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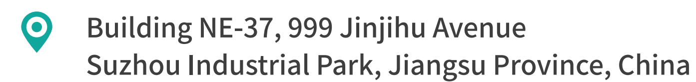
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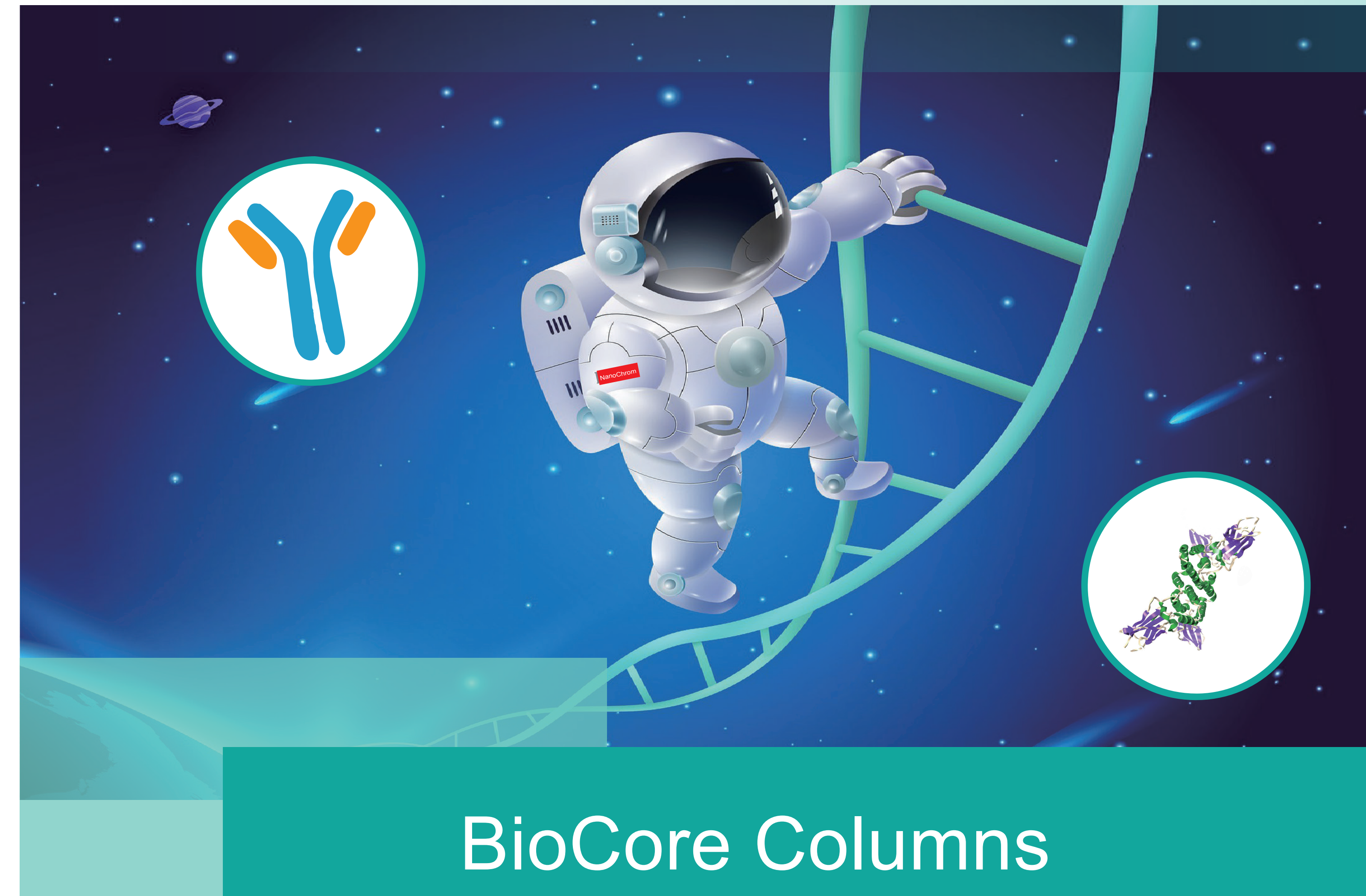


# BioCore Columns

## for antibody and protein separation

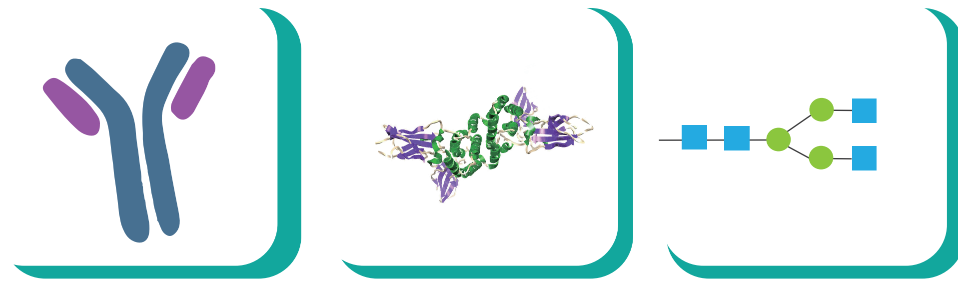
Website

WeChat Official Account

# Introduction

Antibody-based biologics have been a fast-growing area in the bio-therapeutic market for their specificity and efficacy, for the diagnosis and treatment of a wide range of diseases, including auto-immune, cardiovascular, infectious, cancer and inflammation. In the development and production of therapeutic antibodies, the content of impurities, structural variants and post-translational modification variants must be monitored, characterized and quantified, are critical quality attributes (CQAs) to prove the stability and effectiveness of final products. Antibody-based biologics are often of complex microheterogeneity by nature. Thus, their quality control and stability evaluation are highly challenging tasks.

HPLC is an important analytical technique for characterizing and quantifying impurities and variants in antibodies and related substances. Due to the complex nature of these molecules, a variety of separation modes are employed for a thorough characterization, including size exclusion chromatography (SEC) for aggregates and fragments, ion exchange chromatography (IEC) for charged variants, hydrophobic interaction chromatography (HIC) for DAR analysis in ADC, reversed phase chromatography (RPC) for exact mass determination for an antibody and its subunits, hydrophilic interaction chromatography (HILIC) for glycans, and protein A affinity chromatography for titer analysis.





# BioCore Columns Family

The BioCore family is designed to chromatagraphically analyze antibodies, proteins and related substances by liquid chromatography, and serves customers in bio-technology, biopharmaceutical and academic research.

The BioCore family consists of a suite of columns in different separation modes, including BioCore SEC size exclusion columns for aggregates and fragments, BioCore WCX/SCX/WAX/SAX ion exchange columns for charged variants, BioCore HIC hydrophobic interaction columns for DAR analysis in ADC, BioCore RP columns for exact mass determination of intact antibody and subunits, BioCore Glycan HILIC columns for glycan analysis, and BioCore Protein A affinity columns for titer analysis.

| | SEC | IEC | HIC | RPC | HILIC | Affinity |
|---|---|---|---|---|---|---|
| Product | BioCore SEC-150<br>BioCore SEC-300<br>BioCore SEC-500 | BioCore WCX<br>BioCore SCX<br>BioCore WAX<br>BioCore SAX | BioCore HIC | BioCore RP | BioCore Glycan | BioCore Protein A |
| Application | Small-molecule drugs, peptides, proteins, oligos, glycans, etc. | Charged variants in mAbs, bi-specific antibodies, ADCs and proteins | MAbs and ADCs | Intact proteins and protein fragments | N-glycans of proteins | MAbs and Fc fusion proteins |

BioCore columns employ innovative particle technology, advanced column chemistry and rugged packing method to ensure superior performance, quality and ruggedness. All BioCore columns are based on monodispersed spherical particles via innovative production processes, which provide excellent column efficiency and consistency.

BioCore media utilize uniquely designed surface modification and grafting methodology to ensure media bio-compatibility and desired selectivity for separating antibodies, proteins and related substances. Each batch of BioCore media is produced in accordance with a strict quality management standard and tested with relevant biological molecules to ensure performance and batch-to-batch consistency.

Each BioCore column is produced by a well-developed packing method and tested individually to ensure quality and consistency. A certificate of assurance of the media and a column quality assurance report are shipped with every shipped BioCore column.

# BioCore SEC Columns

BioCore SEC is a family of high performance, size exclusion chromatography columns, designed for separating antibodies, proteins, peptides, oligonucleotides and related substances, having a broad application range in bio-tech, biopharmaceutical and academic research.

