

Agilent BioHPLC Column Selection Guide

# YOUR REFERENCE GUIDE TO THE ANALYSIS OF BIOPHARMACEUTICALS AND BIOMOLECULES

The Measure of Confidence



Agilent Technologies



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## BIOCOLUMN SELECTION GUIDELINES

From sample simplification to analysis, Agilent's biomolecule columns and supplies are easy to integrate into your workflow for a complete, reproducible, and high-quality solution.

This comprehensive guide will help you find the right column for your biomolecule separation. 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 Bio-inert LC system with a metal-free sample path and the Agilent 1290 Infinity LC, designed to provide highest speed, resolution, and ultra-sensitivity for UHPLC applications, including those utilizing Agilent wide-pore 300Å ZORBAX StableBond columns. 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 range in size from small lipids to large polynucleotides such as DNA or RNA. They can be monomeric amino acids or polymeric carbohydrates.

In this guide, 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 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 ZORBAX Eclipse Amino Acid Analysis HPLC columns provide a high efficiency solution for rapid 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.

**Broad-distribution polymers** – analysis of lipids, polysaccharides and drug delivery compounds using polymeric columns and standards to determine their molecular weight distribution and composition. These compounds tend to exhibit broad MW distributions, in contrast to other biomolecules that have narrow MW distributions or a defined molecular weight.

## 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 is designed to minimize non-specific 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.

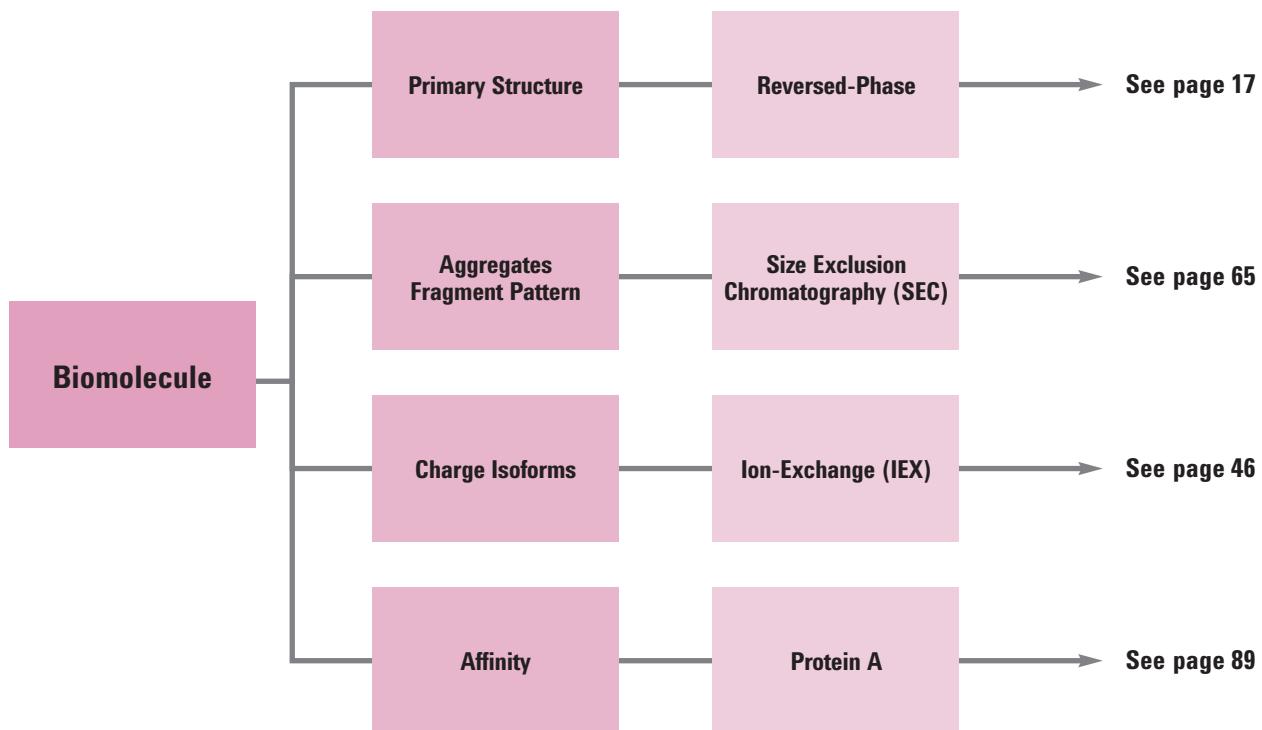
Typically, HPLC has been used to separate biomolecules. Now, advanced techniques such as UHPLC are becoming a popular choice because multiple separation mechanisms are needed in the characterization of biomolecules. Therefore, Agilent offers advanced chemistries developed specifically for the separation of biomolecules using size exclusion, reversed-phase, ion-exchange, and affinity functionalities, all of which are covered in this guide.

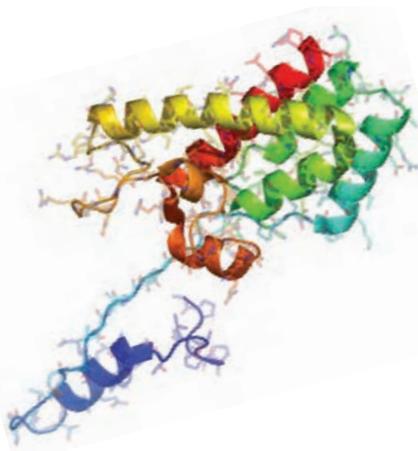


## Column Selection Flow Chart

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

There are a number of guidelines that can be followed to assist in the selection of the optimum column for a biomolecule separation. The starting point will be the size of the molecule, as this will determine 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.





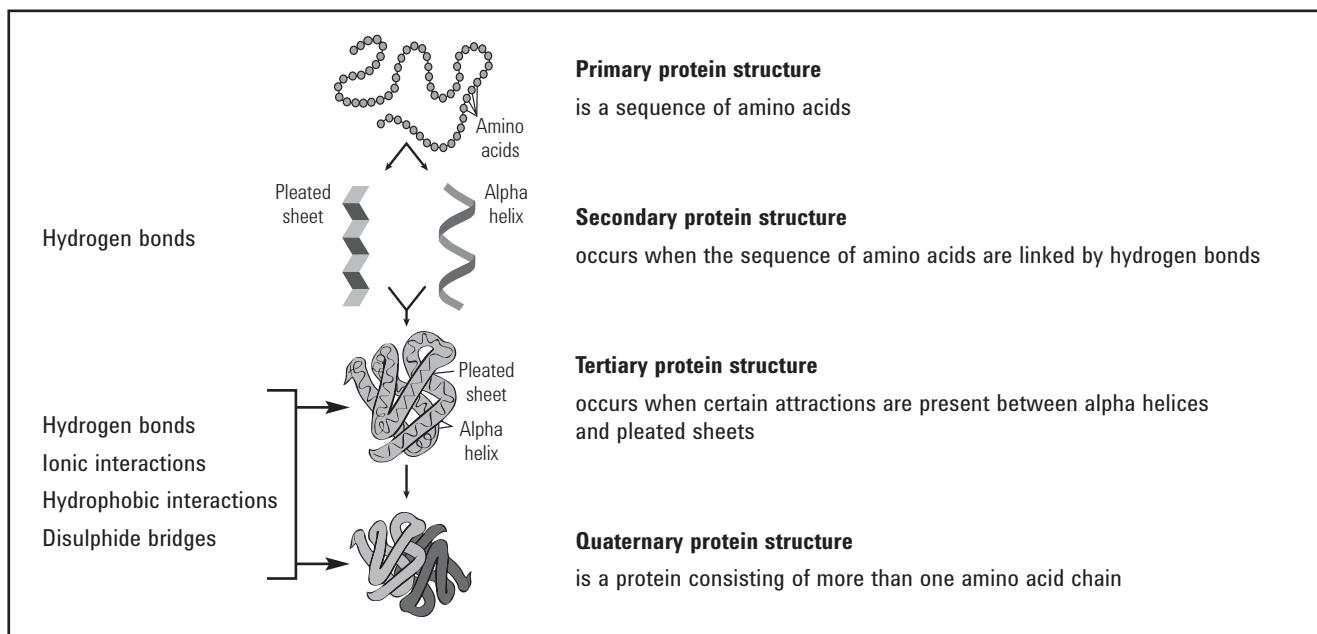
## BIOMOLECULE SEPARATIONS

### Protein Separations

Proteins are complex molecules that require multiple techniques to provide full characterization. They exist as three-dimensional structures and it is this structure that confers 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 disulphide 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.



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

The environment of the protein can influence, stabilize, or disrupt the structure of the protein. 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 ion. Protein structure can also be disrupted by the use of sulphydryl reducing agents to break -S-S- bonds or chaotropic agent, like 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

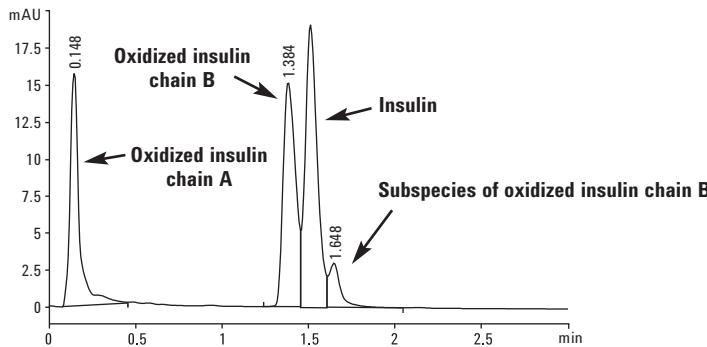
<b>Application</b>	<b>Technique</b>	<b>Agilent Columns</b>	<b>Notes</b>
Primary structure analysis	UHPLC/HPLC reversed-phase separations	ZORBAX 300Å Poroshell 300Å ZORBAX 300Extend-C18 PLRP-S	Reversed-phase separations require (or cause) denaturing of the protein to obtain detailed information about the amino acid sequence and/or amino acid modifications (including post-translational modifications).
Aggregation analysis	Size exclusion separations	Bio SEC-3 Bio SEC-5 ProSEC 300S ZORBAX GFC	Aggregates in protein biopharmaceuticals are of major concern as they can induce an immunogenic response and can influence the composition of the final formulation.
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 (pl). When the solution pH is less than the pl, the protein will be positively charged (acidic), and when the solution pH is greater than the pl, the protein is negatively charged (basic). For ion-exchange analysis, we recommend the eluent pH be at least one pH unit away from its pl. Protein analysis using ion-exchange columns requires buffered mobile phase and either salt gradients or pH gradients for elution.

# Biomolecule Separations

## Higher resolution of oxidation study

**Column:** ZORBAX RRHD 300SB-C18  
857750-902  
2.1 x 50 mm, 1.8  $\mu$ m

Mobile Phase: A: 0.1% TFA  
B: 0.01% TFA + 80% ACN  
Flow Rate: 1.0 mL/min  
Gradient: 33 to 50% B, 0 to 4 min  
Detector: 1290 Infinity LC with diode array detector at 280 nm  
Sample: Insulin, insulin chain A and chain B, oxidized (bovinesigma, 1 mg/mL)

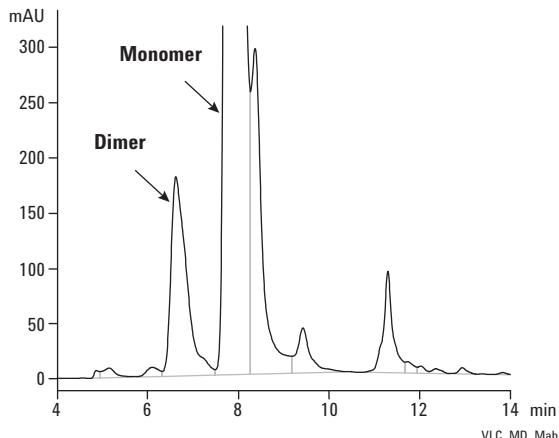


It is evident that the oxidized insulin chains are resolved from insulin in under 2 minutes using the Agilent ZORBAX RRHD 300SB-C18 2.1 x 50 mm, 1.8  $\mu$ m column

## Intact MAb monomer and dimer separation

**Column:** Bio SEC-3, 300 $\text{\AA}$   
5190-2511  
7.8 x 300 mm, 3  $\mu$ m

Buffer: Sodium phosphate buffer, pH 7.0, 150 mM  
Isocratic: 0-100% Buffer A from 0-30 min  
Flow Rate: 1.0 mL/min  
Sample: CHO-humanized MAb, 5 mg/mL – intact  
Injection: 5  $\mu$ L  
Detector: UV 220 nm  
Temperature: Ambient



### Separation of charge variants of human IgG1 with pH gradient

**Column:** Agilent Bio MAb  
2.1 x 150 mm, 5  $\mu$ m

Mobile Phase: A: 10 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 6.0  
B: A + 0.5 M NaCl or just 0.5 M Na<sub>2</sub>HPO<sub>4</sub>, 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.

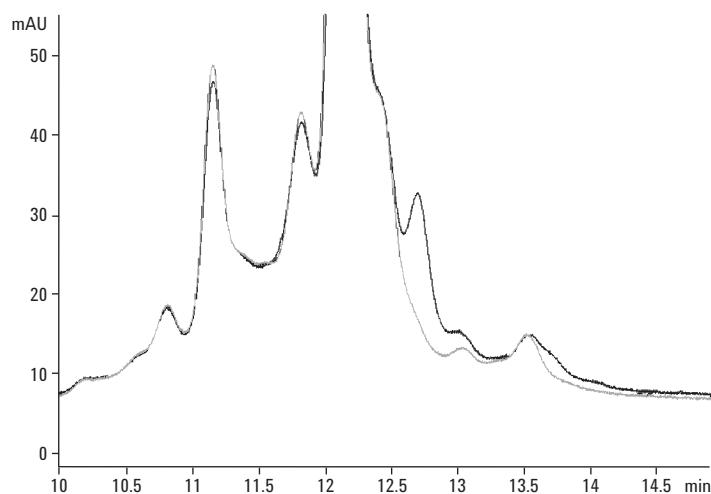
pH Gradient: A: 5 mM Na<sub>2</sub>HPO<sub>4</sub>, buffer pH 5.5 and B: 40 mM Na<sub>2</sub>HPO<sub>4</sub> (not buffered, pH 8.9). 2% B/min at 1 mL/min for 15 min, followed by a column wash with 90% B for 5 min.

Detector: UV at 220 nm

Sample: One mg each/mL in mobile phase A  
Monoclonal antibodies (MAb) -human IgG1 (5 mg/mL stock solution) derived from CHO cells

Instrument: Agilent 1200 SL system with diode array detector

— Before carboxypeptidase B digestion  
— After carboxypeptidase B digestion



MAb c-terminal cleavage: Human IgG1 MAb, 1 mg/mL in 25 mM Na<sub>2</sub>HPO<sub>4</sub> buffer, pH 7.5, was incubated with approximately 25 units of the carboxypeptidase B for 18 hours and 10  $\mu$ L samples were injected.





## 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 that 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. To achieve sufficient resolution for quantification and identification, longer column lengths or higher efficiency particles such as the sub-2 µm ZORBAX RRHD, or superficially porous Poroshell are recommended.

Peptide digests are complex mixtures, and for complete coverage, i.e. resolution of the individual peptides, a high efficiency/high resolution column is required. The peptide fragments can range in size and hydrophobicity, so Agilent offers several columns for peptide mapping. There are three options: pore sizes, particle sizes, and superficially porous and fully porous for UHPLC separations.

### Tips & Tools

Capillary electrophoresis is an alternative technique to liquid chromatography for the separation of complex peptide mixtures. Further information can be found in the following Case Study:

*An orthogonal view of peptide mapping – analysis of bovine serum albumin digest using capillary electrophoresis and quadrupole time-of-flight mass spectrometry (5990-7631EN)*

[www.agilent.com/chem/library](http://www.agilent.com/chem/library)



**Increased resolution for peptide mapping**

**Column:** **ZORBAX 300SB-C18**  
**858750-902**  
**2.1 x 100 mm, 1.8 µm**

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

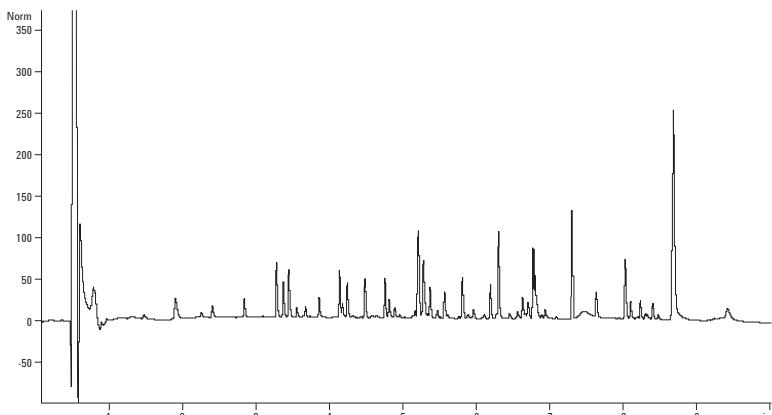
Flow Rate: 0.5 mL/min

Gradient: 2% B for 1 min, 2 to 45% B for 8.8 min,  
               45 to 95% B for 0.2 min, 95% B for 2 min,  
               95 to 2% B for 0.2 min

Temperature: 50 °C

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

Sample: Enzymatic protein digest (MAb)



The longer 100 mm Agilent ZORBAX RRHD 300SB-C18 column provides maximum resolution for protein digests – in this sample the total run time, including washing and equilibration, is under fifteen minutes.

**Peptide Mapping Column Selection**

Recommended column choices determined by system/column pressure maximum and peptide size/hydrophobicity.

Application	Technique	Agilent Columns	Notes
Large peptide fragments/hydrophobic peptide core	400 bar HPLC	Poroshell 300 SB-C18 ZORBAX 300SB-C18, 3.5 µm	Agilent 1200 LC
	600 bar UHPLC	Poroshell 300 SB-C18	Agilent 1260 Infinity LC and 1260 Infinity Bio-inert Quaternary LC
	1200 bar UHPLC	ZORBAX RRHD Poroshell 300 SB-C18, 1.8 µm	Agilent 1290 Infinity LC
Small hydrophobic peptides	400 bar HPLC	Poroshell 120 EC-C18 Poroshell 120 SB-C18	Agilent 1200 LC
	600 bar UHPLC	Poroshell 120 EC-C18 Poroshell 120 SB-C18	Agilent 1260 Infinity LC and 1260 Infinity Bio-inert Quaternary LC
	1200 bar UHPLC	Poroshell 120 EC-C18 Poroshell 120 SB-C18	Agilent 1290 Infinity LC

If you have an Agilent 1290 Infinity LC in your lab we recommend starting with a ZORBAX RRHD 300SB-C18 column to screen your peptide map.

## Separation of Natural and Synthetic Peptides

Purification columns and media are required for the isolation and analysis of natural and synthetic peptides. Purity and recovery determination of the isolated or purified peptide requires the use of high efficiency columns. The primary technique used for the isolation and purification, and analysis, is reversed-phase HPLC.

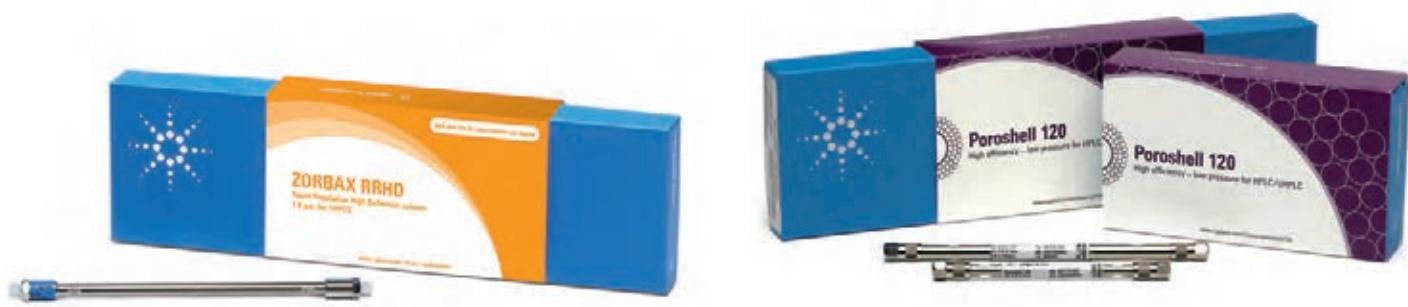
The fractions from a purification or isolation workflow and the final peptide product are analyzed for purity using high efficiency columns. The peptides will vary in size, charge and hydrophobicity and so, as with peptide mapping applications, Agilent offers a range of columns to provide optimum separations of the full range of peptides. For small peptides, typically less than 10 amino acid residues, the smaller pore UHPLC materials are used, but if the peptide is larger, contains more amino acid residues, or exists in a dimeric or multimeric form, then the larger pore size 300Å columns provide better separations due to improved mass transfer.

## Natural and Synthetic Peptides Column Selection

Recommended column choices as determined by system/column pressure maximum for the analysis of natural and synthetic peptides.

Application	Technique	Agilent Columns	Notes
Larger peptides with more than 10 amino acid residues	400 bar HPLC	Poroshell 300 SB-C18 ZORBAX 300SB-C18, 3.5 µm PLRP-S	Agilent 1200 LC
	600 bar UHPLC	Poroshell 300 SB-C18	Agilent 1260 Infinity LC and 1260 Infinity Bio-inert Quaternary LC
	1200 bar UHPLC	ZORBAX RRHD 300SB-C18, 1.8 µm	Agilent 1290 Infinity LC
Peptides with typically less than 10 amino acid residues	400 bar HPLC	Poroshell 120 EC-C18 Poroshell 120 SB-C18 PLRP-S	Agilent 1200 LC
	600 bar UHPLC	Poroshell 120 EC-C18 Poroshell 120 SB-C18	Agilent 1260 Infinity LC and 1260 Infinity Bio-inert Quaternary LC

Reversed-phase columns are also the first choice for purifying large numbers of individual peptides or larger amounts of a particular peptide. High efficiency, small particle pre-packed prep columns are available for the high efficiency purification of small amounts of peptides, and larger particle columns and bulk media for the larger scale purifications, as shown in **Table 1**.



**Table 1. Agilent columns for small- to large-scale peptide purifications.**

Agilent Column	Amount of Peptide Required		
	mg	g	kg
ZORBAX Prep HT 300StableBond		→	
VariTide RPC		→	
PLRP-S		→	

After solid phase synthesis (SPS) using a polystyrene resin such as one of the Agilent StratoSpheres products, the peptide is cleaved from the support and the resultant mixture is separated to obtain the target peptide. A high efficiency column is needed for the purification as the candidate peptide must be resolved from peptides that are very similar in structure. Check [www.agilent.com](http://www.agilent.com) for further information.

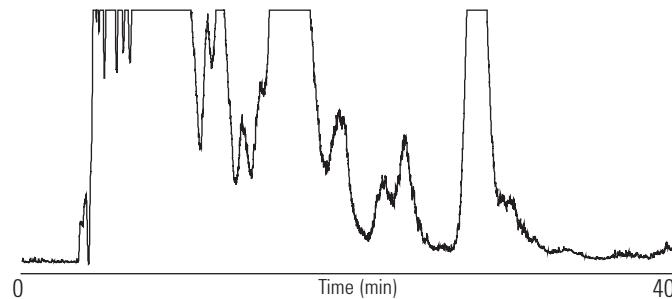
### Preparative scale purification of Leuprolide by concentration overload

**Column:** PLRP-S 100Å, 10 µm  
PL1412-4100

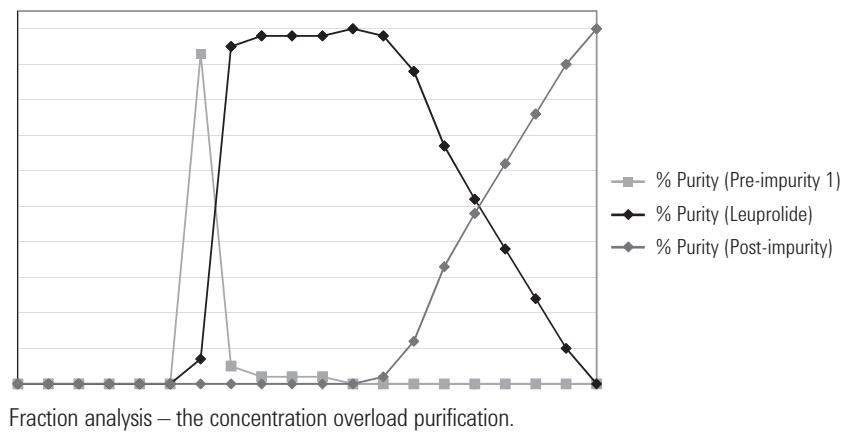
**Bulk Media:** Load & Lock 4001 Column  
PCG93LL500X25

**Mobile Phase:** Isocratic separation  
using 0.1% TFA  
in 28% ACN:72% water

**Flow Rate:** Linear velocity 360 cm/hr



Crude leuprolide separation of 30 mg on-column load.





## 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, i.e. 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 step. 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 some amount of larger oligos produced by double coupling or branching. The sample mixture is complex and high efficiency techniques are required for analysis.

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

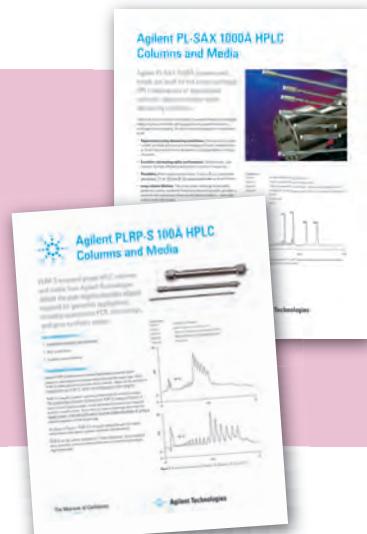
**Trityl-on:** This procedure is relatively simple to perform and separates the full-length target oligo, which still has the DMT group attached, from the deprotected failure sequences. The analytical information obtained is limited and this is generally considered to be a purification method.

**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 complimentary 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	
Deprotected oligonucleotides	Ion-exchange separations of the trityl-off, deprotected oligos	PL-SAX 1000Å	Separates de-protected 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.



**Tips & Tools**

Further information can be found in the following publications:

- Agilent PLRP-S 100Å HPLC Columns and Media* (5990-8187EN)
- Agilent PL-SAX 1000Å HPLC Columns and Media* (5990-8200EN)
- [www.agilent.com/chem/library](http://www.agilent.com/chem/library)



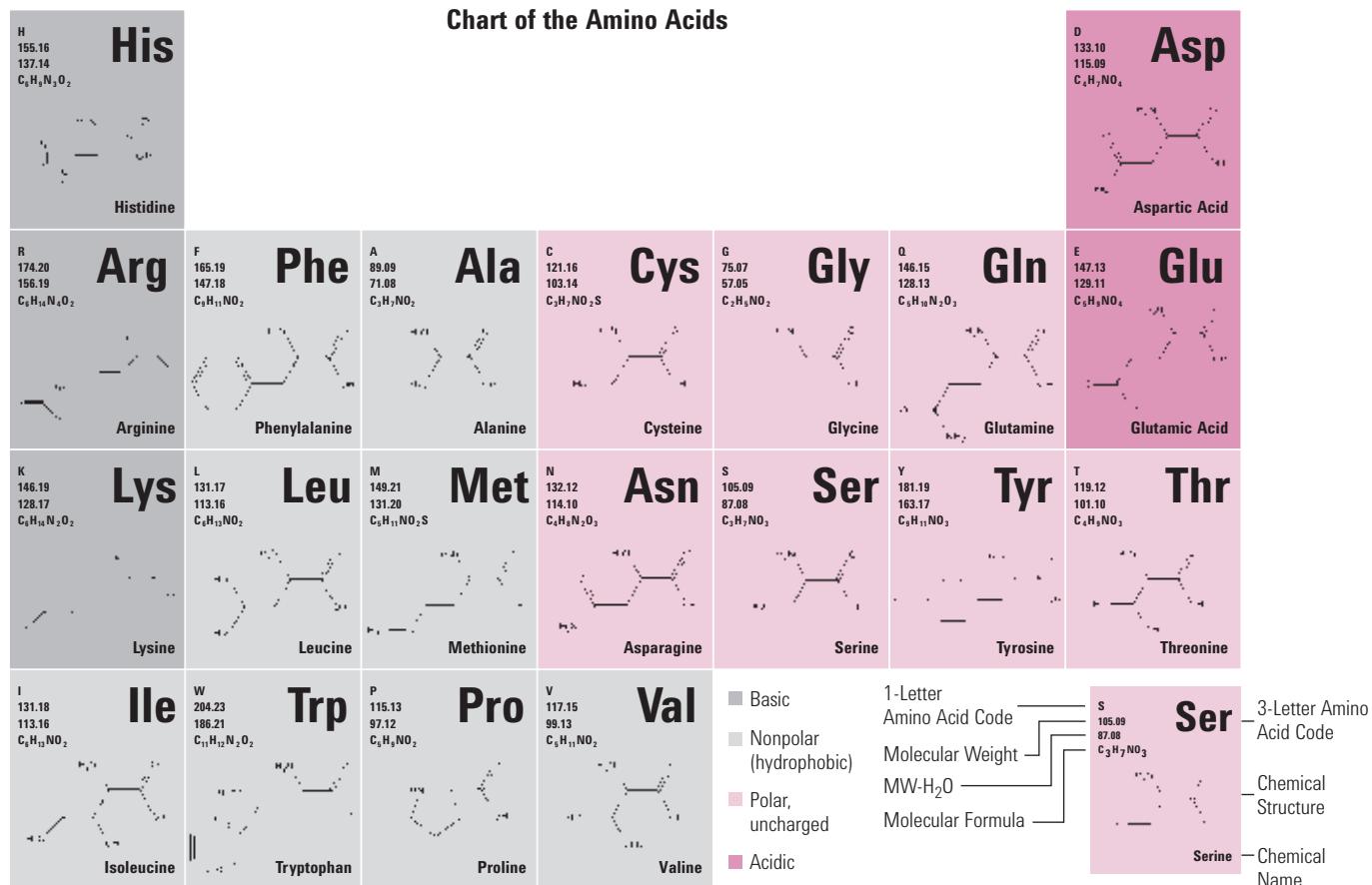
# Amino Acid Analysis

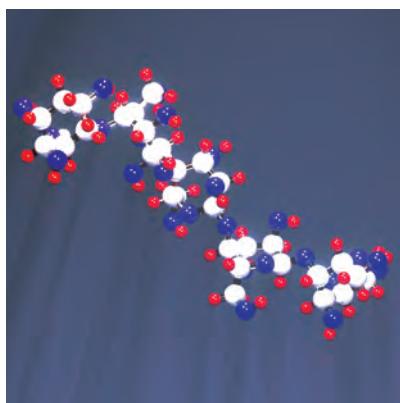
The Agilent ZORBAX Eclipse AAA high efficiency column rapidly separates amino acids following an updated and improved protocol. Total analysis from injection to injection can be achieved in as little as 14 min (9 min analysis time) on shorter, 7.5 cm columns and 24 min (18 min analysis time) on the 15 cm column. Exceptional 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 the Agilent 1200 Infinity LC.

## ZORBAX Eclipse AAA Column Selection

Application	Diameter x Length (mm)	Particle Size ( $\mu\text{m}$ )
Analytical routine sensitivity	4.6 x 150	5.0
Analytical routine sensitivity, high-resolution using FLD	4.6 x 150	3.5
Analytical routine sensitivity, high-throughput	4.6 x 75	3.5
Solvent Saver high sensitivity, high-resolution	3.0 x 150	3.5

## Chart of the Amino Acids





## Broad Distribution Biomolecules

### Carbohydrates, Lipids and PEGs

Aqueous size exclusion chromatography employing columns packed with polymeric media can be extremely useful when investigating biomolecules and their derived species with broad molecular weight distributions. Examples include PEGylated proteins and complex polysaccharides which find use in biopharma applications. The wide pore size distribution of polymeric SEC columns compared to silica-based material are excellent for samples with polydispersities greater than one.

#### Broad Distribution Biomolecule Column Selection

Low MW polymers and oligomers, oligosaccharides, PEGs, lignosulfonates	2 or 3 PL aquagel-OH <ul style="list-style-type: none"><li>• PL aquagel-OH 8 µm</li><li>• PL aquagel-OH 20 5 µm</li><li>• PL aquagel-OH MIXED-M 8 µm</li></ul>	The PL aquagel-OH analytical series has a pH range of 2-10, compatible with organic solvents (up to 50% methanol), mechanical stability up to 140 bar (2030 psi) and low column operating pressures.
Polydisperse biopolymers, polysaccharides, cellulose derivatives	2 or 3 PL aquagel-OH <ul style="list-style-type: none"><li>• PL aquagel-OH MIXED-H 8 µm</li><li>• PL aquagel-OH 60/50/40 8 µm</li></ul>	
Very high MW polymers, hyaluronic acids, starches, gums	PL aquagel-OH 60/50/40 15 µm in series	



## UHPLC/HPLC TECHNIQUES

High-performance liquid chromatography, HPLC, is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. There has been an evolution toward ultra high-performance liquid chromatography (UHPLC) which is widely accepted for high-efficiency separations of small- to medium-sized molecules, and has been used to reduce analysis time and/or to increase resolution. The use of UHPLC has been extended to large biomolecules with the introduction of wide pore chromatographic media in columns that can withstand pressures of 600 to 1200 bar.

On the following pages you will see the wide range of columns that Agilent offers for the HPLC and UHPLC separation of proteins and other biomolecules.

### UHPLC/HPLC Techniques for Biomolecule Analysis

Technique	Advantages	Disadvantages
Reversed-Phase	<ul style="list-style-type: none"><li>• High resolution</li><li>• High capacity</li><li>• Relatively simple</li><li>• Sample concentrated on-column</li><li>• Small particle, 1.8 µm, for UHPLC separations</li><li>• Polymeric media for unsurpassed chemically and thermally stable</li></ul>	<ul style="list-style-type: none"><li>• Denaturing conditions</li><li>• High efficiency silica columns cannot be cleaned using aggressive solvents when performing purifications</li></ul>
Size Exclusion	<ul style="list-style-type: none"><li>• Good recovery of biological activity</li><li>• Non-interactive technique with good sample recovery</li></ul>	<ul style="list-style-type: none"><li>• No sample concentration</li><li>• Limited capacity</li></ul>
Ion-Exchange	<ul style="list-style-type: none"><li>• Good recovery of biological activity</li><li>• High capacity</li><li>• Sample concentrated on-column</li></ul>	<ul style="list-style-type: none"><li>• Limited MS compatibility due to presence of salts</li></ul>
Affinity	<ul style="list-style-type: none"><li>• Highly selective</li><li>• Good recovery of biological activity</li><li>• Sample concentrated on-column</li><li>• Often single step isolation</li></ul>	<ul style="list-style-type: none"><li>• No sample concentration</li><li>• Limited capacity</li></ul>

### Tips & Tools

Don't forget, we have special offers throughout the year.

To learn more, visit [www.agilent.com/chem/specialoffers](http://www.agilent.com/chem/specialoffers)





## REVERSED-PHASE HPLC

### Confidently perform high-resolution separations

Reversed-phase UHPLC/HPLC separates solutes based on differences in hydrophobicity, with the least hydrophobic peak eluting first. This high-resolution technique is capable of separating peptides, proteins and oligonucleotides that differ by only one amino acid or nucleotide residue.

Because HPLC uses organic solvents (such as acetonitrile, methanol, ethanol and propanol) it is also a denaturing technique that disrupts a biomolecule's three-dimensional structure. This allows you to obtain information about a molecule's primary structure and sequence, as well as variations in the sequence to be identified.

Agilent offers the industry's broadest range of wide-pore reversed-phase columns, all backed by technical support experts and application chemists around the globe. This section features the following column innovations:

**ZORBAX 300Å pore silica columns** – an industry first for reversed-phase protein and biomolecule separations – are available in 6 phases, along with a broad array of sizes. For fast UHPLC separations, we also offer a 1.8 µm particle size option that withstands pressures up to 1200 bar, and can be used with high-pressure instruments, such as Agilent's 1290 Infinity LC.

**Agilent Poroshell columns** feature the industry's first solid core/porous shell particle. Our wide-pore Poroshell 300 columns are ideal for fast chromatography, and are available in a variety of phases.

**Agilent PLRP-S columns** contain polymer particles, and can be used to separate peptides and proteins of various sizes and DNA/macromolecular complexes. These columns are unique in that they are 100% organic modified, can withstand temperatures as high as 200 °C, and can be used under conditions from pH 1 to pH 14.

Choose from a range of column sizes, particle sizes (3-8 µm for analytical separations) and pore sizes (100Å to 4000Å). Preparative columns (10-50 µm) are also available, either prepacked in columns or as bulk material.

