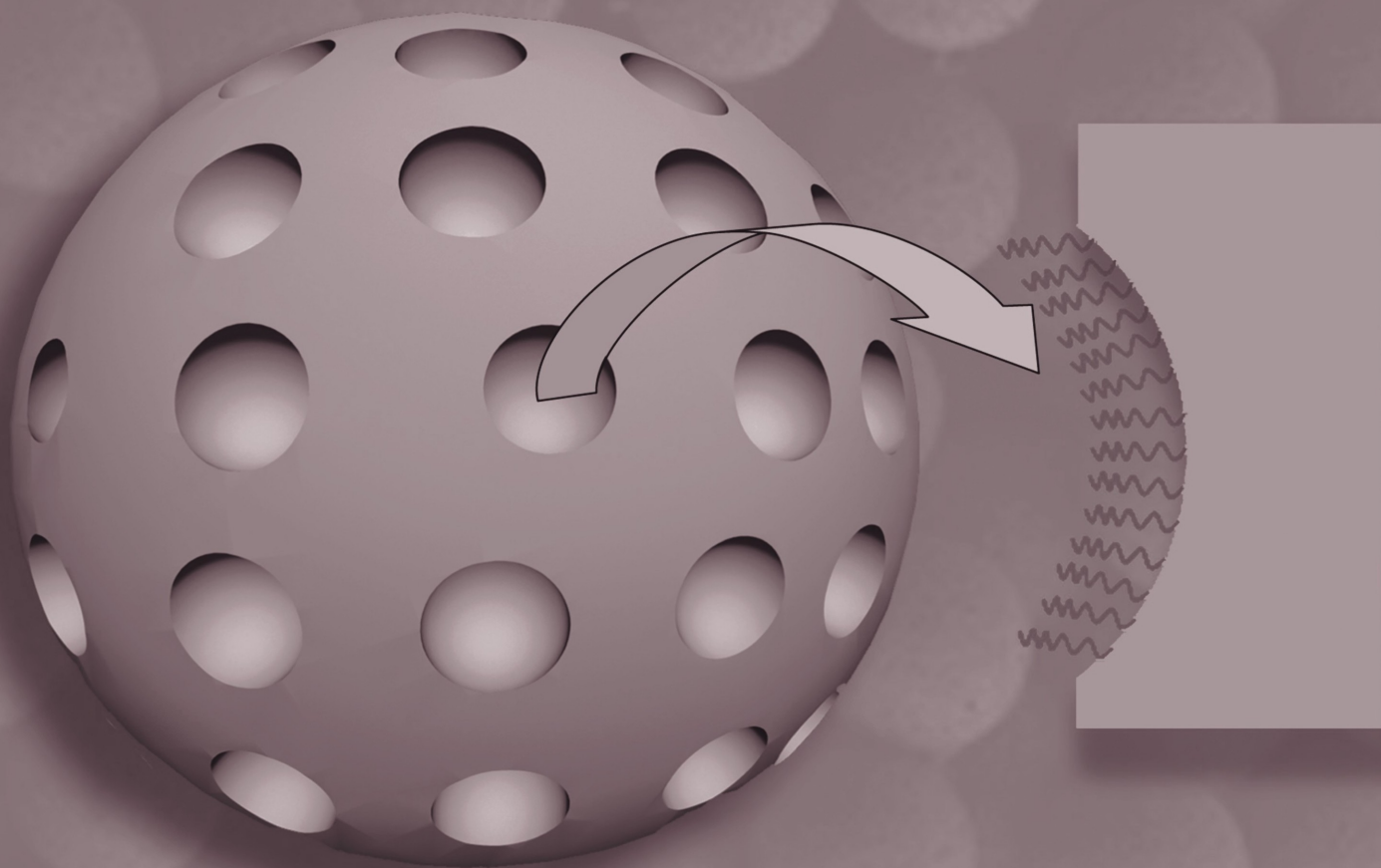


# Size Exclusion Chromatography



*Sepax Technologies*

## Zenix™



**Better Surface Chemistry for Better Separation**

# Sepax Technologies, Inc.

Sepax Technologies, Inc. develops and manufactures products in the area of chemical and biological separations, biosurfaces and proteomics. Sepax product portfolio includes 1) liquid chromatography columns and media, 2) SPE and Flash chromatography columns and tubes, 3) bulk resin for preparative separation and process chromatography, and 4) natural product and Chinese traditional medicine separation and purification.