## Main Features

- Innovative particle technology: monodispersed particles for high efficiency, high mechanical strength for better column lifetime, and high pore volume for high resolution
- Advanced column chemistry for minimal secondary interaction
- Multiple pore size options for broad application range
- Robust column packing for good column lifetime
- Good column-to-column consistency

## Specification

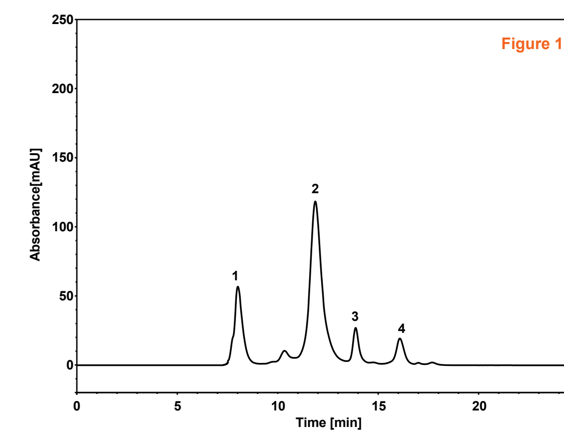
| Product Name | SEC-150 | SEC-300 | SEC-500 |
|---|---|---|---|
| Functional Group | Diol | | |
| Substrate | Monodispersed, high pore volume, porous silica particles | | |
| Particle Size | 1.8, 3 & 5 µm | | |
| Pore Size | 150 Å | 300 Å | 500 Å |
| Pressure Limit | 5000 psi for 1.8 µm<br>2500 psi for 3 µm<br>1500 psi for 5 µm | | |
| Temperature Limit | 40 ℃ | | |
| pH Range | 2-8 | | |
| Calibration Curve (PEG) | 500-15,000 | 1,000-50,000 | 5,000-200,000 |
| Calibration Curve (Glucan) | 1,000-30,000 | 2,000-100,000 | 20,000-500,000 |
| Calibration Curve (Globular Protein) | 5,000-150,000 | 10,000-750,000 | 20,000-1,500,000 |
| Application | Peptides, heparin, glycans, small oligos and small proteins | Aggregates and fragments in antibodies and proteins, DNA/RNA | High order aggregates in antibodies, larger proteins and DNA/RNA |

# Application

Size exclusion chromatography (SEC) is an important analytical technique for determining aggregate and fragment content in antibody biologics, which is one of the most frequently run essays in mAb drug development and drug manufacturing.

Figure 1 illustrates the separation of a mixture of a mAb (~150 kDa), its aggregate (tetramer, ~900 kDa) and fragments (HC, ~50 kDa, and LC, ~25 kDa) on a 5 µm, 7.8×300 mm BioCore SEC-300 column, demonstrating its good suitability for simultaneous separation of mAb, related aggregates and fragments.

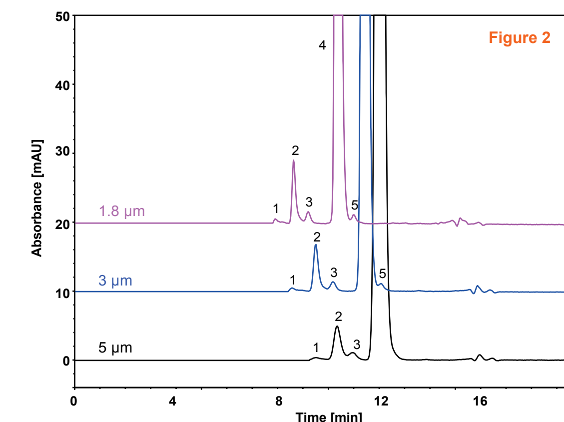
Separation of MAb, Aggregate, Heavy Chain and Light Chain




| | |
|---|---|
| Columns: | **BioCore SEC-300**, 5 µm |
| Dimension: | 7.8×300 mm |
| Mobile Phase: | 300 mM NaCl in 50 mM phosphate buffer, pH 6.8 |
| Flow Rate: | 0.7 mL/min |
| Temperature: | 25 ℃ |
| Injection: | 10 µL |
| Detection: | UV 214 nm |
| Sample: | mAb |
| Peaks: | 1. Aggregate (M.W.=900 kDa)<br>2. MAb (M.W.=150 kDa)<br>3. Heavy Chain (M.W.=50 kDa)<br>4. Light Chain (M.W.=25 kDa) |

For routine analysis, SEC columns packed with 5 µm media are often used. As shown in Figure 2, a 5 µm, 4.6×300 mm SEC-300 column provides suffice separation between the main peak of trastuzumab biosimilar and its aggregates but failing to resolve the fragment adjacent to the main mAb peak. When a 4.6×300 mm SEC-300 column packed with 3 µm media is used, both the resolutions between the mAb and its aggregates and among its aggregates are improved significantly. Moreover, a fragment peak is partially resolved from the main mAb peak. The separation between the mAb and the fragment can be further improved on a 4.6×300 mm SEC-300 column packed with 1.8 µm media.

Separation of MAb, Aggregates and Fragments (from HPLC to UHPLC)

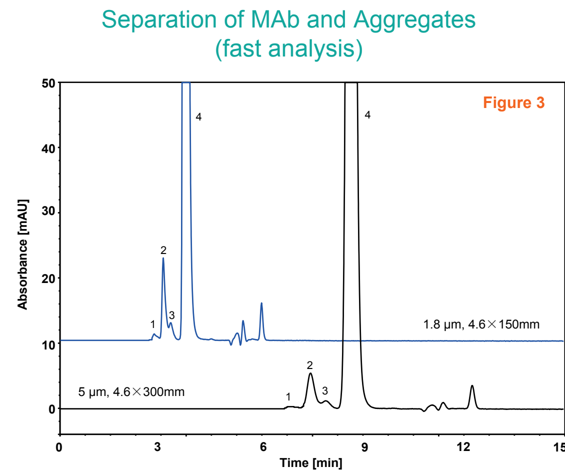



| | |
|---|---|
| Column: | |
| Black: | **BioCore SEC-300**, 5 µm |
| Blue: | **BioCore SEC-300**, 3 µm |
| Red: | **BioCore SEC-300**, 1.8 µm |
| Dimension: | 4.6×300 mm |
| Mobile Phase: | 90/10 v/v 50 mM phosphate buffer, pH6.8/MeCN |
| Flow Rate: | 0.25 mL/min |
| Temperature: | 30 ℃ |
| Injection: | 5 µL |
| Detection: | UV 280 nm |
| Sample: | Trastuzumab Biosimilar (5 mg/mL) |
| Peaks: | 1-3. Aggregates<br>4. MAb<br>5. Fragment |

| Particle Size | N(4) | Rs(1,2) | Rs(2,3) | Rs(3,4) | Rs(4,5) |
|---|---|---|---|---|---|
| 1.8 µm | 22668 | 2.21 | 1.95 | 3.83 | 2.33 |
| 3 µm | 17006 | 2.84 | 1.88 | 3.58 | 1.06 |
| 5 µm | 9616 | 2.29 | 0.94 | 1.68 | / |

Very often, fast analysis is desired for better productivity. Figure 3 compares one 1.8 μm, 4.6×150 mm BioCore SEC-300 with one 5 μm, 4.6×300 mm BioCore SEC-300 column. While the resolutions between aggregates and mAb are comparable, the total analysis time on the 1.8 μm 4.6×150 mm BioCore SEC-300 (1.8 μm is two times faster than that on the 5 μm, 4.6×300 mm column, demonstrating that 1.8 μm UHPLC SEC columns can not only provide superior resolution power, but also support fast analysis.

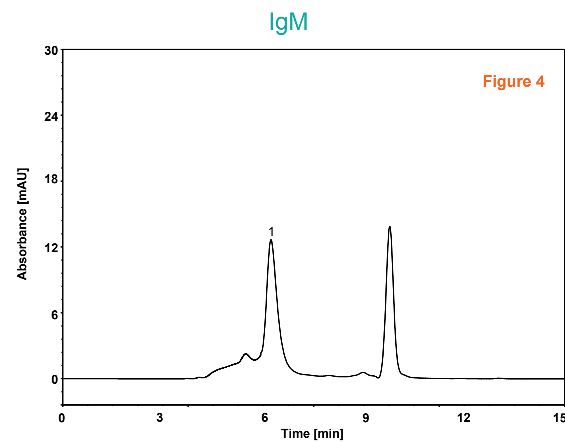
Separation of MAb and Aggregates
(fast analysis)




| | |
|---|---|
| Column: | |
| Black: | **BioCore SEC-300**, 5 μm |
| Blue: | **BioCore SEC-300**, 1.8 μm |
| Dimension: | |
| Black: | 4.6×300 mm |
| Blue: | 4.6×150 mm |
| Mobile Phase: | 300 mM NaCl in 50 mM phosphate buffer, pH6.8 |
| Flow Rate: | 0.35 mL/min |
| Temperature: | 30 ℃ |
| Injection: | 5 μL |
| Detection: | UV 280 nm |
| Sample: | Trastuzumab Biosimilar (5 mg/mL) |
| Peaks: | 1~3. Aggregates |
| | 4. MAb |

Compared with the BioCore SEC-300, BioCore SEC-500 columns employ larger pore (~500 Å) silica particles, thus are suitable for separating larger proteins. IgM is the largest immunoglobulin by size and exists mainly as pentamer in human serum. IgM polymer assembly depend on noncovalent interaction between subunit and disulfide bridging. Figure 4 shows the separation of IgM and its aggregates on a BioCore SEC-500 column.