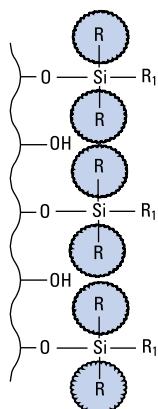
# Reversed-Phase HPLC

## Reversed-Phase Column Selection

Application	Agilent Columns	Notes
Proteins and polypeptides	ZORBAX 300Å, 1.8 µm	Improved packing processes achieve stability up to 1200 bar for use with the Agilent 1290 Infinity LC. RRHD 1.8 µm columns are available in 50 and 100 mm lengths for fast or high resolution – truly high definition – separations of the most complex samples.
	• RRHD 300SB-C18 • RRHD 300SB-C8 • RRHD 300SB-C3 • RRHD 300-Diphenyl	
	ZORBAX 300Å StableBond	Wide-pore, 300Å 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. C18 and C8 are ideal for complex protein and protein digest separations.
	• 300SB-C18 • 300SB-C8 • 300SB-C3 • 300SB-CN	
	ZORBAX 300Å Extend-C18	Incorporate a unique patented bidentate silane, combined with a double-endcapping process that protects the silica from dissolution at high pH – up to pH 11.5.
Peptides and proteins up to 500-1,000 kDa, monoclonal antibodies and intact proteins	Poroshell 300	Poroshell columns use a unique particle made with a layer of porous silica on a solid core of silica. This reduces the diffusion distance for proteins making practical, rapid HPLC separations of peptides and proteins.
Peptides and proteins up to 500-1,000 kDa, monoclonal antibodies and intact proteins	• 300SB-C18 • 300SB-C8 • 300SB-C3 • 300Extend-C18	
	Poroshell 120	The 120Å pore size is ideal for the fast high resolution analysis of small hydrophilic peptides and peptide fragments in protein digests.
Peptides to DNA	PLRP-S	Particles are inherently hydrophobic so an alkyl ligand bonded phase is not required for reversed-phase separations. This gives a highly reproducible material that is free from silanols and heavy metal ions.
	• 100Å • 300Å • 1000Å • 4000Å	
Small molecules/synthesis	PLRP-S 100Å	
Recombinant peptides/proteins	PLRP-S 300Å	
Large proteins	PLRP-S 1000Å	
DNA/high speed separation	PLRP-S 4000Å	



## ZORBAX 300Å StableBond



Sterically Protected 300StableBond Bonded Phase

Agilent ZORBAX 300StableBond columns are an ideal choice for the reproducible separations of proteins and peptides for two key reasons. First, wide-pore, 300Å columns are necessary for an efficient separation of proteins and peptides, or other large molecules, in order 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. 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 1-D or 2-D proteomics separations.

\*DP is available in a 1.8 µm particle size only.

### Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits*	pH Range*	Endcapped	Carbon Load
ZORBAX 300SB-C18	300Å	45 m <sup>2</sup> /g	90 °C	1.0-8.0	No	2.8%
ZORBAX 300SB-C8	300Å	45 m <sup>2</sup> /g	80 °C	1.0-8.0	No	1.5%
ZORBAX 300SB-C3	300Å	45 m <sup>2</sup> /g	80 °C	1.0-8.0	No	1.1%
ZORBAX 300SB-CN	300Å	45 m <sup>2</sup> /g	80 °C	1.0-8.0	No	1.2%

RRHD Diphenyl and HILIC phases due to launch 2012. Visit [www.agilent.com](http://www.agilent.com) for details.

Specifications represent typical values only.

\*300StableBond 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. At mid or high pH, 300Extend-C18 is recommended.



### Tips & Tools

Further information can be found in the following publication:

*Comparison of ZORBAX StableBond 300Å LC Columns to Optimize Selectivity for Antibody Separations Using HPLC and LC/MS* (5989-6840EN)

[www.agilent.com/chem/library](http://www.agilent.com/chem/library)



# Reversed-Phase HPLC

## Higher resolution of intact monoclonal antibody

**Column:** ZORBAX RRHD 300SB-C8  
857750-906  
**2.1 x 50 mm, 1.8  $\mu$ m**

Mobile Phase: A: H<sub>2</sub>O:IPA (98:2) + 0.1% TFA (v/v)  
B: IPA:ACN:H<sub>2</sub>O (70:20:10) + 0.1% TFA (v/v)

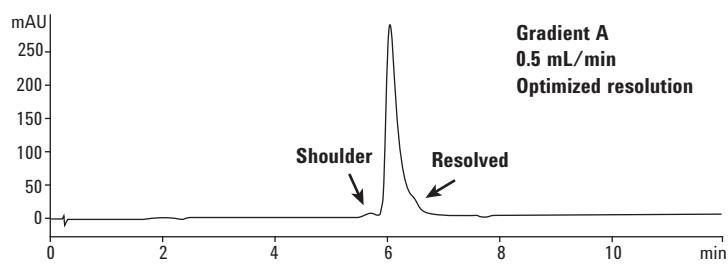
Flow Rate: Between 0.5 mL/min and 1.0 mL/min

Gradient: Multi-segmented and linear elution

Temperature: 80 °C

Detector: Agilent 1290 LC Infinity system with auto injector (ALS), binary pump and thermostatted oven and diode array detector (DAD)

Detection: UV, 225 nm



## Higher resolution of oxidation study

**Column:** ZORBAX RRHD 300SB-C18  
857750-902  
**2.1 x 50 mm, 1.8  $\mu$ m**

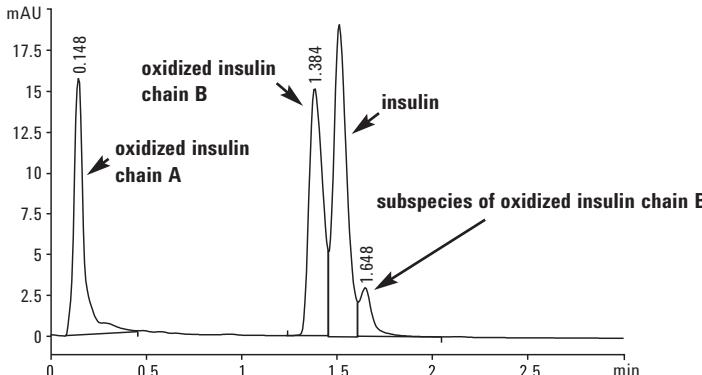
Mobile Phase: A: 0.1% TFA  
B: 0.01% TFA + 80% ACN

Flow Rate: 1.0 mL/min

Gradient: 33 to 50% B, 0 to 4 min

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

Sample: Insulin, insulin chain A and chain B, oxidized (bovinesigma, 1 mg/mL)



It is evident that the oxidized insulin chains are resolved from insulin in under 2 minutes using the Agilent ZORBAX RRHD 300SB-C18 2.1 x 50 mm, 1.8  $\mu$ m column.

## Tips & 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 300Å pore size for proteins up to 100-500 kDa.



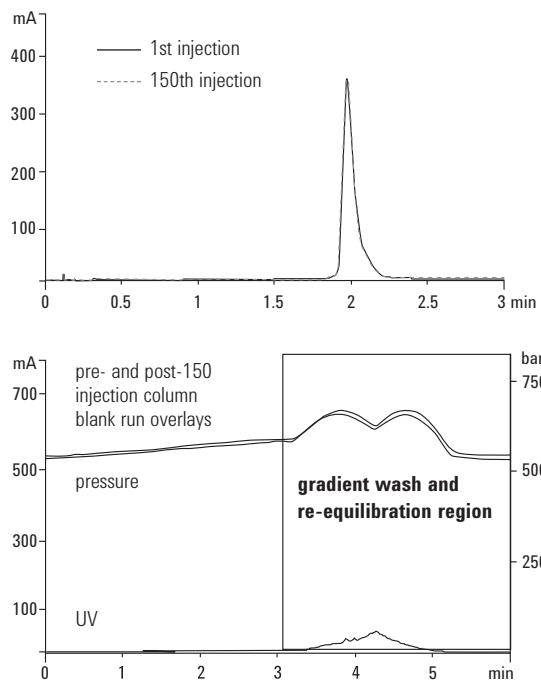
**Improved reproducibility of monoclonal antibodies**

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

Mobile Phase: A: H<sub>2</sub>O:IPA (98.2), 0.1% TFA  
B: IPA:ACN:H<sub>2</sub>O (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 timescale**

Time (min)	% Solvent B
0.00	25
3.00	35
4.00	90
5.00	25

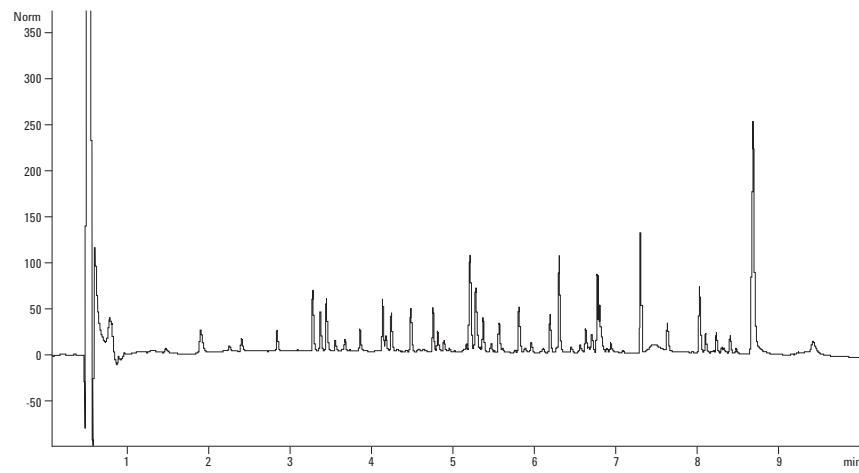


Excellent column reproducibility and protein recovery using Agilent ZORBAX 300SB-C8.

**Increased resolution for peptide mapping**

**Column:** ZORBAX 300SB-C18  
858750-902  
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA  
B: 0.01% TFA + 80% ACN  
Flow Rate: 0.5 mL/min  
Gradient: 2% B for 1 min, 2 to 45% B for 8.8 min, 45 to 95% B for 0.2 min, 95% B for 2 min, 95 to 2% B for 0.2 min  
Temperature: 50 °C  
Detector: 1290 Infinity LC with diode array detector at 280 nm  
Sample: Enzymatic protein digest (MAb)



The longer 100 mm Agilent ZORBAX RRHD 300SB-C18 column provides maximum resolution for protein digests – in this sample the total run time, including washing and equilibration, is under five minutes.

# Reversed-Phase HPLC

## Peptides: Effect of TFA concentration

Column: **ZORBAX 300SB-C8  
883995-906  
4.6 x 150 mm, 5 µm**

Mobile Phase: A: Water and TFA

B: ACN and TFA

Flow Rate: 1.0 mL/min

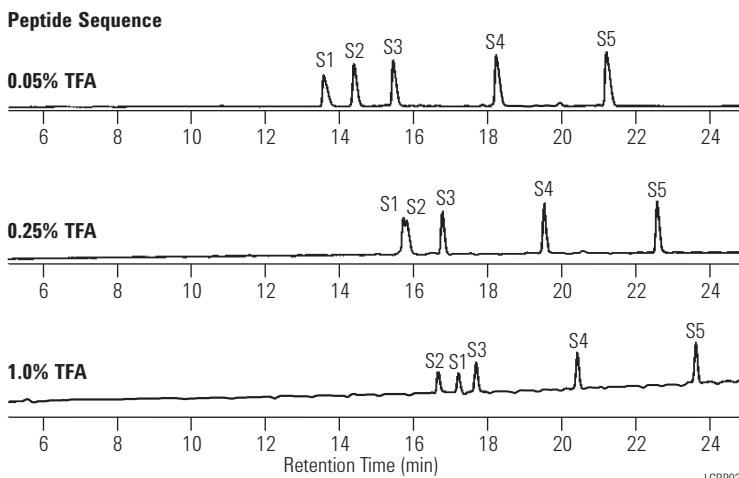
Gradient: 0 min 0% B

30 min 30% B

Temperature: 40 °C

Detector: UV 254 nm

Sample: Peptide Standards S1-S5, decapeptides differing slightly in hydrophobicity, 6 µL



## Peptides/proteins: Effect of elevated temperature

Column: **ZORBAX 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%)

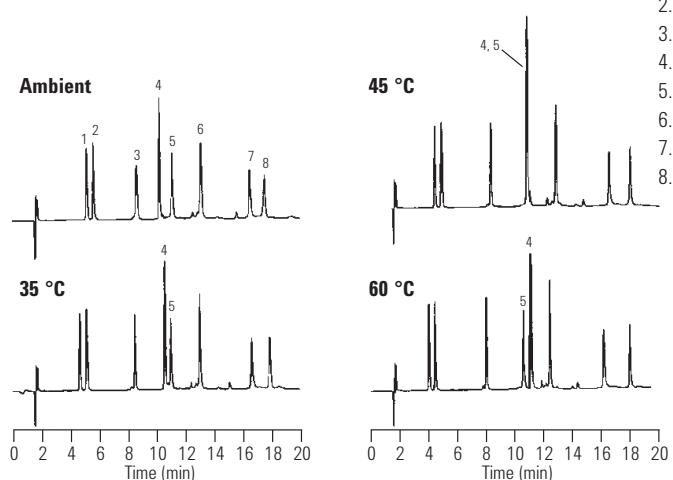
Flow Rate: 1.0 mL/min

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

## Tips & Tools

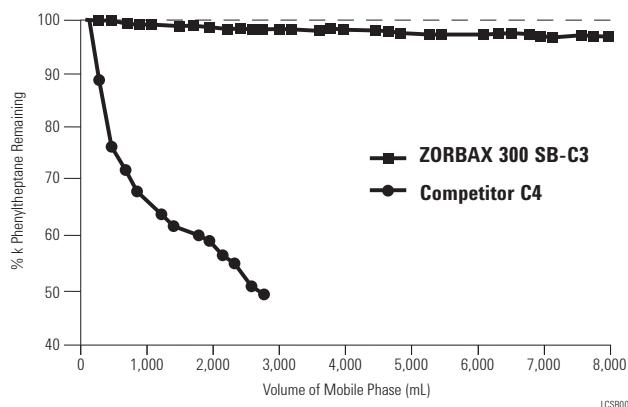
The Agilent 1290 Infinity LC delivers significantly faster results and higher data quality – enabling more informed decisions in shorter time. This higher productivity gives you competitive advantages and provides you a higher return on investment. Calculate for yourself how much you can save by deploying the 1290 Infinity technology. The online method translator and cost savings calculator helps you to transfer your HPLC methods and calculate your cost savings, at [www.agilent.com/chem/hplc2uhplc](http://www.agilent.com/chem/hplc2uhplc)



**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: Gradients 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 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**  
**883995-909**  
**4.6 x 150 mm, 5 µm**

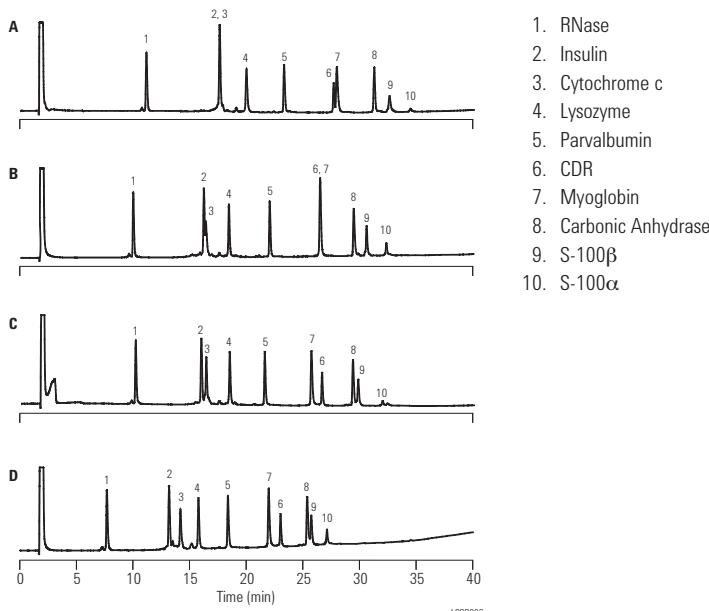
**Column D:** **ZORBAX 300SB-CN**  
**883995-905**  
**4.6 x 150 mm, 5 µm**

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



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.

# Reversed-Phase HPLC

## ZORBAX 300Å StableBond

Hardware Description	Size (mm)	Particle Size ( $\mu\text{m}$ )	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56
<b>Standard Columns (no special hardware required)</b>						
Semi-Preparative	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
Narrow Bore RRHD	2.1 x 50	1.8	857750-902	857750-906		857750-909
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
 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

RRHD Diphenyl and HILIC phases due to launch 2012. Visit [www.agilent.com](http://www.agilent.com) for details.

(Continued)



### Tips & Tools

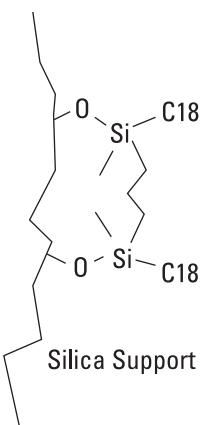
Whatever your instrument, Agilent has a biocolumn to deliver the results you need.



**ZORBAX 300Å StableBond**

<b>Hardware</b>	<b>Description</b>	<b>Size (mm)</b>	<b>Particle Size (<math>\mu\text{m}</math>)</b>	<b>300SB-C18 USP L1</b>	<b>300SB-C8 USP L7</b>	<b>300SB-CN USP L10</b>	<b>300SB-C3 USP L56</b>
<b>PrepHT Cartridge Columns (require endfittings kit 820400-901)</b>							
 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 x 7.5		5	820212-921	820212-918	820212-924	820212-924
 Guard Cartridge Hardware				820444-901	820444-901	820444-901	820444-901
<b>Capillary Glass-lined Columns</b>							
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		
<b>Nano Columns (PEEK fused silica)</b>							
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		

RRHD Diphenyl and HILIC phases due to launch 2012. Visit [www.agilent.com](http://www.agilent.com) for details.



Novel Bidentate C18-C18 Bonding  
for Extend-C18 Bonded Phase

## ZORBAX 300Å 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

Agilent ZORBAX 300Å 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.

### Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits*	pH Range	Endcapped	Carbon Load
ZORBAX 300Å Extend-C18	300Å	45 m <sup>2</sup> /g	60 °C	2.0-11.5	Double	4%

Specifications represent typical values only.

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

### Tips & Tools

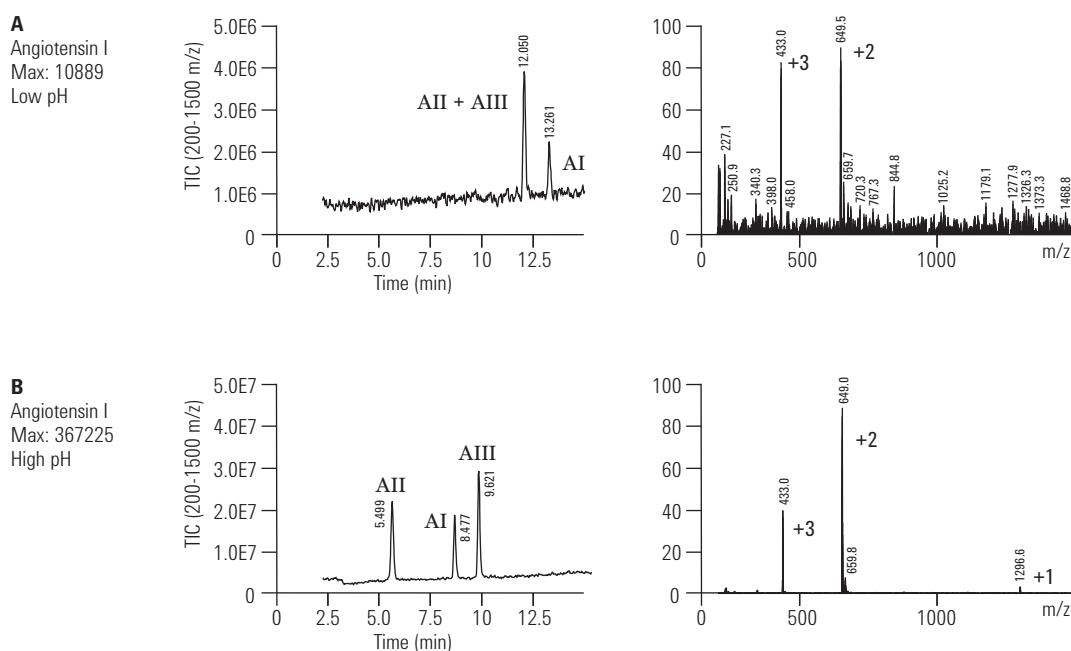
Selecting the right column is only part of the total solution. Don't forget key supplies such as our wide range of LC lamps.



### LC/MS analysis of angiotensin on Extend-C18

**Column:** ZORBAX Extend-C18  
773700-902  
2.1 x 150 mm, 5  $\mu$ m

Mobile Phase:	Acidic Conditions: A: 0.1% TFA in water B: 0.085% TFA in 80% acetonitrile (ACN)	Flow Rate: 0.2 mL/min	Gradient: 15-50% B in 15 min	MS Conditions: Pos. Ion ESI- Vf 70 V, Vcap 4.5 kV, N2- 35 psi, 12 L/min., 325 °C
	Basic Conditions: A: 10 mM NH <sub>4</sub> OH in water B: 10 mM NH <sub>4</sub> OH in 80% ACN	Temperature: 35 °C	Sample: 2.5 $\mu$ L sample (50 pmol each) Angiotensin I, II, III	



LC30003

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.

# Reversed-Phase HPLC

## Long life at high pH with 300Extend-C18

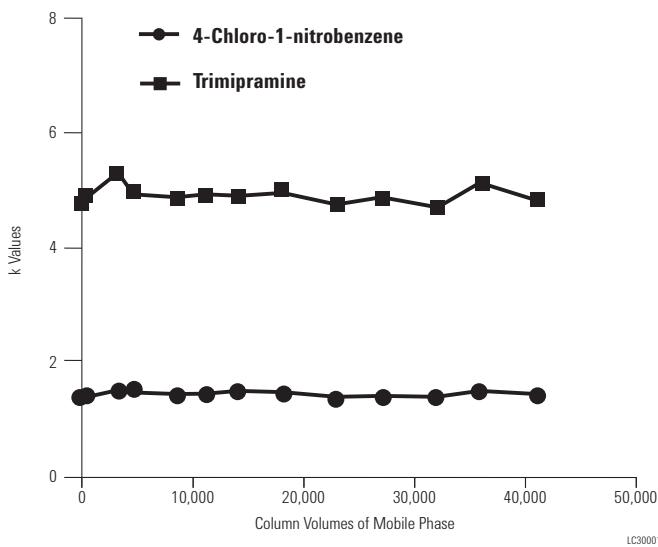
Column: **ZORBAX Extend-C18  
773450-902  
4.6 x 150 mm, 5  $\mu$ m**

Mobile Phase: 20% 20 mM NH<sub>4</sub>OH, 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.



LC30001

## Use ZORBAX Extend-C18 for alternate selectivity at high pH

Column: **ZORBAX Extend-C18  
773700-902  
2.1 x 150 mm, 5  $\mu$ m**

Mobile Phase: A: 0.1% TFA in Water  
B: 0.085% TFA in 80% ACN  
A: 20 mM NH<sub>4</sub>OH in Water  
B: 20 mM NH<sub>4</sub>OH in 80% ACN

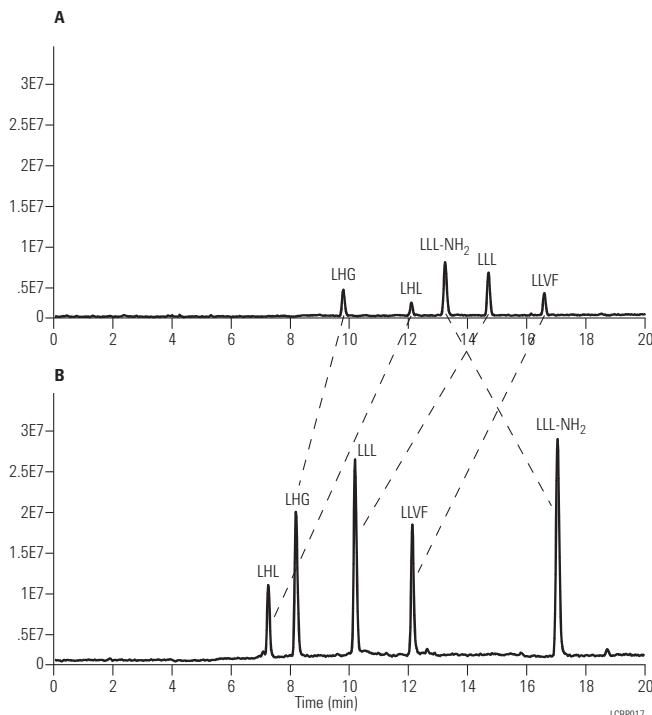
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

MS Conditions: Pos. Ion ESI-Vf 70V, Vcap 4.5 kV  
N<sub>2</sub> – 35 psi, 12 L/min, 300 °C  
4  $\mu$ L (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 result. Just by changing pH, a complimentary 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, so the complimentary separation can be investigated with one column. Better MS sensitivity for this sample is also achieved at high pH.



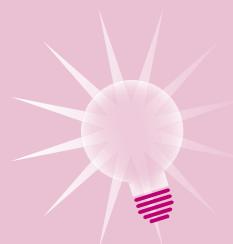
LCBP017

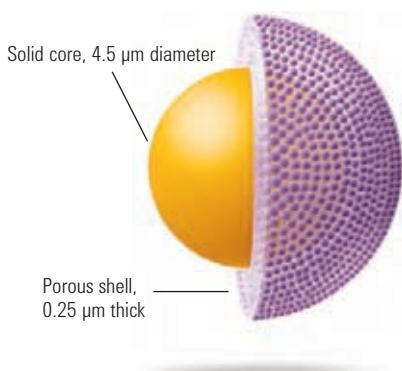
**ZORBAX 300Å Extend-C18**

Hardware Description	Size (mm)	Particle Size ( $\mu\text{m}$ )	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	
<b>ZGC</b>	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-932
<b>ZGC</b>	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-932
<b>ZGC</b>	Guard Hardware Kit			820999-901
<b>Capillary Glass-lined Columns</b>				
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 & 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. Learn how to fix this common problem at [www.agilent.com/chem/troubleshooting](http://www.agilent.com/chem/troubleshooting).





## Poroshell 300

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

Agilent Poroshell 300 columns are ideal for fast separations of proteins and peptides because the superficially porous particle allows for fast flow rates to be used while maintaining sharp, efficient peaks. 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 practical rapid HPLC separations of peptides and proteins up to 500-1,000 kDa possible with 400/600 bar HPLC systems, including the Agilent 1260 Infinity Bio-inert. Poroshell columns bonded 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 be used for analytical protein separations as well as LC/MS separations.



### Column Specifications

Bonded Phase	Pore Size	Temp. Limits*	pH Range	Endcapped
Poroshell 300SB-C18, C8, C3	300Å	90 °C	1.0-8.0	No
Poroshell 300Extend-C18	300Å	40 °C above pH 8 60 °C below 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, 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 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<sub>2</sub>O  
B: 0.07% TFA in ACN

Flow Rate: 3.0 mL/min

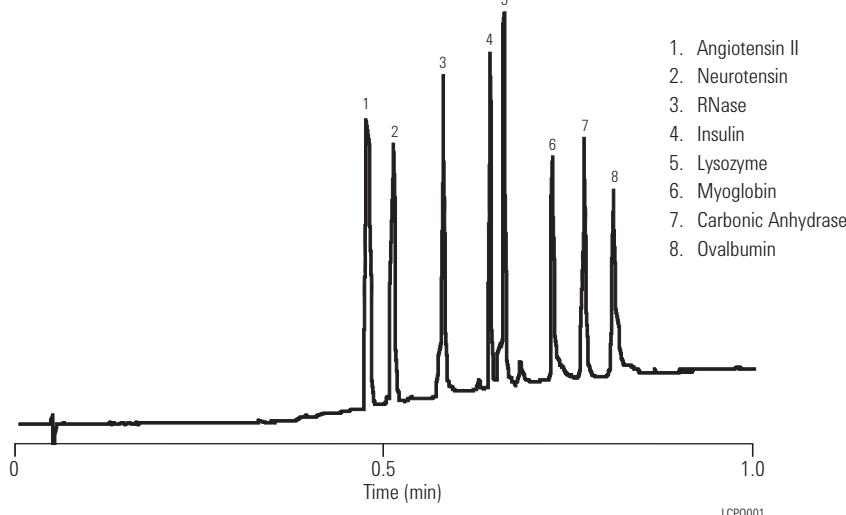
Gradient: 5-100% B in 1.0 min

Temperature: 70 °C, 260 bar pressure

Detector: 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 & Tools

Further information can be found in the following publications:

*Poroshell 300SB-C18* (5988-2100ENUS)

*Rapid HPLC Analysis of Monoclonal Antibody IgG<sub>1</sub> Heavy Chains Using ZORBAX Poroshell 300SB-C8* (5989-0070EN)

*Use of Temperature to Increase Resolution in the Ultrafast HPLC Separation of Proteins with ZORBAX Poroshell 300SB-C8 HPLC Columns* (5989-0589EN)

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

[www.agilent.com/chem/library](http://www.agilent.com/chem/library)



# Reversed-Phase HPLC

## Reduce peptide map analysis time by 90% with Poroshell 300SB

**Column A:** Poroshell 300SB-C18

660750-902

2.1 x 75 mm, 5 µm

**Column B:** ZORBAX 300SB-C18

883750-902

2.1 x 150 mm, 5 µm

Mobile Phase: A: 95% H<sub>2</sub>O, 5% ACN, 0.1% TFA

B: 5% H<sub>2</sub>O, 95% ACN, 0.07% TFA

Flow Rate: 1 mL/min

0.208 mL/min

Gradient: 0-100% B = 12 min

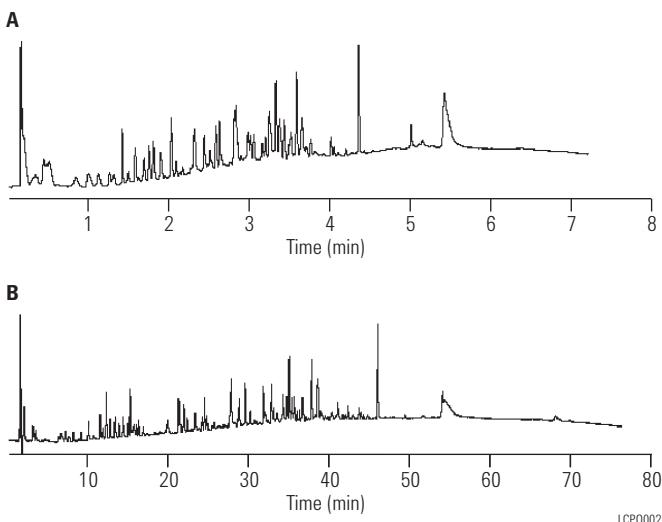
0-100% B = 120 min

Temperature: 70 °C

Sample: 20 µL (0.22 µg/1 µL)

BSA Tryptic Digest

(15 hours, 70 pmol)



LCP0002

A single chromatographic run of a protein tryptic digest can require one hour or more to complete. With Poroshell columns, the same complex separation can be completed in 1/10th the time.

## 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 6000 V

Drying Gas Flow: 12 L/min

Drying Gas Temperature: 350 °C

Nebulizer: 45 psi

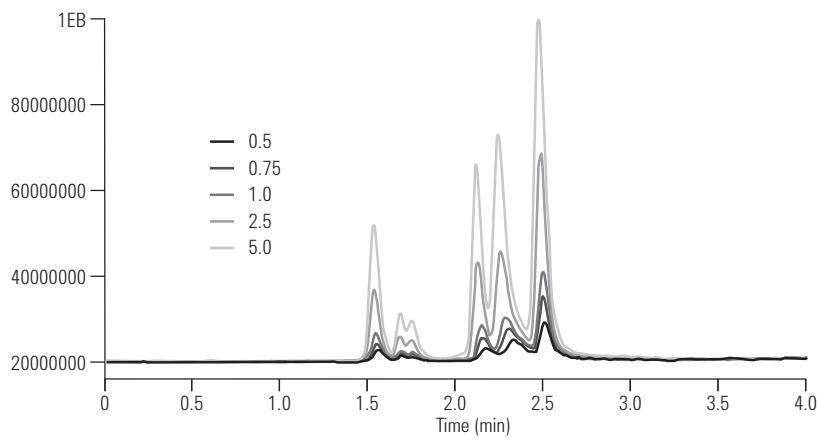
Fragmentor Voltagte: 140 V

Scan: 600-2500

Stepsize: 0.15 amu

Peak width: 0.06 min

Sample: 1 µL



LCP0003

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. Poroshell columns have also been used for rapid MS identification of intact proteins, even in the presence of stabilizers and tissue culture media.

**Monoclonal IgG1 chains:  
Separation on Poroshell 300SB-C8**

**Column:** Poroshell 300SB-C8  
**660750-906**  
**2.1 x 75 mm, 5 µm**

**Mobile Phase:** A: 90% water:  
10% ACN + 3 mL/L of MW 300 PEG  
B: 10% water:  
90% ACN + 3 mL/L of MW 300 PEG

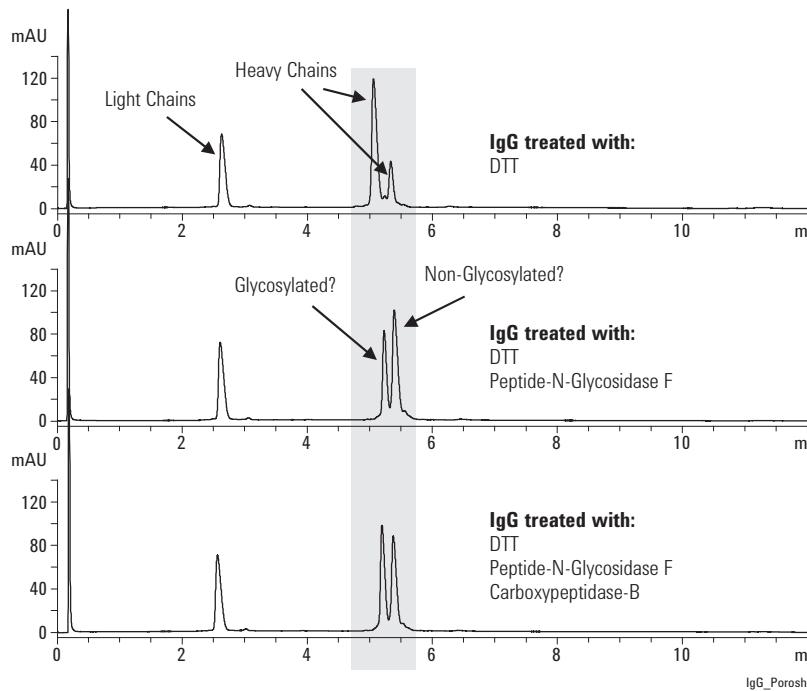
**Flow Rate:** 1.0 mL/min

**Gradient:** 0 min 25% B  
10 min 40% B  
10.1 min 25% B  
12 min 25% B

**Temperature:** 70 °C

**Sample:** Monoclonal IgG1

*Courtesy of:  
Novartis Pharma,  
Biotechnology, Basel  
Dr. Kurt Forrer  
Patrik Roethlisberger*



### Tips & Tools

Agilent offers an extensive selection of certified chromatography sample vials including polypropylene and deactivated and siliconized glass. For more information see 5990-90322EN

[www.agilent.com/chem/library](http://www.agilent.com/chem/library)



# Reversed-Phase HPLC

## Protein elution pattern on ZORBAX Poroshell 300SB-C8

**Column:** Poroshell 300SB-C8  
660750-906  
2.1 x 75 mm, 5  $\mu$ m

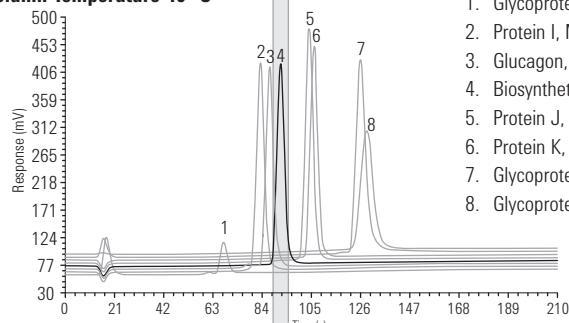
Mobile Phase: A: 0.1% TFA in H<sub>2</sub>O  
B: 0.1% TFA in ACN

Flow Rate: 1.0 mL/min

Gradient: B: 20 to 70% in 3 min

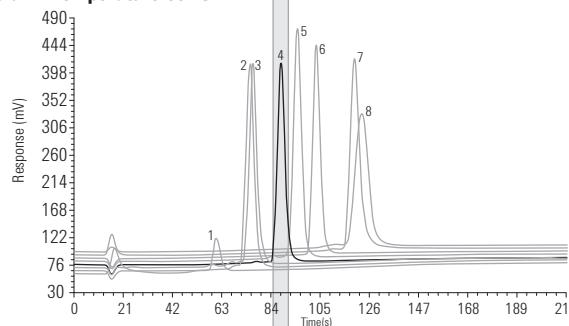
Detector: UV (214 nm)

### Column Temperature 40 °C

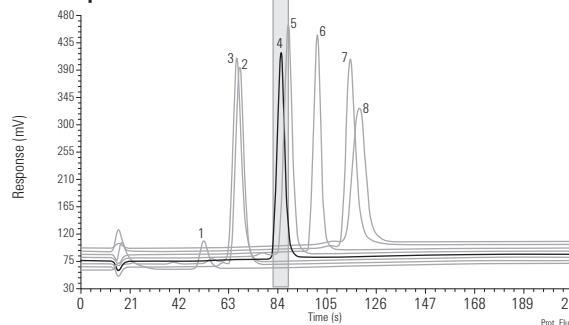


1. Glycoprotein X, MW ~ 22 kDa
2. Protein I, MW ~ 4 kDa
3. Glucagon, MW ~ 3.5 kDa
4. Biosynthetic human insulin, MW ~ 6 kDa
5. Protein J, MW ~ 3 kDa
6. Protein K, MW ~ 6 kDa
7. Glycoprotein Y, MW ~ 45 kDa
8. Glycoprotein Z, MW ~ 30 kDa

### Column Temperature 60 °C



### Column Temperature 75 °C



## Poroshell 300

Hardware Description	Size (mm)	Particle Size ( $\mu$ m)	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



## Poroshell 120

- 120Å pore size for shorter chain peptide mapping
- UHPLC performance on 600 bar systems
- Up to 90% of the efficiency of sub-2 µm
- 2X the efficiency of 3.5 µm
- Up to 50% less pressure than sub-2 µm columns

Agilent Poroshell 120 columns are a 2.7 µm particle with a 1.7 µm solid core and 0.5 µm porous outer layer. This small particle size provides high efficiency, similar to sub-2 µm columns, but with 40-50% less pressure. These high efficiency, high resolution columns can be used on any type of LC. The porous outer layer and solid core limit diffusion distance and improve separation speed while the narrow particle size distribution improves efficiency and resolution. The columns can support high pressure and multiple columns can be used for the highest resolution and efficiency possible. The smaller 120Å pore size is ideal for fast high resolution analysis of small hydrophilic peptides in protein digests.