## *A leader in Biological Separations*

Sepax develops and manufactures wide range of biological separation products using both silica and polymeric resins as the support. The selection of particle size is from 1  $\mu\text{m}$  to 100  $\mu\text{m}$  and pore size from non-porous to 2000  $\text{\AA}$ . Unique and proprietary resin synthesis and surface technologies have been developed for solving the separation challenges in biological area.



## *Bioseparation Products*

### Size Exclusion

SRT<sup>®</sup>

SRT<sup>®</sup>-C

Nanofilm<sup>®</sup>

Zenix<sup>™</sup>

Zenix<sup>™</sup>-C

### Ion-exchange

Proteomix<sup>®</sup>

### Antibody Separation

Antibodix<sup>™</sup>

### Carbohydrate Separation

Carbomix<sup>®</sup>

## Analytical, Semi-prep and Preparative

# Zenix™ SEC Phases

## Highest Efficiency and Resolution Size Exclusion Separation

### General Description

Utilizing proprietary surface technologies, Zenix SEC phases are made of uniform, hydrophilic, and neutral nanometer thick films chemically bonded on high purity and mechanically stabilized silica with the particle size of 3  $\mu\text{m}$ . 3  $\mu\text{m}$  Zenix SEC packings combined with large pore volume achieved highest separation efficiency and resolution. The well-controlled surface chemistry results in excellent lot-to-lot reproducibility. Our unique bonding chemistry, coupled with the maximized bonding density, allows Zenix SEC to provide high stability and negligible non-specific interactions. The available pore sizes of Zenix packings are 100, 150 and 300 $\text{\AA}$ . Typical applications for Zenix SEC columns include separation and analysis of biological molecules and water soluble polymers in aqueous buffers.

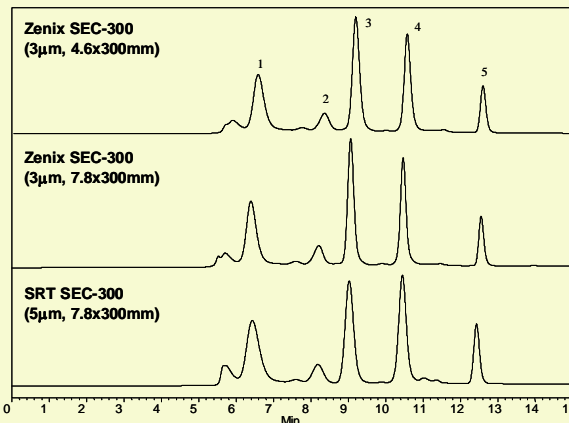
### Featured Characteristics

- Particle size of 3  $\mu\text{m}$
- Selection of pore size: 100, 150 and 300  $\text{\AA}$
- Highest separation efficiency and resolution
- High capacity
- High stability over low and high concentration salt
- Lot-to-lot reproducibility
- High protein recovery with intact biological activity
- Negligible non-specific interactions
- Ideal for separation and analysis of biological molecules: proteins, nucleic acids, oligonucleotides, peptides and virus
- Ideal for separation and analysis of natural polymers, e.g. polysaccharides, synthetic polymers, and nanomaterials, e.g. nanoparticles

### High Separation Efficiency

The advantages of developing small particle size are higher efficiency and higher resolution. When particle size is decreased to 3  $\mu\text{m}$  from 5  $\mu\text{m}$ , the column efficiency is almost doubled. As shown in Fig. 1 and Table 1, the plate numbers of BSA dimer, BSA, ribonuclease A increased from 2720 to 4600, 6590 to 13090, 11160 to 22000 when the particle size decreased from 5  $\mu\text{m}$  to 3  $\mu\text{m}$ . Fig. 2 and Fig. 3 further show that high efficiency has been achieved by 3  $\mu\text{m}$  Zenix columns with various proteins. The efficiency of p-aminobenzoic acid reached to the plate number of 40,000 for 30 cm long Zenix column.

Figure 1. Separation of protein mixture A by Zenix SEC-300 and SRT SEC-300 columns.