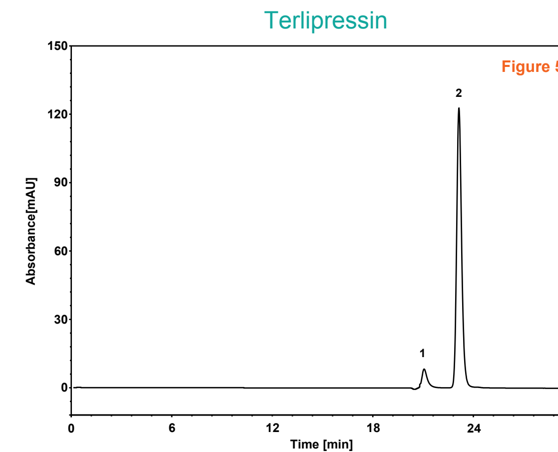
IgM




| | |
|---|---|
| Column: | **BioCore SEC-500**, 3 μm |
| Dimension: | 4.6×150 mm |
| Mobile Phase: | 10/90 v/v MeCN/300 mM NaCl in 50 mM phosphate buffer, pH6.8 |
| Flow Rate: | 0.21 mL/min |
| Temperature: | 30 ℃ |
| Injection: | 1 μL |
| Detection: | UV 280 nm |
| Sample: | IgM in Human Serum |
| Peak: | 1. IgM |

BioCore SEC-150 columns are based on 150 Å pore silica particles, designed for separating peptides and small proteins. Terlipressin is a peptide-like pro-drug to improve kidney function in adults. Terlipressin and impurity can be well separated on a BioCore SEC-150 column, as shown in Figure 5.

Terlipressin




| | |
|---|---|
| Column: | **BioCore SEC-150**, 5 μm |
| Dimension: | 7.8×300 mm |
| Mobile Phase: | 100 mM $Na_2SO_4$ in 100mM phosphate buffer |
| Flow Rate: | 0.5 mL/min |
| Temperature: | 30 ℃ |
| Injection: | 10 μL |
| Detection: | UV 274 nm |
| Peaks: | 1. Impurity U |
| | 2. Terlipressin |

| R.T. (min) | Theoretical Plate (USP) | Tailing Factor (USP) | Resolution (USP) |
|---|---|---|---|
| 23.080 | 29589 | 1.19 | 3.63 |

## Ordering Information

| Particle Size (μm) | Column Dimension L × ID (mm) | Product Name | | |
|---|---|---|---|---|
| | | BioCore SEC-150 | BioCore SEC-300 | BioCore SEC-500 |
| 5 | 300×4.6 | B213-050015-04630S | B213-050030-04630S | B213-050050-04630S |
| | 150×4.6 | B213-050015-04615S | B213-050030-04615S | B213-050050-04615S |
| | 50×4.6 | B213-050015-04605S | B213-050030-04605S | B213-050050-04605S |
| | 300×7.8 | B213-050015-07830S | B213-050030-07830S | B213-050050-07830S |
| | 150×7.8 | B213-050015-07815S | B213-050030-07815S | B213-050050-07815S |
| 3 | 300×4.6 | B213-030015-04630S | B213-030030-04630S | B213-030050-04630S |
| | 150×4.6 | B213-030015-04615S | B213-030030-04615S | B213-030050-04615S |
| | 50×4.6 | B213-030015-04605S | B213-030030-04605S | B213-030050-04605S |
| | 300×7.8 | B213-030015-07830S | B213-030030-07830S | B213-030050-07830S |
| | 150×7.8 | B213-030015-07815S | B213-030030-07815S | B213-030050-07815S |
| 1.8 | 300×4.6 | B213-018015-04630S | B213-018030-04630S | B213-018050-04630S |
| | 150×4.6 | B213-018015-04615S | B213-018030-04615S | B213-018050-04615S |

# BioCore Ion-Exchange Columns

BioCore ion-exchange columns, including BioCore WCX, BioCore SCX, BioCore WAX, BioCoreSAX, are designed for separating charged variants in antibodies, proteins, related substances, and provide a broad application range in the areas including bio-tech, biopharmaceutical and academic research.

## Main Features

- Optimal selectivity for separating charged variants in antibodies and proteins
- Good peak shape and low carryover
- High column efficiency for better resolution power
- Excellent chemical and mechanical stability
- Good column-to-column consistency

## Specification

| Product Name | BioCore WCX | BioCore SCX | BioCore WAX | BioCore SAX |
|---|---|---|---|---|
| Functional Group | Carboxylate | Sulfonate | Tertiary Amine | Quaternary Ammomiun |
| Substrate | Monodispersed, spherical, nonporous PS/DVB particles | | | |
| Particle Size | 5 & 10 µm | | | |
| Pore Size | Nonporous | | | |
| Pressure Limit | 4500 psi for 10 µm<br>5000 psi for 5 µm | | | |
| Temperature Limit | 60 °C | | | |
| pH Range | 2-12 | | | |
| Colum Dimension | 4.6x250 mm<br>4.6x150 mm<br>4.6x50 mm | | | |
| Application | Charged variants in antibodies and proteins | | | |

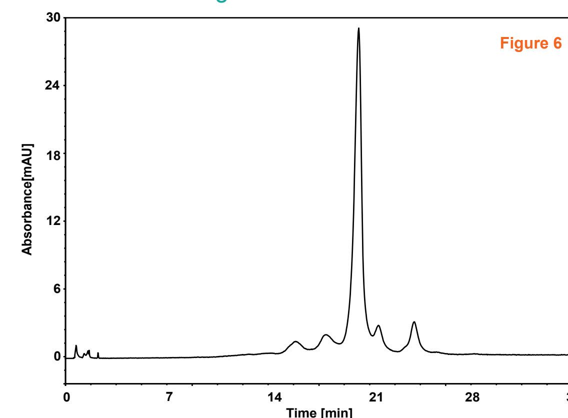
# Application

Charge variants are often considered as a critical quality attribute (CQA) in biopharmaceutical development and need to be closely monitored to ensure the safety and efficacy of biotherapeutics. Depending on the specific conditions of manufacturing or the intrinsic properties of a mAb, charge variants can contain both acidic or basic variants. Because additional charge variation may occur during process development and formulation, charge variant analysis is used across the biopharmaceutical pipeline, from discovery to manufacturing and quality control (QC). LC offers a robust, high-resolution and high-throughput analytical method, thus widely used in biopharmaceutical analysis.

IEX is a commonly used separation mode, which separates molecules based on the difference in the ionic groups on the protein surface. In IEX chromatography, elution is performed using either a salt- or pH-based gradient, which elutes the bound protein by disrupting the electrostatic interactions between the protein and the stationary phase. When traditional salt-based gradients have been employed, significant method development is required to get optimal resolution of charge variants contained in different mAb samples. pH-based gradients have gained popularity, primarily for their more generic applicability across different mAbs.

WCX is the most commonly used IEX mode for separating charged variants in high pI antibodies. Figures 6-8 illustrate three examples of charged variant separation on three mAbs (IgG1, IgG2 and IgG4) using BioCore WCX columns. Figure 9 provides a comparison of charged variant separation using salt-gradient and pH-gradient methods on a BioCore WCX column. Figure 10 shows the charged variant separation of a bispecific antibody on a BioCore WCX column. Figure 11 gives an example of charged variant separation of a pegylated Fab and its native Fab.