### Column Specifications

Bonded Phase	Pore Size	Temp. Limits	pH Range	Endcapped	Carbon Load
SB-C18	120Å	90 °C	1.0-8.0	No	8%
EC-C18	120Å	60 °C	2.0-8.0	Double	10%

Specifications represent typical values only.

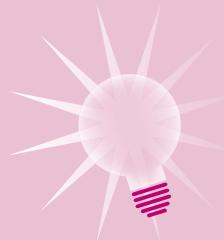


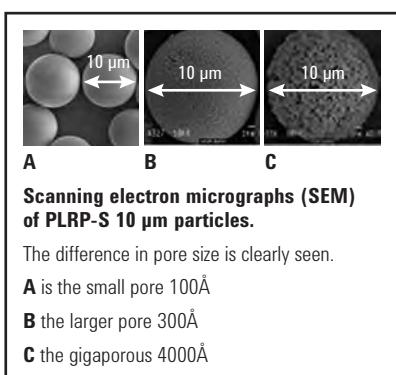
## Poroshell 120

Description	Size (mm)	Particle Size ( $\mu\text{m}$ )	SB-C18 USP L1	EC-C18 USP L1
Analytical	4.6 x 150	2.7	683975-902	693975-902
Analytical	4.6 x 100	2.7	685975-902	695975-902
Solvent Saver	3.0 x 150	2.7	683975-302	693975-302
Solvent Saver	3.0 x 100	2.7	685975-302	695975-302
Narrow Bore	2.1 x 150	2.7	683775-902	693775-902
Narrow Bore	2.1 x 100	2.7	685775-902	695775-902

### Tips & Tools

All Agilent LC conventional columns (non-cartridge) come with a QC chromatogram. Run a standard sample of QC components or key analytes from your lab on each new column before use, and keep this chromatogram in your log book. Re-run this test occasionally to see how your column has aged. Learn how this can help with troubleshooting issues by watching the videos at [www.agilent.com/chem/lctroubleshooting](http://www.agilent.com/chem/lctroubleshooting)





## PLRP-S HPLC Columns

- 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 (100Å-4000Å) 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, 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 nano/capillary separations, including both bottom-up and top-down proteomics, analytical separations, and preparative purifications. In addition, process columns can be packed with bulk media.

### Column Specifications

<b>pH Range</b>	1-14
<b>Buffer Content</b>	Unlimited
<b>Organic Modifier</b>	1-100%
<b>Temperature Limits</b>	200 °C
<b>Maximum Pressure</b>	5-8 μm: 3000 psi (210 bar) 3 μm: 4000 psi (300 bar)

### PLRP-S Applications

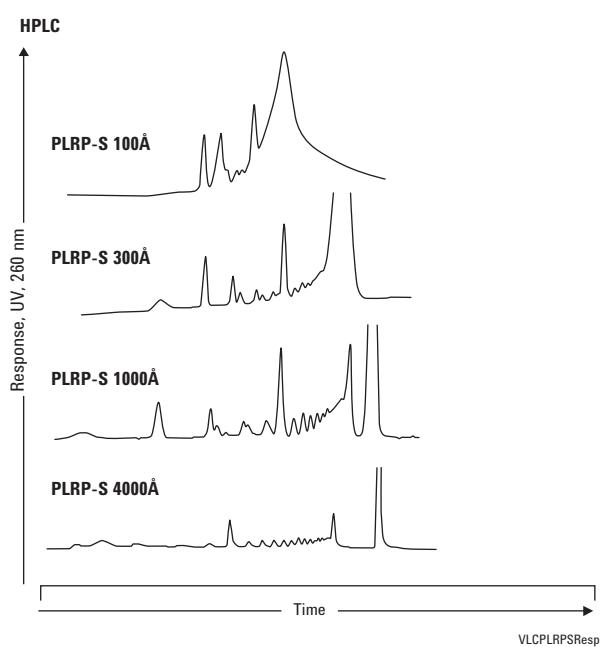
<b>Pore Size</b>	<b>Application</b>
100Å	Small molecules/synthetic biomolecules
300Å	Recombinant peptides/proteins
1000Å	Large proteins
4000Å	DNA/high speed

# Reversed-Phase HPLC

## HPLC of 25 bp DNA ladder

Column: PLRP-S, 2.1 x 150 mm

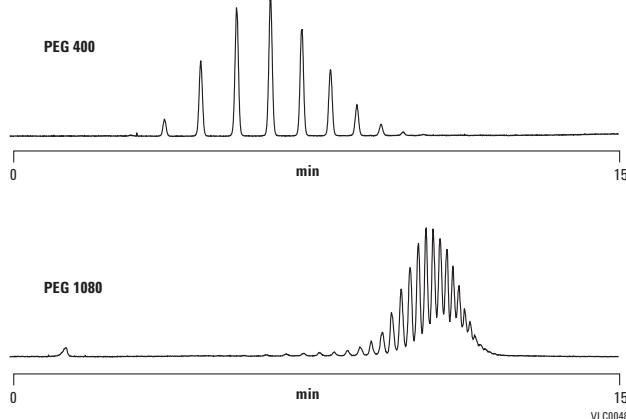
Mobile Phase: A: 0.1 M TEAA  
B: 0.1 M TEAA in 50% water:50% ACN  
Flow Rate: 200 µL/min  
Gradient: 12.5-50% B in 150 min



## Polyethylene glycols

Column: PLRP-S 100Å  
PL1111-3500  
4.6 x 150 mm, 5 µm

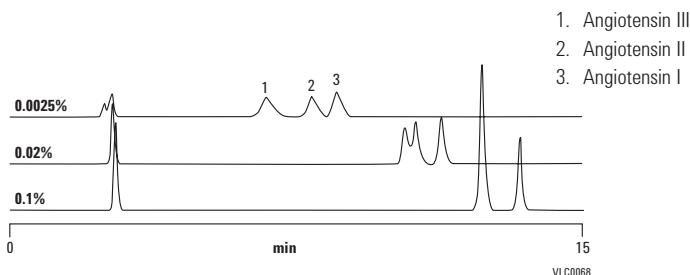
Mobile Phase: A: Water  
B: ACN  
Gradient: 10-30% B in 12 min, held at 30% B for 3 min  
Flow Rate: 1.0 mL/min  
Injection Volume: 10 µL  
Sample Conc: 1 mg/mL  
Detector: ELS (neb=50 °C, evap=70 °C, gas=1.6 SLM)



### Exploiting chemical stability – TFA concentration

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

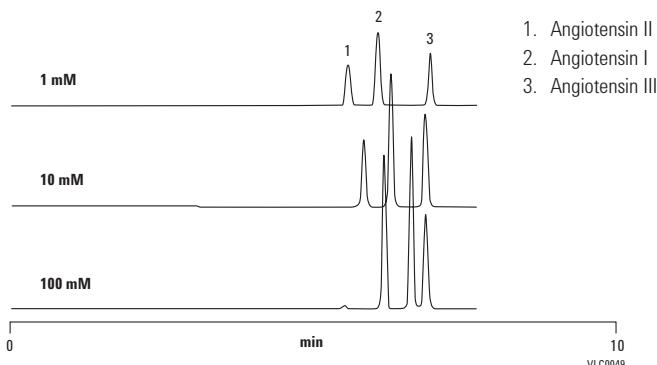
Mobile Phase: A: TFA (various %) in water  
B: TFA (various %) in ACN  
Gradient: Linear 12-40% B in 15 min  
Flow Rate: 1.0 mL/min  
Detector: ELS (neb=75 °C, evap=85 °C, gas=1.0 SLM)



### Exploiting chemical stability – NH<sub>4</sub>OH concentration

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

Mobile Phase: A: NH<sub>4</sub>OH (various mM) in water  
B: NH<sub>4</sub>OH (various mM) in ACN  
Gradient: Linear 10-100% B in 15 min  
Flow Rate: 1.0 mL/min  
Detector: ELS (neb=80 °C, evap=85 °C, gas=1.0 SLM)



# Reversed-Phase HPLC

## Alberta Peptide Institute test mix

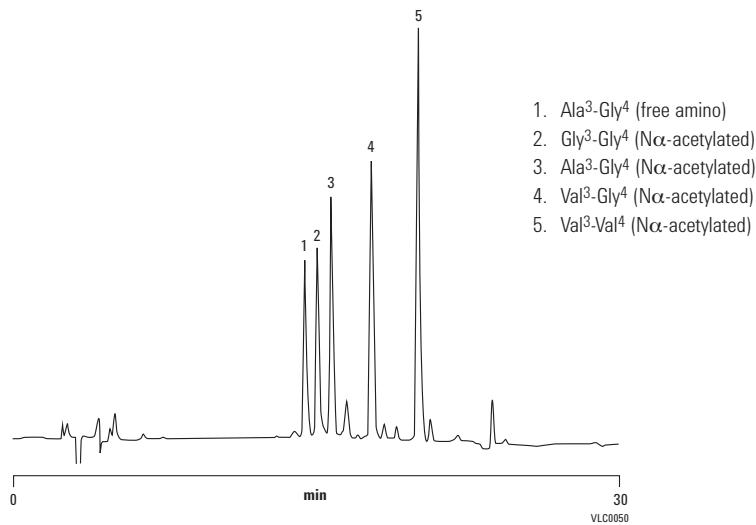
Column: **PLRP-S 100Å  
PL1512-5500  
4.6 x 250 mm, 5 µm**

Mobile Phase: A: 0.1% TFA in 99% water:1% ACN  
B: 0.1% TFA in 70% water:30% ACN

Gradient: 0-100% B in 30 min

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm



## Whey proteins in dairy samples – milk

Column: **PLRP-S 300Å  
PL1512-3801  
4.6 x 150 mm, 8 µm**

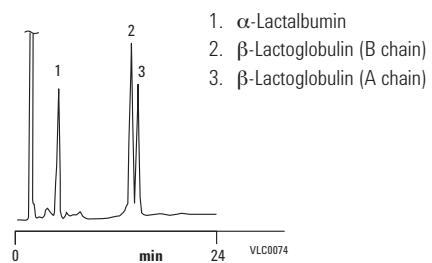
Mobile Phase: A: 0.1% TFA in 99% water:1% ACN  
B: 0.1% TFA in 1% water:99% ACN

Gradient: 36-48% B, 0-24 min, 48-100% B, 24-30 min  
100% B, 30-35 min, 100-36% B, 35-40 min

Flow Rate: 1.0 mL/min

Injection Volume: 10 µL

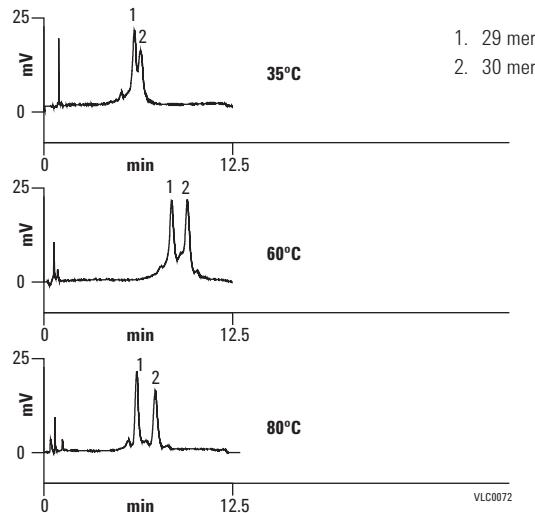
Detector: UV, 220 nm



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

**Column:** PLRP-S 100Å  
PL1512-1300  
4.6 x 50 mm, 3 µm

Mobile Phase: A: 100 mM TEAA  
B: 100 mM TEAA in 25% ACN  
Gradient: 5% change in buffer B over 5 min  
Flow Rate: 1.0 mL/min  
Temperature: 35 °C, 60 °C, or 80 °C  
Detector: UV, 254 nm

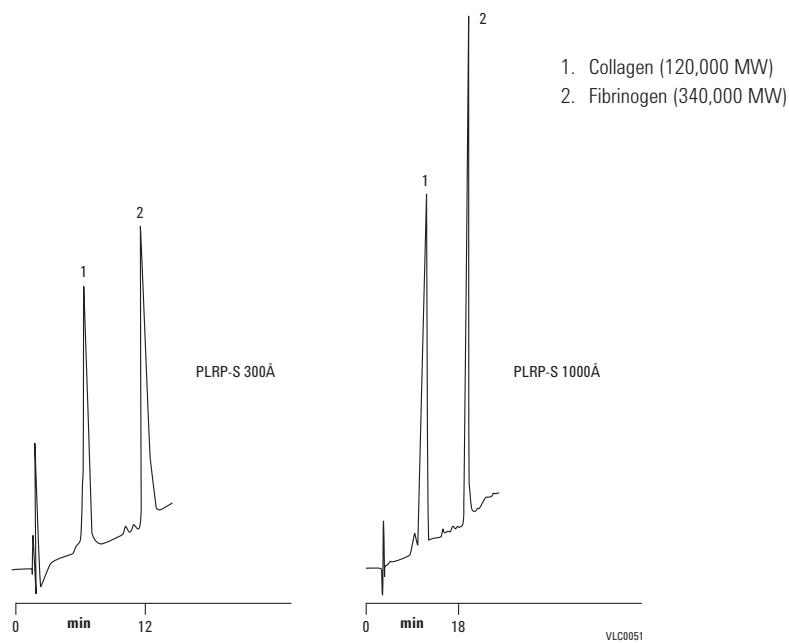


**Large fibrous proteins**

**Column:** PLRP-S 300Å  
PL1512-3801  
4.6 x 150 mm, 8 µm

**Column:** PLRP-S 1000Å  
PL1512-3802  
4.6 x 150 mm, 8 µm

Mobile Phase: A: 0.25% TFA in water  
B: 0.25% TFA in 5% water:95% ACN  
Gradient: 20-60% B in 15 min  
Flow Rate: 1.0 mL/min  
Detector: UV, 220 nm



## PLRP-S HPLC Columns

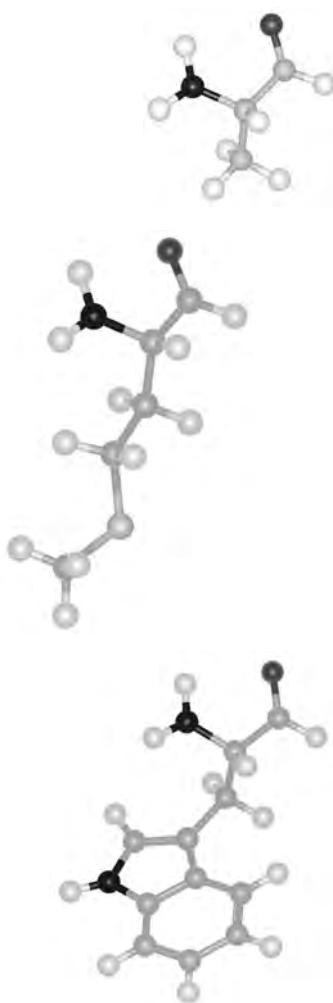
Hardware	Size (mm)	Particle Size ( $\mu\text{m}$ )	PLRP-S 100Å USP L21	PLRP-S 300Å USP L21	PLRP-S 1000Å USP L21	PLRP-S 4000Å USP L21
	4.6 x 250	8	PL1512-5800	PL1512-5801	PL1512-5802	
	4.6 x 150	8	PL1512-3800	PL1512-3801	PL1512-3802	PL1512-3803
	4.6 x 50	8		PL1512-1801	PL1512-1802	PL1512-1803
	4.6 x 250	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		
 PL	PLRP-S Guard Cartridges for 5 x 3 mm, 2/pk		PL1612-1801	PL1612-1801	PL1612-1801	PL1612-1801
 PL	Guard Cartridge holder for 3.0 x 5.0 mm cartridges		PL1310-0016	PL1310-0016	PL1310-0016	PL1310-0016

### Tips & Tools

For microbore columns ordering information, see page 118

For prep columns and media ordering information, see page 125





## ZORBAX Amino Acid Analysis (AAA) Columns and Supplies

### ZORBAX Eclipse Amino Acid Analysis (AAA) Columns

- High resolution and rapid analysis of 24 amino acids
- 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 Agilent 1100/1200 autosampler

The Agilent ZORBAX Eclipse AAA high efficiency column rapidly separates amino acids following an updated and improved protocol. Total analysis from injection-to-injection can be achieved in as little as 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. Exceptional 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 Agilent 1100/1200 HPLC instrument.

### ZORBAX Eclipse Amino Acid Analysis (AAA) Columns

Hardware	Description	Size (mm)	Particle Size ( $\mu\text{m}$ )	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	3.5	961400-302
	Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-931
	Guard Hardware Kit			820888-901

#### Tips & Tools

Further information can be found in the following publication:

*High-Speed Amino Acid Analysis (AAA) on 1.8  $\mu\text{m}$  Reversed-Phase (RP) Columns (5989-6297EN)*

[www.agilent.com/chem/library](http://www.agilent.com/chem/library)



## Amino Acid Standards

Each amino acid standard contains the following amino acids:

- L-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

### Amino Acid Standards, 10 x 1 mL ampoules\*

Description	Part No.
1 nmol/ $\mu$ L	5061-3330
250 pmol/ $\mu$ L	5061-3331
100 pmol/ $\mu$ L	5061-3332
25 pmol/ $\mu$ L	5061-3333
10 pmol/ $\mu$ L	5061-3334
Amino acids supplement kit Includes 1 g each of norvaline, sarcosine, asparagine, glutamine, tryptophan, and 4-hydroxyproline	5062-2478

\*Consider shelf-life and buy limited quantities, P/N 5062-2478 ships as 1 g vials

### Amino Acid Separations Reagents

Description	Part No.
OPA reagent, 10 mg/mL each in 0.4 M borate buffer o-phthalaldehyde (OPA) and 3-mercaptopropionic acid, 6 x 1 mL ampoules	5061-3335
FMOC reagent, 2.5 mg/mL in acetonitrile, 9-fluorenylmethylchloroformate, 1 mL, 10 ampoules	5061-3337
Borate buffer, 100 mL	5061-3339
DTDPA (Dithiodipropionic) reagent, for analysis of cysteine, 5 g	5062-2479

### High resolution of 24 amino acids using ZORBAX Eclipse-AAA protocol

**Column:** ZORBAX Eclipse AAA  
963400-902  
4.6 x 150 mm, 3.5  $\mu$ m

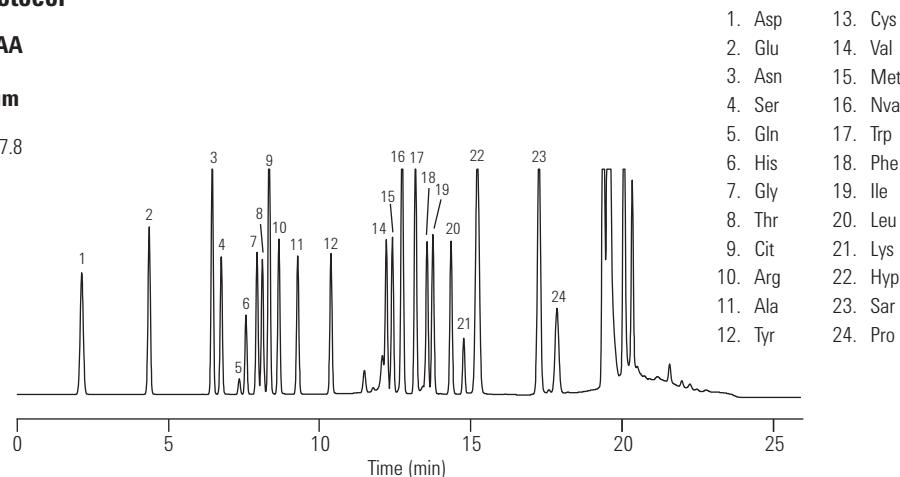
Mobile Phase: A: 40 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.8  
B: ACN:MeOH:Water,  
45:45:10 v/v

Flow Rate: 2 mL/min

Temperature: 40 °C

Detector: Fluorescence

Sample: 24 Amino Acids

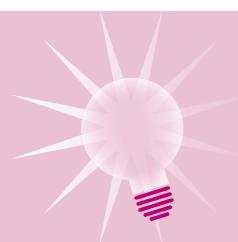


LCPAH01

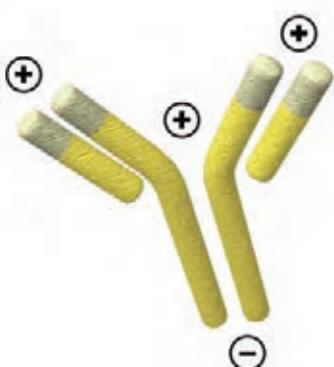
This high resolution separation of 24 amino acids is done in 18 minutes. If the Rapid Resolution 4.6 x 75 mm Eclipse AAA column is selected, these amino acids are resolved in 9 minutes.

### Tips & Tools

Quick Reference Guides list the common supplies you should have on hand to keep your Agilent 1200 series LC operating at peak efficiency. Download your free copy at [www.agilent.com/chem/getguides](http://www.agilent.com/chem/getguides)



## ION-EXCHANGE CHROMATOGRAPHY



### 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 biopharmaceuticals.

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.

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

- **Agilent non-porous Bio IEX columns** are designed for high-resolution, high-efficiency, and high-recovery separations.
- **Agilent Bio MAb columns** are optimized for separating charge isoforms of monoclonal antibodies.
- **Agilent 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 to separating antibodies, viruses, and DNA.

**Ion-Exchange Column Selection**

<b>Application</b>	<b>Agilent Columns</b>	<b>Notes</b>
Monoclonal antibodies	Agilent Bio MAb	Thorough characterization of monoclonal antibodies includes the identification and monitoring of acidic and basic isoforms. Agilent Bio MAb HPLC columns feature a unique resin specifically designed for high-resolution charge-based separations of monoclonal antibodies.
Peptides and proteins	Agilent Bio IEX	Agilent Bio Ion-Exchange columns are packed with polymeric, nonporous, ion-exchange particles. Bio IEX columns are designed for high resolution, high recovery and highly efficient separations.
Proteins, peptides and deprotected synthetic oligonucleotides	PL-SAX • 1000Å • 4000Å	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 delivers separations at high resolution with the 30 µm media used for medium pressure liquid chromatography.
Globular proteins and peptides	PL-SAX 1000Å	
Very large biomolecules/high speed	PL-SAX 4000Å	
Small peptides to large proteins	PL-SCX • 1000Å • 4000Å	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 delivers separations at higher resolution with the 30 µm media used for medium pressure liquid chromatography.
Globular proteins	PL-SCX 1000Å	
Very large biomolecules/high speed	PL-SCX 4000Å	
Antibodies (IgG, IgM), plasmid DNA, viruses, phages and other macro biomolecules	Bio-Monolith • Bio-Monolith QA • Bio-Monolith DEAE • Bio-Monolith SO <sub>3</sub> • Bio-Monolith Protein A	Strong cation-exchange, strong and weak anion-exchange, and Protein A phases. Bio-Monolith HPLC columns are compatible with preparative LC systems, including Agilent 1100 and 1200 HPLC systems.
Viruses, DNA, large proteins	Bio-Monolith QA	
Plasmid DNS, bacteriophages	Bio-Monolith DEAE	
Proteins, antibodies	Bio-Monolith SO <sub>3</sub>	



## Tips & Tools

Capillary electrophoresis is an alternative technique to liquid chromatography for the separation of charged isoforms. Further information can be found in the following Technical Note:

*Capillary electrophoresis focusing on the Agilent Capillary Electrophoresis system  
(5989-9852EN)*

[www.agilent.com/chem/library](http://www.agilent.com/chem/library)



## Agilent Bio MAb HPLC Columns

- A packing support composed of a rigid, spherical, highly cross-linked polystyrene divinylbenzene (PS/DVB) non-porous bead
- Particles grafted with a hydrophilic, polymeric layer, virtually eliminating non-specific binding of antibody proteins
- A different process is used to layer the weak cation-exchange phase to the particle making it a higher density than the Agilent 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. Agilent Bio MAb HPLC columns feature a unique resin specifically designed for high-resolution, charge-based separations of monoclonal antibodies. Compatible with aqueous solution buffers, acetonitrile/acetone/methanol and water mixtures. Commonly used buffers: 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

**Consistent ion-exchange MAb separation**

**Column:** Bio MAb, PEEK  
**5190-2411**  
**2.1 x 250 mm, 5  $\mu$ m**

**Buffer:** A: Sodium phosphate buffer, 20 mM  
 B: Buffer A + 400 mM NaCl

**Gradient:** 15-35% Buffer B from 0-30 min

**Flow Rate:** 0.65 mL/min

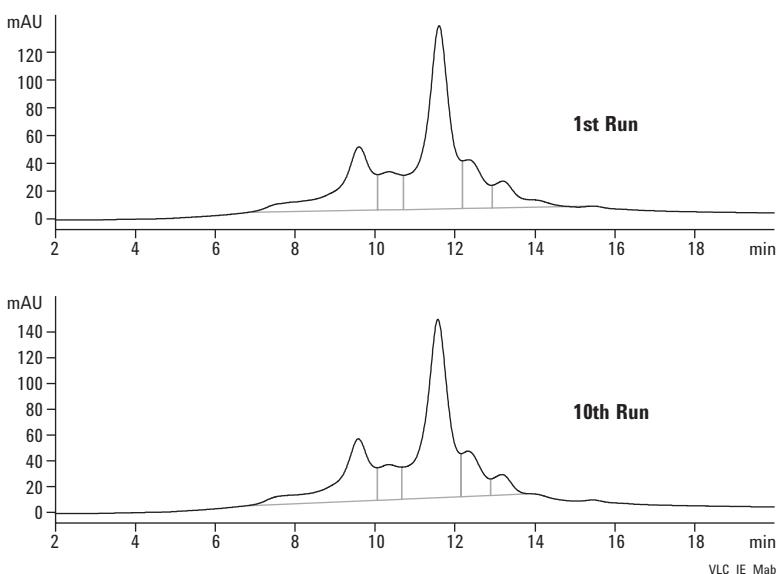
**Sample:** CHO-humanized MAb, 1 mg/mL

**Injection:** 2.5  $\mu$ L

**Detector:** UV 220 nm

**Temperature:** Ambient

To provide a metal free flow path Bio MAb PEEK columns are available

**Virtually eliminate retention time variations**

**Column:** Bio MAb, stainless steel  
**5190-2413**  
**4.6 x 250 mm, 10  $\mu$ m**

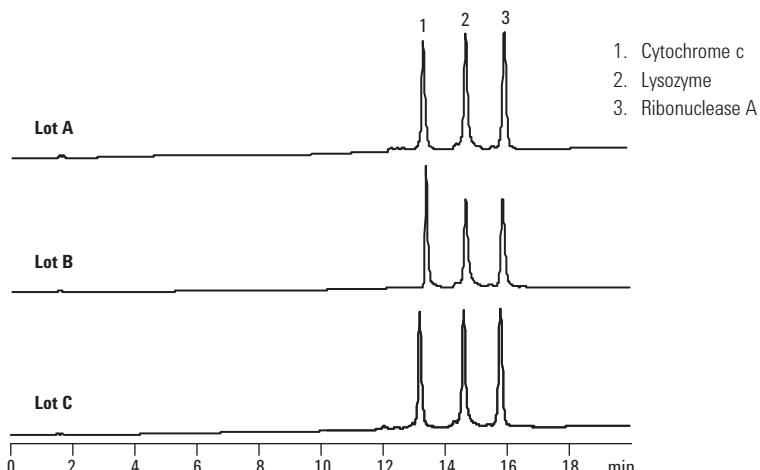
**Mobile Phase:** A: 10 mM phosphate, pH 6.0  
 B: A + 1.0 M NaCl

**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.

# Ion-Exchange Chromatography

## Charge isoform analysis of monoclonal antibodies

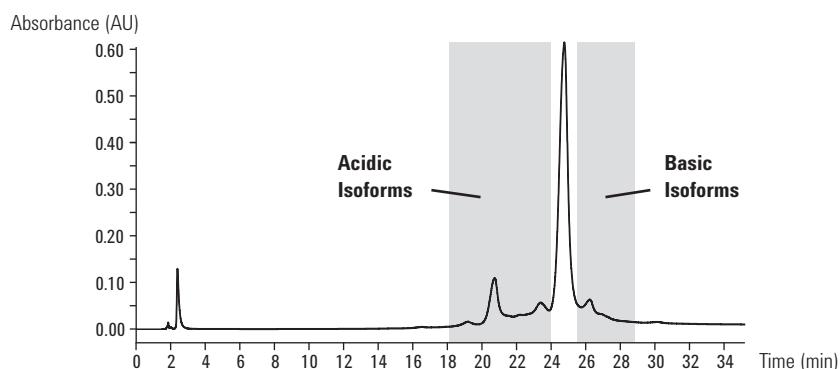
Column: **Bio MAb, PEEK  
5190-2407  
4.6 x 250 mm, 5 µm**

Mobile Phase: A: 10 mM Sodium Phosphate, pH 7.50  
B: A + 100 mM NaCl, pH 7.50

Flow Rate: 0.8 mL/min

Gradient: 15-95% B in 60 min

Sample: 5 µL, 5 mg/mL, MAb



High resolution separation of acidic and basic charge variants using the Agilent Bio MAb NP5 column

## Agilent Bio MAb HPLC Columns

Size (mm)	Particle Size (µm)	Bio MAb PEEK	Bio MAb Pressure Limit	Bio MAb Stainless Steel	Bio MAb Pressure Limit
4.6 x 250	10	5190-2415	275 bar, 4000 psi	5190-2413	275 bar, 4000 psi
4.6 x 50, Guard	10	5190-2416	275 bar, 4000 psi		
4.6 x 250	5	5190-2407	413 bar, 6000 psi	5190-2405	413 bar, 6000 psi
4.6 x 50, Guard	5	5190-2408	413 bar, 5800 psi		
4.6 x 50	3			5190-2403	551 bar, 8000 psi
4.6 x 50	1.7			5190-2401	689 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	689 bar, 8700 psi
2.1 x 250	10	5190-2419	275 bar, 4000 psi		
2.1 x 50, Guard	10	5190-2420	275 bar, 4000 psi		
2.1 x 250	5	5190-2411	413 bar, 5800 psi		
2.1 x 50, Guard	5	5190-2412	413 bar, 5800 psi		



## Agilent 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 anchoring) 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 anchoring to increase capacity

Agilent Bio IEX 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 offers 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 non-porous particles sizes.

### Column Specifications

Bonded Phase	ID	Particle Size	pH Stability	Operating Temperature Limit	Flow Rate
SCX (Strong cation-exchange) - SO <sub>3</sub> H	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 <sub>3</sub> ) <sub>3</sub>					
WAX (Weak anion-exchange) - N(C <sub>2</sub> H <sub>5</sub> ) <sub>2</sub>					

### Tips & Tools

More information is a click away. We have a variety of educational primers, application notes, maintenance guides, and literature available from Agilent for free.

To learn more, visit [www.agilent.com/chem/OnlineLibrary](http://www.agilent.com/chem/OnlineLibrary)



# Ion-Exchange Chromatography

## Exceptional separating power

**Column:** Agilent Bio SCX, stainless steel  
5190-2423  
4.6 x 50 mm, 3 µm

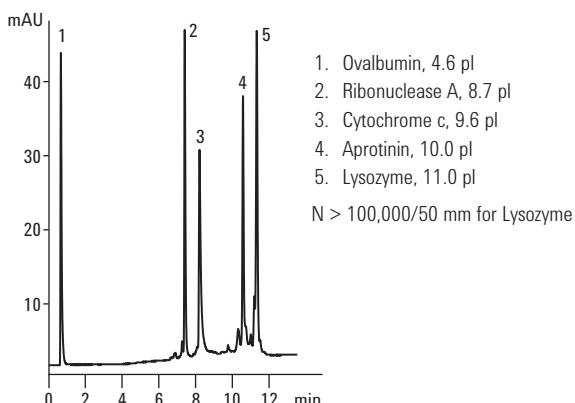
Buffer: 10 mM Phosphate, pH 6.0

Flow Rate: 0.5 mL/min

Gradient: 0-1.0 M NaCl, 15 min

Detector: 280 nm

The hydrophilic, polymeric layer and densely packed ion-exchange functional groups provide extremely sharp peak shapes and high resolution of a mixture of proteins with a broad range of isoelectric points (pI).