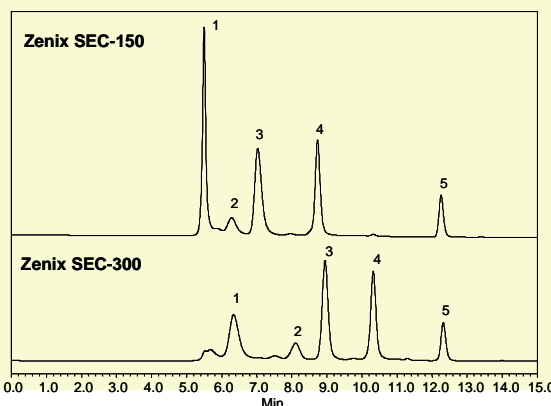


Column: Zenix SEC-300 and SRT SEC-300  
Mobile phase: 150 mM Sodium Phosphate, pH 7  
Flow rate: 1.0 mL/min for 7.8x300 mm  
0.35 mL/min for 4.6x300 mm  
Temperature: Ambient (~23° C)  
Detection: UV 214nm  
Injection: 10  $\mu\text{L}$  (3  $\mu\text{L}$  for 4.6x300 mm)  
Sample: 1) Thyroglobulin (1.0 mg/mL), 670 kD; 2) BSA dimer, 132 kD; 3) BSA (1.0 mg/mL), 66 kD; 4) Ribonuclease A (1.0 mg/mL), 13.7 kD, and 5) Uracil (2.5  $\mu\text{g/mL}$ ), 120D.

Table 1. Efficiency of Zenix SEC-300 and SRT SEC-300 columns

Peak	Protein	Zenix 300 (4.6x300)	Zenix 300 (7.8x300)	SRT 300 (7.8x300)
1	Thyroglobulin	2180	1730	1120
2	BSA Dimer	4390	4600	2720
3	BSA	10280	13090	6590
4	Ribonuclease A	16490	22000	11160
5	Uracil	33640	38500	27860

Figure 2. Separation of protein mixture A by Zenix SEC-150 and 300 columns with 7.8 mm ID.

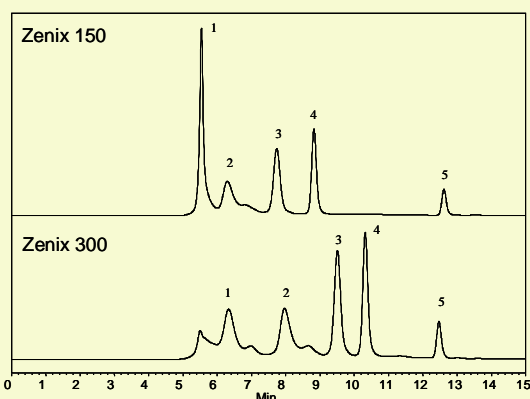


Columns: Zenix SEC (7.8x300 mm, 3  $\mu$ m)  
 Mobile phase: 150 mM Sodium Phosphate, pH 7  
 Flow rate: 1.0 mL/min  
 Backpressure: 1,375 psi for Zenix 300  
 1,100 psi for Zenix 150  
 Temperature: Ambient (~23° C)  
 Detection: UV 214nm  
 Injection: 10  $\mu$ L  
 Sample: 1) Thyroglobulin (1.0 mg/mL), 670 kD; 2) BSA dimer, 132 kD; 3) BSA (1.0 mg/mL), 66 kD; 4) Ribonuclease A (1.0 mg/mL), 13.7 kD, and 5) Uracil (2.5  $\mu$ g/mL), 120D.

Table 2. Efficiency of 7.8x300 mm Zenix SEC-150 and 300 columns

Peak	Compound	Zenix 150	Zenix 300
1	Thyroglobulin	18050	2460
2	BSA Dimer	2420	5110
3	BSA	5620	13090
4	Ribonuclease A	17600	22000
5	Uracil	42000	38500

Figure 3. Separation of protein mixture B by Zenix SEC-150 and 300 columns with 7.8 mm ID.



Columns: Zenix SEC (7.8x300 mm, 3  $\mu$ m)  
 Mobile phase: 150 mM Sodium Phosphate, pH 7  
 Flow rate: 1.0 mL/min  
 Backpressure: 1,375 psi for Zenix 300  
 1,100 psi for Zenix 150  
 Temperature: Ambient (~23° C)  
 Detection: UV 214nm  
 Injection: 10  $\mu$ L  
 Sample: 1) Thyroglobulin, 670 kD; 2)  $\gamma$ -Globulin, 158 kD; 3) Ovalbumin, 44 kD; 4) Ribonuclease A, 13.7 kD; 5) p-Aminobenzoic acid, 137 D.