IgG1 on BioCore WCX

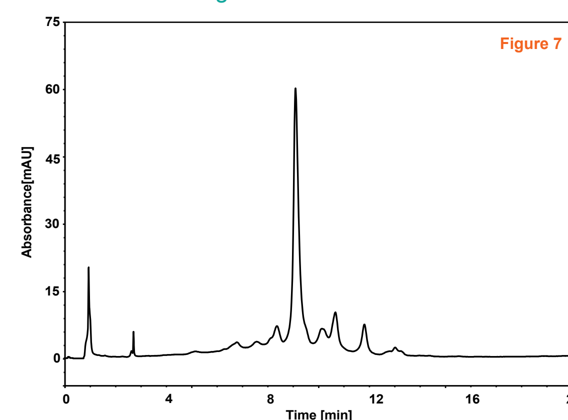



Column: **BioCore WCX**, 10 µm
Dimension: 4.6×250 mm
Mobile Phase: A) 20 mM MES, pH6.5
B) 150 mM NaCl in 20 mM MES, pH6.5
Gradient:

| t(min) | %A | %B |
|---|---|---|
| -15 | 95 | 5 |
| 0 | 95 | 5 |
| 0.1 | 95 | 5 |
| 40 | 80 | 20 |
| 40.1 | 0 | 100 |
| 43 | 0 | 100 |

Flow Rate: 1.0 mL/min
Temperature: 30 ℃
Injection: 10 µL
Detection: UV 280 nm
Sample: IgG1 (~2.5 mg/mL in mobile phase A)

IgG2 on BioCore WCX

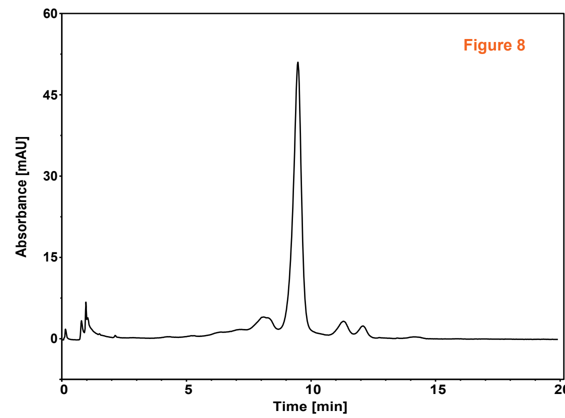



Column: **BioCore WCX**, 5 µm
Dimension: 4.6×150 mm
Mobile phase : A) 20 mM phosphate buffer, pH6.5
B) 300 mM NaCl in 20 mM phosphate buffer, pH6.5
Gradient:

| t (min) | %A | %B |
|---|---|---|
| -15 | 85 | 15 |
| 0 | 85 | 15 |
| 0.1 | 85 | 15 |
| 20 | 70 | 30 |
| 20.1 | 0 | 100 |
| 23 | 0 | 100 |

Flow rate: 1.0 mL/min
Temperature: 30 ℃
Injection: 25 µL
Detection: UV 280 nm
Sample: IgG2 (~1 mg/mL in mobile phase A)

### IgG4 on BioCore WCX



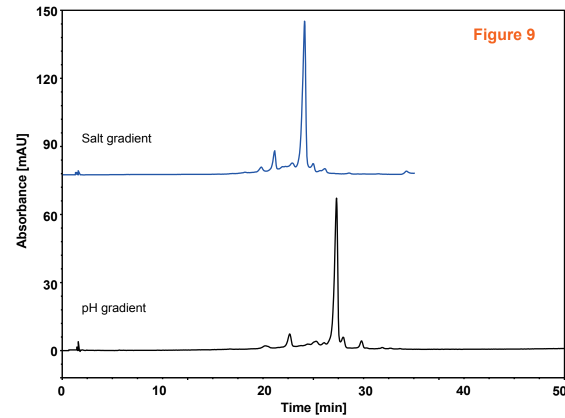

Column: **BioCore WCX**, 10 μm
Dimension: 4.6×150mm
Mobile phase: A) 20 mM phosphate buffer, pH6.5
B) 300 mM NaCl in 20 mM phosphate buffer, pH6.5

Gradient:

| t(min) | %A | %B |
|---|---|---|
| -15 | 95 | 5 |
| 0 | 95 | 5 |
| 0.1 | 95 | 5 |
| 20 | 80 | 20 |
| 20.1 | 0 | 100 |
| 23 | 0 | 100 |

Flow rate: 1 mL/min
Temperature: 30 ℃
Injection: 25 μL
Detection: UV 280 nm
Sample: IgG4 (1mg/mL in $H_2O$)

### Salt-Gradient vs pH-Gradient on BioCore WCX




Column: **BioCore WCX**, 10 μm
Dimension: 4.6×250 mm
Mobile Phase: A) 10 mM Tris, pH8.5
B) 100 mM NaCl in 10 mM Tris, pH8.5

Salt gradient:

| t (min) | %C | %D |
|---|---|---|
| -15 | 100 | 0 |
| 0 | 100 | 0 |
| 2 | 100 | 0 |
| 32 | 60 | 40 |
| 32.1 | 0 | 100 |
| 35 | 0 | 100 |

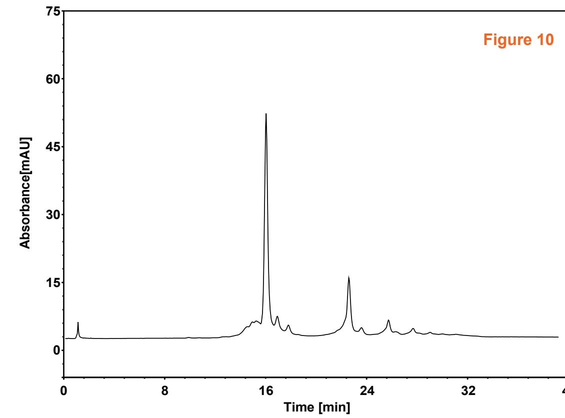
Mobile Phase: A) pH gradient buffer A, pH5.0
B) pH gradient buffer B, pH10.8

pH gradient:

| t (min) | %A | %B |
|---|---|---|
| 0 | 40 | 60 |
| 2 | 40 | 60 |
| 60 | 2 | 98 |
| 64 | 2 | 98 |
| 65 | 40 | 60 |
| 72 | 40 | 60 |

Flow Rate: 1.0 mL/min
Temperature: 30 ℃
Injection: 10 μL
Detection: UV 280 nm
Sample: Trastuzumab Biosimilar (5 mg/mL)

### Bispecific Antibody on BioCore WCX




Column: **BioCore WCX**, 10 μm
Dimension: 4.6×150 mm
Mobile Phase: A) 20 mM phosphate buffer, pH6.5
B) 300 mM NaCl in 20 mM phosphate buffer, pH6.5

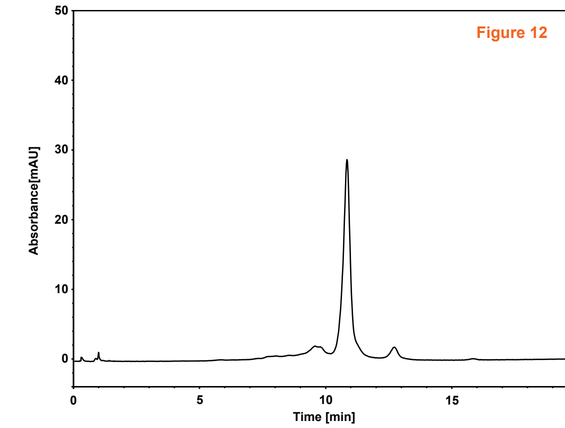
Gradient:

| t (min) | %A | %B |
|---|---|---|
| 0 | 100 | 0 |
| 40 | 65 | 35 |
| 40.1 | 0 | 100 |
| 45 | 0 | 100 |
| 45.1 | 100 | 0 |
| 60 | 100 | 0 |

Flow Rate: 1.0 mL/min
Temperature: 30 ℃
Injection: 20 μL
Detection: UV 280 nm
Sample: Bispecific Antibody (~5.0 mg/mL in mobile phase A)

The BioCore SCX offers selectivity different from the BioCore WCX, thus can complement BioCore WCX for separating mAb charged variants (Figure 12). In addition, The charged variants of a fusion protein (Figure 13) can be resolved well on a BioCore SCX column.