## Separation of protein standards on Agilent 3 µm ion-exchange columns by cation-exchange chromatography

**Column A:** Agilent Bio SCX, NP 3, 4.6 x 50 mm, SS

**Column B:** Agilent Bio WCX, NP 3, 4.6 x 50 mm, SS

**Column C:** Agilent Bio MAb, NP 3, 4.6 x 50 mm, SS

Mobile Phase: A: 10 mM NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, pH 5.70

B: A + 1 M NaCl

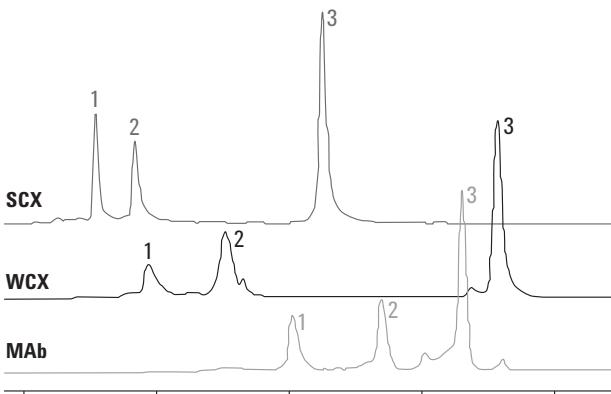
Flow Rate: 0.5 mL/min

Gradient: 0 min - 100% A : 0% B  
25 min - 0% A : 100% B

Temperature: Ambient

Detector: Agilent Infinity 1260 Bio-inert HPLC system -  
with diode array detector at 220 nm

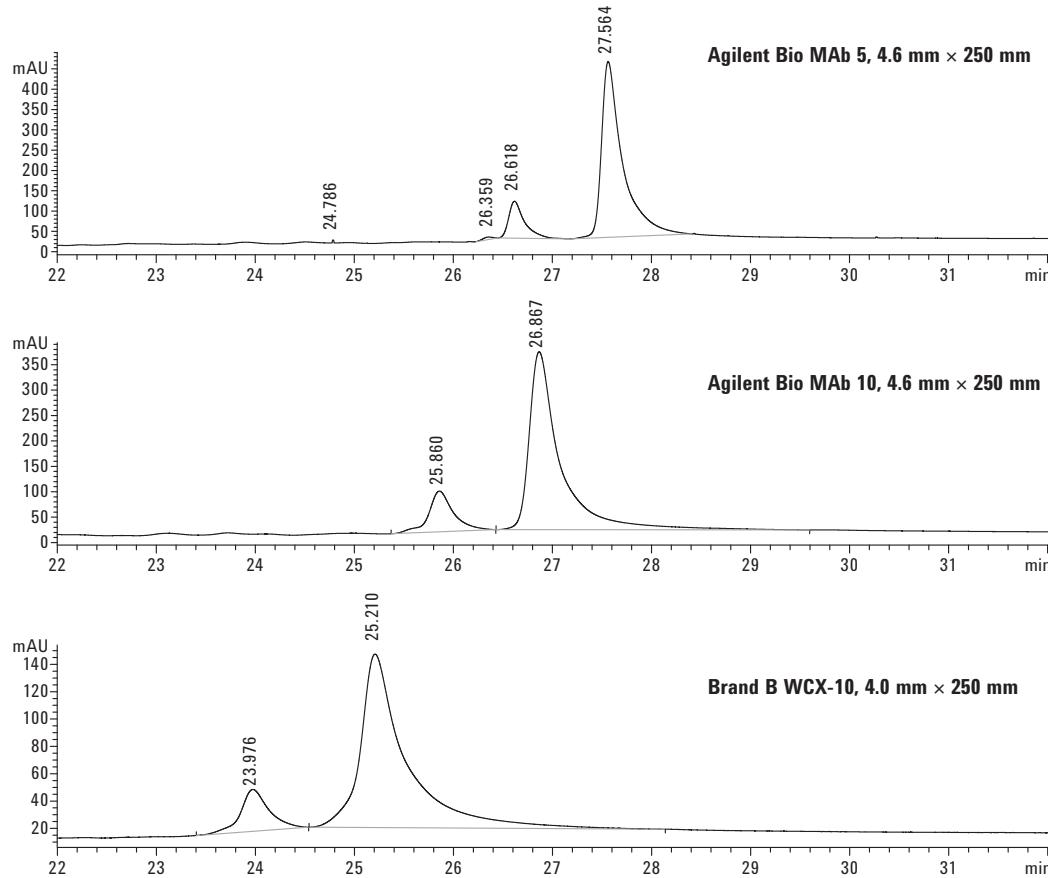
Sample: Cytochrome c, ribonuclease A, lysozyme and protein mix



## Illustration that Bio WCX, SCX and MAb columns are capable of producing protein separations

Agilent column	Peak number	Peak name	RT [min]	Height [mAU]	Area [mAU*s]	Plates	Width [min]	Resolution
Bio WCX NP, 3 µm	1	Cytochrome c	7.86	124	1833	7844	0.2089	-
	2	RNase A	9.03	241	3358	10800	0.2044	3.32
	3	Lysozyme	13.13	636	7274	44488	0.1466	13.73
Bio SCX NP, 3 µm	1	RNase A	7.06	396	2616	39847	0.0832	-
	2	Cytochrome c	7.66	297	2778	28920	0.1060	1.08
	3	Lysozyme	10.49	763	7186	44828	0.1167	1.37
Bio MAb NP, 3 µm	1	Cytochrome c	10.04	203	2369	21814	0.1600	-
	2	RNase A	11.37	256	2690	33314	0.1467	3.11
	3	Lysozyme	12.59	652	6616	56734	0.1244	5.28

**Weak cation-exchange chromatography for P128 therapeutic protein sample  
on the Agilent 1260 Bio-inert Quaternary LC system using different cation-exchange columns**



# Ion-Exchange Chromatography

## Agilent Bio IEX HPLC Columns, PEEK

Size (mm)	Particle Size ( $\mu\text{m}$ )	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	5190-2435	5190-2455	5190-2475	5190-2495
4.6 x 50, Guard	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, Guard	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, Guard	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, Guard	5	400 bar, 5800 psi	5190-2432	5190-2452	5190-2472	5190-2492

## Agilent Bio IEX HPLC Columns, Stainless Steel

Size (mm)	Particle Size ( $\mu\text{m}$ )	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	5190-2433	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 50	3	551 bar, 8000 psi	5190-2423	5190-2443	5190-2463	5190-2483
4.6 x 50	1.7	600 bar, 10000 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	600 bar, 10000 psi	5190-2424	5190-2444	5190-2464	5190-2484
4.0 x 10, Guard	1.7	275 bar, 4000 psi	5190-2422	5190-2442	5190-2462	5190-2482

## Tips & Tools

We offer a variety of e-Seminars and on-site training to help you be a more effective chromatographer, at [www.agilent.com/chem/education](http://www.agilent.com/chem/education)





## PL-SAX Strong Anion-Exchange Columns

- Small particles deliver excellent chromatographic performance
- Wide range of particle sizes and 2 pore sizes for flexible analysis to scale-up purification
- Exceptional stability for long column lifetime

PL-SAX -N(CH<sub>3</sub>)<sub>3</sub><sup>+</sup> 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	Pore Size	pH Stability	Operating Temperature Limit
Strong Anion-Exchange	2.1, 4.6, 7.5, 25, 50 and 100	5, 8, 10 and 30	1000Å and 4000Å	1-14	80 °C

# Ion-Exchange Chromatography

## Standard ion-exchange protein separation

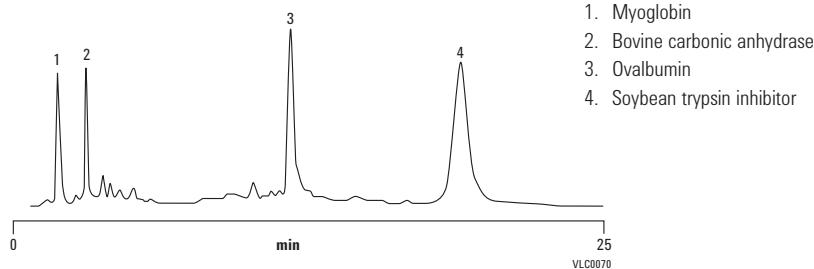
**Column:** **PL-SAX 1000Å**  
**PL1551-1502**  
**4.6 x 50 mm, 5 µm**

Mobile Phase: A: 10 mM Tris HCl pH 8  
B: A+0.35 M NaCl pH 8

Gradient: 0-100% B in 20 min

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm



## Analysis of choline kinase on PL-SAX 4000Å

**Column:** **PL-SAX**  
**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

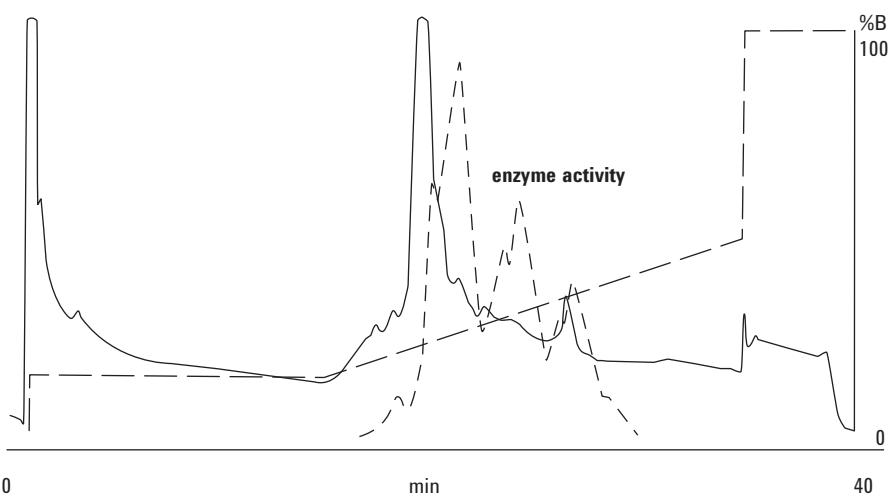
2.0 mM  $\beta$ -Mercaptoethanol

0.2 mM Phenylmethylsulfonyl  
fluoride

B: A + 1 M KCl

Flow Rate: 3.0 mL/min

Detector: UV, 280 nm



Separation courtesy of T Porter, Purdue University, USA

**Analysis of representative whey proteins**

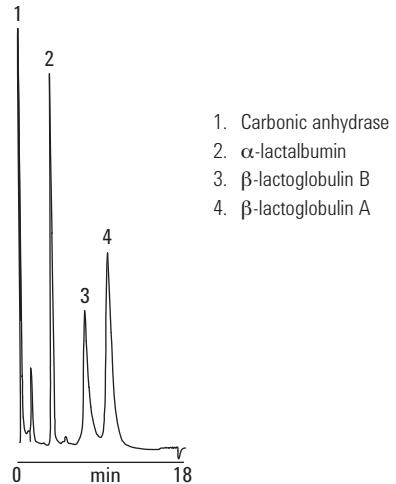
**Column:** **PL-SAX 1000Å**  
**PL1551-1802**  
**4.6 x 50 mm, 8 µm**

Mobile Phase: A: 0.02 M Tris HCl, pH 7  
 B: A + 0.5 M CH<sub>3</sub>COONa, pH 7

Flow Rate: 1.0 mL/min

Gradient: Linear 0-50% B in 10 min

Detector: UV, 280 nm



1. Carbonic anhydrase
2.  $\alpha$ -lactalbumin
3.  $\beta$ -lactoglobulin B
4.  $\beta$ -lactoglobulin A

**High resolution separation of a Poly-T-Oligonucleotide size standard spiked with 10mer, 15mer, 30mer and 50mer (main peaks)**

**Column:** **PL-SAX 1000Å**  
**PL1551-1802**  
**4.6 x 50 mm, 8 µm**

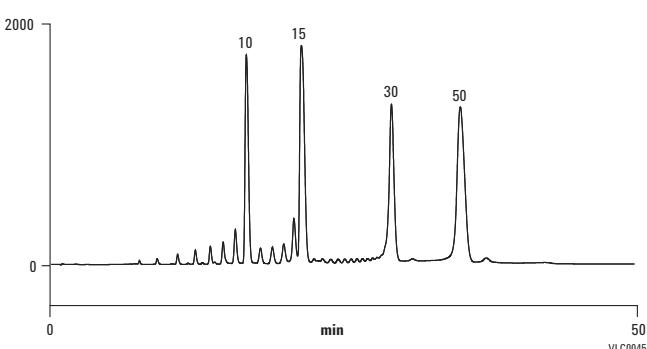
Mobile Phase: A: 7:93 v/v ACN: 0.1 M TEAA, pH 8.5  
 B: 7:93 v/v ACN: 0.1 M TEAA, 1 M ammonium chloride, pH 8.5

Gradient: 0-40% B in 10 min, followed by 40-70% B in 14 min and 70-100% B in 25 min

Flow Rate: 1.5 mL/min

Temperature: 60 °C

Detector: UV, 220 nm



VLC0045

# Ion-Exchange Chromatography

## PL-SAX Strong Anion-Exchange Columns

Size (mm)	Particle Size ( $\mu\text{m}$ )	Pressure Limit	PL-SAX 1000Å	PL-SAX 4000Å
1.0 x 50	5	207 bar, 3000 psi	PL1351-1502	PL1351-1503
2.1 x 50	5	207 bar, 3000 psi	PL1951-1502	PL1951-1503
4.6 x 50	5	207 bar, 3000 psi	PL1551-1502	PL1551-1503
2.1 x 50	8	207 bar, 3000 psi	PL1951-1802	PL1951-1803
2.1 x 150	8	207 bar, 3000 psi	PL1951-3802	PL1951-3803
4.6 x 50	8	207 bar, 3000 psi	PL1551-1802	PL1551-1803
4.6 x 150	8	207 bar, 3000 psi	PL1551-3802	PL1551-3803
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
25 x 50	10	207 bar, 3000 psi	PL1251-1102	PL1251-1103
25 x 150	10	207 bar, 3000 psi	PL1251-3102	PL1251-3103
50 x 150	10	207 bar, 3000 psi	PL1751-3102	PL1751-3103
100 x 300	10	207 bar, 3000 psi	PL1851-2102	PL1851-2103
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
25 x 150	30	207 bar, 3000 psi	PL1251-3702	PL1251-3703
50 x 150	30	207 bar, 3000 psi	PL1751-3702	PL1751-3703
100 x 300	30	207 bar, 3000 psi	PL1851-3102	PL1851-3103

## PL-SAX Strong Anion-Exchange Bulk Media

Size	Particle Size ( $\mu\text{m}$ )	PL-SCX 1000Å	PL-SCX 4000Å
100 g	10	PL1451-4102	PL1451-4103
1 kg	10	PL1451-6102	PL1451-6103
100 g	30	PL1451-4702	PL1451-4703
1 kg	30	PL1451-6702	PL1451-6703



## 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  $\text{-SO}_3^-$  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 $\text{\AA}$  and 4000 $\text{\AA}$ , to provide good mass transfer characteristics for a range of solute sizes. The 5  $\mu\text{m}$  media delivers separations at higher resolution with the 30  $\mu\text{m}$  media used for medium pressure liquid chromatography.

### Column Specifications

Bonded Phase	ID	Particle Size	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 $\text{\AA}$ and 4000 $\text{\AA}$	1-14	80 °C

### Standard protein separation

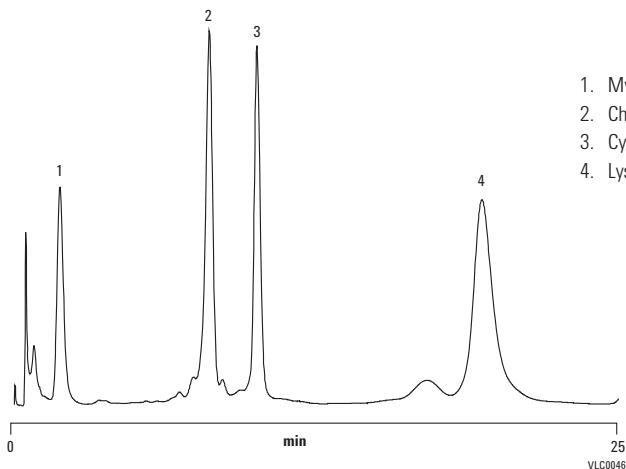
**Column:** **PL-SCX 1000 $\text{\AA}$**   
**PL1545-1502**  
**4.6 x 50 mm, 5  $\mu\text{m}$**

Mobile Phase: A: 20 mM  $\text{KH}_2\text{PO}_4$ , pH 6.0  
B: A + 1 M NaCl

Gradient: 0-100% B in 20 min

Flow Rate: 1.0 mL/min

Detector: UV, 280 nm



1. Myoglobin
2. Chymotrypsinogen A
3. Cytochrome c
4. Lysozyme

## Tips & Tools

PL-SAX and PL-SCX columns and bulk media are also available for Prep to Process, see page 127.



## PL-SCX Strong Cation-Exchange Columns

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

## PL-SCX Strong Cation-Exchange Bulk Media

Size	Particle Size ( $\mu\text{m}$ )	PL-SCX 1000Å	PL-SCX 4000Å
100 g	10	PL1445-4102	PL1445-4102
1 kg	10	PL1445-6102	PL1445-6103
100 g	30	PL1445-4702	PL1445-4703
1 kg	30	PL1445-6702	PL1445-6703



## Agilent 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 mm 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

Agilent 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 HPLC and preparative LC systems, including Agilent 1100 and 1200 HPLC systems.

### Agilent 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.	<ul style="list-style-type: none"> <li>• Adenovirus process monitoring and quality control</li> <li>• IgM purification monitoring and quality control</li> <li>• Monitoring DNA impurity removal</li> <li>• Monitoring endotoxin removal</li> <li>• HSA Purity</li> </ul>	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.	<ul style="list-style-type: none"> <li>• Process monitoring and quality control of bacteriophage manufacturing and purification</li> <li>• Process monitoring and quality control of plasmid DNA purification</li> </ul>	5069-3636
Bio-Monolith SO <sub>3</sub>	The sulfonyl bonded phase (Strong Cation-Exchange) is fully charged over a working pH range of 2-13, binding positively charged biomolecules.	<ul style="list-style-type: none"> <li>• Fast and high resolution analytical separations of large molecules such as proteins and antibodies</li> <li>• Hemoglobin A1c fast analytics</li> </ul>	5069-3637

### Tips & Tools

Easily scale your method to a different particle size or different column dimensions with Agilent's Method Translation Tool. Calculate time and solvent savings too at [www.agilent.com/chem/lcmethodtranslator](http://www.agilent.com/chem/lcmethodtranslator)



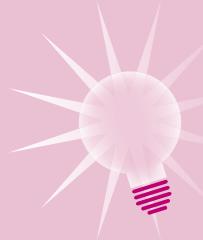
# Ion-Exchange Chromatography

## Column Specifications

<b>Dimensions</b>	5.2 mm x 4.95 mm
<b>Column volume</b>	100 µL
<b>Maximum pressure</b>	150 bar (15 MPa, 2200 psi)
<b>Temperature min/max</b>	Working: 4-40 °C Storage: 4-30 °C
<b>Recommended pH</b>	Working range: 2-13 Cleaning-in-place: 1-14
<b>Materials of construction</b>	Hardware: Stainless steel Packing: poly (glycidyl methacrylate-co-ethylene dimethacrylate) highly porous monolith
<b>Color ring identifier</b>	Bio-Monolith QA: Blue Bio-Monolith DEAE: Green Bio-Monolith SO <sub>3</sub> : Red
<b>Shelf life/expiration date</b>	SO <sub>3</sub> , QA, DEAE: 24-36 months

## Tips & Tools

Agilent Bio-Monolith Protein A Affinity columns are found on page 89.



### Baseline expansion of a separation of protein standards

**Column:** Agilent Bio-Monolith CM15,  
5.5 x 15 mm

Mobile Phase: A: 10 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 6.0  
B: A + 0.5 M NaCl or just 0.5 M Na<sub>2</sub>HPO<sub>4</sub>, pH 6.0

Flow Rate: 2 mL/min

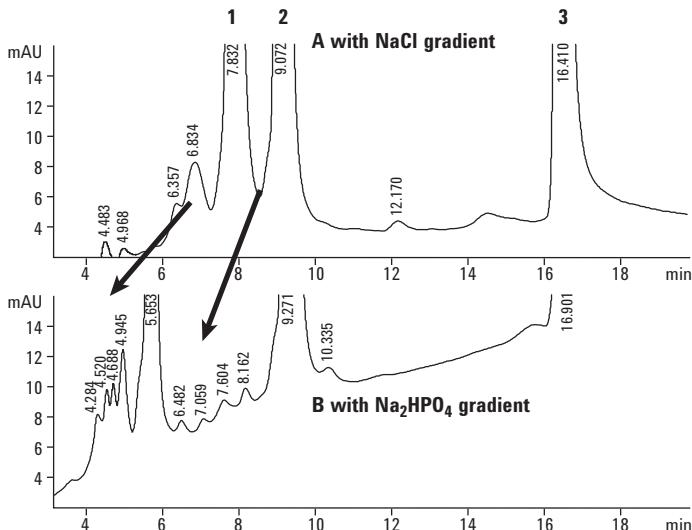
Gradient: 0.5 min hold with mobile phase A followed by a linear gradient to 45% B in 15 min (elapsed time 15.5 min); then 60% B at 15.6 min continued to 20 min. Column flushed with 100% B for 15 min before re-equilibration for the next run.

pH Gradient: A: 5 mM Na<sub>2</sub>HPO<sub>4</sub>, buffer pH 5.5 and B: 40 mM Na<sub>2</sub>HPO<sub>4</sub> (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 at 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: Agilent 1200 SL with diode array detector



B shows a better resolution of protein contaminants.

# Ion-Exchange Chromatography

## Bio-Monolith DEAE column monitors phage production during fermentation

**Column:** **DEAE**  
**5069-3636**  
**5.2 x 4.95 mm**

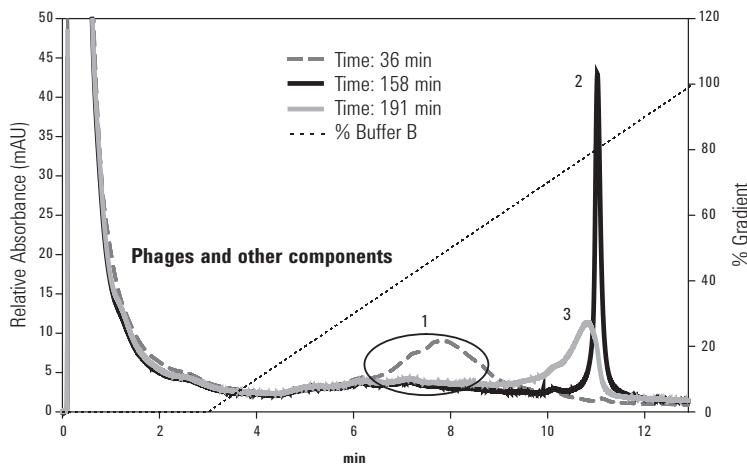
Mobile Phase: A: 125 mM Phosphate buffer, pH 7.0  
B: 125 mM Phosphate buffer + 1 M NaCl, pH 7.0

Flow Rate: 1 mL/min

Gradient: 100% buffer A (2.5 min)  
0-100% buffer B (10 min)  
100% buffer A (2 min)

Detector: UV at 280 nm

Instrument: High pressure gradient HPLC system,  
Agilent 1200



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





## SIZE EXCLUSION CHROMATOGRAPHY (SEC)

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.

### 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. Aggregation formations occur through a variety of mechanisms, including disulfide bond formation and non-covalent 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 discreet molecular weight, SEC can be used to detect and quantitate monomers, dimers, aggregates and fragments. SEC can also separate oligonucleotide mixtures.

For biopolymers of varying sizes, like starches and other polysaccharides, SEC can provide data on molecular weight distribution and branching (with the proper detectors).

# Size Exclusion Chromatography (SEC)

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:

- **Bio SEC-3 and Bio SEC-5 columns** are available in a variety of pore sizes, and are well suited for protein analysis – 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 best for preparative SEC of proteins, because of their larger column size and higher flow rates.
- **PL aquagel-OH columns** can be used to analyze biopolymers of broad molecular weights, such as PEGs, oligo- and polysaccharides, starches, and gums.

## Size Exclusion Chromatography (SEC)

Application	Agilent Columns	Notes
Peptides, proteins	Agilent Bio SEC-3	Higher resolution and faster separations from 3 µm particles, with 100Å, 150Å, and 300Å pore sizes.
Large biomolecules and samples with multiple molecular weight components	Agilent Bio SEC-5	More pore size options (100Å, 150Å, 300Å, 500Å, 1000Å, and 2000Å) to cover a wider range of analytes.
Globular proteins, antibodies	ProSEC 300S	Single column option for protein analysis in high salt conditions.
Proteins, globular proteins	ZORBAX GF-250/450	Higher flow rate capabilities and larger column size for SEC semi-prep and prep.
Low MW polymers and oligomers, oligosaccharides, PEGs, lignosulfonates	2 or 3 PL aquagel-OH <ul style="list-style-type: none"><li>• PL aquagel-OH 8 µm</li><li>• PL aquagel-OH 20 5 µm</li><li>• PL aquagel-OH MIXED-M 8 µm</li></ul>	The PL aquagel-OH analytical series has a pH range of 2-10, compatibility with organic solvent (up to 50% methanol), mechanical stability up to 140 bar (2030 psi), and low column operating pressures.
Polydisperse biopolymers, polysaccharides, cellulose derivatives	2 or 3 PL aquagel-OH <ul style="list-style-type: none"><li>• PL aquagel-OH MIXED-H 8 µm</li><li>• PL aquagel-OH 60/50/40 8 µm</li></ul>	
Very high MW polymers, hyaluronic acids, starches, gums	PL aquagel-OH 60/50/40 15 µm in series	



## Agilent Bio SEC-3

- Exceptional loading capacity, stability, and reproducibility for size-based biomolecule separations
- Sharper peaks, higher resolution, and better protein recovery
- Faster separations than large-particle SEC columns
- Compatibility with most aqueous buffers
- Excellent stability in high-salt and low-salt conditions

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

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

### Column Specifications

Pore Size	Particle Size	MW Range	pH Range	Max Pressure	Flow Rate
100Å	3 µm	100-100,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
150Å	3 µm	500-150,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
300Å	3 µm	5,000-1,250,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)

### Tips & 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.



# Size Exclusion Chromatography (SEC)

## Calibration curves – Bio SEC-3

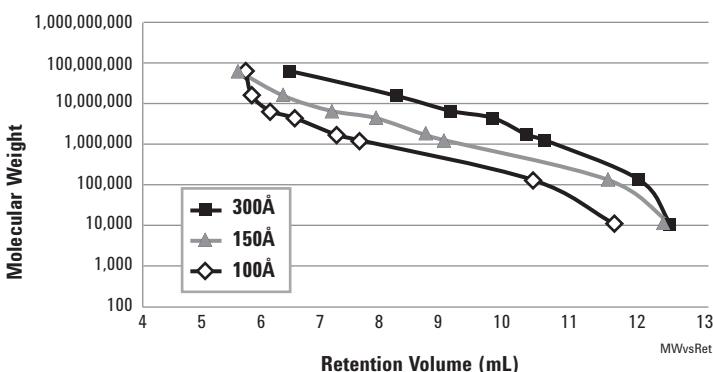
Column: **Bio SEC-3**  
**7.8 x 300 mm, 3  $\mu$ m**

Mobile Phase: 150 mM Na phosphate, pH 7.0

Flow Rate: 1.0 mL/min

Detector: UV

Proteins	MWt	300Å	150Å	100Å
Thyroglobulin	670000	6.34	5.50	5.63
Gamma globulin	158000	8.03	6.24	5.74
BSA	67000	8.90	7.00	6.03
Ovalbumin	45000	9.57	7.70	6.41
Myoglobin	17000	10.12	8.50	7.10
Ribonuclease A	12700	10.40	8.80	7.46
Vitamin B-12	1350	11.90	11.40	10.20



## Intact MAb monomer and dimer separation

Column: **Bio SEC-3, 300Å**  
**5190-2511**  
**7.8 x 300 mm, 3  $\mu$ m**

Buffer: Sodium phosphate buffer, pH 7.0, 150 mM

Isocratic: 0-100% Buffer A from 0-30 min

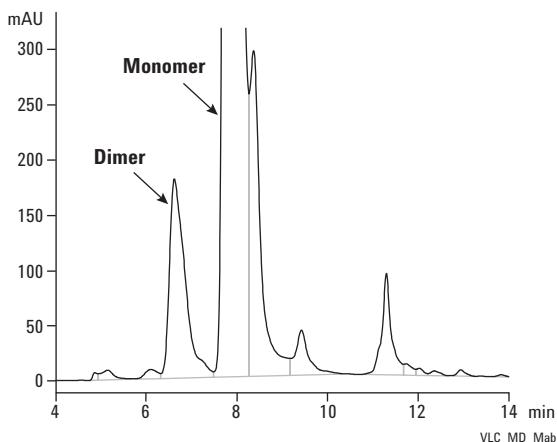
Flow Rate: 1.0 mL/min

Sample: CHO-humanized MAb, 5 mg/mL – intact

Injection: 5  $\mu$ L

Detector: UV 220 nm

Temperature: Ambient



**Comparison of Agilent Bio SEC-3  
and competitor column in the analysis  
of a monoclonal antibody**

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

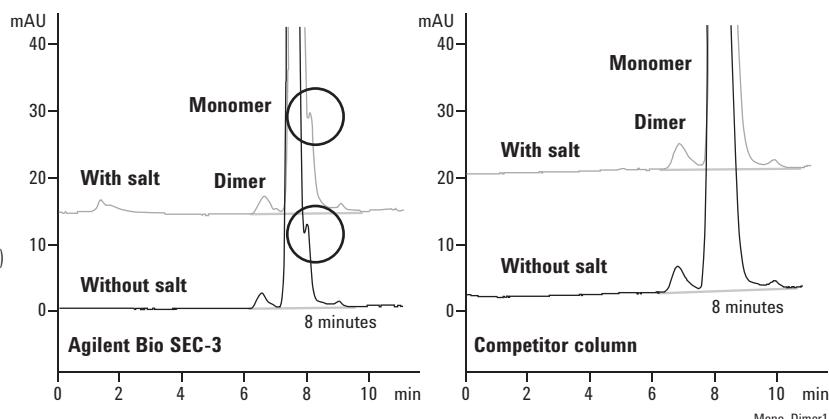
Column: Competitor 7.8 x 300 mm

Mobile Phase: 150 mM sodium phosphate  
+ 100 mM Na sulfate (with salt)  
150 mM sodium phosphate (without salt)

Flow Rate: 1.0 mL/min

Detector: 220 nm

Sample: MAb (2 mg/mL)



The Agilent Bio SEC-3 column reveals the presence of MAb fragments missed by the competitor column.

**Monoclonal Antibody Monomer and Dimer Analysis  
using Agilent Bio SEC-3 and a Competitor Column**

Eluent	Column	Resolution Ratio Monomer:Dimer	Monomer Efficiency	Percentage Dimer
With salt	Agilent	2.04	7,518	0.59
With salt	Competitor	1.88	3,967	0.59
Without salt	Agilent	2.08	7,942	0.60
Without salt	Competitor	1.92	4,164	0.57

# Size Exclusion Chromatography (SEC)

## 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, 100Å  
5190-2503  
4.6 x 300 mm, 3 µm

**Column B:** Bio SEC-3, 150Å  
5190-2508  
4.6 x 300 mm, 3 µm

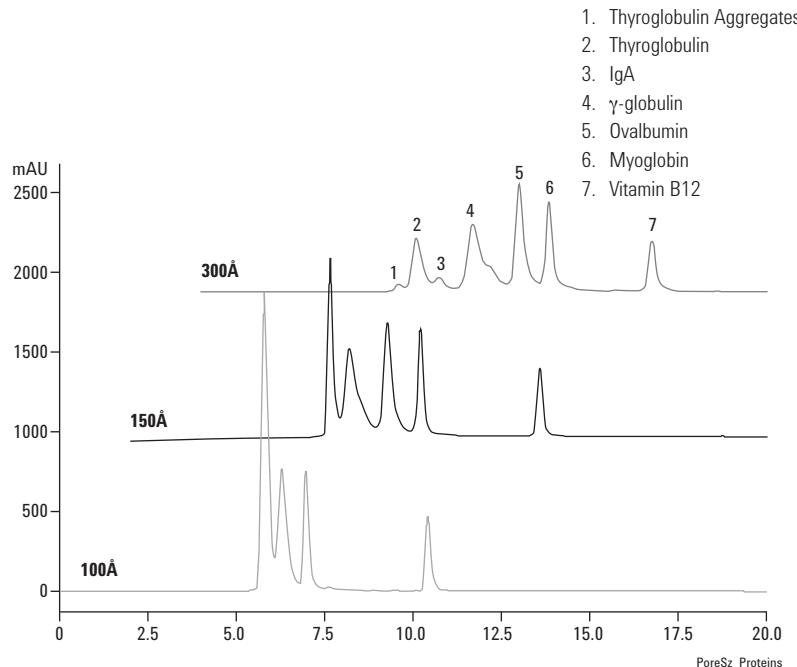
**Column C:** Bio SEC-3, 300Å  
5190-2513  
4.6 x 300 mm, 3 µm

Mobile Phase: 50 mM Na<sub>2</sub>HPO<sub>4</sub>, 50 mM NaH<sub>2</sub>PO<sub>4</sub>  
+ 0.15 M NaCl, pH 6.8

Flow Rate: 0.35 mL/min

Detector: UV @ 220 nm

Sample: BioRad Gel Filtration Standards Mix



### Pore size choice: Mouse IgG

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

**Column B:** Bio SEC-3, 150Å  
5190-2508  
4.6 x 300 mm, 3 µm

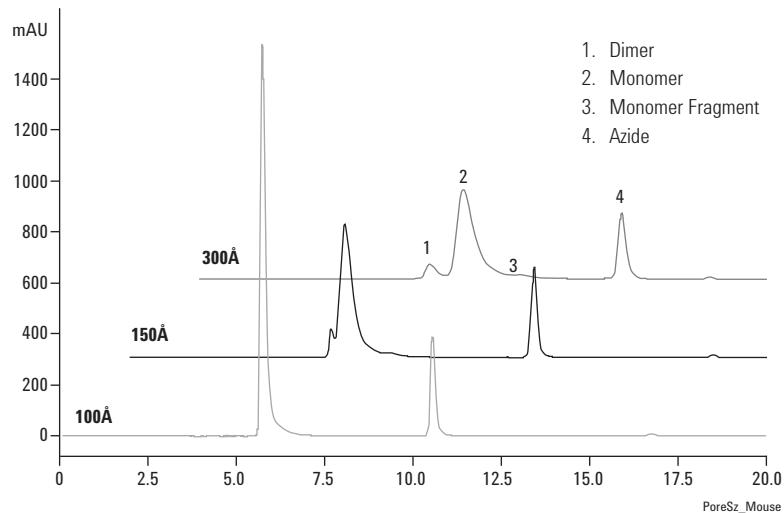
**Column C:** Bio SEC-3, 300Å  
5190-2513  
4.6 x 300 mm, 3 µm

Mobile Phase: 50 mM Na<sub>2</sub>HPO<sub>4</sub>, 50 mM NaH<sub>2</sub>PO<sub>4</sub>  
+ 0.15 M NaCl, pH 6.8

Flow Rate: 0.35 mL/min

Detector: UV @ 220 nm

Sample: Mouse IgG



## Column Length

Where the separation time is a critical parameter shorter columns packed with the higher efficiency 3 µm media are used. With the shorter columns higher flow rates are used to reduce the analysis time but without compromising the quality of the data – quantitation of monoclonal antibody monomer and dimer.

### Agilent Bio SEC-3 column length comparison, 150 mm

**Column:** **Bio SEC-3, 300Å**  
**5190-2512**  
**7.8 x 150 mm, 3 µm**

Mobile Phase: 150 mM sodium phosphate

Flow Rate: 1.0 mL/min (56 bar), 1.5 mL/min (75 bar)

Detector: 220 nm

Sample: MAb (2 mg/mL)

### Agilent Bio SEC-3 column length comparison, 300 mm

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

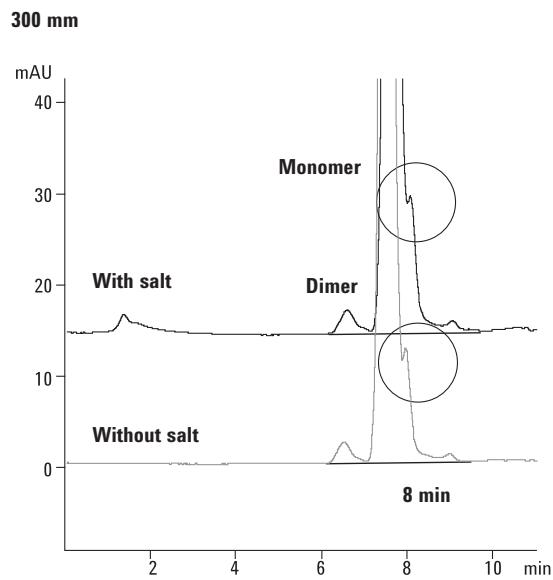
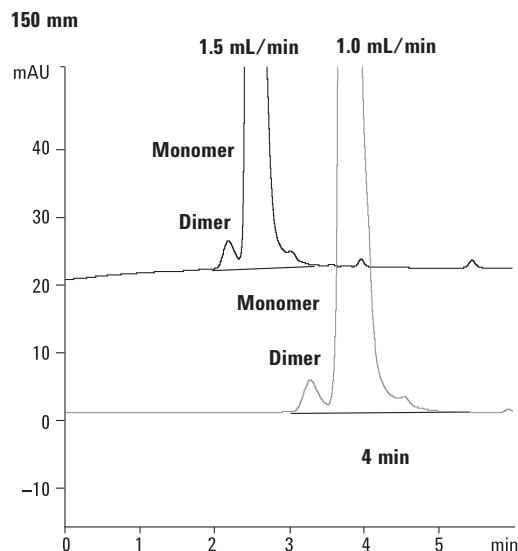
Mobile Phase: 150 mM sodium phosphate + 100 mM Na sulfate (with salt)

150 mM 300 mm  
150 mM sodium phosphate (without salt)

Flow Rate: 1.0 mL/min

Detector: 220 nm

Sample: MAb (2 mg/mL)



# Size Exclusion Chromatography (SEC)

## Agilent Bio SEC-3

Size (mm)	Particle Size ( $\mu\text{m}$ )	Bio SEC-3 100 $\text{\AA}$ USP L33	Bio SEC-3 150 $\text{\AA}$ USP L33	Bio SEC-3 300 $\text{\AA}$ USP L33
7.8 x 300	3	5190-2501	5190-2506	5190-2511
7.8 x 150	3	5190-2502	5190-2507	5190-2512
4.6 x 300	3	5190-2503	5190-2508	5190-2513
4.6 x 150	3	5190-2504	5190-2509	5190-2514
7.8 x 50, Guard	3	5190-2505	5190-2510	5190-2515





## Agilent Bio SEC-5

- Maximum recovery for a broad range of size-based, biomolecule separations
- Outstanding reproducibility and column lifetime
- Excellent stability, even under high-pH, high-salt, and low-salt conditions
- Compatibility with most aqueous buffers

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

Bio SEC-5 columns are available in 5 µm particles with 100Å, 150Å, 300Å, 500Å, 1000Å, and 2000Å nominal pore sizes.

