Size (µm)	Bio SEC-5 100 Å USP L33	Bio SEC-5 150 Å USP L33	Bio SEC-5 300 Å USP L33	Bio SEC-5 500 Å USP L33	Bio SEC-5 1000 Å USP L33	Bio SEC-5 2000 Å USP L33
21.2 x 300	5	5190-6863	5190-6864	5190-6865	5190-6866	5190-6867	5190-6868
Prep guards							
21.2 x 50	5	5190-6869	5190-6870	5190-6871	5190-6872	5190-6874	5190-6875

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Understanding cellular metabolism is critical to understanding many cellular processes. With Agilent you can analyze metabolism in live cells, in real time, and generate the most physiologically relevant data.

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If you are involved in the detection of Chemical Warfare Agents (CWA), Biological Warfare Agents (BWA), Toxic Industrial Chemicals (TIC), or in the monitoring of food, air, soil, and water, Agilent has a fast, flexible and reliable solution to meet your needs.

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Agilent metabolomics solutions are used for disease research, analytical toxicology, environmental analysis, agriculture, biofuel development, and nutrition. Metabolomics results are combined with gene expression and/or proteomics studies to provide a comprehensive understanding of the biology.

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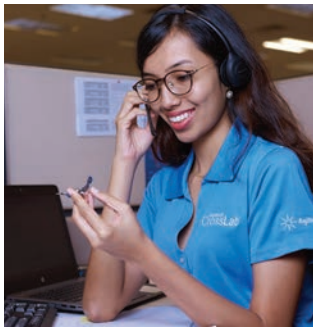

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