### IgG4 on BioCore SCX



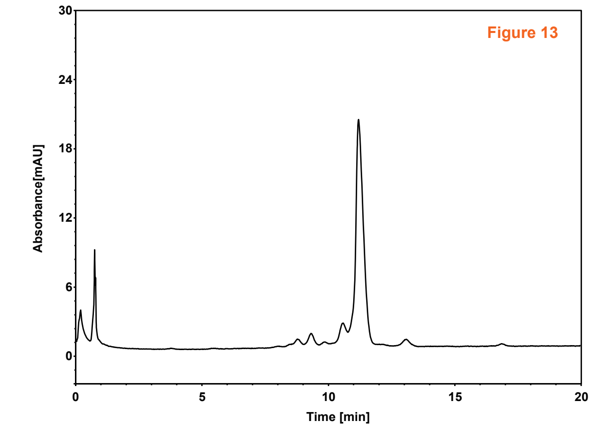

Column: **BioCore SCX**, 10 μm
Dimension: 4.6×150 mm
Mobile Phase: A) 20 mM MES, pH6.5
B) 300 mM NaCl in 20 mM MES, pH6.5

Gradient:

| t(min) | %A | %B |
|---|---|---|
| -15 | 93 | 7 |
| 0 | 93 | 7 |
| 20 | 79 | 21 |
| 20.1 | 0 | 100 |
| 23 | 0 | 100 |

Flow Rate: 1.0 mL/min
Temperature: 30 ℃
Injection: 10 μL
Detection: UV 280 nm
Sample: IgG4 (1.0 mg/mL in $H_2O$)

### Fusion Protein on BioCore SCX




Column: **BioCore SCX**, 10 μm
Dimension: 4.6×150 mm
Mobile Phase: A) 20 mM MES, pH6.0
B) 300 mM NaCl in 20 mM MES, pH6.0

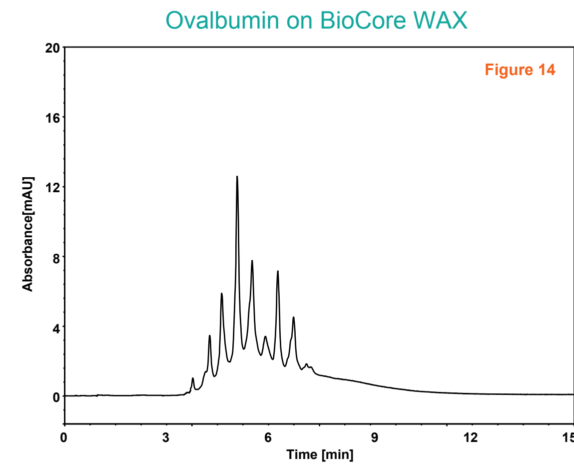
Gradient:

| t(min) | %A | %B |
|---|---|---|
| -15 | 72 | 28 |
| 0 | 72 | 28 |
| 25 | 45 | 55 |
| 25.1 | 0 | 100 |
| 28 | 0 | 100 |

Flow Rate: 1.0 mL/min
Temperature: 30 ℃
Injection: 10 μL
Detection: UV 280 nm
Sample: Fusion Protein (2.5 mg/mL in mobile phase A)

BioCore anion-exchange columns are often employed to separate charged variants in low pI proteins, such as ovalbumin using BioCore WAX, (Figure 14) as well as a glycoprotein (Figure 15), a recombinant fusion protein (Figure 16), and a hGH-L-vFc protein (Figure 17) using the BioCore SAX.

### Ovalbumin on BioCore WAX

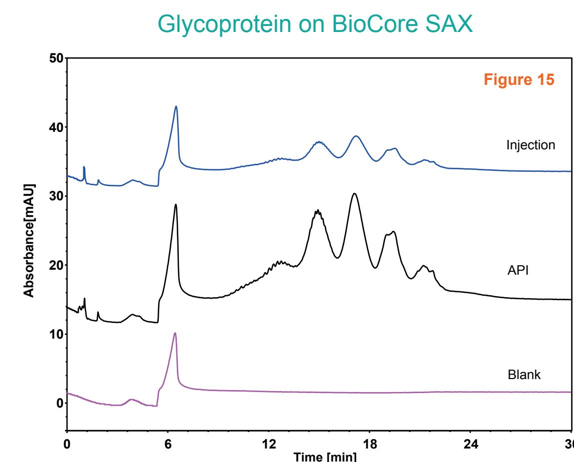



Column: **BioCore WAX**, 5 µm
Dimension: 4.6×150 mm
Mobile Phase: A) 20 mM Tris, pH8.0
B) 500mM NaCl in 20 mM Tris, pH8.0
Gradient:

| t(min) | %A | %B |
|---|---|---|
| 0 | 1 | 0 |
| 0.1 | 1 | 0 |
| 15 | 50 | 50 |
| 15.1 | 99 | 100 |
| 20 | 99 | 100 |
| 20.1 | 1 | 0 |
| 30 | 1 | 0 |

Flow Rate: 1.0 mL/min
Temperature: 30 ℃
Injection: 10 µL
Detection: UV 280 nm
Sample: Ovalbumin (5 mg/mL)

### Glycoprotein on BioCore SAX

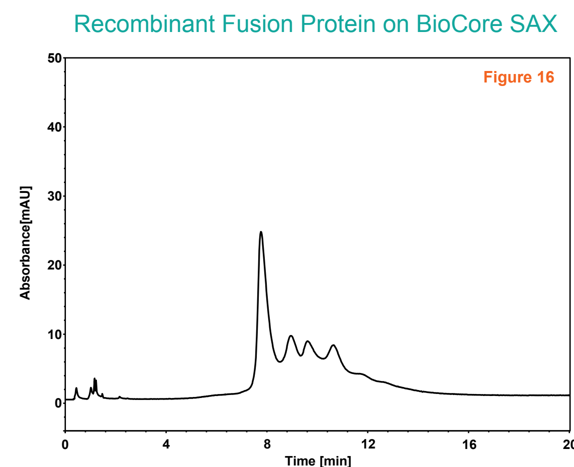



Column: **BioCore SAX**, 10 µm
Dimension: 4.6×250 mm
Mobile Phase: A) 20 mM phosphate buffer, pH3.0
B) 300 mM NaCl in 20 mM phosphate buffer, pH3.0
Gradient:

| t(min) | %A | %B |
|---|---|---|
| -15 | 100 | 0 |
| 0 | 100 | 0 |
| 20 | 0 | 100 |
| 23 | 0 | 100 |

Flow Rate: 1.0 mL/min
Injection: 5 µL
Temperature: 30 ℃
Detection: UV 280 nm
Sample: API (40 mg/mL)
Injection (10 mg/mL)

### Recombinant Fusion Protein on BioCore SAX

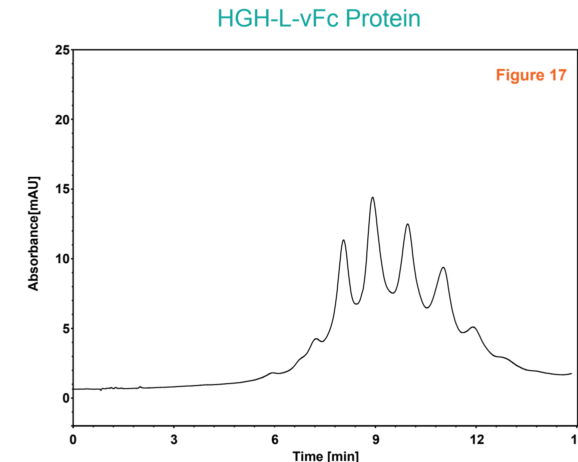



Column: **BioCore SAX**, 10 µm
Dimension: 4.6×150 mm
Mobile Phase: A) 20 mM MES, pH6.5
B) 300 mM NaCl in 20 mM MES, pH6.5
Gradient:

| t(min) | %A | %B |
|---|---|---|
| -15 | 70 | 30 |
| 0 | 70 | 30 |
| 20 | 40 | 60 |
| 20.1 | 0 | 100 |
| 23 | 0 | 100 |

Flow Rate: 0.8 mL/min
Injection: 10 µL
Temperature: 20 ℃
Detection: UV 280 nm
Sample: Recombinant Fusion Protein (1 mg/mL in $H_2O$)

### HGH-L-vFc Protein




Column: **BioCore SAX**, 10 µm
Dimension: 4.6×150 mm
Mobile Phase: A) 20 mM phosphate buffer, pH6.5
B) 300 mM NaCl in 20 mM phosphate buffer, pH6.5
Gradient:

| t (min) | %A | %B |
|---|---|---|
| 0 | 80 | 20 |
| 20 | 50 | 50 |
| 20.1 | 0 | 100 |
| 25 | 0 | 100 |
| 25.1 | 80 | 20 |
| 35 | 80 | 20 |

Flow Rate: 1.0 mL/min
Temperature: 30 ℃
Injection: 5 µL
Detection: UV 220 nm
Sample: HGH-L-vFc Protein (pI=5.9-6.2, 8 mg/mL)

## Ordering Information

| Particle Size (µm) | Column Dimension L × ID (mm) | Product Name | | | |
|---|---|---|---|---|---|
| | | BioCore WCX | BioCore SCX | BioCore WAX | BioCore SAX |
| 10 | 250×4.6 | B311-100000-04625P | B411-100000-04625P | B511-100000-04625P | B611-100000-04625P |
| | 150×4.6 | B311-100000-04615P | B411-100000-04615P | B511-100000-04615P | B611-100000-04615P |
| | 50×4.6 | B311-100000-04605P | B411-100000-04605P | B511-100000-04605P | B611-100000-04605P |
| 5 | 250×4.6 | B311-050000-04625P | B411-050000-04625P | B511-050000-04625P | B611-050000-04625P |
| | 150×4.6 | B311-050000-04615P | B411-050000-04615P | B511-050000-04615P | B611-050000-04615P |
| | 50×4.6 | B311-050000-04605P | B411-050000-04605P | B511-050000-04605P | B611-050000-04605P |

# BioCore HIC-Butyl

BioCore HIC-Butyl is a family of high-performance, hydrophobic interaction chromatography columns that separate monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs) based on their differences in surface hydrophobicity.