### Column Specifications

Pore Size	Particle Size	MW Range	pH Range	Max Pressure	Flow Rate
100Å	5 µm	100-100,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
150Å	5 µm	500-150,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
300Å	5 µm	5,000-1,250,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
500Å	5 µm	15,000-5,000,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
1000Å	5 µm	50,000-7,500,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
2000Å	5 µm	>10,000,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)

# Size Exclusion Chromatography (SEC)

## Comparison Between Agilent Bio SEC-3 and Agilent Bio SEC-5

### Analysis of monoclonal antibody

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

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

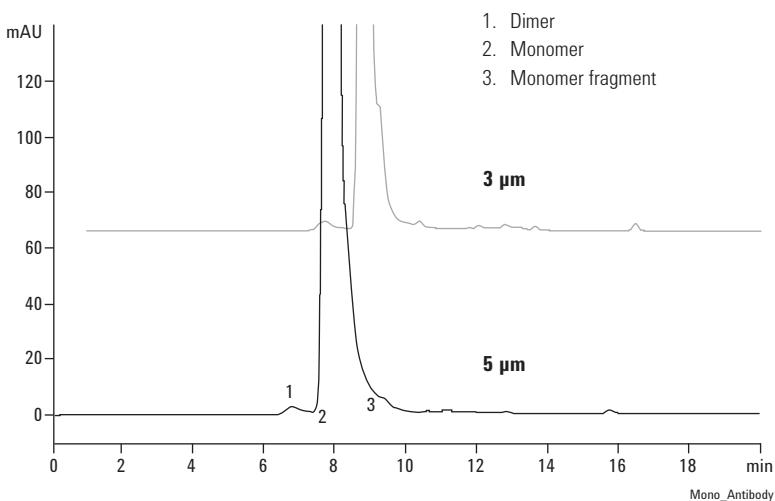
Mobile Phase: 150 mM Sodium Phosphate, pH 7

Flow Rate: 1 mL/min

Detector: UV @ 220 nm

Sample: Humanized monoclonal antibody

The 3 µm column gives higher definition of fragmentation pattern.



### Tips & Tools

Agilent carries a full line of instrument parts and supplies for all Agilent LC and MS instruments. For more information go to [www.agilent.com/chem/supplies](http://www.agilent.com/chem/supplies)



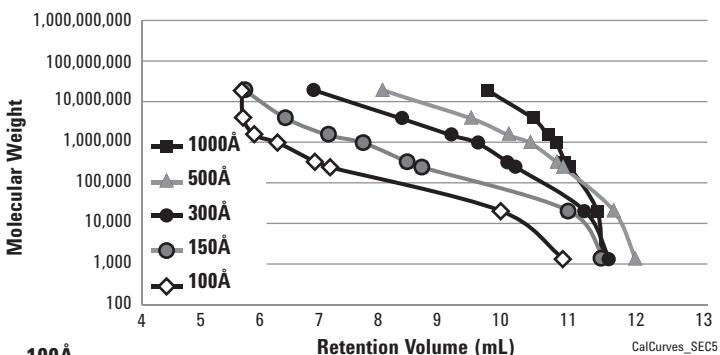
**Calibration curves – Bio SEC-5**

**Column:** **Bio SEC-5**  
**7.8 x 300 mm, 5  $\mu$ m**

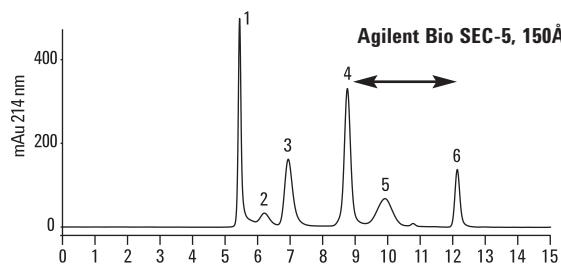
Mobile Phase: 150 mM Na phosphate, pH 7.0

Flow Rate: 1.0 mL/min

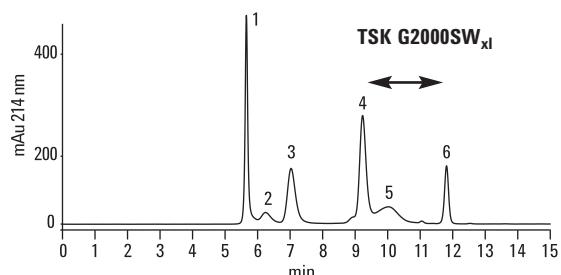
Detector: UV



Proteins	MW	1000Å	500Å	300Å	150Å	100Å
Thyroglobulin	670000	10.07	8.23	7.03	5.82	5.77
Gamma globulin	158000	10.88	9.80	8.57	6.55	5.79
BSA	67000	11.13	10.44	9.44	7.29	6.00
Ovalbumin	45000	11.28	10.83	9.89	7.90	6.40
Myoglobin	17000	11.44	11.28	10.42	8.66	7.05
Ribonuclease A	12700	11.52	11.41	10.58	8.93	7.32
Vitamin B-12	1350	12.00	12.59	11.78	11.49	10.30

**Side-by-side comparison**

- 1. Thyroglobulin, 5.43 min
- 2. BSA dimer, 6.19 min
- 3. BSA monomer, 6.93 min
- 4. Ribonuclease A, 8.74 min
- 5. Poly-DL-alanine (1-5 kDa), 9.90 min
- 6. Uracil, 12.13 min



- 1. Thyroglobulin, 5.64 min
- 2. BSA dimer, 6.23 min
- 3. BSA monomer, 7.02 min
- 4. Ribonuclease A, 9.22 min
- 5. Poly-DL-alanine (1-5 kDa), 10.02 min
- 6. Uracil, 11.81 min

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

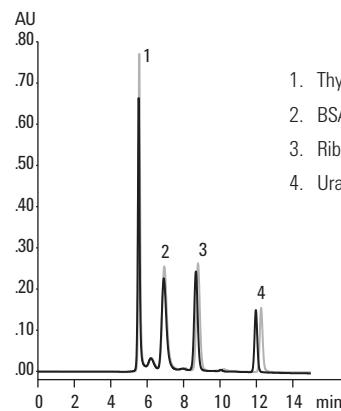
# Size Exclusion Chromatography (SEC)

## Exceptional lot-to-lot reproducibility

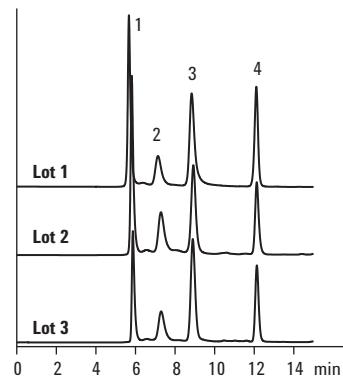
Column: **Bio SEC-5, 150Å  
5190-2521  
7.8 x 300 mm, 5 µm**

Mobile Phase: 150 mM Phosphate  
Buffer, pH 7.0

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



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



— 3rd run  
— After 300 injections

## Agilent Bio SEC-5

Size (mm)	Particle Size (µm)	Bio SEC-5 100Å USP L33	Bio SEC-5 150Å USP L33	Bio SEC-5 300Å USP L33	Bio SEC-5 500Å USP L33	Bio SEC-5 1000Å USP L33	Bio SEC-5 2000Å USP L33
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
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
7.8 x 50, Guard	5	5190-2520	5190-2525	5190-2530	5190-2535	5190-2540	5190-2545



## ProSEC 300S

- Mechanically robust polymer particles that do not bleed during use
- Single column with extended linear resolving range
- Column dimensions for use with multi-detector systems

The Agilent 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 MW Range	pH Range	Flow Rate	Max Pressure
ProSEC 300S	300Å	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.
4.6 x 250	5	PL1547-5501
7.5 x 300	5	PL1147-6501
<b>Guard Columns</b>		
4.6 x 50	5	PL1547-1501
7.5 x 50	5	PL1147-1501

# Size Exclusion Chromatography (SEC)

## Calibration of the ProSEC 300S column with globular proteins

Mobile Phase: 50 mM KH<sub>2</sub>PO<sub>4</sub>-K<sub>2</sub>HPO<sub>4</sub> (@ pH 6.8) containing 0.3 M NaCl

Flow Rate: 1.0 mL/min

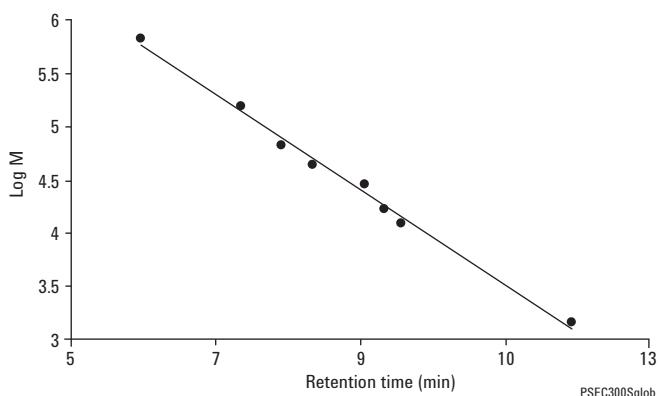
Detector: UV, 280 nm

Sample: Protein samples

### Molecular weights of the proteins.

#### Mw/Daltons      Protein

670,000	Thyroglobulin
155,000	$\gamma$ -Globulin
66,430	Bovine serum albumin
44,287	Ovalbumin
29,000	Carbonic anhydrase
16,700	Myoglobin
12,384	Cytochrome c
1,423	Bacitracin



## Analysis of Bovine Serum Albumin by light scattering using ProSEC 300S columns

Column: **ProSEC 300S**  
**PL1147-6501**  
**7.5 x 300 mm, 5  $\mu$ m**

Mobile Phase: Water + 120 mM NaCl, 2.7 mM KCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub>

Flow Rate: 1.0 mL/min

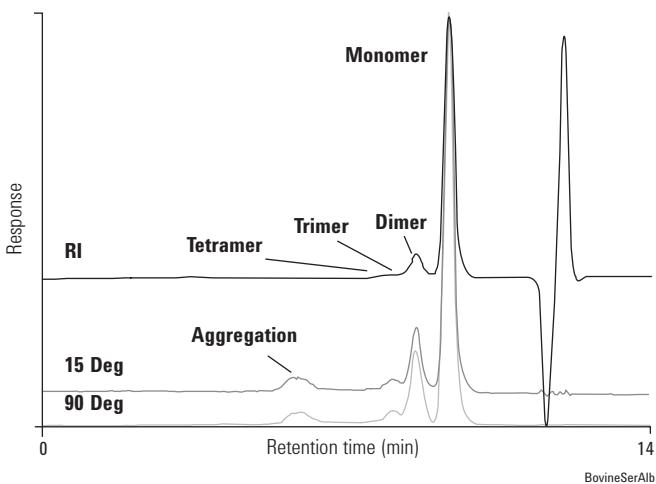
Detector: Differential refractive index  
+ 15/90 dual angle light scattering

Sample: Bovine serum albumin

### Molecular Weights

#### Monomer      **66,900 Daltons, 88.5%**

Dimer	34,900 Daltons (2.02 x monomer molecular weight), 9.8%
Trimer	197,000 Daltons (2.94 x monomer molecular weight), 1.2%
Tetramer	279,300 Daltons (5.17 x monomer molecular weight), 0.5%

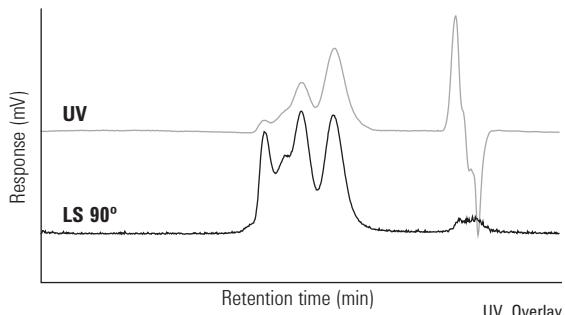


Overlay of differential refractive index and dual angle light scattering sample.

**Overlay of UV and light scattering 90° for a sample of  $\gamma$ -globulins, illustrating monomer, dimer, and trimer peaks**

Column: ProSEC 300S  
PL1147-6501  
7.5 x 300 mm, 5  $\mu$ m

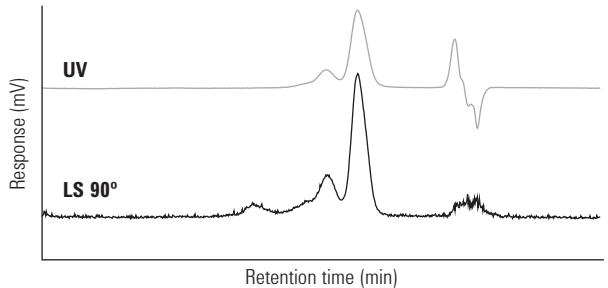
Mobile Phase: 0.1 M KH<sub>2</sub>PO<sub>4</sub> containing 0.3 M NaCl, pH 8.0  
Flow Rate: 1.0 mL/min  
Temperature: 5 °C  
Detector: UV at 310 nm + PL-LS 15/90°  
Sample: Proteins



**Overlay of UV and light scattering 90° for a sample of BSA, illustrating monomer, dimer, trimer and aggregate peaks**

Column: ProSEC 300S  
PL1147-6501  
7.5 x 300 mm, 5  $\mu$ m

Mobile Phase: 0.1 M KH<sub>2</sub>PO<sub>4</sub> containing 0.3 M NaCl, pH 8.0  
Flow Rate: 1.0 mL/min  
Temperature: 5 °C  
Detector: UV at 310 nm + PL-LS 15/90°  
Sample: Proteins



### Tips & Tools

Further information can be found in the following publications:

*ProSEC 300S Protein Characterization Columns* (5990-7468EN)

*Analysis of Globulins using Agilent ProSEC 300S Columns* (5990-7851EN)

*Static Light Scattering Analysis of Globular Proteins with Agilent ProSEC 300S Columns* (5990-7939EN)

[www.agilent.com/chem/library](http://www.agilent.com/chem/library)





## ZORBAX GF-250 and GF-450 Gel Filtration Columns

- High efficiency and reproducibility with short analysis time
- Semi-prep and prep column dimensions
- Compatible with organic modifiers and denaturants
- Wide usable pH range (3-8)

Agilent 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 3-8. 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 a highly 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.

### Column Specifications

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

Specifications represent typical values only.

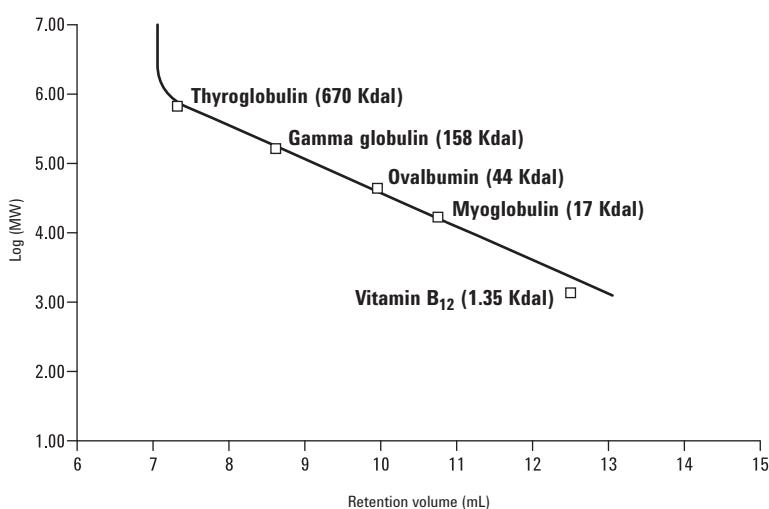
**Retention volume versus log (MW)  
for the Bio-Rad standards separated  
on an Agilent ZORBAX GF-250 column**

**Column:** ZORBAX GF-250  
884973-901  
9.4 x 250 mm, 4  $\mu$ m

Mobile Phase: 200 mM Sodium phosphate, pH 7.0

Temperature: Ambient

Detector: UV (254 nm)



**Separations of proteins on preparative columns**

**Column A:** ZORBAX GF-250

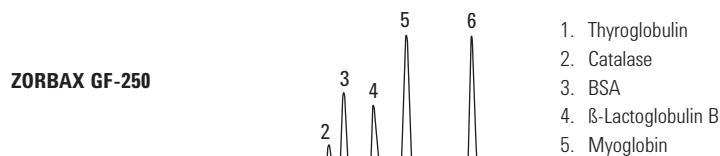
**Column B:** ZORBAX GF-450

Mobile Phase: 0.2 M Na<sub>2</sub>HPO<sub>4</sub>, pH 7.0

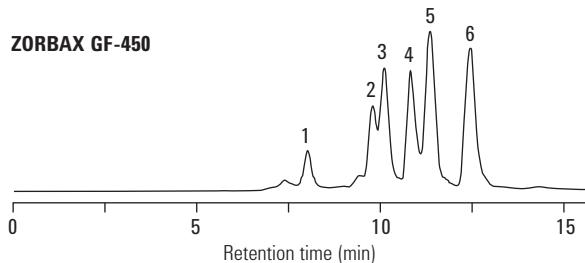
Flow Rate: 5.0 mL/min

Detector: 280 nm

Sample: 200  $\mu$ L



1. Thyroglobulin
2. Catalase
3. BSA
4.  $\beta$ -Lactoglobulin B
5. Myoglobin
6. Tyr-Gly-Gly



# Size Exclusion Chromatography (SEC)

## ZORBAX GF-250 (USP L33) and GF-450 (USP L35) Gel Filtration Columns

Hardware Description		Size (mm)	Particle Size ( $\mu\text{m}$ )	Part No.
GF-250, 150 $\text{\AA}$		9.4 x 250	4	884973-901
GF-250, 150 $\text{\AA}$		4.6 x 250	4	884973-701
GF-450, 300 $\text{\AA}$		9.4 x 250	6	884973-902
<b>Guard Columns (hardware required)</b>				
 GF-250 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
 GF-250 Diol, Guard Cartridge, 4/pk		4.6 x 12.5	6	820950-911
 Prep Guard Hardware Kit				840140-901
 Guard Hardware Kit				820999-901
<b>PrepHT Columns</b>				
 PrepHT GF-250, 150 $\text{\AA}$		21.2 x 250	6	877974-901
 PrepHT GF-450, 300 $\text{\AA}$		21.2 x 250	6	877974-910
 PrepHT endfittings, 2/pk				820400-901
 PrepHT GF-250, Guard Cartridge, 2/pk		17 x 7.5	5	820212-911
 PrepHT GF-450, Guard Cartridge, 2/pk		17 x 7.5	5	820212-911
 Guard Cartridge Hardware				820444-901



## Agilent PL aquagel-OH SEC Columns for Biomolecule Analysis

### Biomolecule SEC with durability and versatility

Aqueous size exclusion chromatography (SEC) is widely used for the determination of molecular weight distributions of naturally occurring water soluble polymers, such as macromolecular biomolecules. This includes natural products and derivatives which may be used as excipients and active ingredients in pharma applications.

The PL aquagel-OH series provides a chemically and physically stable matrix for reliable aqueous SEC separations. The columns are packed with macroporous copolymer beads with an extremely hydrophilic polyhydroxyl functionality. The "neutral" surface and the capability to operate across a wide range of eluent conditions provide for high performance analyses of compounds with neutral, ionic and hydrophobic moieties, alone or in combination. PL aquagel-OH is available for analytical and preparative applications.

The excellent stability of the PL aquagel-OH packing material allows the eluent to be tailored to suit the polymer, while retaining the high column efficiency. For ionic interactions, the eluent can be modified by the addition of salt and/or the adjustment of pH. For water soluble polymers with a hydrophobic character, only the addition of a weak organic solvent (methanol) is required to inhibit hydrophobic interactions.

### PL aquagel-OH analytical columns

Available with mixed and individual pore sizes, and 5, 8 and 15 µm particle sizes, to cover a very wide range of molecular weights.

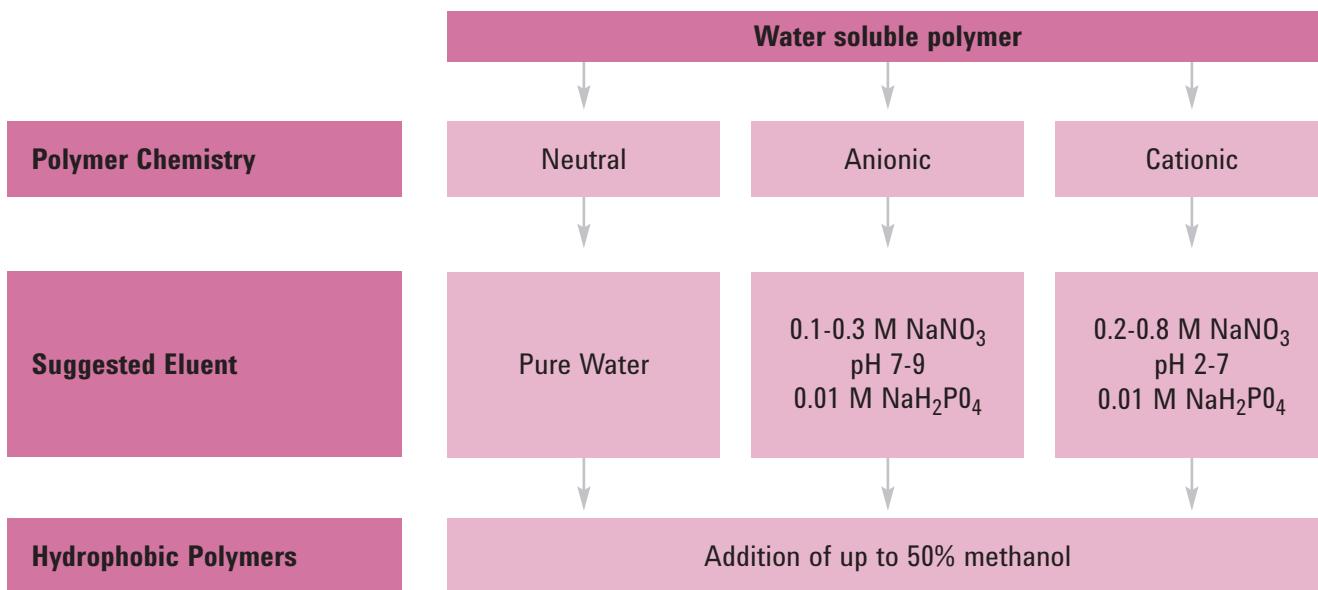
### PL aquagel-OH preparative columns

For rapid and convenient scale-up from analytical separations. The columns are packed with the same robust macroporous particles as the analytical column range.



# Size Exclusion Chromatography (SEC)

## Guide to eluent selection for PL aquagel-OH applications



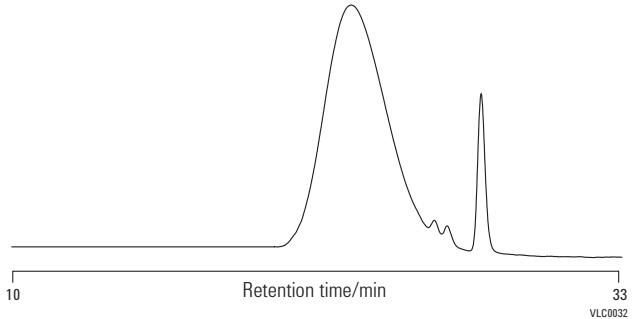
### Polyvinyl alcohol

Column: **3 x PL aquagel-OH MIXED  
PL1149-6800  
7.5 x 300 mm, 8 µm**

Mobile Phase: 0.2 M NaNO<sub>3</sub>, 0.01 M NaH<sub>2</sub>PO<sub>4</sub>, pH 7

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



**Heparin**

**Column:** **2 x PL aquagel-OH 30  
PL1120-6830  
7.5 x 300 mm, 8 µm**

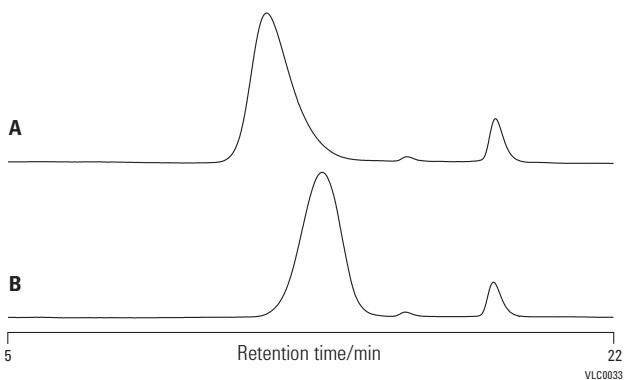
Mobile Phase: 0.2 M NaNO<sub>3</sub>, 0.01 M NaH<sub>2</sub>PO<sub>4</sub>, pH 7

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)

**Correlation of the SEC results  
with the polymer specification**

Sample	Mn	Mw
A	22,500	28,200
B	9,350	10,770

**Hyaluronic acid**

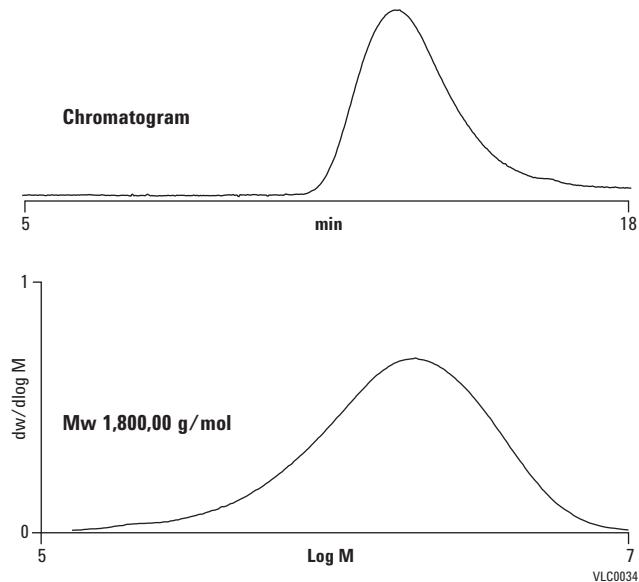
**Column:** **PL aquagel-OH 60 15 µm  
PL1149-6260  
7.5 x 300 mm, 15 µm**

**Column:** **PL aquagel-OH 40 15 µm  
PL1149-6240  
7.5 x 300 mm, 15 µm**

Mobile Phase: 0.2 M NaNO<sub>3</sub>, 0.01 M NaH<sub>2</sub>PO<sub>4</sub>, pH 7

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



# Size Exclusion Chromatography (SEC)

## PL aquagel-OH Analytical

Description	Size (mm)	MW Range (g/mol) (PEG/PEO)	Guaranteed Efficiency (p/m)	Part No.
PL aquagel-OH 20 5 µm	7.5 x 300	100-10,000	> 55,000	PL1120-6520
PL aquagel-OH 20 8 µm	7.5 x 300	100-20,000	> 35,000	PL1149-6820
PL aquagel-OH 30 8 µm	7.5 x 300	100-30,000	> 35,000	PL1120-6830
PL aquagel-OH 40 8 µm	7.5 x 300	10,000-200,000	> 35,000	PL1149-6840
PL aquagel-OH 40 15 µm	7.5 x 300	10,000-200,000	> 15,000	PL1149-6240
PL aquagel-OH 50 8 µm	7.5 x 300	50,000-1,000,000	> 35,000	PL1149-6850
PL aquagel-OH 50 15 µm	7.5 x 300	50,000-1,000,000	> 15,000	PL1149-6250
PL aquagel-OH 60 8 µm	7.5 x 300	200,000-> 10,000,000	> 35,000	PL1149-6860
PL aquagel-OH 60 15 µm	7.5 x 300	200,000-> 10,000,000	> 15,000	PL1149-6260
PL aquagel-OH MIXED-H 8 µm	7.5 x 300	100-10,000,000	> 35,000	PL1149-6800
PL aquagel-OH MIXED-M 8 µm	7.5 x 300	> 600,000	> 35,000	PL1149-6801

### Tips & Tools

Key PL aquagel-OH columns for biomolecule applications –  
for full product information see publication 5990-7995EN.

[www.agilent.com/chem/library](http://www.agilent.com/chem/library)





## Standards for Biomolecule Analysis – Polyethylene Glycol/Oxide

### Most popular standards for aqueous SEC

- Available in individual, kit, and EasiVial formats
- Combine glycols and oxides to extend the MW range and cover more applications
- MWs selected to provide equidistant calibration points for greater accuracy

These hydrophilic polymers are both suitable for aqueous SEC. The oxides are available in high molecular weights, while the glycols cover the lower molecular weight range.

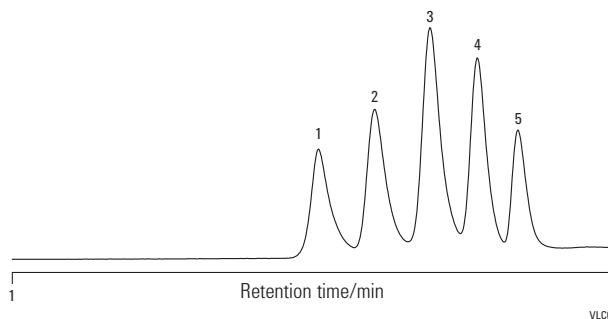
#### Polyethylene Glycol/Oxide standards

**Column:** PL aquagel-OH MIXED-H 8 µm  
PL1149-6800  
7.5 x 300 mm, 8 µm

Mobile Phase: Water

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



#### Specifications

EasiVial Color	EasiVial PEG/PEO	EasiVial PEG
Red	600	282
	12,000	1,000
	125,000	6,000
	1,200,000	35,000
Yellow	200	194
	4,000	600
	60,000	3,750
	1,000,000	21,000
Green	100	106
	1,500	420
	25,000	2,000
	460,000	12,000

# Size Exclusion Chromatography (SEC)

## Polyethylene Glycol/Oxide Calibration Kits

**PEG-10 (10 x 0.5 g)  
PL2070-0100**

**PEO-10 (10 x 0.2 g)  
PL2080-0101**

### Constituent Polymer Nominal Mp (g/mol)

106	20,000
194	30,000
400	50,000
600	70,000
1,000	100,000
2,000	200,000
4,000	300,000
7,000	400,000
13,000	700,000
20,000	1,000,000

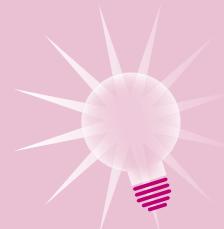
## Calibration Kits

Description	Vial Volume (mL)	Quantity (vials/kit)	Part No.
EasiVial PEG/PEO	2	30/pk	PL2080-0201
EasiVial PEG/PEO	4	30/pk	PL2080-0200

## Tips & Tools

For more information and full details of individual standards, please refer to the *GPC/SEC Standards Product Guide* (5990-7996EN).

[www.agilent.com/chem/library](http://www.agilent.com/chem/library)





## AFFINITY CHROMATOGRAPHY

Affinity chromatography is a powerful technique which takes advantage of highly specific molecular interactions, frequently between specific proteins (e.g. antigen/antibody). Agilent offers several specialty affinity products, a monolithic Protein A column 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.

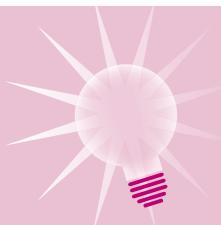
### Agilent Bio-Monolith Protein A HPLC Columns

- Designed for the analytical separation of all IgG (human and mouse), except for IgG class3
- 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

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

#### Tips & Tools

Agilent Ion-Exchange Bio-Monolith columns are found on page 61



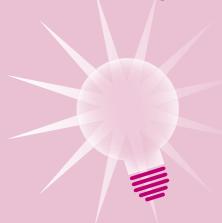
# Affinity Chromatography

## Tips & Tools

Further information can be found in the following application note:

*Rapid Human Polyclonal IgG Quantification Using the Agilent Bio-Monolith Protein A HPLC Column (5989-9733EN)*

[www.agilent.com/chem/library](http://www.agilent.com/chem/library)



## Column Specifications

<b>Dimensions</b>	5.2 mm x 4.95 mm
<b>Column volume</b>	100 µL
<b>Maximum pressure</b>	150 bar (15 MPa, 2200 psi)
<b>Temperature min/max</b>	Working: 4-40 °C Storage: 4-30 °C
<b>Recommended pH</b>	Working range: 2-13 Cleaning-in-place: 1-14
<b>Materials of construction</b>	Hardware: Stainless steel Packing: poly(glycidyl methacrylate-co-ethylene dimethacrylate) highly porous monolith
<b>Color ring identifier</b>	Bio-Monolith Protein A: White
<b>Shelf life/expiration date</b>	Protein A: 12 months

## Bio-Monolith Protein A

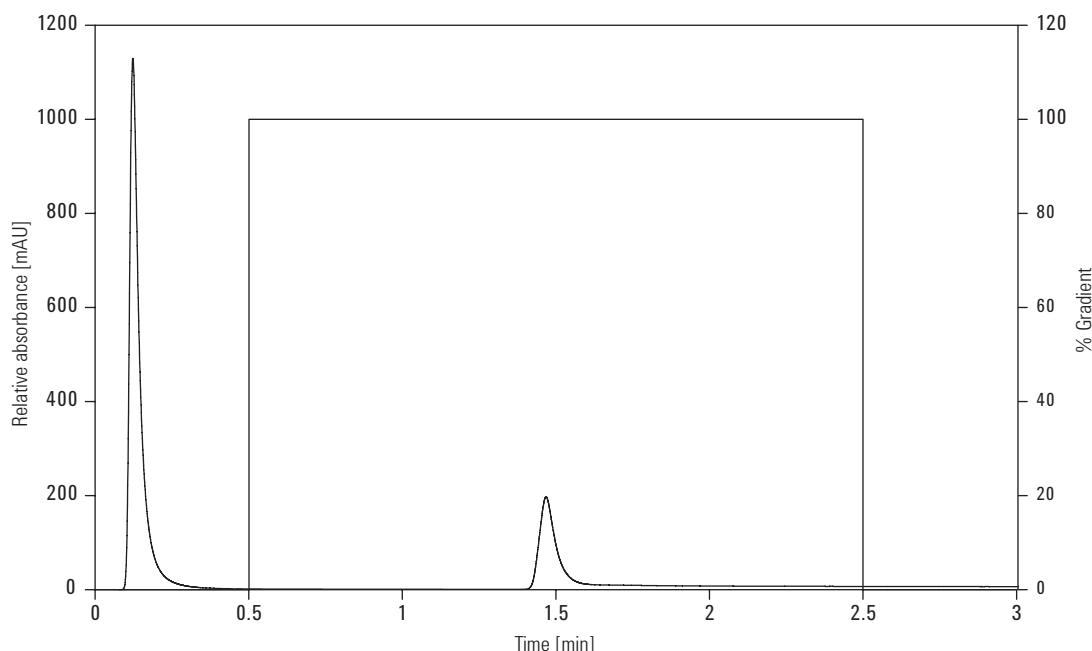
Column	Description	Key Applications	Part No.
Bio-Monolith Protein A	The Protein A affinity column is designed for the analytical separation of all IgG (human and mouse), except for IgG class3.	• Quantitative determination of IgG (fermentation titer calculation)	5069-3639

**Rapid human polyclonal IgG quantification using the Agilent Bio-Monolith Protein A HPLC column**

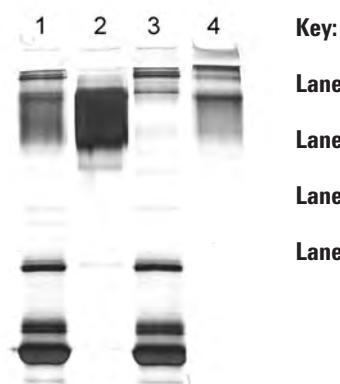
**Column:** Protein A  
5069-3639  
5.2 x 4.95 mm

Mobile Phase: PBS buffer, pH 7.4  
0.5 M acetic acid, pH 2.6

Flow Rate: 1 mL/min  
Detector: A high pressure gradient HPLC system, Agilent 1200 - UV at 280 nm  
Sample: Human Plasma diluted with binding buffer (PBS buffer, pH 7.4)



The selectivity of the Bio-Monolith Protein A column for the IgG from human plasma. IgG binds to protein A, a 100% buffer B step gradient is applied, and IgG elutes at 1.5 min.