Table 3. Efficiency of 7.8x300 mm Zenix SEC-150 and 300 columns

Peak	Protein	Zenix 150	Zenix 300
1	Thyroglobulin	12850	1450
2	$\gamma$ -Globulin	2860	3650
3	Ovalbumin	6780	11760
4	Ribonuclease A	17730	21690
5	p-Aminobenzoic acid	41900	39400

## High Stability

The proprietary stationary phases of Zenix SEC packings utilize densely bonded chemistry on the silica surface, which greatly hinders the diffusion of the molecules that would attack the bond of silica-stationary phase layer, thus enabling high stability over a wide range of pH from 2 to 8.5.

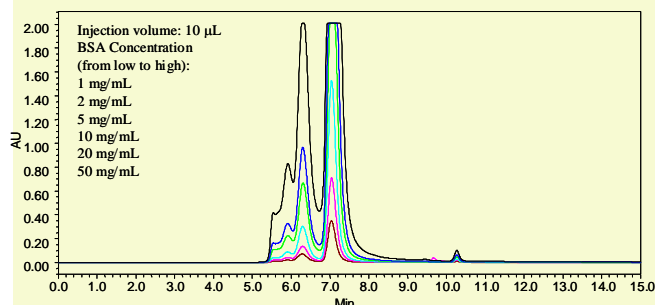
## Mobile Phase Compatibility

Zenix SEC phases are compatible with most aqueous buffers, such as ammonium acetate, phosphate, trizma and so on. Zenix SEC phases can tolerate high concentration of salts, such as 2.0 M. Furthermore, Zenix SEC columns are stable in both organic solvents, such as methanol, ethanol, THF, DMF, DMSO, and so on; as well as the mixture of water and organic solvents.

## High Loading Capacity

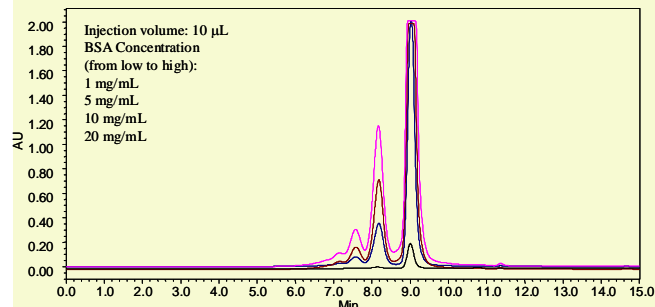
Loading capacity is critical for size exclusion separation and purification. Figure 4 shows high loading capacity for BSA as one example (>500  $\mu$ g for an analytical column).

Figure 4. BSA loading test on a Zenix SEC-150 column.



Column: Zenix SEC-150 (3  $\mu$ m, 7.8x300 mm)  
 Mobile phase: 150 mM Sodium Phosphate, pH 7.0  
 Flow Rate: 1.0 mL/min  
 Injection volume: 10  $\mu$ L  
 Detection: UV214 nm

Figure 5. BSA loading test on a Zenix SEC-300 column.



Column: Zenix SEC-300 (3  $\mu$ m, 7.8x300 mm)  
 Mobile phase: 150 mM Sodium Phosphate, pH 7.0  
 Flow Rate: 1.0 mL/min  
 Injection volume: 10  $\mu$ L  
 Detection: UV214 nm

## Lot-to-Lot Reproducibility

The controlled surface chemistry used to synthesize Zenix SEC phases makes the surface coating highly reproducible, leading to consistent column manufacturing. Separation variation from batch to batch is controlled to be within 5% for retention time.