## Main Features

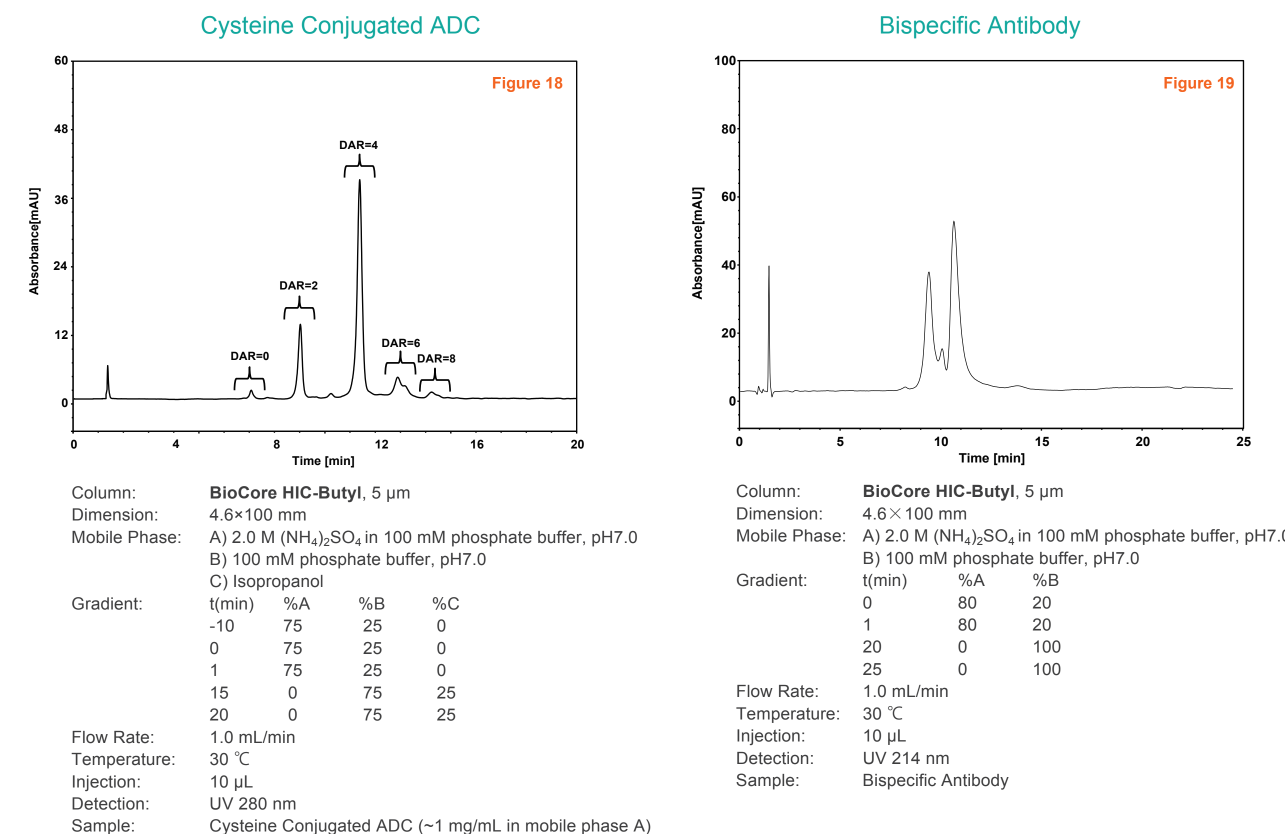
- Optimal selectivity for the DAR analysis in ADCs
- Minimal undesired interactions for low carryover
- Excellent mechanical strength for column robustness
- Good column-to-column consistency

## Specification

| Product Name | BioCore HIC-Butyl |
|---|---|
| Functional Group | Butyl |
| Substrate | Monodispersed, wide-pore silica particles |
| Particle Size | 5 µm |
| Pore Size | 1000 Å |
| Pressure Limit | 6000 psi |
| Temperature Limit | 60 ℃ |
| pH Range | 2-8 |
| Colum Dimension | 4.6x250 mm<br>4.6x100 mm<br>4.6x50 mm |
| Application | DAR analysis in ADCs, oxidation variants in mAbs |

# Application

Hydrophobic interaction chromatography (HIC) is a traditional technique used for the separation, purification, and characterization of proteins. As the number of antibody-drug conjugates (ADCs) continues to increase in development of bio-therapeutics, HIC and RP methods, both utilizing changes in hydrophobicity for separation, are often used for ADC characterization and analysis. Unlike RP technique, HIC uniquely allows for protein analysis under mild nondenaturing conditions that preserve the native structure and activity of the molecules, thus analysis of the ADC in its native form is advantageous. Figure 18 presents a HIC method for the drug antibody ratio (DAR) analysis of a cysteine-conjugated antibody using an ammonium sulfate buffer system on a BioCore HIC-Butyl column. Figure 19 demonstrates the suitability of the BioCore HIC-Butyl for separating variants in a bispecific antibody.




## Ordering Information

| Product Name | Particle Size (µm) | Column Dimension L × ID (mm) | Part Number |
|---|---|---|---|
| BioCore HIC-Butyl | 5 | 250×4.6 | B713-050100-04625S |
| | | 100×4.6 | B713-050100-04610S |
| | | 50×4.6 | B713-050100-04605S |

# BioCore RP-Butyl

BioCore RP-Butyl is a family of high-performance, reversed-phase columns, designed for the separation and determination of exact mass of antibodies / proteins and related substances.

## Main Features

- High column efficiency and low carryover
- Excellent mechanical strength
- Good MS compatibility
- Good column-to-column consistency

## Specification

| Product Name | BioCore RP-Butyl |
|---|---|
| Functional Group | Butyl |
| Substrate | Monodispersed, spherical PS/DVB particles |
| Particle Size | 5 μm |
| Pore Size | Nonporous |
| Pressure Limit | 4500 psi |
| Temperature Limit | 100 ℃ |
| pH Range | 2-12 |
| Colum Dimension | 3.0×100 mm<br>3.0×50 mm |
| Application | Intact protein and protein fragment analysis |

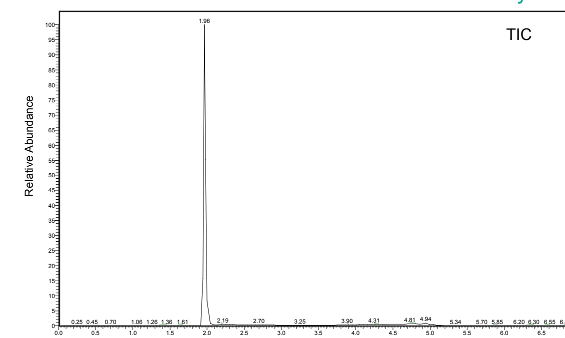
## Ordering Information

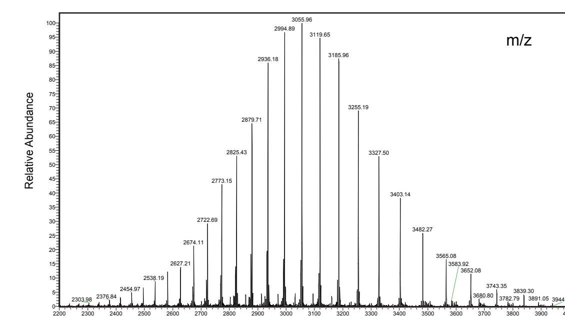
| Product Name | Particle Size (μm) | Column Dimension L × ID (mm) | Part Number |
|---|---|---|---|
| BioCore RP-Butyl | 5 | 150×4.6 | B821-050000-04615S |
| | | 100×4.6 | B821-050000-04610S |
| | | 50×4.6 | B821-050000-04605S |
| | | 150×3.0 | B821-050000-03015S |
| | | 100×3.0 | B821-050000-03010S |
| | | 50×3.0 | B821-050000-03005S |
| | | 150×2.1 | B821-050000-02115S |
| | | 100×2.1 | B821-050000-02110S |
| | | 50×2.1 | B821-050000-02105S |

# Application

Reversed-phase HPLC is one of most important techniques for protein separations and the method of choice for peptide separation. In protein characterization, RP-HPLC is commonly used for separating variants in proteins or antibodies by their difference in hydrophobicity. When combined with high resolution mass spectrometry (MS), RP-HPLC can be utilized for determination of the exact mass for the whole proteins or their subunits (e.g., heavy chain, light chain, Fab, Fc). Figures 20 and 21 provide examples of exact mass determination by LC-MS using a BioCore RP-Butyl column.