**Key:**

**Lane 1:** Whole serum prior to separation

**Lane 2:** IgG standard

**Lane 3:** Peak 1 (flow-through fraction)

**Lane 4:** Peak 2 (Protein A-bound fraction; i.e. IgG1 and IgG2)

SDS PAGE analysis of fractions from the separation.



## 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

In order to more easily isolate and identify proteins in biological samples, such as serum, plasma, and cerebro-spinal fluid (CSF), the Agilent 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.

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, a range of validated reagents for sample preparation in biomarker discovery and other proteomics applications are also available, including a complex standard, proteomics grade trypsin, and fixed formalin paraffin embedded (FFPE) protein extraction solution. For your convenience, these reagents are fully compatible with Agilent LC/MS methods and require no additional sample pretreatments. Large volume requirements can also be addressed with our custom configurations.

### Tips & Tools

Learn more about Agilent's complete services portfolio at [www.agilent.com/chem/services](http://www.agilent.com/chem/services)





## Multiple Affinity Removal System

The Multiple Affinity Removal System from Agilent 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 14 high abundant proteins found in human biological fluids and 3 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's optimized buffers, convenient spin filters and concentrators, the Agilent Multiple Affinity Removal System creates an automated, integrated depletion solution compatible with most LC instruments (columns), and bench top centrifuges (spin cartridges).

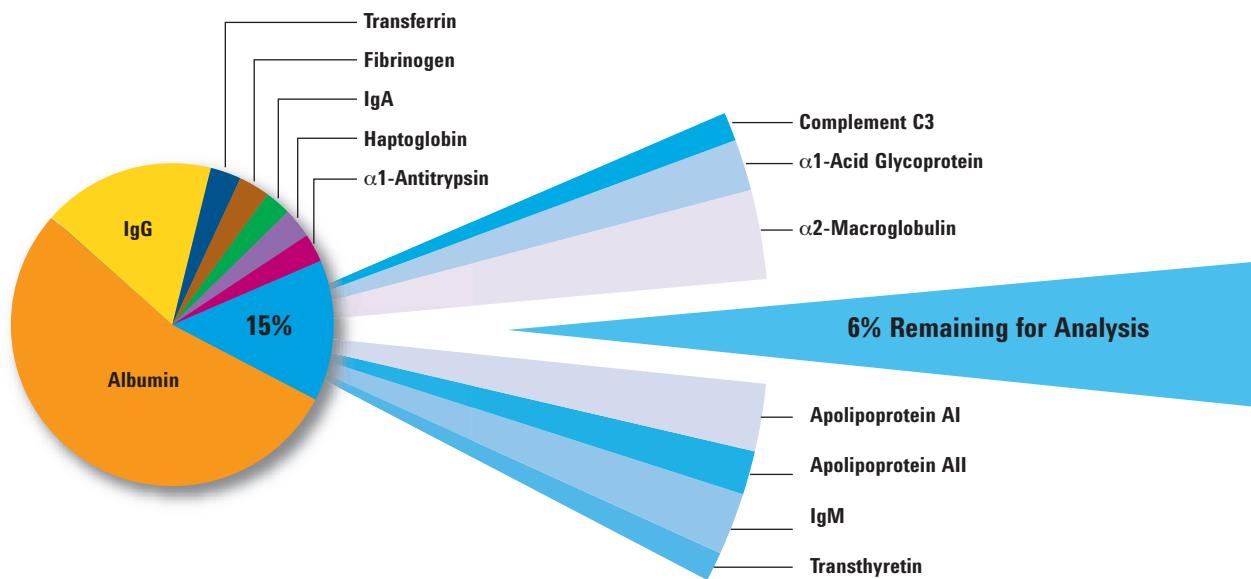
Samples depleted using the Multiple Affinity Removal System are ready for downstream analyses such as 2-D gel electrophoresis, LC/MS, and other analytical techniques.

# Affinity Chromatography

## Multiple Affinity Removal System Selection Guide

Product	Proteins Removed	Total Protein Removed	Dimension	Load Capacity	Part No.
MARS Human-14	Albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein Al, apolipoprotein All, 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 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 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-6 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 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	5188-5289
			4.6 x 50 mm	37 - 50 µL	5188-5217
			4.6 x 100 mm	75 - 100 µL	5188-5218

### Illustration of high abundance proteins removed by Agilent Multiple Affinity Removal Columns and Spin Cartridges



# Affinity Chromatography



LC Column Reagent Starter Kit, 5185-5986



Luer-Lok adapters, 5188-5249



Luer-Lok syringe, 5188-5250



Luer-Lok needles, 5188-5253

## Multiple Affinity Removal System Starter Kits

The LC Column and Spin Cartridge Reagent Starter Kits include all the required supplies to use with 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.

### Multiple Affinity Removal System Starter Kits

Description	Part No.
LC Column Reagent Starter Kit	5185-5986
Includes:	
Buffer A, 1 L, for loading, washing, and equilibrating	5185-5987
Buffer B, 1 L, for eluting	5185-5988
Spin filters, 0.22 µm cellulose acetate, 25/pk	5185-5990
Spin concentrators, 5K MWCO, 4 mL, 25/pk	5185-5991
Mult Aff Rem Spin Cartridge Reagent Kit	5188-5254
Includes:	
Buffer A, 1 L, for loading, washing, and equilibrating	5185-5987
Buffer B, 1 L, for eluting	5185-5988
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	5188-5250
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
High concentration sample dilution buffer, 50 mL,	5188-8283



## 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 (like immunodepleted serum or plasma proteins).

- Greater than 95-99% protein sample recovery has been observed with immunodepleted serum using the Agilent 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 (4000 psi)
- Compatible with water and all common organic solvents

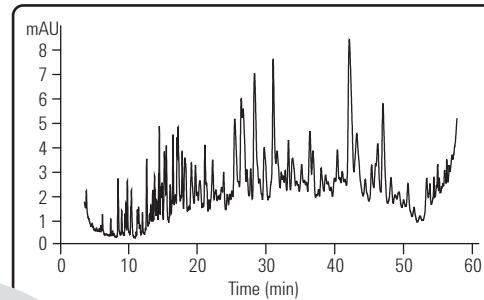
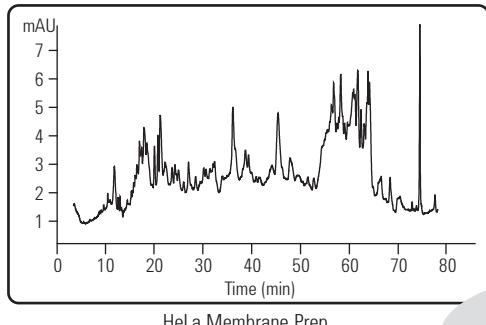
### mRP-C18 High-Recovery Protein Columns

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

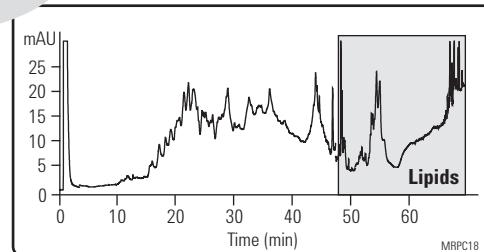
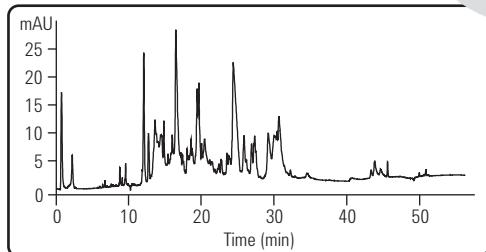
# Affinity Chromatography

## Protein Fractionation of Complex Samples on the mRP Column

4.6 x 50 mm mRP-C18



Highest Recovery



# METHOD DEVELOPMENT

## ZORBAX Column Methods

This ZORBAX Column Selection Strategy for Proteins and Peptides provides some critical details on method development for proteins or polypeptides.

### Choose the Initial Column and Conditions for Peptides, Polypeptides, and Proteins

#### Peptides, Polypeptides, Proteins MW <50 kDa

#### Peptides, Polypeptides, Proteins MW <1,000 kDa

#### **Stable Bond 300SB-C8**

300SB columns are wide-pore columns with unbeatable lifetime in TFA-containing mobile phases. This makes them an ideal first choice for separations of peptides and proteins.

- C8 is an excellent starting bonded phase because of its moderate hydrophobicity
- C18 and C8 are generally selected for peptides and protein digests but can also be used for proteins
- C3, C4 and CN are generally selected for larger, hydrophobic polypeptides and proteins but can also be used for peptides
- PLRP-S when chemical and thermal stability are needed

#### **Poroshell 300SB-C18**

Poroshell 300SB columns use an innovative particle technology to deliver rapid protein separations. Short analysis times with efficient peaks are easily obtained with Poroshell columns.

- C18 is a good starting bonded phase choice with Poroshell for most peptides, polypeptides and proteins because the retention is maximized
- C8 is generally selected for moderate size proteins but can be used with polypeptides or very large proteins
- C3 is generally selected for antibodies or large proteins but can be used for peptides and polypeptides

### Initial Separation Conditions

**Column:** **StableBond 300SB-C8 4.6 x 150 mm, 3.5 or 5 µm**  
**883995-906**  
**863973-906**

**Mobile Phase:** A: 95% H<sub>2</sub>O: 5% ACN with 0.1% TFA  
B: 5% H<sub>2</sub>O: 95% ACN with 0.085% TFA

**Gradient:** 0-60% B in 60 min

**Temperature:** 35-40 °C

**Flow Rate:** 1 mL/min

**Column:** **Poroshell 300SB-C18 2.1 x 75 mm, 5 µm**  
**660750-902**

**Mobile Phase:** A: 95% H<sub>2</sub>O: 5% ACN with 0.1% TFA  
B: 5% H<sub>2</sub>O: 95% ACN with 0.085% TFA

**Gradient:** 0-60% B in 60 min

**Temperature:** 35-40 °C

**Flow Rate:** 2 mL/min

# Method Development

## Start at Low pH with Simple Aqueous/Organic Gradient

Typically, a water/acetonitrile with 0.1% TFA gradient is used to elute all components of interest. A typical high resolution gradient on a 300Å pore size column requires 30-50 min. A Poroshell 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.

## 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 ZORBAX 300StableBond and Poroshell 300SB, while neutral solvents and dilute bases can be used with ZORBAX 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	
5% HOAc/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.

**StableBond 300SB - up to 80 °C**

**Poroshell 300SB - up to 80 °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.

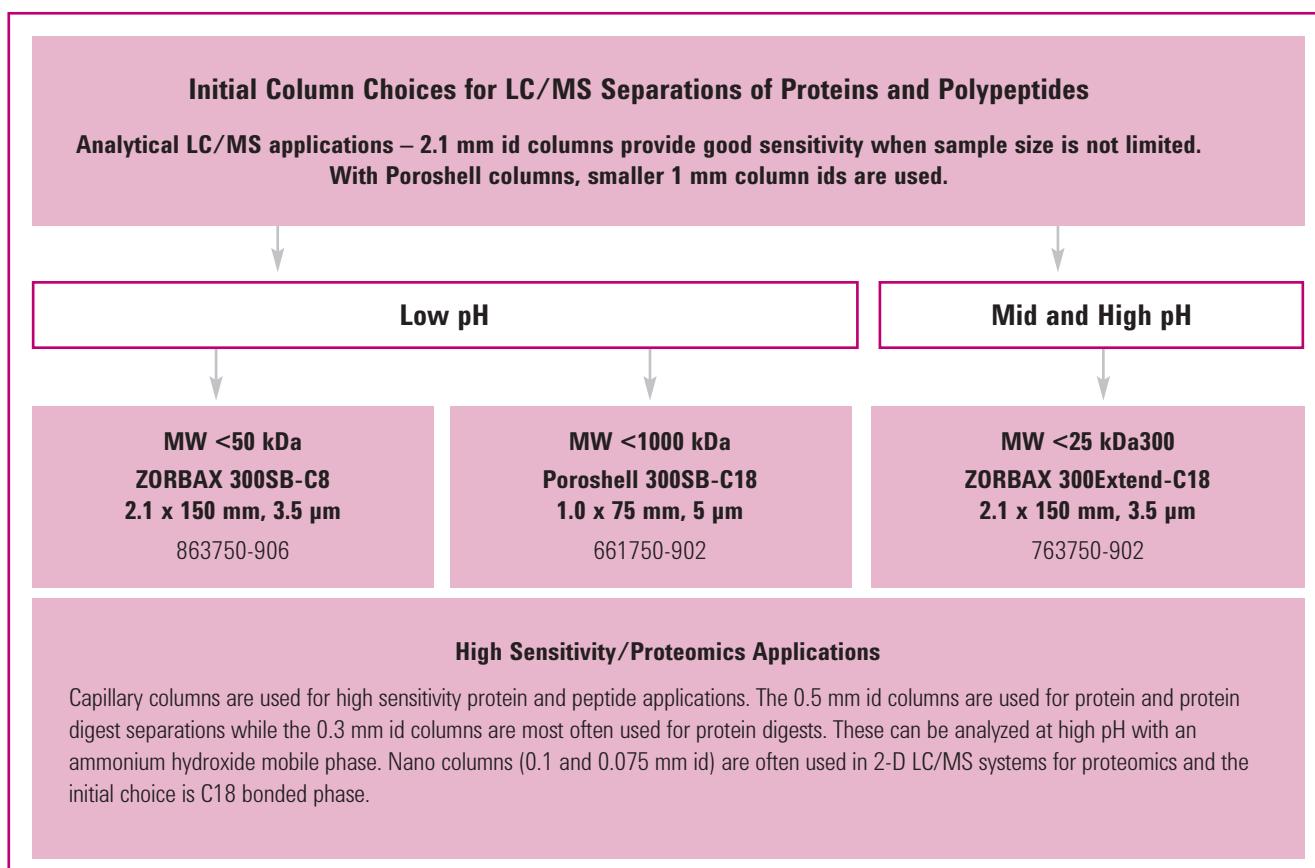
Column:	ZORBAX 300Extend-C18 4.6 x 150 mm, 5 µm 773995-902	Gradient:	5-60% B in 30 min
Mobile Phase:	A: 20 mM NH <sub>4</sub> OH in H <sub>2</sub> O B: 20 mM NH <sub>4</sub> OH in 80% ACN	Temperature:	25-30 °C (<60 °C)
		Flow Rate:	1 mL/min

### Starting Column Choices for Analytical Separations of Peptides, Polypeptides, and Proteins



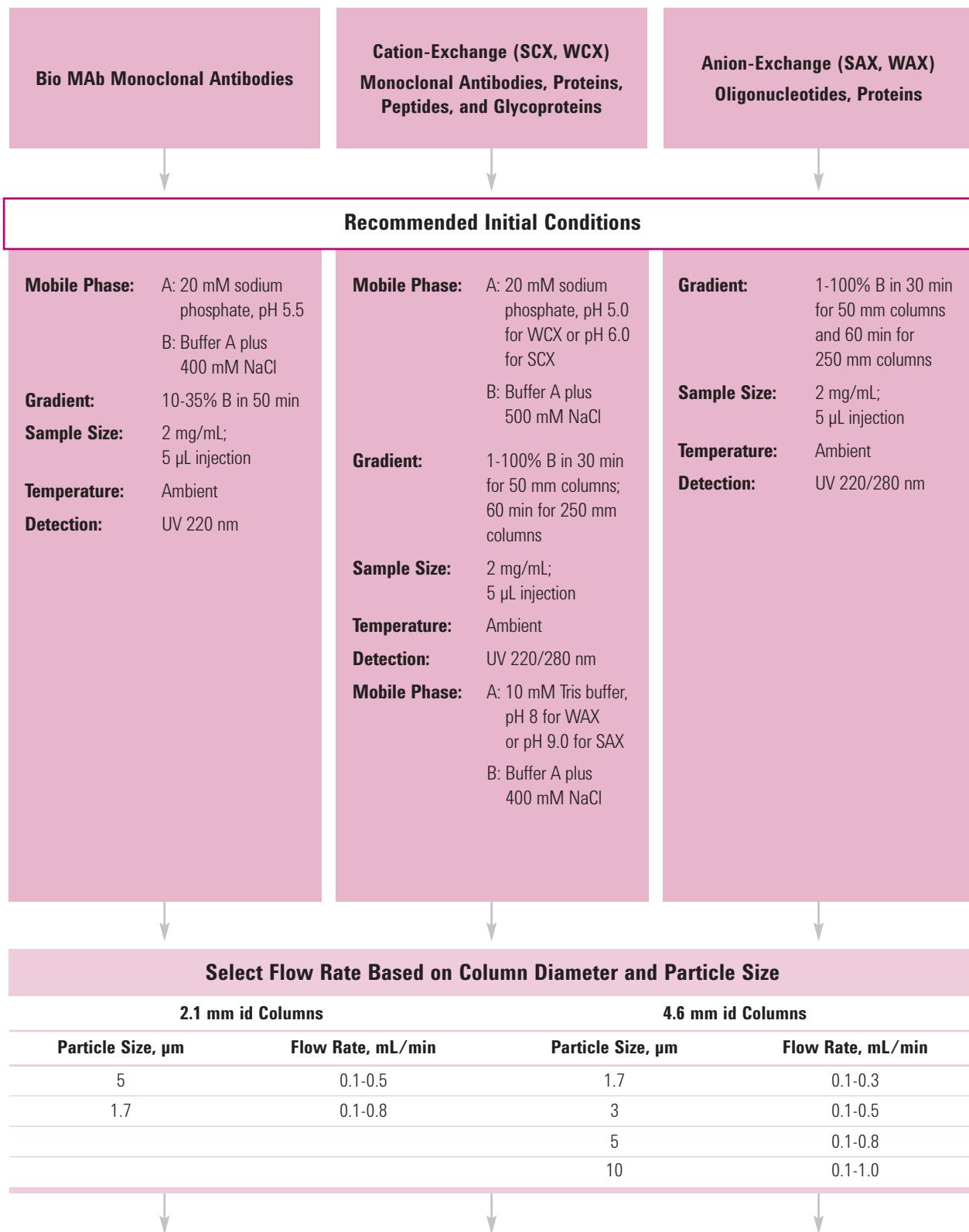
### Reversed-Phase LC/MS Methods

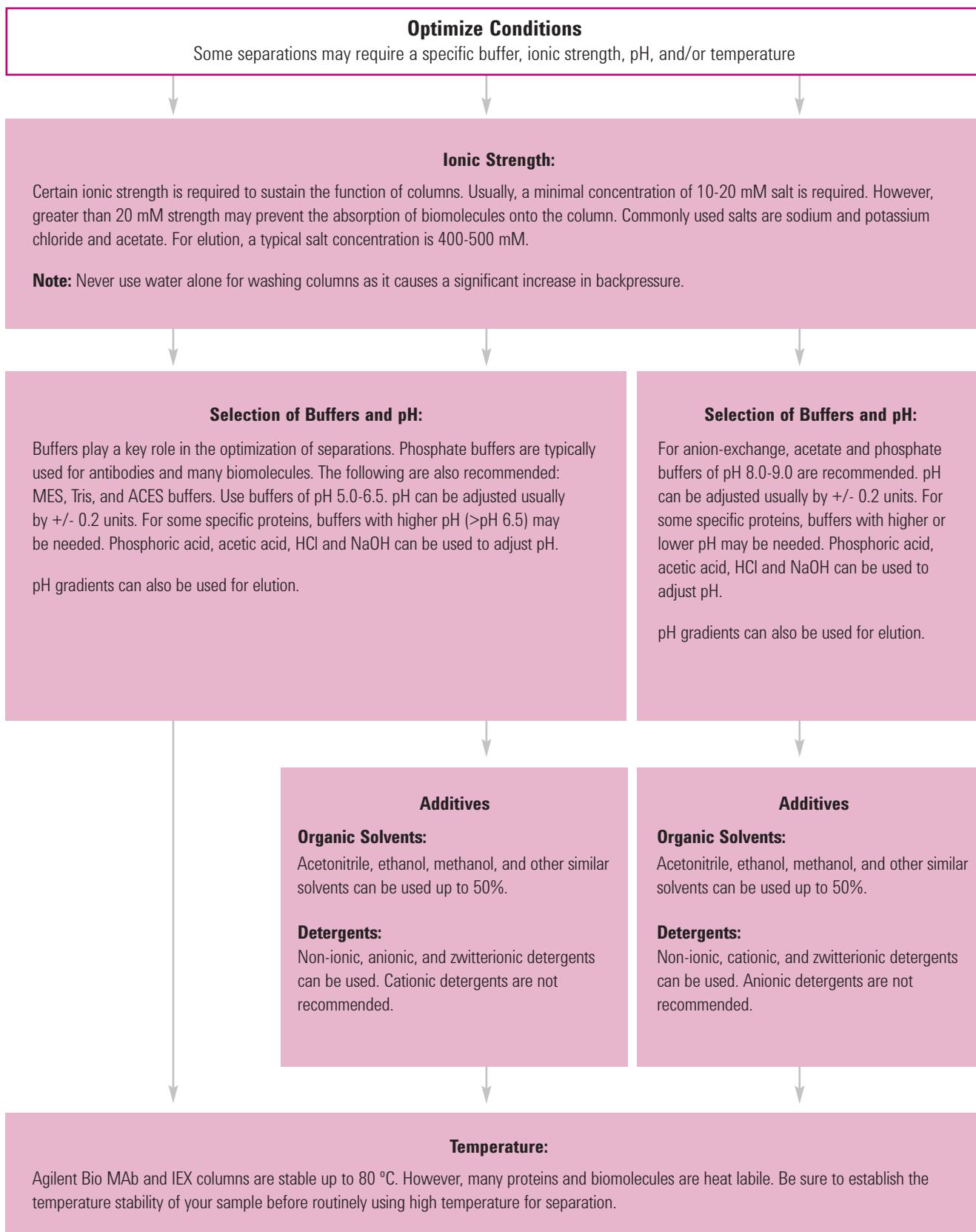
LC/MS of proteins and peptides is used to provide information for protein characterization, to accurately identify post-translational modifications of proteins, and to determine the molecular weight of synthetic and natural peptides. LC/MS is also used to provide protein identification in 2-D separations for proteomics applications. Therefore, LC/MS of proteins and peptides is a critical separation area, which requires some special column and mobile phase recommendations. In general, smaller column sizes are 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.



# Method Development

## Bio Ion-Exchange Column Methods





## SEC Column Methods

### Choose Initial Columns and Conditions for Size-Based Separation of Biomolecules, Aggregation Analysis – Peptides, Polypeptides, and Proteins

Peptides, Polypeptides, Proteins

MW >0.1-1,250 kDa

Peptides, Polypeptides, Proteins

MW >0.1-10,000 kDa

### Select Column Based on Molecular Weight Range and Pore Size

#### Agilent Bio SEC-3 (3 µm)

Pore Size	MW range, kDa
100Å	0.1-100
150Å	0.5-150
300Å	5-1,250

#### Agilent Bio SEC-5 (5 µm)

Pore Size	MW range, kDa
100Å	0.1-100
150Å	0.5-150
300Å	5-1,250
500Å	15-5,000
1000Å	50-7,500
2000Å	>10,000

### Recommended Initial Separation Conditions

- Column:** Agilent Bio SEC (3 µm and 5 µm)  
**Mobile Phase:** 150 mM phosphate buffer, pH 7.0\*  
**Gradient:** Isocratic in 30-60 min range

- Temperature:** Recommended: 10-30 °C, Maximum: 80 °C  
**Flow Rate:** 0.1-0.4 mL/min for 4.6 mm id columns  
0.1-1.25 mL/min for 7.8 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 application note: *Defining the Optimum Parameters for Efficient Size Separations of Proteins* (5990-8895EN)

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 in strength 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:**

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

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

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

Other similar salts (e.g. KCl) and guanidine hydrochloride can also be used

**pH Range:**

2.0-8.5

**Potential organic solvent additions include:**

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

5% DMSO in 50 mM sodium phosphate, pH 7.0

**Temperature:**

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

Maximum temperature of Bio SEC columns is 80 °C

## High Sensitivity Capillary Column Methods

### Mobile Phase Considerations

#### Low pH

TFA is generally not used for LC/MS separations of proteins and peptides. 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. In some cases, the TFA can be displaced post-column with an alternative acid, such as propionic acid.

#### Mid and High pH

LC/MS can also be done at high pH with 10-20 mM NH<sub>4</sub>OH as a mobile phase additive.



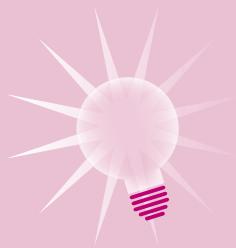
## CAPILLARY AND NANO COLUMNS

- 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
- Packings/phases for both small and large molecules (80Å and 300Å pore sizes, respectively)
- Ideal for 1-D and 2-D (proteomics) applications

### Tips & Tools

Agilent offers a variety of 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](http://www.agilent.com/chem/education)



Agilent ZORBAX Capillary (0.5 and 0.3 mm id) and Nano (0.1 and 0.075 mm id) columns are now available in a wide variety of phases, pore sizes, and dimensions. These columns are ideal for very sample-limited applications because they provide enhanced sensitivity by reducing on-column sample dilution. This high sensitivity can be provided with exceptional reproducibility using Agilent columns and low dispersion HPLC instruments. The fastest growing application for capillary and nano columns is 2-D LC/MS for complex proteomics samples. Agilent provides all the columns needed for the 2-D separation – the SCX columns for the first dimension, the reversed-phase trapping column, and the reversed-phase column for the second dimension.



# Capillary and Nano Columns

## ZORBAX Nano columns for high sensitivity protein digest analysis by LC/MS

Column: ZORBAX 300SB-C18  
5065-9911  
0.075 x 150 mm, 3.5  $\mu$ m

Mobile Phase: A: Water + 0.1% Formic acid,  
B: ACN + 0.1% Formic acid

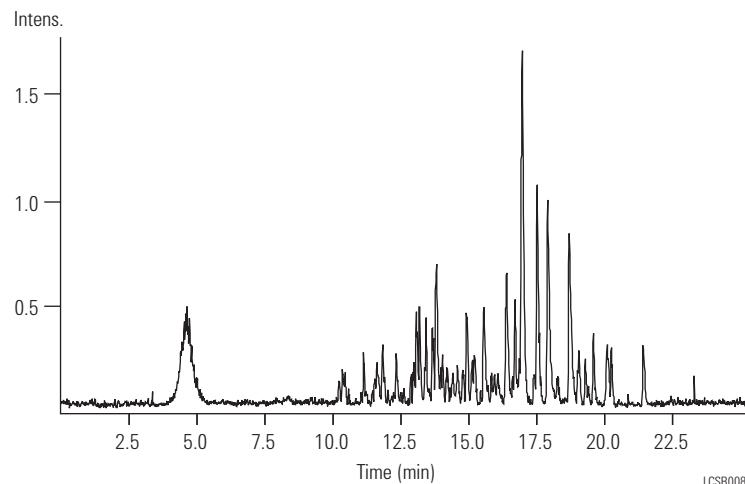
Flow Rate: 600 nL/min

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

Detector: Positive Ion Nano Electrospray MS

Sample: 100 fm (1  $\mu$ L) Digest of 8 Proteins

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

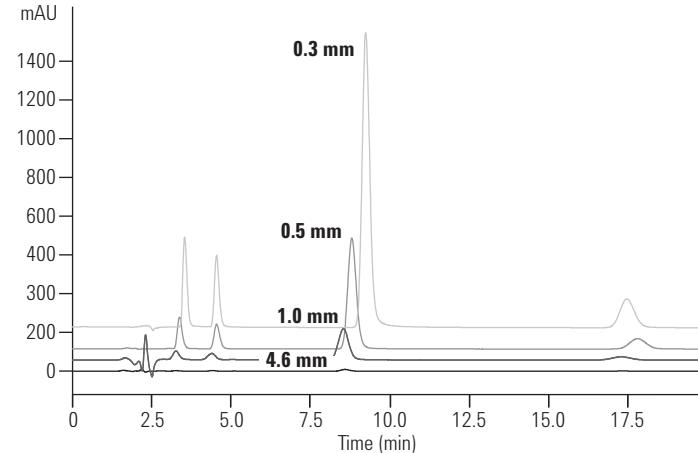
Column: ZORBAX SB-C18  
5064-8255  
0.3 x 150 mm, 5  $\mu$ m

Column: ZORBAX SB-C18  
5064-8256  
0.5 x 150 mm, 5  $\mu$ m

Column: ZORBAX SB-C18  
863600-902  
1.0 x 150 mm, 3.5  $\mu$ m

Column: ZORBAX SB-C18  
883975-902  
4.6 x 150 mm, 5  $\mu$ m

Sample: 200 ng Biphenyl



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 by LC/MS

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

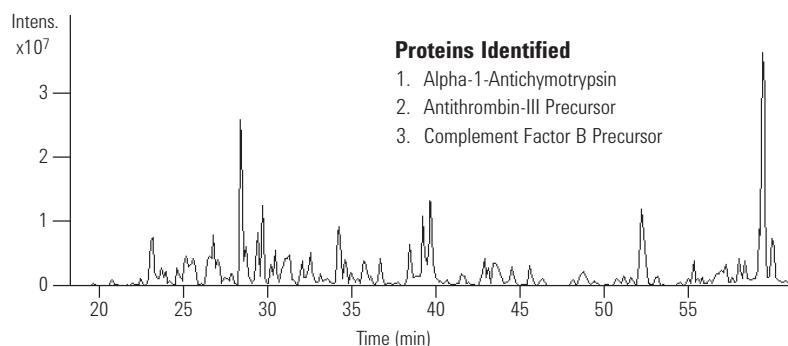
**Mobile Phase:** A: Water + 0.1% Formic acid  
 B: Acetonitrile + 0.1% Formic acid

**Flow Rate:** 6  $\mu$ L/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 1-D in gel digest

#### Base Peak Chromatogram



#### Proteins Identified

1. Alpha-1-Antichymotrypsin
2. Antithrombin-III Precursor
3. Complement Factor B Precursor

#### 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 1-D 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  $\mu$ m

**Mobile Phase:** A: Water + 0.1% Formic acid  
 B: Acetonitrile + 0.1% Formic acid

**Flow Rate:** 5.5  $\mu$ L/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: 4000 V

Drying gas flow: 7 L/min

Drying gas temperature: 250 °C

Nebulizer: 15 psi

Capillary Exit Volt: 50 V Max

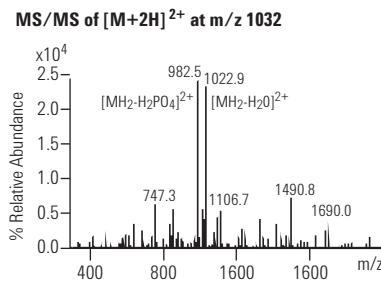
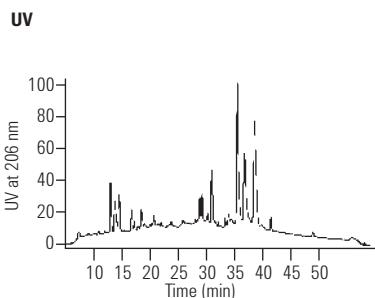
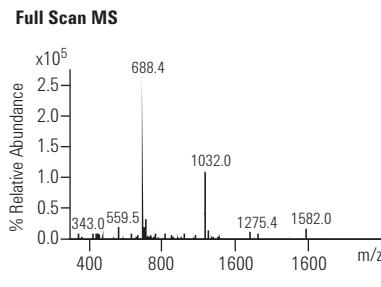
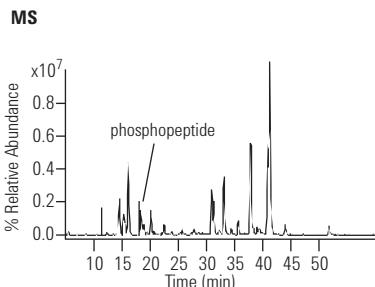
Accum Time: 300 ms

Total Averages: 3

Isolation Width: 3 m/z

Frag Amplitude: 1.0 V

**Sample:** Beta casein digest, 100 nL (4 pmol)



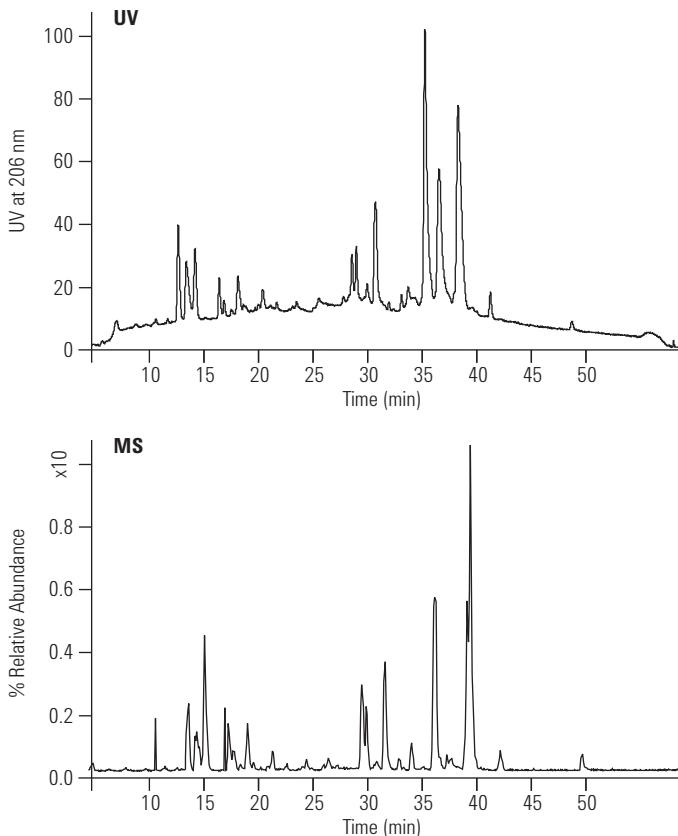
LCBP037

## Capillary columns for HPLC analyses with UV and MS detection

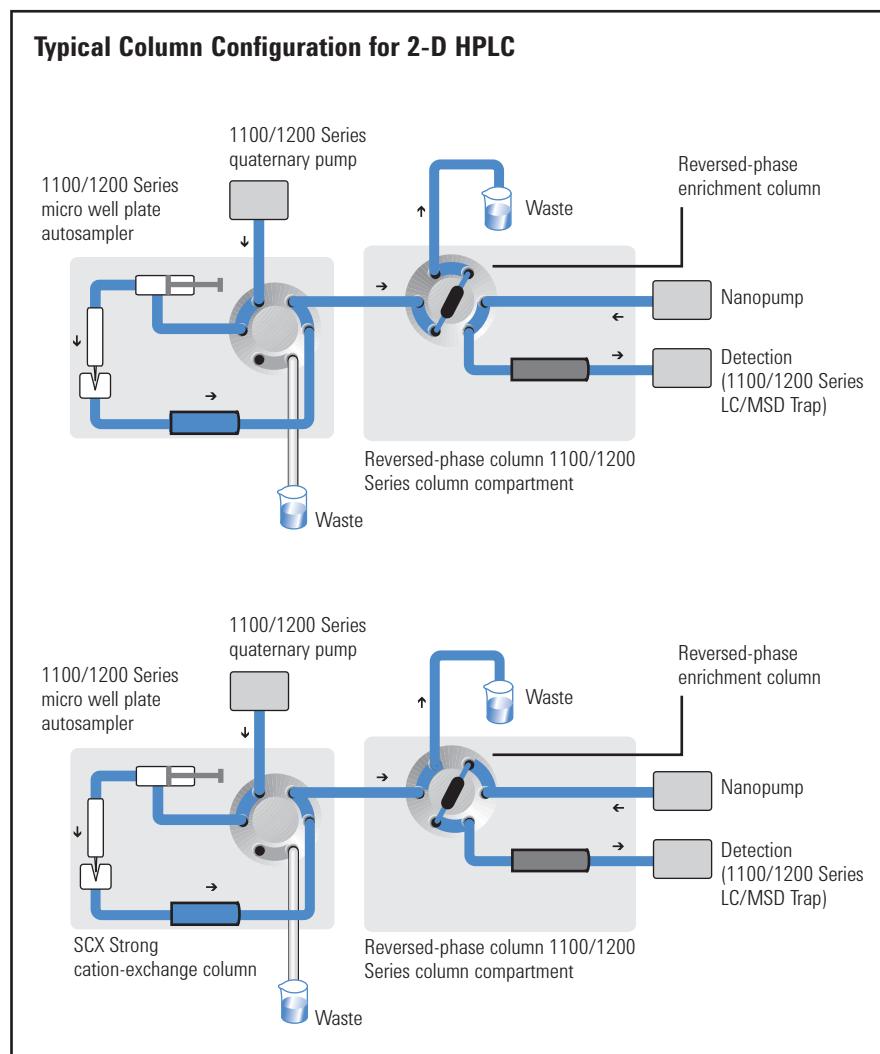
**Column:** ZORBAX 300SB-C18  
5064-8263  
**0.3 x 150 mm, 5 µm**

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: 206 nm  
MS Conditions: LC/MS: Pos. Ion ESI  
with LC/MSD trap-Vcap 4000 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: 100 nL  
Beta Casein Digest (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.



