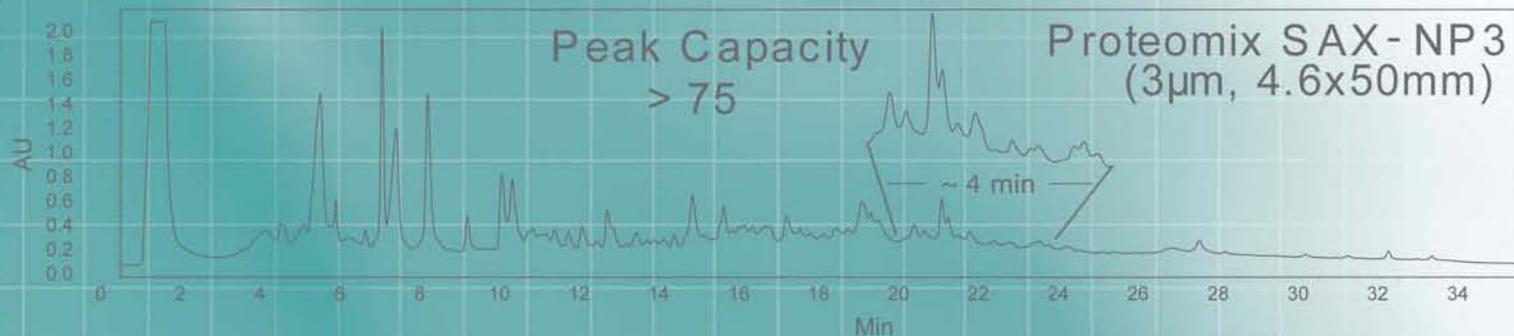
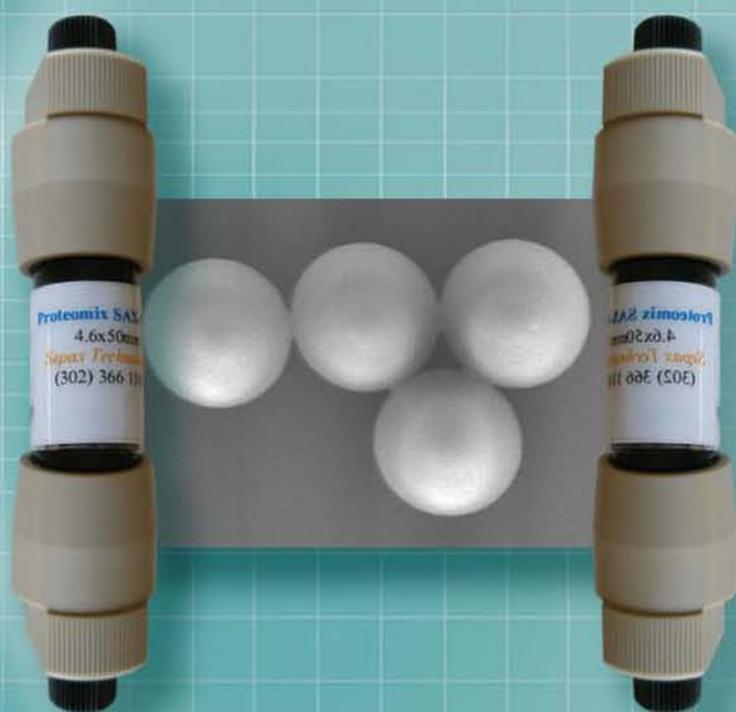