### Exact Mass of a Monoclonal Antibody



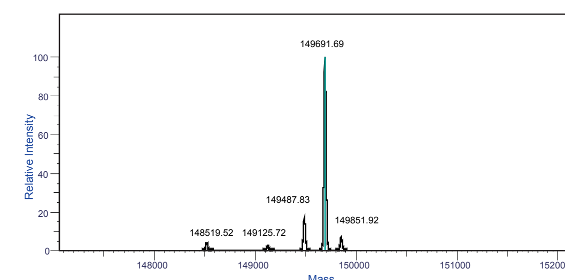




Figure 20

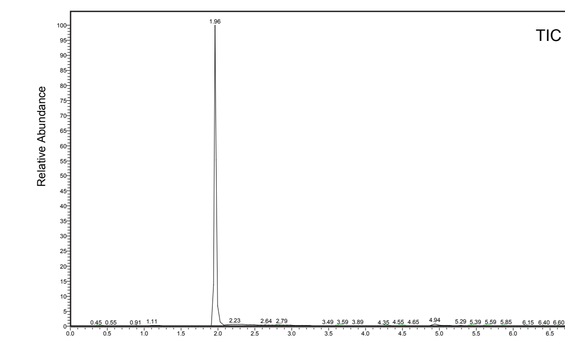
Column: **BioCore RP-Butyl**, 5 μm
Dimension: 3.0×50 mm
Mobile Phase: A) 0.1% HCOOH in $H_2O$
B) 0.1% HCOOH in MeCN

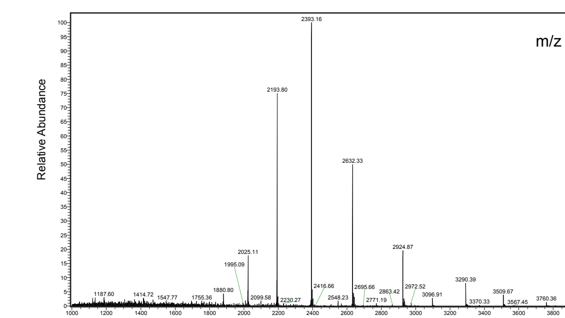
Gradient:

| t(min) | %A | %B |
|---|---|---|
| 0 | 95 | 5 |
| 1 | 95 | 5 |
| 1.1 | 95 | 5 |
| 1.2 | 5 | 95 |
| 4 | 5 | 95 |
| 4.1 | 95 | 5 |
| 7 | 95 | 5 |

Flow Rate: 0.6 mL/min
Temperature: 60 ℃
Injection: ~1 μg
Detection: QE
Sample: Monoclonal Antibody

### Exact Mass of a Recombinant Protein



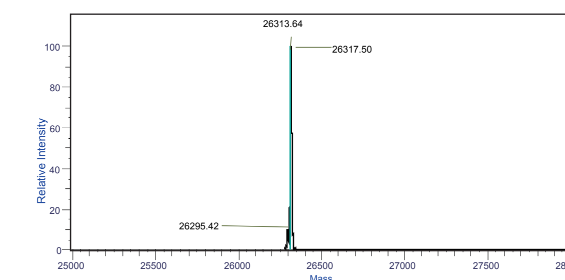




Figure 21

Column: **BioCore RP-Butyl**, 5 μm
Dimension: 3.0×50 mm
Mobile Phase: A) 0.1% HCOOH in $H_2O$
B) 0.1% HCOOH in MeCN

Gradient:

| t(min) | %A | %B |
|---|---|---|
| 0 | 95 | 5 |
| 1 | 95 | 5 |
| 1.1 | 95 | 5 |
| 1.2 | 5 | 95 |
| 4 | 5 | 95 |
| 4.1 | 95 | 5 |
| 7 | 95 | 5 |

Flow Rate: 0.6 mL/min
Temperature: 60 ℃
Injection: ~1 μg
Detection: QE
Sample: Recombinant Protein

# BioCore Glycan

BioCore Glycan is a family of high-performance HILIC columns designed for profiling N-glycans present in proteins/antibodies and related substances.

## Main Features

- Desired selectivity for separating fluorescently labeled N-glycans in proteins
- High resolution and stability
- Good MS compatibility
- Good column-to-column consistency

## Specification

| Product Name | BioCore Glycan |
| --- | --- |
| Functional Group | Polyamide |
| Substrate | Monodispersed, spherical silica particles |
| Particle Size | 3 µm |
| Pore Size | 180 Å |
| Pressure Limit | 6000 psi |
| Temperature Limit | 80 °C |
| pH Range | 2-9 |
| Colum Dimension | 2.1×150 mm<br>2.1×100 mm<br>3.0×150 mm<br>3.0×100 mm |
| Application | N-glycans analysis in antibodies and proteins |

## Ordering Information

| Product Name | Particle Size (µm) | Column Dimension L x ID (mm) | Part Number |
| --- | --- | --- | --- |
| BioCore Glycan | 3 | 150×2.1 | B913-030018-02115S |
| | | 100×2.1 | B913-030018-02110S |
| | | 150×3.0 | B913-030018-03015S |
| | | 100×3.0 | B913-030018-03010S |
| | | 150×4.6 | B913-030018-04615S |
| | | 100×4.6 | B913-030018-04610S |

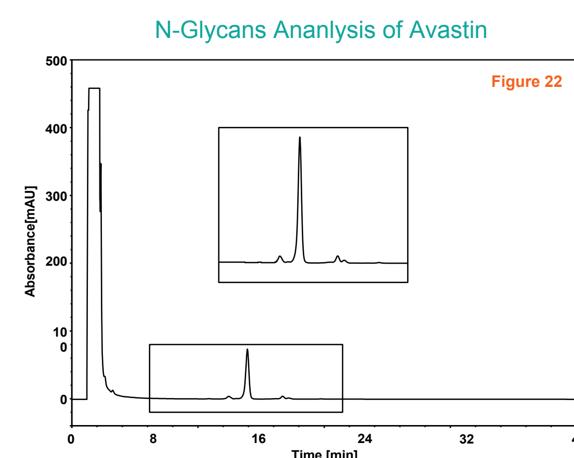
# Application

Biopharmaceuticals are highly complex molecules with remarkable heterogeneity. Protein glycosylation is an inherent source of this heterogeneity, which affects the safety, efficacy, and half-life of therapeutic glycoproteins. Therefore, analysis of the glycan pattern is an important issue for characterization and quality control in the biopharmaceutical industry.

For the analysis of protein N-glycans, the sample-preparation procedure consists of the release of the N-glycans by PNGase-F, followed by fluorescence labeling and removal of excess label. Subsequently, labeled glycans are usually analyzed by hydrophilic-interaction liquid chromatography (HILIC) with a fluorescence detector.

Figures 22 and 23 demonstrate the N-glycan profiling with 2-AB labeling of Avastin and a recombinant protein obtained from a BioCore Glycan column.

N-Glycans Ananlysis of Avastin

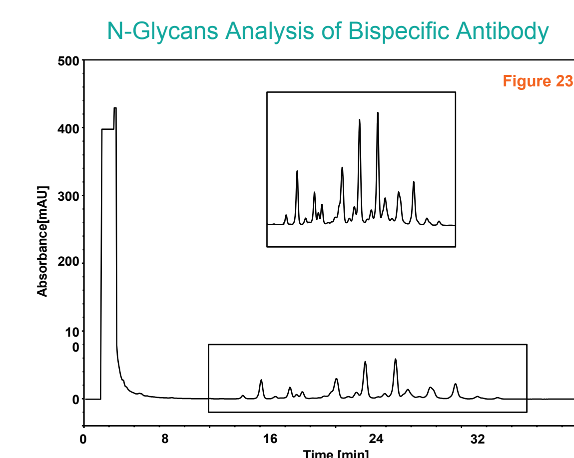



Column: **BioCore Glycan**, 3 µm
Dimension: 3.0×100 mm
Mobile Phase: A) 50 mM $CH_3COONH_4$ in $H_2O$, pH4.4
B) MeCN
Gradient:

| t (min) | %A | %B |
| --- | --- | --- |
| 0 | 25 | 75 |
| 2 | 25 | 75 |
| 37 | 46 | 54 |
| 37.1 | 25 | 75 |
| 40 | 25 | 75 |

Flow Rate: 0.4 mL/min
Temperature: 65 ℃
Injection: 30 µL
Detection: Ex/Em= 250 nm/428 nm
Sample: N-Glycans of Avastin

N-Glycans Analysis of Bispecific Antibody




Column: **BioCore Glycan**, 3 µm
Dimension: 3.0×100 mm
Mobile Phase: A) 50 mM $CH_3COONH_4$ in $H_2O$, pH4.4
B) MeCN
Gradient:

| t (min) | %A | %B |
| --- | --- | --- |
| 0 | 25 | 75 |
| 2 | 25 | 75 |
| 37 | 46 | 54 |
| 37.1 | 25 | 75 |
| 40 | 25 | 75 |

Flow Rate: 0.4 mL/min
Temperature: 65 ℃
Injection: 30 µL
Detection: Ex/Em= 250 nm/428 nm
Sample: N-Glycans of a Recombinant Protein

# BioCore Protein A

BioCore Protein A is a family of high-performance affinity chromatography columns, designed for fast titer analysis of monoclonal antibodies (mAb) and Fc fusion proteins.