## High Protein Recovery

Zenix SEC phases are hydrophilic and neutral. Proteins and other biological molecules have negligible nonspecific interactions with Zenix stationary phases. The protein adsorption to the silica surface is suppressed, leading to high recovery of intact proteins, maintaining the protein activity after separation. More than 95% recovery is

## Sample Loading Recommendation

ID	2.1x300 mm	4.6x300 mm	7.8x300 mm	10x300mm	21.2x300mm	30x300mm	50x300mm
Type	Nano	Narrow-bore	Regular	Semi prep	Prep	Process	Process
V-injection	0.1-2 $\mu$ L	0.5-10 $\mu$ L	1-100 $\mu$ L	1-250 $\mu$ L	0.01-1.0 mL	0.1-5ml	0.5-20ml
Mass (BSA)	0.1-10 $\mu$ g	0.5-50 $\mu$ g	1-500 $\mu$ g	1-750ug	0.1-5.0mg	0.1-10.0mg	0.1-25.0mg
Standard Flow rate (Maximum)	0.067mL/min	0.35mL/min	1.0mL/min	1.65 mL/min (2.0 mL/min)	7.5 mL/min (10mL/min)	15 ml/min (25 ml/min)	41 ml/min (60 ml/min)
Sensitivity	Highest	Higher	High	N/A	N/A	N/A	N/A
Back pressure	~1,200 psi	~1,200psi	~1,200psi	700-900 psi	700-900 psi	700-900 psi	700-900psi
Instrument Type	Capillary	Regular	Regular	Prep	Prep	Process	Process

## Zenix SEC Technical Specifications

Phase	Zenix SEC-100	Zenix SEC-150	Zenix SEC-300
Material	Neutral, hydrophilic film bonded silica		
Particle size	3 $\mu$ m	3 $\mu$ m	3 $\mu$ m
Pore size	~ 100 Å	~ 150 Å	~ 300 Å
Protein MW range (native)	100 - 100,000	500 - 150,000	5,000 - 1,250,000
pH stability	2 – 8.5 (pH 8.5-9.5 can be tolerated temporarily.)		
Backpressure for 7.8x300 mm (1.0 mL/min)	~ 1,500 psi	~ 1,375 psi	~ 1,100 psi
Backpressure for 4.6x300 mm(0.35 mL/min)	~ 1,400 psi	~ 1,250 psi	~ 1,000 psi
Maximum backpressure	~ 4,500 psi	~ 4,500 psi	~ 3,500 psi
Salt concentration range	20 mM - 2.0 M		
Maximum temperature	~ 80 °C		
Mobile phase compatibility	Aqueous and organic		

Zenix phases have wide applications for separation, identification and purification of proteins, protein variants, peptide fragments, phosphorylated, sialylated, pegylated, and other derivatized proteins. They are well suited for studies such as molecular weight estimation, purification and analysis of biological molecules.

achieved for BSA and lysozyme, the representatives for acidic and basic proteins, respectively.

## Column Dimension Availability

Available Zenix SEC column dimensions are 0.75, 1.0, 2.1, 3.0, 4.6, 7.8, 10, 21.2 and 30 mm I.D., and 20, 30, 50, 100, 150, 250, 300 and 600 mm length. Sepax also offers custom-made columns. Both stainless steel and PEEK tubes are available.

## Pore size vs. MW exclusion limit

Phases(3 $\mu$ m)	Pore Size	Protein MW Exclusion Limit
Zenix SEC-100	100 Å	100,000
Zenix SEC-150	150 Å	150,000
Zenix SEC-300	300 Å	1,250,000

## Applications

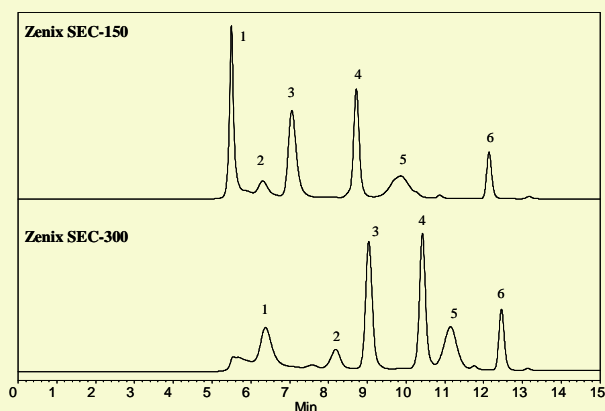
Separation and Analysis
<i>Proteins</i>
<i>Monoclonal antibodies</i>
<i>Cell lysates</i>
<i>Nucleic acids</i>
<i>Nucleotides</i>
<i>Peptides</i>
<i>Water soluble polymers</i>
<i>Nanoparticles</i>
<i>Nanotubes</i>