# Ion Exchange Chromatography



*Sepax Technologies*

## Proteomix<sup>®</sup>



**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

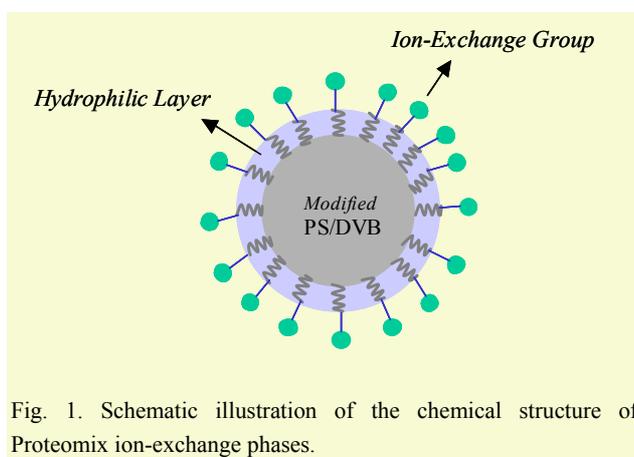
# Proteomix<sup>®</sup> Ion-exchange Phases

## General Description

Proteomix ion-exchange columns are specially designed for high resolution, high efficiency and high recovery separations of proteins, oligonucleotides, carbohydrates, and peptides. The packing support is composed of a rigid, spherical, highly cross-linked poly(styrene divinylbenzene) (PS/DVB) bead. Both porous and non-porous PS/DVB beads are provided. The porous PS/DVB resins have particle sizes of 5 and 10  $\mu\text{m}$  with the pore size of 500  $\text{\AA}$ . The non-porous resins have particle sizes of 1.7, 3, 5 and 10  $\mu\text{m}$ . The PS/DVB resin surface is grafted with a highly hydrophilic, neutral polymer layer with the thickness in the range of nanometer. The hydrophobic PS/DVB resin surface is totally covered by such a hydrophilic coating that eliminates non-specific bindings with biological analytes, leading to high efficiency and high recovery separations for biomolecules. On the top of the hydrophilic layer, a layer of ion-exchange functional groups is attached via chemical bonding. A proprietary chemistry was developed to synthesize a densely packed, uniform ion-exchange layer.

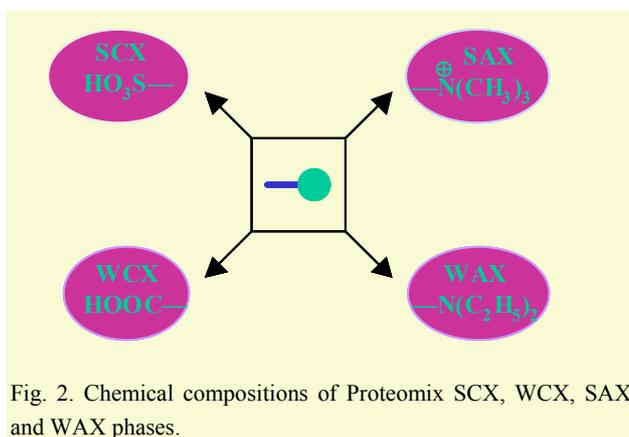
## Chemical Structure of Proteomix Resins

The chemical structure of Proteomix ion-exchange phases is composed of a rigid PS/DVB core, a densely packed, nanometer thick, hydrophilic coating, and a uniform ion-exchange layer, as shown in Figure 1.



## Protomix Ion-Exchange Phases

As shown in Figure 2, Proteomix ion-exchange phases are composed of SCX, WCX, SAX, and WAX, which are strong cation exchanger with sulfonate functional groups, weak cation exchanger with carboxylate functional groups, strong anion exchanger with quaternary ammonium functional groups, weak anion exchanger with tertiary amine functional, respectively. All ion-exchange functional groups are chemically bonded to the top of the hydrophilic coating for both porous and non-porous PS/DVB resins.