## 2-D LC/MS Analyses Using ZORBAX Capillary and Nano LC Columns



Flow path of the Agilent 1100 Series Nanoflow Proteomics Solution system.

1. Sample loading, elution from SCX and trapping on enrichment column
2. Valve switch in column compartment, elution from enrichment column; separation on RP, and MS analysis

## Proteins in a complex sample by 2-D 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  
Nanopump: A = Water, 0.1% Formic acid,  
B = ACN, 0.1% Formic acid

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, octopole 1:12 V,  
octopole 2:3.5 V, trap drive: 80 V. ICC: on, averages: 4, max accu time:  
150 ms; target 60,000, ion mode positive, MS/MS mode.

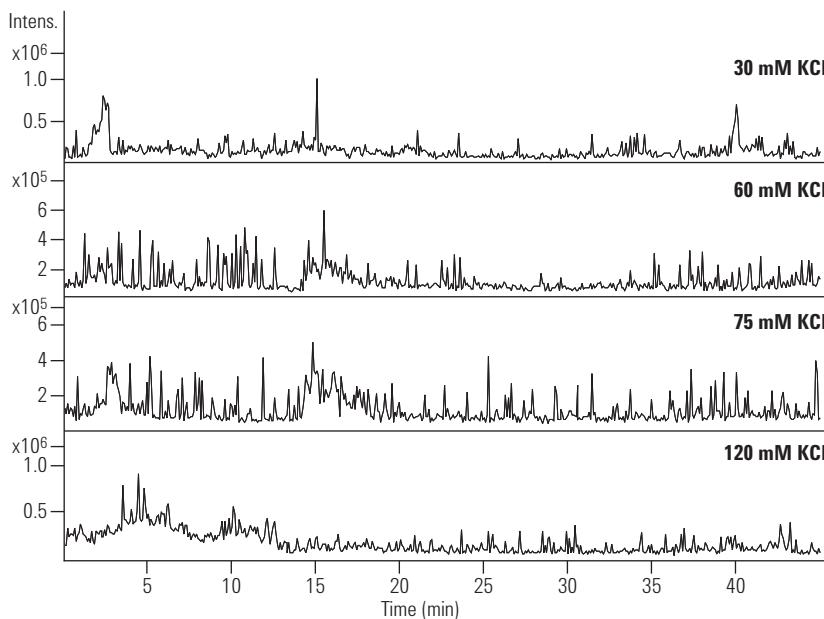
Flow Rate: Quaternary Pump: 30 µL/min  
Nanopump: 300 nL/min

Sample: Tryptic Digest of bovine serum albumin

Gradient: Quaternary Pump: Isocratic  
Nanopump:  
6 min = 3% B, 120 min = 60% B, 125 min = 80% B,  
130 min = 80% B, 131 min = 3% B, 140 min = 3% B

Volume: 1 to 8 µL

Salt Step Elution: 8 mL of 10 mM-100 mM KCl (10 mM increments),  
125 mM, 150 mM, 200 mM, 300 mM, 500 mM, 1 M.



LCCN004

Tryptic digest of bovine serum albumin (BSA). The base peak chromatograms show a selection of fractions from a 2-D 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 has Bio-SCX Series II columns designed for optimized 2-D separations of peptides and proteins using LC/MS. This packing is based on ultra-pure 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 2-D analysis of peptides and proteins.

### Column Specifications

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

### ZORBAX Bio-SCX Series II

Description	Size (mm)	Particle Size (µm)	Bio-SCX Series II
Capillary	0.3 x 35	3.5	5065-9912
Capillary	0.8 x 50	3.5	5065-9942



## ZORBAX Bio-SCX Series II provides more retention of small peptides

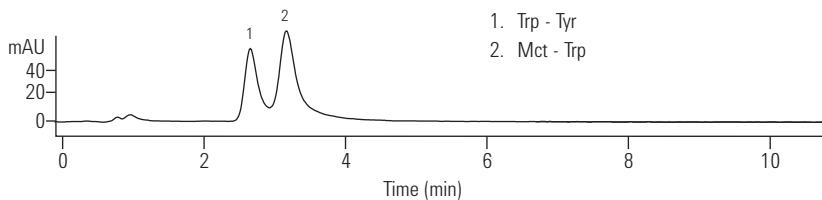
Column: **ZORBAX Bio SCX Series II**  
**5065-9912**  
**0.3 x 35 mm, 3.5  $\mu$ m**

Mobile Phase: 95% 40 mM NaCl: 5% ACN,  
0.3% Formic acid

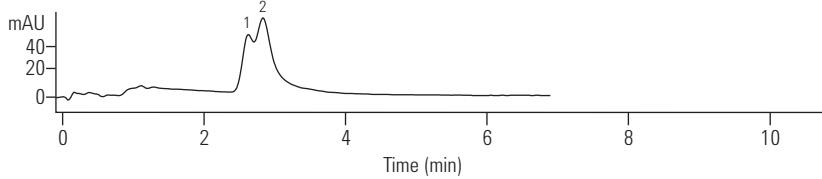
Flow Rate: 5  $\mu$ L/min

Detector: 230 nm

Sample: Synthetic Dipeptides



1. Trp - Tyr  
2. Mct - Trp



LCIE002

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

**ZORBAX HPLC Capillary Columns (glass-lined stainless steel)**

Description	Size (mm)	Particle Size ( $\mu\text{m}$ )	300SB-C18	300SB-C8	Poroshell 300SB-C8	300Extend C18	Bio-SCX Series II
Capillary	0.8 x 50	3.5					5065-9942
Capillary	0.5 x 250	5	5064-8266				
Capillary	0.5 x 150	5	5064-8264				
Capillary	0.5 x 75	5			5065-4468		
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	0.3 x 35	3.5				5065-9912	
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	5065-4427	

**ZORBAX Nano HPLC Columns (PEEK)**

Description	Size (mm)	Particle Size ( $\mu\text{m}$ )	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

Agilent MicroBore (1.0 mm id) columns are a good choice when sample sizes are limited. They can improve detection limits 5 times over 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  $\sim 50 \mu\text{L}/\text{min}$ ). 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 use 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 stop depth to provide a perfect zero dead volume connection every time.

### Separation of a tryptic digest on ZORBAX MicroBore 300SB-C18

**Column:** **ZORBAX 300SB-C18**  
**863630-902**  
**1.0 x 150 mm, 3.5  $\mu\text{m}$**

Mobile Phase: Gradient: 2-60% B in 60 min

A: 0.1% TFA

B: 0.075% TFA/80% ACN

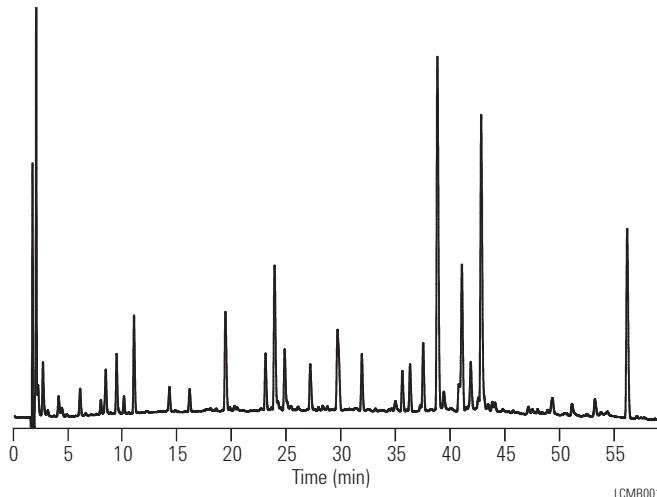
Flow Rate: 50  $\mu\text{L}/\text{min}$

Temperature: 50 °C

Detector: 215 nm

Sample: 2  $\mu\text{L}$   
Tryptic Digest of rhGH

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 100Å 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

## Peak Identification

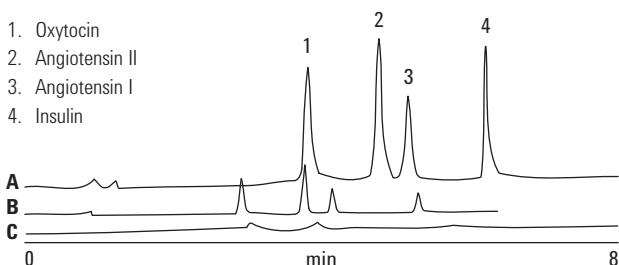
**A.** 1.0 mm id (flow rate 47 µL/min)**B.** 2.1 mm id (flow rate 200 µL/min)**C.** 4.6 mm id (flow rate 1 mL/min)

1. Oxytocin

2. Angiotensin II

3. Angiotensin I

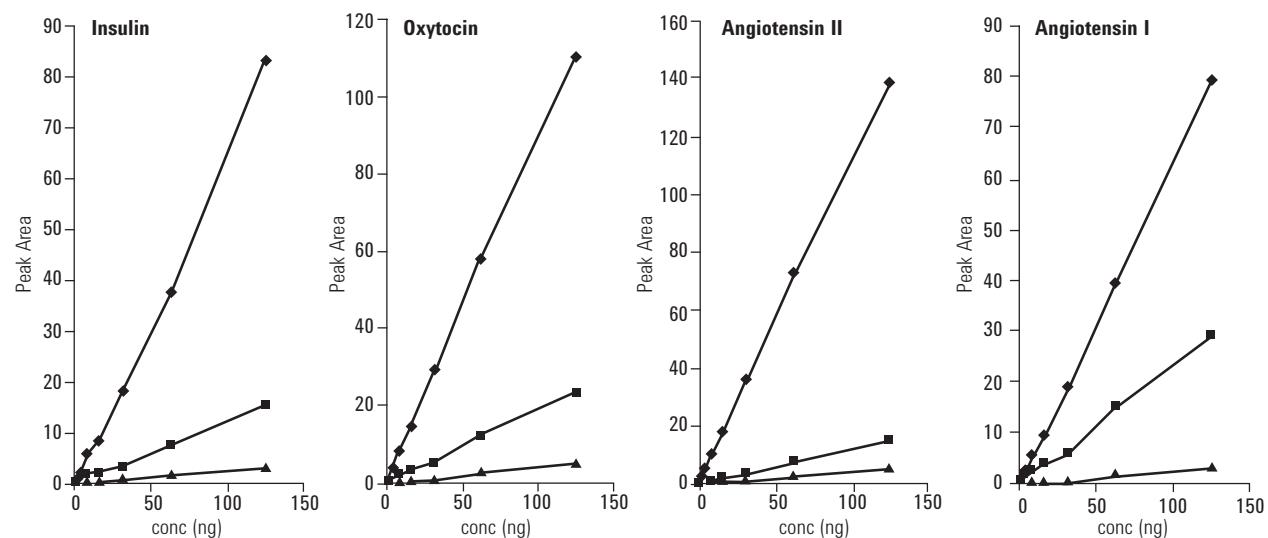
4. Insulin



Peptide separation on Agilent PLRP-S 100Å 5 µm columns

## Peak Identification

- ◆ 1.0 mm
- 2.1 mm
- ▲ 4.6 mm



Standard curve data-point graphs on Agilent PLRP-S columns

## MicroBore (1.0 mm id) Columns

### MicroBore (1.0 mm id)

Description	Size (mm)	Particle Size ( $\mu\text{m}$ )	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 ( $\mu\text{m}$ )	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 ( $\mu\text{m}$ )	PLRP-S 100Å USP L21	PLRP-S 300Å USP L21	PLRP-S 1000Å USP L21	PLRP-S 4000Å USP L21
MicroBore	1.0 x 50	3	PL1312-1300	PL1312-1301		
MicroBore	1.0 x 150	3	PL1312-3300			
MicroBore	1.0 x 50	5	PL1312-1500		PL1312-1502	
Description	Size (mm)	Particle Size ( $\mu\text{m}$ )	PL-SCX 1000Å	PL-SCX 4000Å	PL-SCX 1000Å	PL-SCX 4000Å
MicroBore	1.0 x 50	5	PL1351-1502	PL1351-1503	PL1345-1502	PL1345-1503



## 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  $\mu\text{g}$  and  $\text{mg}$  amounts of a biopharmaceutical drug candidate and fully porous bulk media, to pack development and process columns to purify multiple 100 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. Figure 1 shows prep column/media options and the quantity of product that can be purified.

BioPharmaceutical Lifecycle		Discovery	Development	Production		
		$\mu\text{g}$ high efficiency	$\text{mg}$	$\text{g}$	$\text{kg}$ high throughput	multi-kg
Reversed-Phase	mRP-C18	→				
	ZORBAX Prep HT 300Å StableBond		→			
	VariTide RPC		→			
	PLRP-S 100Å, 300Å, 1000Å, 4000Å		→			
Ion-Exchange	PL-SAX		→			
	PL-SCX		→			
Size Exclusion	ZORBAX GF-250/450		→			

Figure 1: 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 purifications with maximum recovery.	mRP-C18
All Biomolecules	Reversed-Phase	High efficiency 300Å 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 column, 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.  <ul style="list-style-type: none"> <li>• 3 µm and 5 µm for high efficiency</li> <li>• 8 µm, 10 µm, 10-15 µm, 15-20 µm, 30 µm and 50 µm particles for larger scale and low pressure purification</li> </ul>	PLRP-S
All Biomolecules	Ion-Exchange	A fully porous strong anion-exchanger  <ul style="list-style-type: none"> <li>• 5 µm particle size for high efficiency separations</li> <li>• 8 µm, 10 µm and 30 µm particles for larger scale medium and low pressure purification</li> </ul> A fully porous strong cation-exchanger  <ul style="list-style-type: none"> <li>• 5 µm particle size for high efficiency separations</li> <li>• 8 µm, 10 µm and 30 µm particles for larger scale medium and low pressure purification</li> </ul>	PL-SAX PL-SCX



### Tips & Tools

Further information can be found in the following publication:

*Biomolecule Purification* (5990-8335EN)

[www.agilent.com/chem/library](http://www.agilent.com/chem/library)





## ZORBAX PrepHT

- Easy scale-up from analytical to preparative scale with ZORBAX phases
- Fast preparative separations, up to 2000 mg
- 5 to 7 µm particles for high efficiency and high yield
- Easy to install finger-tight connections seal up to 5000 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 Agilent ZORBAX PrepHT columns. These are available in a variety of bonded phases – StableBond 300Å, 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 300Å StableBond

Hardware Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56
<b>PrepHT Cartridge Columns (require endfittings kit 820400-901)</b>						
PL PrepHT Cartridge	21.2 x 250	7	897250-102	897250-106	897250-105	897250-109
PL PrepHT Cartridge	21.2 x 150	7	897150-102	897150-106		897150-109
PL PrepHT Cartridge	21.2 x 150	5	895150-902	895150-906		895150-909
PL PrepHT Cartridge	21.2 x 100	5	895100-902	895100-906		895100-909
PL PrepHT Cartridge	21.2 x 50	5	895050-902	895050-906		895050-909
PL PrepHT endfittings, 2/pk			820400-901	820400-901	820400-901	820400-901
PL PrepHT Guard Cartridge, 2/pk	17 x 7.5	5	820212-921	820212-918	820212-924	820212-924
PL Guard Cartridge Hardware			820444-901	820444-901	820444-901	820444-901



## PLRP-S FOR REVERSED-PHASE 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. A Type II Drug Master File and regulatory support files are available for process materials, and facility audits are routinely conducted.



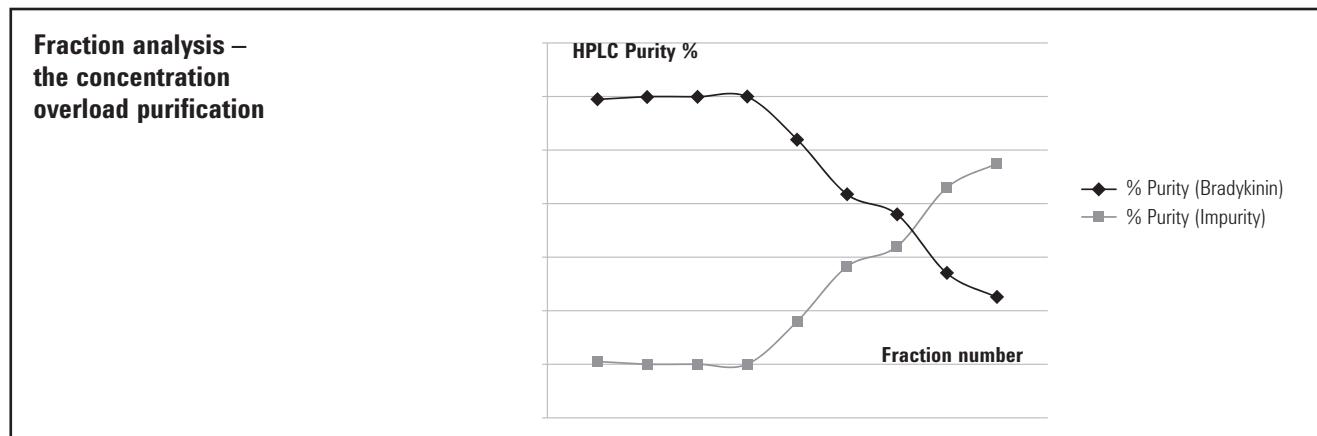
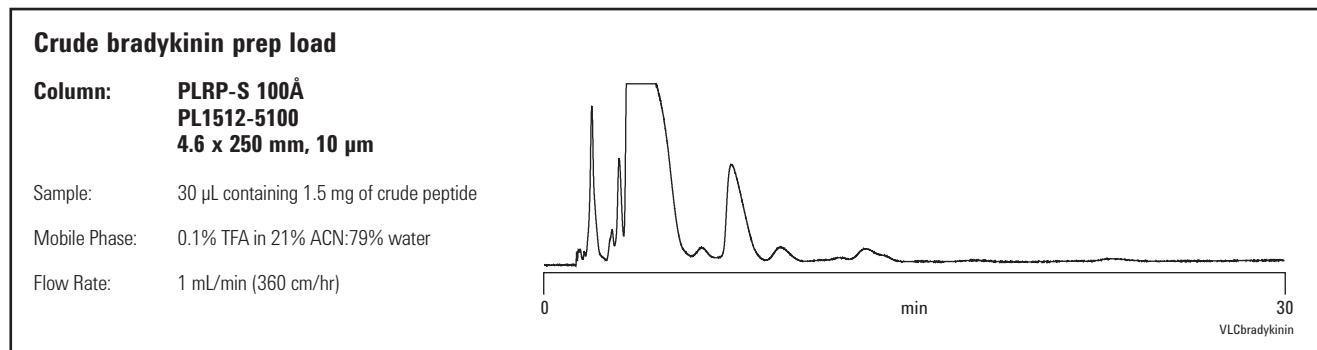
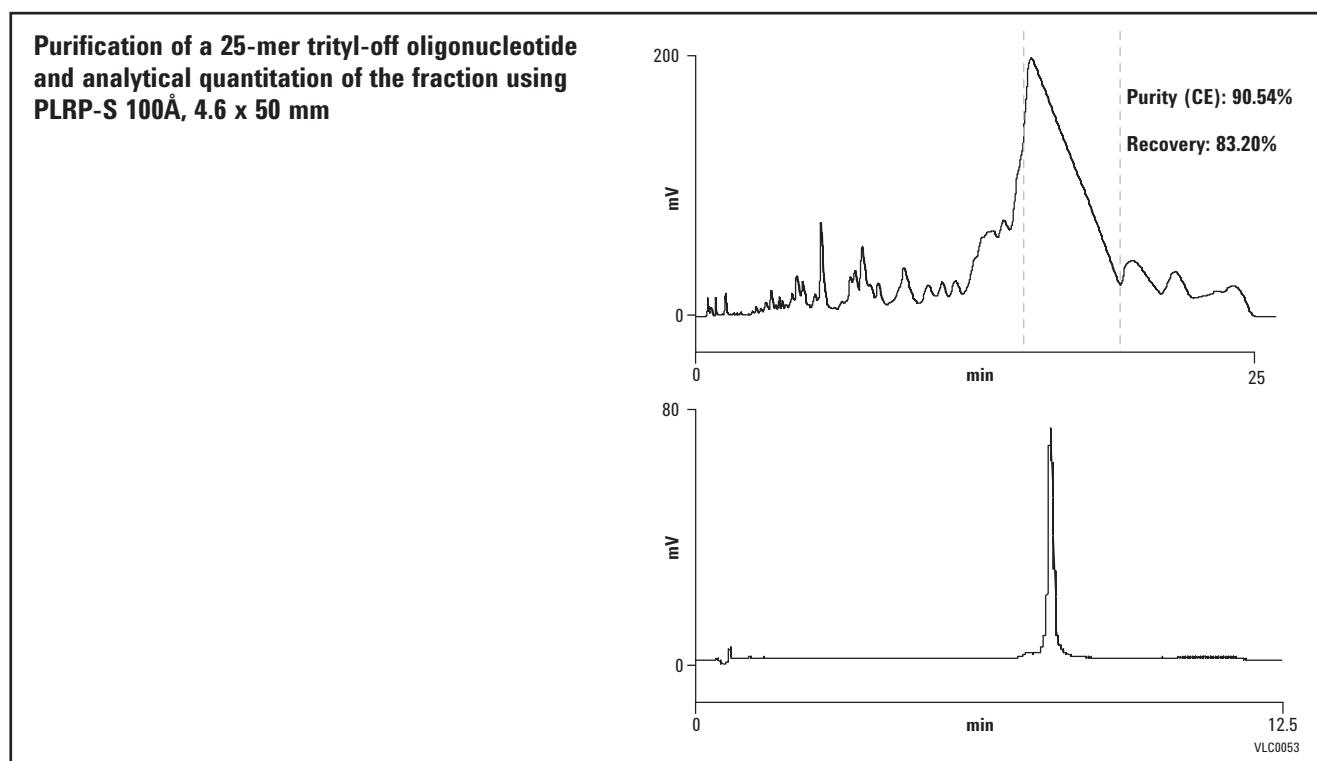
**PLRP-S Prep to Process Application Guide**

<b>Application</b>	<b>PLRP-S Media Pore Size</b>			
	<b>100Å</b>	<b>300Å</b>	<b>1000Å</b>	<b>4000Å</b>
Synthetic biomolecules, peptides, and oligonucleotides	◆	◆		
Recombinant biomolecules, peptides, and proteins	◆	◆		
Large biomolecules, antibodies, DNA fragments			◆	◆
Small molecules, unstable compounds including metal sensitivity	◆			

**Column Specifications**

<b>pH Range</b>	1-14
<b>Buffer Content</b>	Unlimited
<b>Organic Modifier</b>	1-100%
<b>Temperature Limits</b>	200 °C
<b>Maximum Pressure</b>	5-8 µm: 3000 psi (210 bar) 3 µm: 4000 psi (300 bar)

# Prep to Process PLRP-S



**Prep to Process PLRP-S**

<b>Size (mm)</b>	<b>Particle Size (<math>\mu\text{m}</math>)</b>	<b>PLRP-S 100Å</b>	<b>PLRP-S 300Å</b>	<b>PLRP-S 1000Å</b>	<b>PLRP-S 4000Å</b>
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
<b>PLRP-S Method Development Columns</b>					
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 ( $\mu\text{m}$ )	Unit	PLRP-S 100Å	PLRP-S 200Å	PLRP-S 300Å	PLRP-S 1000Å	PLRP-S 4000Å
50	1 kg	PL1412-6K00	PL1412-6K05	PL1412-6K01	PL1412-6K02	
	100 g	PL1412-4K00	PL1412-4K05	PL1412-4K01	PL1412-4K02	
30	1 kg				PL1412-6702	PL1412-6703
	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	PL1412-6102	PL1412-6103
	100 g	PL1412-4100		PL1412-4101	PL1412-4102	PL1412-4103
8	1 kg	PL1412-6800		PL1412-6801		

For larger quantities, please contact your local Agilent sales office.



## 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 clean-up. 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 1000Å and 4000Å 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 reduces 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 1000Å pore size is for high-capacity purifications and the 4000Å gigaporous particles with improved mass transfer are intended for large biomolecules and high-speed, high-resolution purifications.



## Column Specifications

	PL-SAX	PL-SCX
<b>Matrix</b>	Fully polymeric	Fully polymeric
<b>Pore Sizes</b>	1000Å, 4000Å	1000Å, 4000Å
<b>Particle Sizes</b>	10 µm, 30 µm	10 µm, 30 µm
<b>Bead Form</b>	Rigid spherical	Rigid spherical
<b>Functionality</b>	Quaternary amine	Sulfonic acid
<b>Pressure Stability</b>	3000 psi	3000 psi
<b>Temperature Stability</b>	80 °C	80 °C
<b>pH Range</b>	1-14	1-14
<b>Eluent Compatibility</b>	All anion-exchange buffers	All cation-exchange buffers
<b>Packed Bed Density</b>	0.39 g/mL	0.39 g/mL

### Purification of a large oligonucleotide

**Column:** PL-SAX 1000Å, 8 µm

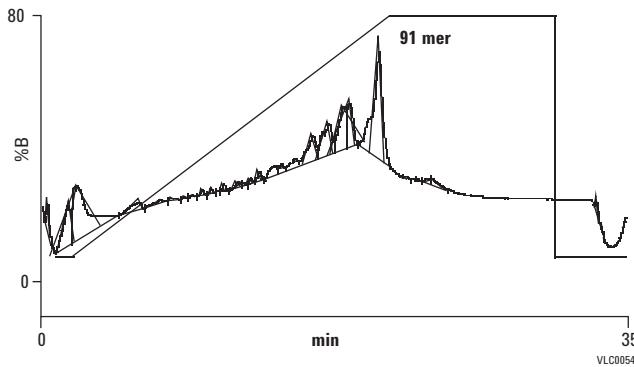
Mobile Phase: A: 93% 0.1 M TEAA, pH 7:7% ACN  
B: 93% 0.1 M TEAA, 3.24 M ammonium acetate, pH 7:7% ACN

Gradient: 0-100% B in 20 min

Flow Rate: 1.5 mL/min

Temperature: 60 °C

Detector: UV, 290 nm



### Preparative fractionation of a culture filtrate containing amyloglucosidases on Agilent PL-SAX 4000Å

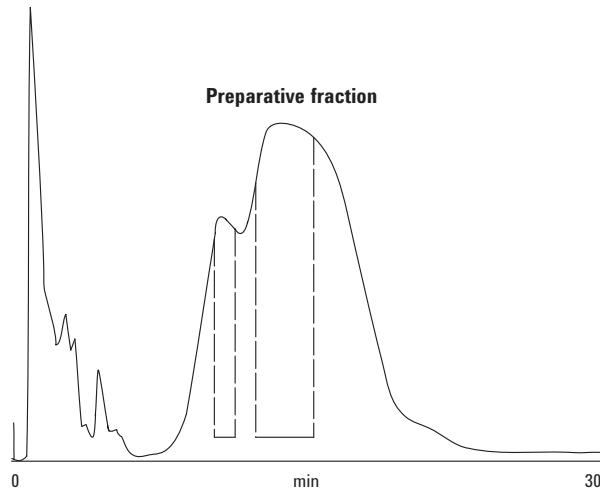
**Column:** PL-SAX  
PL1551-1803  
4.6 x 50 mm, 8 µm

Mobile Phase: A: 0.01 M Tris HCl, pH 8  
B: A + 0.5 M NaCl, pH 8

Flow Rate: 4.0 mL/min

Gradient: Linear 0-100% B in 2 min

Detector: UV, 280 nm



**Prep to Process PL-SAX and PL-SCX**

Dimensions	Particle Size ( $\mu\text{m}$ )	PL-SAX 1000Å	PL-SAX 4000Å	PL-SCX 1000Å	PL-SCX 4000Å
100 x 300	30	PL1851-3102	PL1851-3103	PL1845-3102	PL1845-3103
100 x 300	10	PL1851-2102	PL1851-2103	PL1845-2102	PL1845-2103
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

**PL-SAX and PL-SCX Bulk Media**

Particle Size ( $\mu\text{m}$ )	Unit	PL-SAX 1000Å	PL-SAX 4000Å	PL-SCX 1000Å	PL-SCX 4000Å
30	1 kg	PL1451-6702	PL1451-6703	PL1445-6702	PL1445-6703
	100 g	PL1451-4702	PL1451-4703	PL1445-4702	PL1445-4703
10	1 kg	PL1451-6102	PL1451-6103	PL1445-6102	PL1445-6103
	100 g	PL1451-4102	PL1451-4103	PL1445-4102	PL1445-4103

For larger quantities, please contact your local Agilent sales office.



## 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 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 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**

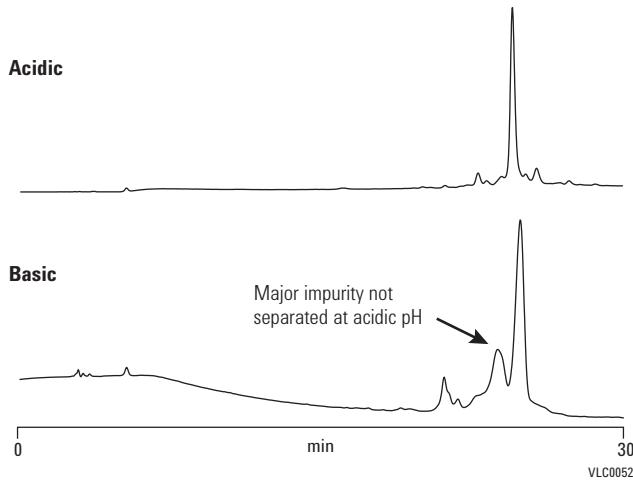
**Column:** VariTide RPC  
PL1512-5A05  
4.6 x 250

Mobile Phase: Acidic  
A: 0.1% TFA in 95% water: 5% ACN  
B: 0.1% TFA in 50% water: 50% ACN  
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**

- Pre-packed 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 pre-packed 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	PL3540-D603VP
500 mg per 6 mL tube	~ 25 mL 0.1% TFA	50/pk	PL3540-C603VP
1 g per 20 mL tube	~ 50 mL 0.1% TFA	25/pk	PL3540-P603VP
25 g			PL3549-3603VP

## LOAD & LOCK PREPARATIVE HPLC COLUMN PACKING SYSTEMS



Agilent offers a complete range of Load & Lock column systems for laboratory and process preparative LC. They are designed to let you easily and quickly pack your own preparative high efficiency columns. This is the right solution for applications ranging in scale from development (multigrams) to production (multi-kilo) of pharmaceutical compounds, peptides, and natural products. Our Load & Lock columns have a unique fluid/sample distribution system to maximize productivity. The system provides dynamic axial compression (DAC) and static "locked" axial compression (SAC) and is designed for easy operation to deliver greater convenience.

### Laboratory Load & Lock Columns

- Mobile packing station supports three different column sizes
- Runs on compressed air with no need for a power supply
- Quick and easy packing and unpacking within minutes

Agilent's 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. Three different column sizes are supported: 1 in, 2 in and 3 in id. Because the station is powered by compressed air, it is the perfect solution for hazardous environments. The quick-release single bolt clamp offers speedy and easy packing and unpacking within minutes.

### Load & Lock Preparative HPLC Column Packing Systems

Description	Water Jacket	Size (mm)	Part No.
Load & Lock 4001 Column	No	25 x 500	PCG93LL500X25
	Yes	25 x 500	PCG93LL500X25WJ
	Spare parts kit		PCG931AAKIT
Load & Lock 4002 Column	No	50 x 500	PCG93LL500X50
	Yes	50 x 500	PCG93LL500X50WJ
	Spare parts kit		PCG932AAKIT
Load & Lock 4003 Column	No	75 x 500	PCG93LL500X75
	Yes	75 x 500	PCG93LL500X75WJ
	Spare parts kit		PCG933AAKIT
Mobile packing station (air driven hydraulic)			PCG93LLSTAND123

# CARTRIDGE COLUMN SYSTEMS

## Cartridge Selection Guide

Icon*	Type of Cartridge	Features	Benefits
	ZORBAX Guard Cartridge: Standalone system	High efficiency, standalone, low-dead-volume cartridge	Seals up to 400 bar
		Polymeric cartridge designed for leak-tight seals against metal surfaces	No gaskets required More solvent-resistant than PEEK
		Reusable fittings	Adapt for connections to 1/16 in LC fittings
	ZORBAX Semi-Preparative Guard HPLC Hardware Kit: Standalone system	Easy, low-dead-volume assembly	Seals up to 2000 psi (135 bar, 13.5 MPa)
		Tubing (polyphenylene sulfone) designed for leak-tight seals against metal surfaces	No gaskets required
		Reusable fittings	Adapt for connections to 1/16 in LC fittings
	ZORBAX and Agilent Prep Preparative Cartridge Column and Guard HPLC System: Standalone and integral hardware options	Easy, low-dead-volume assembly	Extends column lifetime
		Reusable fittings	Permits rapid column changes
		Hardware options for integral and external guards	Can use with 21.2 and 30 mm id columns
	Polymeric Analytical Column and Guard Cartridge	High efficiency	Inexpensive
		Low dead volume	Rapid cartridge changes
		Reusable holder	Extends column lifetime

\*Look for these icons to help you select the proper guard cartridges and columns.

# Appendices

## Cartridge/Guard Cartridge Systems Compatibility Guide\*

Icon	Column Type	Guard Cartridge Holder	ID (mm)	Phases
	Standard fitting	Column guard cartridge (standalone) cartridge holder 820999-901	2.1 3.0 4.6	ZORBAX
	Semi-preparative column	Semi-prep guard cartridge (standalone) cartridge holder 840140-901	9.4	ZORBAX
	PrepHT	Guard cartridge 820444-901	21.2	ZORBAX Agilent Prep
	Analytical	Guard cartridge holder (PL1310-0016) and PLRP-S guard cartridges, 2/pk (PL1612-1801)	3.0	PLRP-S
			3.0	PL-SAX
			3.0	PL-SCX

\*Standalone guard cartridges fit all cartridge and standard fitting columns available from Agilent. All columns without icons are standard fitting columns.

## USP DESIGNATIONS – BIOHPLC COLUMNS ONLY

For a full listing of USP designations for all HPLC columns, please consult the Agilent catalog or [www.agilent.com](http://www.agilent.com).

The US Pharmacopeia (USP) is a standard source for many pharmaceutical methods that specifies columns by packing materials rather than by manufacturer. Listed below are the recommended Agilent Technologies columns suitable for most LC methods listed with the USP.