## Main Features

- High specificity for efficient capture of a broad selection of antibodies and antibody fragments
- High dynamic binding capacity for a wide linear range
- High mechanical strength for faster analysis and better column lifetime
- Low ligand leakage for higher purity

## Specification

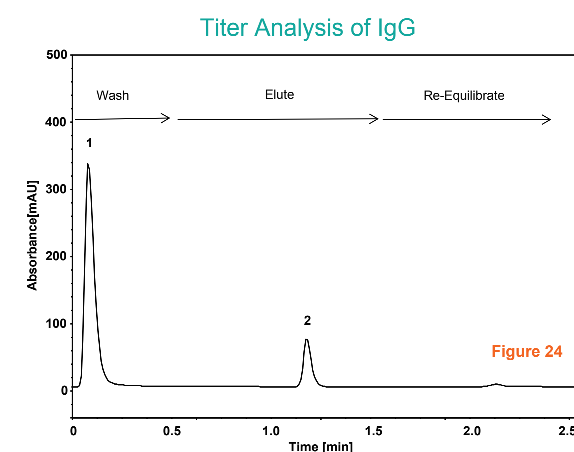
| Product Name | BioCore Protein A |
|---|---|
| Functional Group | pH stable rProtein A |
| Substrate | Monodispersed, porous, spherical PS/DVB particles |
| Particle Size | 15 µm |
| Pore Size | 1000 Å |
| Pressure Limit | 1450 psi |
| Temperature Limit | 2-40 ℃ |
| pH Range | 2-12 |
| Dynamic Binding Capacity | ≥20 mg/mL (IgG) |
| Linear Range (≥0.99) | 0-200 µg (2.1X30 mm) |



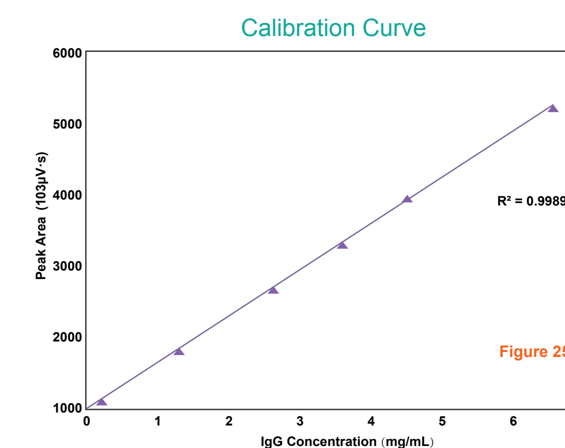
# Application

In early development of recombinant monoclonal antibodies (mAbs) for biotherapeutics, a large number of harvest cell culture (HCC) samples need to be screened for immunoglobulin G (IgG) titer. Affinity chromatography employing protein A ligand is often used for the mAb concentration determination, as well as for downstream purification.

Titer analysis is required for accurate determination of amount of mAbs in the sample. Figure 24 shows a 20 µL injection of an antibody sample onto the BioCore Protein A column. The unbound material elutes first (large peak) at pH7.0. Then, the antibody is released using a low pH wash (pH 2.5). The BioCore Protein A column gives a sharp peak for the mAb with fast, efficient elution. The complete cycle time, including equilibration, is 2.5 minutes. The mAb titer is determined by back calculating the integrated IgG peak area against a previously generated calibration curve (see Figure 25). The BioCore Protein A provides accurate titer analysis with linearity over a wide concentration range (0.2 mg/mL to 6.25 mg/mL).



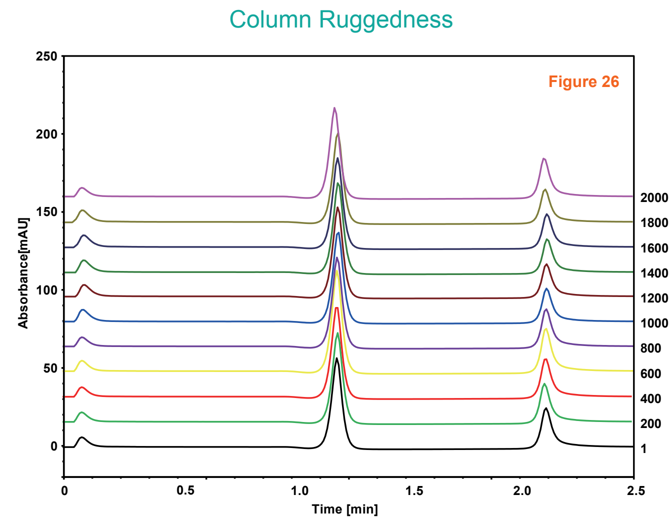

| | | | |
|---|---|---|---|
| Column: | **BioCore Protein A**, 15 µm | | |
| Dimension: | 2.1×30 mm | | |
| Mobile Phase : | 150 mM NaCl in 50 mM phosphate buffer, pH7.0<br>150 mM NaCl in 50 mM phosphate buffer, pH2.5 | | |
| Gradient: | t(min) | %A | %B |
| | 0 | 100 | 0 |
| | 0.5 | 100 | 0 |
| | 0.51 | 0 | 100 |
| | 1.5 | 0 | 100 |
| | 1.51 | 100 | 0 |
| | 2.5 | 100 | 0 |
| Flow Rate: | 2.0 mL/min | | |
| Temperature: | 30 ℃ | | |
| Injection: | 20 µL | | |
| Detection: | UV 280 nm | | |
| Sample: | Harvest Cell Culture (IgG~2.4 mg/mL) | | |
| Peaks: | 1. Unbound Components<br>2. IgG | | |




| | | | |
|---|---|---|---|
| Column: | **BioCore Protein A**, 15 µm | | |
| Dimension: | 2.1×30 mm | | |
| Mobile Phase: | A) 150 mM NaCl in 50 mM phosphate buffer, pH 7.0<br>B) 150 mM NaCl in 50 mM phosphate buffer, pH 2.5 | | |
| Gradient: | t (min) | %A | %B |
| | 0 | 100 | 0 |
| | 0.5 | 100 | 0 |
| | 0.51 | 0 | 100 |
| | 1.5 | 0 | 100 |
| | 1.51 | 100 | 0 |
| | 2.5 | 100 | 0 |
| Flow Rate: | 2.0 mL/min | | |
| Temperature: | 30 ℃ | | |
| Injection: | 20 µL | | |
| Detection: | UV 280 nm | | |
| Sample: | IgG (0.2-6.25 mg/mL in $H_2O$) | | |

The BioCore Protein A column illustrates satisfactory ruggedness when tested continuously for 2,000 cycles and every hundred cycles a set of calibration standards (from 0.2 mg/mL to 6.25 mg/mL) are analyzed. As shown in Figure 26, the retention time, peak area, and peak width of IgG remain virtually unchanged.

Column Ruggedness




Column: **BioCore Protein A**, 15 µm
Dimension: 2.1×30 mm
Mobile Phase : 150 mM NaCl in 50 mM phosphate buffer, pH7.0
150 mM NaCl in 50 mM phosphate buffer, pH2.5
Gradient:

| t(min) | %A | %B |
|---|---|---|
| 0 | 100 | 0 |
| 0.5 | 100 | 0 |
| 0.51 | 0 | 100 |
| 1.5 | 0 | 100 |
| 1.51 | 100 | 0 |
| 2.5 | 100 | 0 |

Flow Rate: 2.0 mL/min
Temperature: 30 ℃
Injection: 20 µL
Detection: UV 280 nm
Sample: IgG (1.0 mg/mL in $H_2O$)



## Ordering Information

| Product Name | Particle Size (µm) | Column Dimension L x ID (mm) | Part Number |
|---|---|---|---|
| BioCore Protein A | 15 | 100×2.1 | B111-150100-02110S |
| | | 50×2.1 | B111-150100-02105S |
| | | 30×2.1 | B111-150100-02103S |
| | | 100×4.6 | B111-150100-04610S |
| | | 50×4.6 | B111-150100-04605S |
| | | 30×4.6 | B111-150100-04603S |