## Highlights of Proteomix Non-Porous Ion-exchange Resins

- Combined high capacity and high separation efficiency in one material
- Particle size selection of 1.7, 3, 5 and 10  $\mu\text{m}$
- Mono-dispersed particles
- Tolerate high pressure operation: 4,000, 6,000, 8,000 and 12,000 psi for 10, 5, 3 and 1.7  $\mu\text{m}$  resins, respectively
- Wide pH range: 2-12
- High resolving power for slightly differed structures of biological species
- 1.7 and 3  $\mu\text{m}$  particles are best suitable for whole protein separation in proteome studies
- Suitable for separations of peptides, carbohydrates and glycans, proteins, polynucleotides, cell lysates and multi-dimensional separations

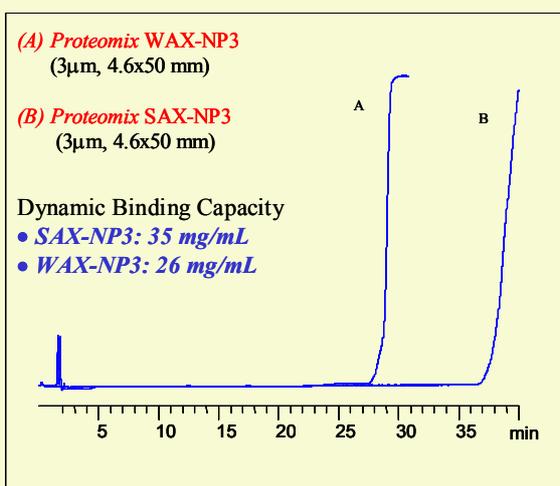
## Non-Porous Proteomix Ion-exchangers – Technology Advancements

### High Capacity of Proteomix Non-Porous Resins

It is well known that the non-porous resin maximizes the mass transfer and minimizes the lateral diffusion, resulting in high speed and high efficiency separations. However, due to the low surface area, the separation capacity is low. The low capacity issue creates a great challenge to the non-porous resins for many applications. The Proteomix non-porous ion-exchange resins developed by Sepax proprietary technologies have a breakthrough on the improvement of their capacities. This new technology enables the capacity of the non-porous resins increased to the level comparable to that of the porous resins. Figure 3 shows examples of dynamic binding capacities of the 3  $\mu\text{m}$  non-porous Proteomix SAX and WAX resins: 35 and 26 mg/mL, respectively. Figure 4 shows an example of the dynamic binding capacity of 3  $\mu\text{m}$  Proteomix SCX non-porous resin. Its dynamic binding capacity reaches as high as 53.5 mg/mL.

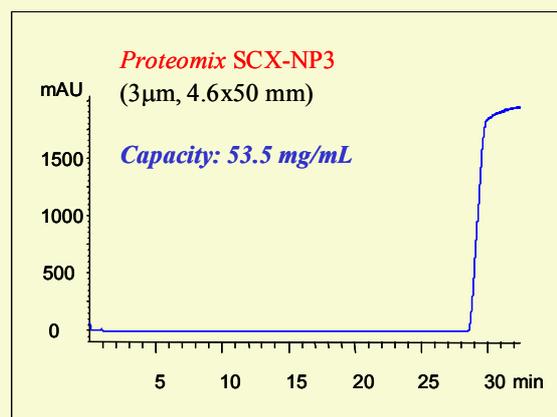
High dynamic binding capacity leads to high sample loading without deterioration of the separation efficiency and the resolution. Figure 5 shows the elution of cytochrome C with the loading up to 200  $\mu\text{g}$  for a 1.7  $\mu\text{m}$  Proteomix SCX non-porous column without any deterioration of the separation.

Fig. 3. The dynamic binding capacity tests for Proteomix anion-exchange resins by flowing BSA into the columns.