### Separation of protein and peptide mixture

Zenix SEC columns offer a number of benefits. First, Zenix offers higher capacity, 6.7 mL and 6.9 mL for Zenix SEC-150 and 300, respectively, calculated from the total permeation peak (uracil) to total exclusion peak (thyroglobulin). Secondly Zenix offers higher resolution. Poly-DL-alanine (from Sigma) is a peptide with the MW of 1-

5 kD. For size exclusion chromatography, an empirical rule is that a baseline separation can be achieved for two compounds if their MWs difference is two fold (2x). Both Zenix SEC-150 and 300 columns well separated ribonuclease A (13.7kD) and poly-DL-alanine (1-5 kDa), as shown in Fig. 6. Thirdly Zenix column shows a good separation profile of Poly-DL-alanine, indicating Zenix packing does not have non-specific interactions with Poly-DL-alanine.

Figure 6. Separation of a mixture of proteins and peptide by using Zenix SEC-150 and 300 columns.

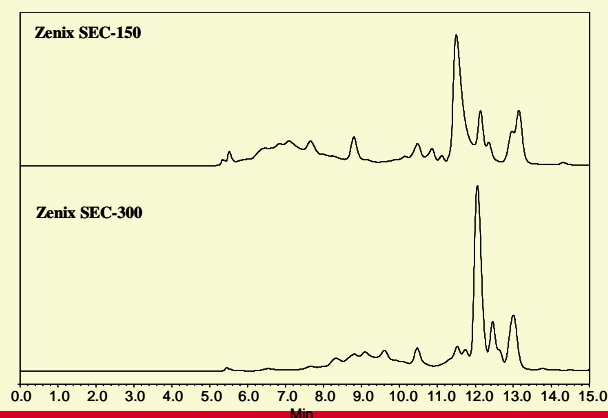


Columns: Zenix SEC (3  $\mu$ m, 7.8x300 mm)  
 Mobile phase: 150 mM Sodium Phosphate, pH 7  
 Flow rate: 1.0 mL/min  
 Temperature: Ambient (~23° C)  
 Detection: UV 214nm  
 Injection: 10  $\mu$ L  
 Sample: 1) Thyroglobulin, 670kD; 2) BSA monomer, 66kD; 3) Ribonuclease A, 13.7kD; 4) poly-DL-alanine, 1-5 kD; 5) Uracil, 120D.

### Separation of *E. coli* Lysate

Zenix SEC-150 and 300 columns are used to separate the *E. Coli* lysate. The elution profiles in Fig. 7 showed both columns achieved high resolution separation. However, Zenix SEC-150 is more suitable for separation of *E. coli* lysate due to the small molecule weight of the lysate.

Figure 7. Separation of *E. coli* lysate with various pore size Zenix columns

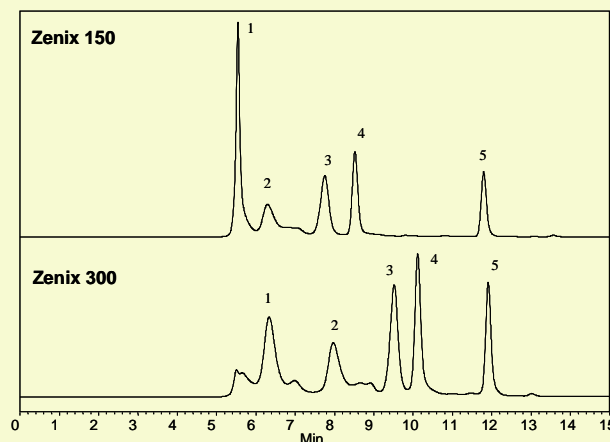


Columns: Zenix SEC (3  $\mu$ m, 7.8x300 mm)  
 Mobile phase: 0.15 M Sodium Phosphate, pH 7.0  
 Flow rate: 1.0 mL/min  
 Detection: UV 214 nm  
 Injection: 10  $\mu$ L  
 Sample: *E. coli* lysate (2.5 mg/mL)

### Separation of Biorad standard protein mixture

Biorad protein mixture of thyroglobulin,  $\gamma$ -globulin, ovalbumin, myoglobin and vitamin B12 have the molecular weight in the range of 660,000 – 1,355, which has been used to characterize the performance of SEC columns. The separation of Biorad protein mixture by Zenix 150 and 300 are shown in Figure 8 with the peak efficiency shown in Table 4. The plate number of myoglobin is more than 20,000 with Zenix SEC-300 column, which was not achieved by any other SEC columns.