<b>USP Method</b>	<b>USP Packing Materials</b>	<b>Column</b>	<b>Particle Size (µm)</b>
L1	Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 1.5 to 10 µm in diameter	Poroshell 120 EC-C18	2.7
		Poroshell 120 SB-C18	2.7
		ZORBAX 300SB-C18	1.8, 3.5, 5, 7
		ZORBAX 300 Extend C18	3.5, 5
L7	Octyl silane chemically bonded to totally porous microsilica particles, 1.5 to 10 µm in diameter	Poroshell 120 EC-C8	2.7
		ZORBAX 300SB C-8	1.8, 3.5, 5, 7
L10	Nitrile groups chemically bonded to porous silica particles, 3 to 10 µm in diameter	ZORBAX 300 SB-CN	3.5, 5, 7
L14	Silica gel 10 µm in diameter with a chemically bonded, strongly basic quaternary ammonium anion-exchange coating	ZORBAX SAX	5
L17	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 7 to 11 µm in diameter	Hi-Plex H	8
		Bio SCX NP10	10
L21	A rigid, spherical styrene-divinylbenzene copolymer, 5 to 10 µm in diameter	PLRP-S 100Å	3, 5, 8, 10
		PLRP-S 300Å	3, 5, 8, 10
		PLRP-S 1000Å	5, 8, 10
		PLRP-S 4000Å	5, 8, 10
L25	Packing having the capacity to separate compounds with a MW range from 1,000 to 5,000 da (as determined by the polyethylene oxide), applied to neutral, ionic and cationic water-soluble polymers	PL aquagel-OH	5, 8
L33	Packing having the capacity to separate proteins by molecular size over a range of 4,000 to 400,000 da. It is spherical, silica-based, and processed to provide pH stability	ZORBAX GF-250	4
		Bio SEC-3	3
		Bio SEC-5	5
		ProSEC 300S	5
L35	A zirconium-stabilized spherical silica packing with a hydrophilic (diol-type) molecular monolayer bonded phase	ZORBAX GF-250	4
		ZORBAX GF-450	6
L50	A strong cation-exchange resin made of porous silica with sulfopropyl groups, 5 to 10 µm in diameter	ZORBAX 300SCX	5
L56	Propyl silane chemically bonded to totally porous silica particles, 3 to 10 µm in diameter	ZORBAX SB-C3	1.5, 3, 5

# Appendices

## BioHPLC Columns Literature

Title	Column/ Product	Application	Publication Number	Publication Type
Rapid Analysis of Adenovirus Type 5 Particles with Bio-Monolith Anion-Exchange HPLC Columns to Support the Development of a High-Titre Manufacturing Platform	Bio-Monolith QA	Adenovirus	5990-5524EN	Application Note
Separation of Two Sulfurated Amino Acids with other Seventeen Amino Acids by HPLC with Pre-Column Derivatization	Eclipse Plus-C18	Amino acid analysis	5990-5977EN	Application Note
Rapid, Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids	ZORBAX Eclipse AAA	Amino acid analysis	5980-1193EN	Application Note
High-Speed Amino Acid Analysis (AAA) on 1.8 µm Reversed-Phase (RP) Columns	ZORBAX Eclipse Plus	Amino acid analysis	5989-6297EN	Application Note
Improved Amino Acid Methods using Agilent ZORBAX Eclipse Plus C18 Columns for a Variety of Agilent LC Instrumentation and Separation Goals	ZORBAX Eclipse Plus	Amino acid analysis	5990-4547EN	Application Note
Rapid and Precise Determination of Cellular Amino Acid Flux Rates using HPLC with Automated Derivatization with Absorbance Detection	ZORBAX Eclipse Plus	Amino acid analysis	5990-3283EN	Application Note
Agilent PL-SAX 1000Å HPLC Columns and Media	PL-SAX	Analysis/Prep - Oligonucleotides	5990-8200EN	Flyer
Compliance for Biopharmaceutical Laboratories	Reference	Compliance	5990-7001EN	Primer
Macroporous Reversed-Phase C18 High-Recovery Protein Fractionation HPLC Column	mRP-C18	Human serum, Biomarkers	5989-2714EN	Brochure
Rapid Human Polyclonal IgG Quantification using the Agilent Bio-Monolith Protein A HPLC Column	Bio-Monolith	IgG	5989-9733EN	Application Note
Rapid IgM Quantification in Cell Culture Production and Purification Process Monitoring using the Agilent Bio-Monolith QA Column	Bio-Monolith QA	IgM	5989-9674EN	Application Note
Optimization of Protein Separations on Weak Cation-Exchange Columns – a Study of the Particle Size, Buffer Salts and Gradients	Bio IEX	MAbs	5990-8833EN	Technical Poster
Characterization of Monoclonal Antibodies on the Agilent 1260 Infinity Bio-inert Quaternary LC by Size Exclusion Chromatography using the Agilent BioSEC Columns	Bio SEC	MAbs	5990-6414EN	Application Note

(Continued)



**BioHPLC Columns Literature**

<b>Title</b>	<b>Column/ Product</b>	<b>Application</b>	<b>Publication Number</b>	<b>Publication Type</b>
Agilent BioHPLC Columns for the Characterization of Monoclonal Antibodies	Biocolumns	MAbs	5990-7753EN	Flyer
Fast Separation of Monoclonal Antibody and Dimer by SEC with Agilent Bio SEC	Bio SEC	MAbs	5990-8613EN	Application Note
Choosing a ZORBAX Poroshell Phase (C3, C8, or C18) for Fast Separation of Monoclonal Antibodies	Poroshell 300	MAbs	5989-0071EN	Application Note
Determination of the Glycosylation Status of Intact Recombinant Human Antibodies using Time of Flight Mass Spectrometry	Poroshell 300	MAbs	N/A	Technical Poster
High Speed and Ultra-High Speed Peptide Mapping of Human Monoclonal IgG on Poroshell 300SB-C18, C8, and C3	Poroshell 300	MAbs	5989-0590EN	Application Note
Rapid HPLC Analysis of Monoclonal Antibody IgG1 Heavy Chains using ZORBAX Poroshell 300SB-C8	Poroshell 300	MAbs	5989-0070EN	Application Note
Comparison of ZORBAX StableBond 300Å LC Columns to Optimize Selectivity for Antibody Separations Using HPLC and LC/MS	ZORBAX 300SB	MAbs	5989-6840EN	Application Note
Reversed-Phase Separation of Intact Monoclonal Antibodies (MAb) using Agilent ZORBAX RRHD 300SB-C8	ZORBAX RRHD 300SB-C8	MAbs	5990-9016EN	Application Note
Increased UV-Sensitivity in Combination with Novel WCX Column Separation for Better Detectability of Charge State Variants of Biotherapeutic Proteins	Bio MAb	MAbs and other proteins	N/A	Technical Poster
Agilent HPLC Column Selection Guide	HPLC columns	Many	5990-4435EN	Selection Guide
The LC Handbook: Guide to LC Columns and Method Development	LC columns	Method development	5990-7595EN	Primer
Agilent PLRP-S 100Å HPLC Columns and Media	PLRP-S	Oligonucleotides	5990-8187EN	Flyer
HPLC Purification of 26-bp Serial Analysis of Gene Expression Ditags	PLRP-S	Oligonucleotides	5990-7739EN	Application Note
Improved Column Lifetime with Thermally Stable Polymer Columns for Oligonucleotide Ion-Pair RP HPLC	PLRP-S	Oligonucleotides	5990-7764EN	Application Note
Ion-Pair Reversed-Phase Purification of De-Protected Oligonucleotides – Choice of Pore Size	PLRP-S	Oligonucleotides	5990-7763EN	Application Note
Use Temperature to Enhance Oligonucleotide Mass Transfer and Improve Resolution in Ion-Pair RP HPLC	PLRP-S	Oligonucleotides	5990-7765EN	Application Note
High Resolution Separations of Oligonucleotides using PL-SAX Strong Anion-Exchange HPLC Columns	PL-SAX	Oligonucleotides	5990-8297EN	Application Note
Fast Impurity Profiling of Synthetic Oligonucleotides with the Agilent 1290 Infinity LC System and Agilent 6530 Accurate-Mass QTOF LC/MS Plus C18 RRHD	ZORBAX Eclipse Plus C18 RRHD	Oligonucleotides	5990-5825EN	Application Note

(Continued)

# Appendices

## BioHPLC Columns Literature

Title	Column/ Product	Application	Publication Number	Publication Type
Agilent PLRP-S Media and Load & Lock Columns – The Future of Prep/Process Chromatography	Prep/Process	Oligonucleotides, Peptides, Proteins	5990-8201EN	Flyer
Agilent PLRP-S 50 µm HPLC Media	PLRP-S	Oligonucleotides, Peptides, Small proteins	5990-8188EN	Flyer
Reduce Tubing Volume to Optimize Column Performance	Small diameter columns	Optimizing instrument performance	5990-4964EN	Application Note
Using the High-pH Stability of ZORBAX Poroshell 300Extend-C18 to Increase Signal-to-Noise in LC/MS	ZORBAX 300 Extend-C18	Optimizing instrument performance	5989-0683EN	Application Note
Increase Sensitivity with Microbore Polymeric HPLC Columns from Agilent	PLRP-S (Microbore)	Peptide hormone, small proteins, small molecules	5990-8666EN	Technical Overview
Decreasing Analysis Time Using Poroshell 300SB-C18 in Analysis of a Protein Digest	Poroshell 300	Peptide mapping	5988-6081EN	Application Note
Rapid Peptide Mapping Method with High Resolution using a sub 2-µm Column	ZORBAX 300SB-C18	Peptide mapping	5990-4712EN	Application Note
Increased Peak Capacity for Peptide Analysis with the Agilent 1290 Infinity LC System	ZORBAX Eclipse Plus	Peptide mapping	5990-6313EN	Application Note
Trypsin-Digested Monoclonal Antibody and BSA using Agilent ZORBAX RRHD 300SB-C18	ZORBAX RRHD 300SB-C18	Peptide mapping	5990-8244EN	Application Note
Preparative Scale Purification of Bradykinin by Concentration Overload	PLRP-S	Peptide purification	5990-7736EN	Application Note
Preparative Scale Purification of Bradykinin by Volume Overload	PLRP-S	Peptide purification	5990-7741EN	Application Note
Preparative Scale Purification of Depherelin by Concentration Overload	PLRP-S	Peptide purification	5990-7742EN	Application Note
Preparative Scale Purification of Leuprolide by Concentration Overload	PLRP-S	Peptide purification	5990-7735EN	Application Note
Superior Resolution of Peptides on SepTech ST150 10-C18 using Acetonitrile-Free Gradient Elution	SepTech	Peptide purification	5990-7761EN	Application Note
Agilent PLRP-S Media for HPLC Analysis of Peptides	PLRP-S	Peptides	5990-8667EN	Technical Overview

(Continued)

**BioHPLC Columns Literature**

<b>Title</b>	<b>Column/ Product</b>	<b>Application</b>	<b>Publication Number</b>	<b>Publication Type</b>
Analysis of Peptides on a PLRP-S 100Å 10 µm with ELS Detection and Acetonitrile-Free Eluents	PLRP-S	Peptides	5990-7760EN	Application Note
Investigation into the Alternatives to Acetonitrile for the Analysis of Peptides	PLRP-S	Peptides	5990-7740EN	Application Note
Investigation into the Alternatives to Acetonitrile for the Analysis of Peptides on a SepTech ST150 10-C18	SepTech	Peptides	5990-7951EN	Application Note
Investigation into the Alternatives to Acetonitrile for the Analysis of Peptides on a VariTide RPC	VariTide RPC	Peptides	5990-8145EN	Application Note
Fast Monitoring of Bacteriophage Production During Fermentation Using the Agilent Bio-Monolith HPLC Column	Bio-Monolith	Phage production, process monitoring	5990-3247EN	Application Note
Physicochemical Characterization of a Therapeutic Protein by Peptide Mapping, SEC and IEX using the Agilent 1260 Infinity Bio-inert Quaternary LC System	Bio MAb, Bio SEC, ZORBAX Eclipse Plus, Poroshell 120	Protein analysis	5990-6192EN	Application Note
Optimization of the Agilent 1100 HPLC System for Superior Results with ZORBAX Poroshell Columns	Poroshell 300	Protein analysis	5988-9998EN	Application Note
Using Poroshell 300SB-C18 for High-Sensitivity, High-Throughput Protein Analysis on the Agilent LC/MSD	Poroshell 300-C18	Protein analysis	5988-7031EN	Application Note
Analysis of Albumin Proteins using ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7852EN	Application Note
Analysis of Complex Bacterial Cell Division Proteins by Size Exclusion Chromatography (SEC)	ProSEC 300S	Protein analysis	5990-8143EN	Application Note
Analysis of Globulins using ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7851EN	Application Note
Analysis of Hsp47, a Collagen Chaperone, by Size Exclusion Chromatography (SEC)	ProSEC 300S	Protein analysis	5990-8142EN	Application Note

(Continued)

# Appendices

## BioHPLC Columns Literature

Title	Column/ Product	Application	Publication Number	Publication Type
Analysis of Various Globular Proteins using ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7850EN	Application Note
Effect of pH on Protein Size Exclusion Chromatography	ProSEC 300S	Protein analysis	5990-8138EN	Application Note
Globular Proteins and the Calibration of ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7767EN	Application Note
Light Scattering Analysis of BSA with ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7766EN	Application Note
Static Light Scattering Analysis of Globular Proteins with Agilent ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7939EN	Application Note
LC Handbook and Compliance Guide to Recombinant Protein Characterization	N/A	Protein analysis	5990-8561EN	Primer
Agilent ZORBAX 300SB-C18 1.8 µm Rapid Resolution High Definition Columns for Proteins	ZORBAX 300SB-C18	Protein analysis	5990-7989EN	Technical Overview
Analysis of Oxidized Insulin Chains using Reversed-Phase Agilent ZORBAX RRHD 300SB-C18	ZORBAX RRHD 300SB-C18	Protein analysis	5990-7988EN	Application Note
ACN-free HPLC Analysis and Prep Purification of ACP Fragment	PLRP-S	Protein purification	5990-7762EN	Application Note
Isocratic Purification of Synthetic Acyl Carrier Protein Fragment 65-74	PLRP-S	Protein purification	5990-7737EN	Application Note
Agilent PL-SAX Anion-Exchange Media for Amyloglucosidase Purification and Analysis	PL-SAX	Protein purification	5990-8664EN	Technical Overview
Progressive Denaturation of Globular Proteins in Urea	ProSEC 300S	Protein purification	5990-8141EN	Application Note
Separation of High MW Fibrous Proteins	PLRP-S	Protein separation	5990-8137EN	Application Note
Fast Protein Separations Using Agilent Poroshell 300	Poroshell 300	Protein separation	5989-9899EN	Application Note

(Continued)

**BioHPLC Columns Literature**

<b>Title</b>	<b>Column/ Product</b>	<b>Application</b>	<b>Publication Number</b>	<b>Publication Type</b>
Fast Separation of Large and Heterogeneous Proteins using ZORBAX Poroshell C18, C8, and C3 Phases	Poroshell 300	Protein separation	5989-0015EN	Application Note
Use of Temperature to Increase Resolution in the Ultrafast HPLC Separation of Proteins with ZORBAX Poroshell 300SB-C8 HPLC Columns	Poroshell 300-C8	Protein separation	5989-0589EN	Application Note
The Effect of NaCl Concentration on Protein Size Exclusion Chromatography	ProSEC 300S	Protein separation	5990-8139EN	Application Note
The Effect of Temperature on Protein Size Exclusion Chromatography	ProSEC 300S	Protein separation	5990-8140EN	Application Note
Infinitely Better for Bio-Molecule Analysis	Agilent 1260 Infinity Bio-inert Quaternary LC System	Proteins	5990-6220EN	Brochure
Defining the Optimum Parameters for Efficient Size Separations of Proteins	Bio SEC	Proteins	5990-8832EN	Technical Poster
Defining the Optimum Parameters for Efficient Size Separations of Proteins	Bio SEC	Proteins	5990-8895EN	Application Note
Compliance for Biopharmaceutical Laboratories	Many	Proteins	5990-7001EN	Primer
Gradient Purification of Synthetic Acyl Carrier Protein Fragment 65-74	PLRP-S	Proteins	5990-7738EN	Application Note
Fast Agilent HPLC for Large Biomolecules	PLRP-S, PL-SAX, PL-SCX	Proteins	5990-8663EN	Technical Overview
Agilent Anion-Exchange Media for Proteins – Loading vs Resolution – Effect of Flow Rate and Example Protein Separations	PL-SAX	Proteins	5990-8777EN	Technical Overview
Purity Assessment Following Affinity Separation	PL-SAX	Proteins	5990-8436EN	Technical Overview
Agilent PL-SCX Cation-Exchange Media for Large Biomolecules	PL-SCX	Proteins	5990-8665EN	Technical Overview

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# Appendices

## BioHPLC Columns Literature

Title	Column/ Product	Application	Publication Number	Publication Type
Poroshell 300SB-C18 for Fast, High Protein Separation	Poroshell 300	Proteins	5988-2100ENUS	Brochure
Progressive Denaturation of Globular Proteins in Urea	ProSEC 300S	Proteins	5990-8141EN	Application Note
ProSEC 300S Columns Protein Characterization Columns	ProSEC 300S	Proteins	5990-7468EN	Flyer
Static Light Scattering Analysis of Globular Proteins with Agilent ProSEC 300S Columns	ProSEC 300S	Proteins	5990-7939EN	Application Note
Confidently Separate and Characterize Biomolecules with Agilent BioHPLC Columns	Bio SEC, Bio IEX, Bio MAb	Proteins	5990-5195EN	Brochure
Increase your Productivity with Agilent ZORBAX RRHD 300Å 1.8 µm Columns	ZORBAX RRHD 300SB-C18, C8	Proteins, Peptides	5990-8124EN	Flyer
High Purity, High Recovery, High Throughput – Agilent Technologies Offers Two New Lines of Preparative HPLC Columns	Agilent Prep HT	Purification/Prep	5989-2350EN	Brochure
Biomolecule Purification – Purification Columns and Media for Peptides, Oligonucleotides, and Proteins	PLRP-S, PL-SAX, PL-SCX	Purification/Prep	5990-8335EN	Brochure
The Influence of Silica Pore Size on Efficiency, Resolution and Loading in Reversed-Phase HPLC	SepTech	Purification/Prep	5990-8298EN	Application Note
Analysis of Protein Primary Structure when using Wide-Pore sub-2-µm Particles and UHPLC	ZORBAX RRHD 300SB-C18	Purification/Prep	5990-8830EN	Technical Poster
Polyethylene Glycol/Oxide Standards and the Calibration of Agilent ProSEC 300S Columns	ProSEC 300S	SEC	5990-8147EN	Application Note

# BIOPHARM DEFINITIONS

## A

### **active starting material**

The raw material that is identified as directly related to the active chemical comprising the product, and is defined at the first stage during chemical synthesis at which part or most of the critical moieties are present. Defining active starting material defines the step at which compliance with cGMP requirements begins during manufacturing. For biopharmaceuticals, this term is not used.

### **affinity**

Attraction between particles or substances; relatively speaking, a measure of the attraction of one molecule toward another.

### **aggregate**

A clustered mass, as of protein molecules; or to cluster together in such a way. Aggregates of cells (solid, fluffy or pelletized) can clog the pores of filters or other fermentation apparatus.

### **albumins**

Protein constituents of blood plasma and serum also found in muscle, egg white, and milk.

### **alkylation**

The introduction by substitution or addition, of an alkyl group into an organic compound; alkylating agents are various substances that contain an alkyl radical and that can, therefore, replace a hydrogen atom in an organic compound; alkylation is used to prevent refolding of already reduced proteins during peptide mapping.

### **alpha helix ( $\alpha$ -helix)**

A coil or spiral element of protein secondary structure.

### **amino acids**

A class of 20 naturally occurring hydrocarbon molecules that combine to form proteins in living things. They include alanine (A), aspartic acid or asparagine (B), cysteine (C), glutamic acid (Z) or glutamine (Q), phenylalanine (P), glycine (G), histidine (H), isoleucine (I), lysine (K), leucine (L), methionine (M), proline (P), arginine (R), serine (S), threonine (T), valine (V), tryptophan (W), and tyrosine (Y). (Those are the so-called normal amino acids; others have been synthesized and are used in medicinal chemistry). They are incorporated into proteins by transfer RNA according to the genetic code.

### **amphoteric**

A substance that has both acid and base properties; amphoteric molecules can accept or donate protons to act as an acid or a base.

### **antibody**

Protein molecules produced by the immune system. Antibodies recognize foreign molecules or structures such as bacteria, viruses and various antigens to which the body has been exposed.

### **antigen**

Any agent that reacts specifically with an antibody. Each antigen may contain more than one site capable of binding to a particular antibody. See immunogen.

### **antigenicity**

The capacity of a substance to induce the formation of antibodies or to elicit an immune response when injected into an animal.

### **API**

Active product intermediate; the chemical entity that has the drug activity and structure, but is not yet formulated with excipients.

## B

### **base pair**

Two bases on different strands of nucleic acid that join together. In DNA, cytosine (C) always pairs with guanine (G) and adenine (A) always links to thymine (T). In RNA molecules, adenine joins to uracil (U).

### **beta sheet ( $\beta$ -sheet)**

A structure resulting from the regular, accordion-like folding of polypeptide chains; the chief alternative to the alpha helix.

### **bioactivity**

A protein's ability to function correctly after it has been delivered to the active site of the body (in vivo).

### **biogeneric**

A biopharmaceutical that is produced and licensed by a different firm than the one that originally licensed the molecule. A biogeneric is used for the same indications and may be produced by a substantially similar process, or one that is different, but results in comparable product.

## biologics

Products of living organisms used in the prevention or treatment of disease.

## biosimilar

A biopharmaceutical that is produced using a different cell line or master cell bank and/or different process, yet meets criteria for comparability in clinical activity. A biosimilar may differ in its purity/impurity profile, and its potency may differ in a definable way. See also biogeneric and follow-on biologic.

## BLA

Biologics license application; the required application for marketing a biologic product in the United States. Most biotechnology-derived drugs are approved through a BLA, rather than an NDA, although some biologics, such as recombinant insulin and human growth hormone, considered to be simpler in structure and well-characterized, have been approved under NDAs.

## bulk active ingredient

Also bulk drug substance, the active ingredient that is formulated with excipients to produce the drug product formulation. Biopharmaceuticals are produced "in bulk" through bioprocessing.

## C

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### CBER

Center for Biologics Evaluation and Research at the FDA. CBER regulates vaccines, gene therapy, cellular products, allergenic extracts, antitoxins, antivenins, venoms, blood, and blood products (clotting factors and plasma derived products).

### CDER

Center for Drug Evaluation and Research. The largest of FDA's six centers, CDER regulates prescription and over-the-counter drugs. Following a transfer of responsibility for biologics that began in June 2003, CDER now also regulates therapeutic proteins and monoclonal antibodies for in vivo use, which were formerly regulated by CBER.

### cGMP

Current good manufacturing practice; see GMP.

## change control

A system by which changes to facilities, equipment, and processes are documented and approved. The change control system ensures that changes are evaluated and approved prior to implementation to maintain the facilities, equipment, and processes in a validated state.

## chaotropic

Disrupting the structure of water, macromolecules, or living systems to promote activities that would have been inhibited by the water, molecules, or systems. Urea and guanidine hydrochloride are common chaotropic agents used with proteins.

## characterization

Precisely deciphering and describing an entity's properties (physical and chemical properties in the case of a molecular entity; genetic and stability properties in the case of a cell line).

## C-terminal

Carboxyl-terminal; the carboxyl terminus of a protein chain, with a free carboxyl group.

## D

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## deamidation

Removal of one or more amide groups from the Gln or Asn residue in a protein, converting the residues to Glu, Asp, or isoAsp. Depending on the protein, this may have no effect, or major effects, on potency, stability, or solubility.

## denaturation

A condition in which a protein unfolds or its polypeptide chains are disordered, rendering the molecules less soluble and usually nonfunctional.

## denature

To unfold a protein or break it up, changing its usual three-dimensional structure. Proteins can be denatured by chemical action, heat, or even agitation of a protein solution.

## denatured protein

A protein having unfolded or disordered polypeptide chains, which render the molecule less soluble and usually nonfunctional. Sometimes a denatured protein can be refolded (renatured).

**dimer**

A polymer made up of two identical molecules. When three molecules link up, the resultant polymer is called a trimer. Larger polymers are usually referred to by placing a number before the "-mer" suffix: 4-mer, 5-mer, 6-mer, and so on.

**disulfide bond**

A covalent bond formed between sulfur atoms of different cysteines in a protein; such bonds (links, bridges) help hold proteins together.

**DNA**

Deoxyribonucleic acid, the nucleic acid based on deoxyribose (a sugar) and the nucleotides G, A, T, and C. Double-stranded DNA has a corkscrew-ladder shape (the Double helix") and is the primary component of chromosomes, which thus carry inheritable characteristics of life. See nucleotides and nucleic acids.

**drug substance**

(or active pharmaceutical ingredient); the active drug chemical or biological substance in purified bulk form. The drug substance is further processed to derive a drug product.

**E****efficacy**

The ability of a substance (such as a protein therapeutic) to produce a desired clinical effect; its strength and effectiveness; usefulness; the power to produce an effect.

**enzymes**

Proteins that catalyze biochemical reactions by causing or speeding up reactions without being changed in the process themselves.

**epitope**

A molecular region on the surface of an antigen that elicits an immune response and can combine with the specific antibody produced by such a response; also called a determinant or an antigenic determinant.

**excipient**

A type of raw material that is present in the drug product and thus has direct patient contact; includes inert materials such as bulking agents, stabilizing agents, preservatives, salts, solvents or water. An excipient must be evaluated for safety in animals, unless it has been approved as GRAS or is on a list of approved excipients.

**express**

To translate a cell's genetic information, stored in its DNA (gene), into a specific protein.

**expression system**

A host organism combined with a genetic vector (such as a virus or circular DNA molecule called a plasmid) that is loaded with a gene of interest. The expression system provides the genetic context in which a gene will function in the cell - that is, the gene will be expressed as a protein.

**expression vector**

A virus, plasmid, cosmid, or artificial chromosome that delivers foreign genes to a host, creating a recombinant organism that will express the desired protein.

**F****Fab**

Antigen-binding fragment of an immunoglobulin. An IgG Fab is prepared by enzymatic cleavage of the intact tetrameric IgG, and reduction of the inter-chain disulfide links, and binds one mole of antigen per mole. See F(ab)'2.

**F(ab)'2**

Dimeric antigen-binding fragment of an immunoglobulin. An IgG F(ab)'2 is prepared by enzymatic digestion of an intact IgG, which removes the Fc portion of the molecule. F(ab)'2 binds two moles of antigen per mole. See Fab.

**FAb**

Antibodies are Y-shaped molecules. The "arms" of each Y are the FAb regions (fragment antigen binding sites) that bind to antigens; the stem of the Y is the Fc region, which attracts microbe-engulfing cells to destroy what has been bound. If the active part of an antibody can be identified, sometimes only that part may be needed as a therapeutic molecule (facilitating production and processing by reducing the size and lessening the chances of an immune response in patients who receive the drug). This fragment may be "conjugated" to another molecule (such as PEG) for stability or other reasons.

**Fc**

Portion of an immunoglobulin molecule that carries various effector functions, such as the ability to bind complement. Important in immunological activities, and separable from the antigen-binding portion by enzymatic or chemical cleavage. See Fab.

# Appendices

## FDA

Food and Drug Administration.

## folding

A process in which a protein spontaneously forms into its correct, knotted tertiary structure that is held in place by chemical bonds and by attractive forces between atoms.

## follow-on biologic

Another term for biosimilar or biogenetic.

## G

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### gene

The unit of inheritance consisting of a sequence of DNA occupying a specific position within the genome. Three types of genes have been identified: structural genes encoding particular proteins; regulatory genes controlling the expression of the other genes; and genes for transfer RNA or ribosomal RNA instead of proteins.

### gene therapy

Treats, cures, or prevents disease by changing the expression of a person's genes or inserting genes into the genome. In its infancy, current gene therapy is primarily experimental, with most human clinical trials only in the research stages. Gene therapy can target somatic (body) or germ (egg and sperm) cells. In somatic gene therapy, the recipient's genome is changed, but the change is not passed along to the next generation. In germ-like gene therapy, the parent's egg and sperm cells are changed with the goal of passing on the changes to their offspring.

## GLP

Good laboratory practices; according to 21 CFR Part 58, regulations to ensure quality of nonclinical laboratory studies related to safety. All activity is recorded, trained staff uses only established procedures, and records and samples are maintained.

### glycoproteins

Proteins that contain sugar side chains added as a post-translational process; presence of sugar side chains often affects activity and in vivo stability.

### glycosylation

Adding one or more carbohydrate molecules onto a protein (a glycoprotein) after it has been built by the ribosome; a post-translational modification.

## GMPs

Good manufacturing practices; according to 21 CFR Parts 210, 211, 600, 610, and (for devices) 820, current good manufacturing practices (cGMPs) influence the manner in which biopharmaceuticals and other drugs and medical devices are produced. Standard operating procedures must be followed, processes must be validated, equipment must be qualified, and properly trained staff must maintain a clean/sterile environment.

## Golgi body

A cell organelle consisting of stacked membranes where post-translational modifications of proteins are performed; also called Golgi apparatus.

## H

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### hybridization

The process of joining complementary strands of DNA to make an RNA-DNA hybrid; the partial pairing of DNA single strands from genetically different sources.

### hydrophilic

Having an affinity for water; attracting, dissolving in, or absorbing water; readily absorbing moisture; having strongly polar molecular groups that readily interact with water.

### hydrophobic

Insoluble in water; the extent of insolubility; not readily absorbing water, resisting or repelling water, wetting, or hydration; or being adversely affected by water.

## I-J

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### immunogen

A substance that provokes an immune response - that is, the body recognizes it as a foreign agent that must be expelled or destroyed.

### in vitro

Performed using laboratory apparatus rather than a living animal.

### in vivo

Involving living animals or humans as test subjects.

### intermediates

Substances formed in the middle stages of a series of processing steps; "stepping stones" between a parent substance and a final product.

**K-L****ligase**

An enzyme that causes molecular fragments (such as DNA, RNA, or peptides) to link together; DNA ligase is used with restriction enzymes to create recombinant DNA.

**light-scattering analysis**

Analytical method that gives information about the size and shape of molecules based on how they disperse ultraviolet and visible light.

**M****MAb**

Monoclonal antibody; a highly specific, purified antibody that recognizes only a single epitope.

**microheterogeneity**

In BioPharm, usually small differences in the amino acid sequence or structure of a polypeptide chain. For example, to produce a recombinant protein in *E. coli*, a Methionine (Met) must be added to one end of the protein sequence to act as a signal that initiates protein synthesis. In most cases, that Met is removed once the protein is made. Sometimes the Met is removed for only some of the molecules. The purified product is then a mixture of a protein with the native sequence and a protein with the native sequence plus the extra amino acid.

**mRNA**

Messenger RNA; which serves as a template for protein synthesis. It is made as a complement to a DNA sequence and then transported from the cell nucleus to the ribosomes.

**multimer**

Any small polymer; in BioPharm, usually a protein made up of more than one polypeptide chain.

**N****native**

The natural non-denatured state exhibiting biological activity; in BioPharm context, it usually refers to a molecule's normal three-dimensional structure under optimal conditions.

**NDA**

The New Drug Application; CDER's equivalent of the BLA. It is used for small-molecules and some biopharmaceuticals (such as hormones and small peptides), which are regulated by CDER rather than CBER.

**N-terminal**

Amino-terminal or amine terminus; the amine terminus of a protein chain (with a free  $\alpha$ -amino group).

**nucleic acids**

DNA or RNA: chainlike molecules composed of nucleotides.

**nucleotides**

Molecules composed of a nitrogen-rich base, phosphoric acid, and a sugar. The bases can be adenine (A), cytosine (C), guanine (G), thymine (T), or uracil (U).

**O****oxidation**

Chemical reaction in which a compound or atom loses valence electrons; due to reaction with an oxidizing agent (e.g. oxygen, peroxides, metal ions or others). Many proteins are prone to oxidation on exposure to air (such as oxidation of the Methionine (Met) amino acid into methionine sulfide or sulfone). See also redox.

**P****PAT**

Process analytical technology; an FDA initiative that seeks to encourage industry to develop and use new analytical technology and multivariate analyses as part of risk management during process development, manufacturing, and quality control testing. PAT includes on-line, real-time analyses, process control tools, continuous improvement and knowledge management tools, and statistical tools.

**PEGylation**

Covalent attachment of polyethylene glycol molecule(s) to a protein molecule via selected amino acid side groups, for example free amino or sulphhydryl groups. May be done to decrease toxicity or improve its solubility and circulating half-life in the body.

**peptide bond**

The carbon-nitrogen covalent bond (link) between an amino group of one amino acid and a carboxyl group of another, formed by removing water and resulting in the group RCO-NH. This linkage does not allow free rotation, and it is the important bond that connects amino acid monomers to form the polymer known as a polypeptide.

## peptide mapping

Bioanalytical method in which proteins are selectively cleaved by enzymes to create a characteristic pattern of peptides that is elucidated through chromatographic separations and spectroscopic or spectrometric detection.

## peptides

Proteins consisting of fewer than 40 amino acids.

## phosphorylation

Addition of a phosphate ( $\text{PO}_4$ ) group to a molecule, usually enzymatically done by transferring a phosphate group from ATP (adenosine triphosphate).

## pl

Isoelectric point, the pH at which a substance has no net charge; above which a substance acts as a base and below which it acts as an acid. A solution of proteins or amino acids has its minimum conductivity and viscosity at the isoelectric point. The pl is a pH value for a given substance; for example, the pl of gelatin is pH 4.7. pl can be used to identify and characterize proteins.

## plasmid

Hereditary material that is not part of a chromosome. Plasmids are circular and self-replicating and found (naturally in bacteria and some yeasts) in the cytoplasm of cells. They can be used as vectors for introducing up to 10,000 base-pairs of foreign DNA into recipient cells.

## polishing

The final purification step(s) in a biopharmaceutical manufacturing process, usually involving an affinity or other refined chromatography method. Often this step uses the most expensive technique in the process because it handles the smallest amount of material.

## post-translational modification

After a DNA sequence has been interpreted and a protein has been created, it may be modified by the addition of sugar (glycosylation) or other molecules. This protein processing is done by the Golgi bodies after proteins have been constructed by ribosomes.

## protease

An enzyme that cleaves the peptide bonds linking amino acids in protein molecules, classified according to the most prominent functional molecular group (such as serine or cysteine) at the active site; also called proteinase.

## protein variants

Proteins with the same amino acid sequences but different folds or different carbohydrate residues. They must be separated from the therapeutic proteins.

## proteins

Complex organic macromolecules whose structures are coded in an organism's DNA. Each is a chain of more than 40 amino acids in peptide linkages that folds back upon itself in a particular way. Proteins are the principal constituent of all cell protoplasms (the entire contents of a live cell). Each protein has a unique, genetically defined amino acid sequence that determines its specific shape and function (as enzymes, structural elements, hormones, and immunoglobulins, involved in oxygen transport, muscle contraction, or electron transport, for instance).

## proteolysis

Separation (cleavage) of peptide bonds in proteins by proteases (enzymes that recognize and cut specific peptide bonds) or other means.

## Q-R

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## recombinant

Refers to DNA (or the protein resulting from such DNA) that has been genetically engineered to contain genetic material from another organism. Genetically altered micro-organisms are usually referred to as recombinant, whereas plants and animals so modified are called *transgenic* (see transgenics).

## redox

Equilibrium reaction of oxidation/reduction, for example, thiodisulfide exchange, a step used during refolding of recombinant proteins that contain cysteine (Cys) residues, in order to form correct pairing of sulphydryl groups (-SH) and form stable disulfide (S-S) bonds.

## RNA

Ribonucleic acid, the nucleic acid based on ribose (a sugar) and the nucleotides G, A, U, and C. It translates the information encoded by DNA into amino acid sequences the cell uses to make proteins. Similar to DNA but based on ribose, and with the base uracil (U) in place of thymine (T). Various forms of RNA are found: mRNA (messenger RNA); tRNA (transfer RNA); and rRNA (ribosomal RNA). Most RNA molecules are single-stranded, although they can form double-stranded units.

**S****secondary structure**

In proteins, the folding, twisting, coiled, sometimes spring-like chain that results when hydrogen bonds form between the adjacent parts of a molecule, as in an alpha helix or beta sheet.

**SOPs**

Standard operating procedures; detailed (step-by-step), instructions to achieve uniformity in the performance of a specific process or piece of equipment, which are approved by the quality control unit and used for GMP operations.

**T-U****tertiary structure**

The three-dimensional folding (its normal state) of a polypeptide chain in a protein molecule.

**transgenics**

The alteration of plant or animal DNA so that it contains a gene from another organism. There are two types of cells in animals and plants, germ-line cells (the sperm and egg in animals, pollen and ovule in plants) and somatic cells (all of the other cells). Germ-line DNA is altered in transgenic animals and plants so those alterations are passed on to offspring. That is done to produce therapeutics, to study disease, and to improve livestock strains. Transgenic plants have been created for increased resistance to disease and insects as well as to make biopharmaceuticals.

**translation**

The process by which information transferred from DNA by RNA specifies the sequence of amino acids in a polypeptide (protein) chain.

**tRNA**

Transfer RNA, a type of RNA with triplet nucleotide sequences that complement the nucleotide coding sequences of mRNA. In protein synthesis, tRNA bonds with amino acids and transfers them to the ribosomes, where proteins are assembled according to the genetic code carried by mRNA.

**unfolding**

A form of protein degradation in which the three-dimensional structure of a molecule unravels to something that more closely resembles a basic chain of amino acids.

**V-Z****vaccines**

Preparations that elicit an immune response (production of antibodies) to protect a person or animal from a disease-causing agent.

**virus**

The simplest form of life: RNA or DNA wrapped in a shell of protein, sometimes with a means of injecting that genetic material into a host organism (infection). Viruses cannot reproduce on their own, but require the aid of a host (bacteria, plant, or animal). The host cell's synthesis is often inhibited by the infecting virus, which may or may not result in disease (more than 200 viruses are known to produce human disease). An individual virus particle is called a virion, and virions vary in structure, complexity, and size (ranging from 20-25 nm or less to 2,000 nm or more). Six classes of virus are defined by whether they are single or double stranded, DNA or RNA, or positive or negative.

**well-characterized**

A chemical entity whose identity, purity, impurities, potency, and quantity can be determined and controlled; most well-characterized biologics are recombinant DNA-derived proteins or monoclonal antibodies.

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