Figure 8. Separation of Biorad protein mixture by Zenix SEC-150 and 300 columns.



Columns: Zenix SEC (3  $\mu$ m, 7.8x300 mm)  
 Mobile phase: 0.15 M Sodium Phosphate, pH 7.0  
 Flow rate: 1.0 mL/min  
 Detection: UV214 nm  
 Injection: 10  $\mu$ L  
 Sample: 1) Thyroglobulin, 670 kD; 2)  $\gamma$ -Globulin, 158 kD; 3) Ovalbumin, 44 kD; 4) Myoglobin, 16.9 kD; 5) Vitamin B12, 1355 D.

Table 4. Efficiency of Zenix SEC-150 and 300 columns

Peak	Protein	Zenix 150 7.8x300mm	Zenix 300 7.8x300mm
1	Thyroglobulin	12420	1760
2	$\gamma$ -Globulin	2860	3650
3	Ovalbumin	6620	11760
4	Myoglobin	15020	20810
5	Vitamin B12	34370	35460

## Ordering Information

### Zenix SEC-100 (3 $\mu\text{m}$ , 100 Å)

ID x Length (mm)	P/N
21.2x300	SEP213100-21230
21.2x250	SEP213100-21225
21.2x150	SEP213100-21215
21.2x100	SEP213100-21210
21.2x50 (Guard)	SEP213100-21205
10x300	SEP213100-10030
10x250	SEP213100-10025
10x150	SEP213100-10015
10x100	SEP213100-10010
10x50 (Guard)	SEP213100-10005
<b>7.8x300</b>	<b>SEP213100-7830</b>
7.8x250	SEP213100-7825
7.8x150	SEP213100-7815
7.8x50 (Guard)	SEP213100-7805
<b>4.6x300</b>	<b>SEP213100-4630</b>
4.6x250	SEP213100-4625
4.6x150	SEP213100-4615
4.6x50 (Guard)	SEP213100-4605

### Zenix SEC-150 (3 $\mu\text{m}$ , 150 Å)

ID x Length (mm)	P/N
21.2x300	SEP213150-21230
21.2x250	SEP213150-21225
21.2x150	SEP213150-21215
21.2x100	SEP213150-21210
21.2x50 (Guard)	SEP213150-21205
10x300	SEP213150-10030
10x250	SEP213150-10025
10x150	SEP213150-10015
10x100	SEP213150-10010
10x50 (Guard)	SEP213150-10005
<b>7.8x300</b>	<b>SEP213150-7830</b>
7.8x250	SEP213150-7825
7.8x150	SEP213150-7815
7.8x50 (Guard)	SEP213150-7805
<b>4.6x300</b>	<b>SEP213150-4630</b>
4.6x250	SEP213150-4625
4.6x150	SEP213150-4615
4.6x50 (Guard)	SEP213150-4605

### Zenix SEC-300 (3 $\mu\text{m}$ , 300 Å)

ID x Length (mm)	P/N
21.2x300	SEP213300-21230
21.2x250	SEP213300-21225
21.2x150	SEP213300-21215
21.2x100	SEP213300-21210
21.2x50 (Guard)	SEP213300-21205
10x300	SEP213300-10030
10x250	SEP213300-10025
10x150	SEP213300-10015
10x100	SEP213300-10010
10x50 (Guard)	SEP213300-10005
<b>7.8x300</b>	<b>SEP213300-7830</b>
7.8x250	SEP213300-7825
7.8x150	SEP213300-7815
7.8x50 (Guard)	SEP213300-7805
<b>4.6x300</b>	<b>SEP213300-4630</b>
4.6x250	SEP213300-4625
4.6x150	SEP213300-4615
4.6x50 (Guard)	SEP213300-4605

**\*\*Precolumn Filter 2.0  $\mu\text{m}$  PEEK SEP102000-P356**

## How to Order

[www.dichrom.com](http://www.dichrom.com)

Contact dichrom GmbH by  
Phone: +49-2365-69926-0  
Fax: +49-2365-69926-11  
E-mail: [info@dichrom.com](mailto:info@dichrom.com)

Gräwenkolkstraße 66  
45770 Marl  
Deutschland

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