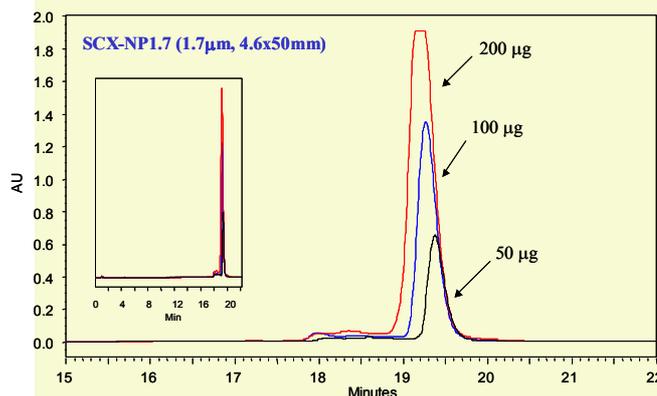
Column: 4.6x50 mm  
Mobile Phase: 10 mM Tris/HCl, pH 8.0  
Flow Rate: 0.25 mL/min  
Detection: 280 nm  
Temperature: 25  $^{\circ}\text{C}$   
Sample: BSA (3 mg/mL)

Fig. 4. The dynamic binding capacity test for Proteomix SCX-NP3 resin by flowing lysozyme into the column.



Mobile Phase: 10 mM phosphate, pH 6.0  
Flow Rate: 0.5 mL/min  
Detection: 280 nm  
Temperature: 25  $^{\circ}\text{C}$   
Sample: Lysozyme (3 mg/mL)

Fig. 5. Elution profiles of cytochrome C at various loadings.



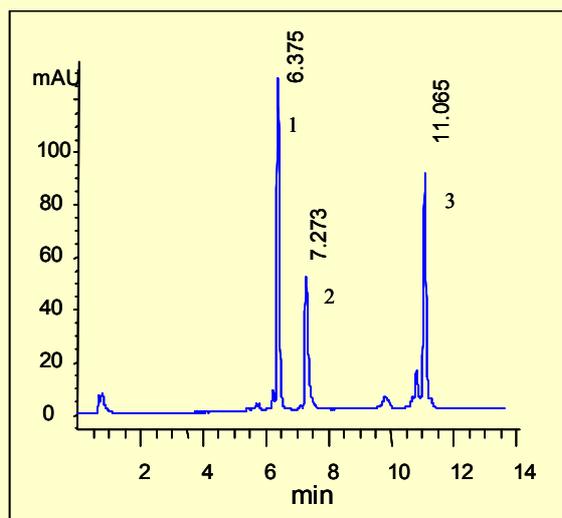
Column: Proteomix SCX-NP1.7 (1.7  $\mu\text{m}$ , 4.6x50 mm)  
Mobile Phase: A, 20 mM PBS, pH 6.0; B, A+1.0 M NaCl  
Gradient: 0-70%B (21 min)  
Flow Rate: 0.35 mL/min  
Detection: 280 nm  
Temperature: 25  $^{\circ}\text{C}$   
Sample: Cytochrome C (20 mg/mL)

### High Separation Efficiency, Resolution and Selectivity

Proteomix SCX-NP, WCX-NP, SAX-NP, and WAX-NP resins have three unique features. First, their nanometer thick hydrophilic layer completely eliminates the non-specific interactions with the biological analytes. Second, non-porous beads minimize the biological analytes' lateral diffusion and suppress their diffusion into the chromatographic bed. Third, a uniform layer of ion-exchange functional groups synthesized by Sepax's proprietary technology greatly improved their ion-exchange

capacities. Such innovatively designed Proteomix SCX-NP, WCX-NP, SAX-NP, and WAX-NP phases result in the highest efficiency separations for proteins, oligonucleotides, carbohydrates, and peptides. Figure 6 is a typical test chromatogram for separation of three proteins: ribonuclease A, cytochrome C, and lysozyme by a 4.6 × 50 mm, Proteomix SCX-NP column (3 μm). The efficiency of lysozyme reaches 100,000 of plates with 5 cm long column. Such a high efficiency separation is unprecedented.

Fig. 6. Separation of a protein mixture by a Proteomix SCX-NP column.



Column: Proteomix SCX-NP3 (3 μm, 4.6x50 mm)  
 Mobile phase: A, 10 mM phosphate, pH 6.0  
 B, A + 1.0 M NaCl  
 Gradient: 0-70%B in 15 min  
 Flow rate: 0.5 mL/min  
 Sample: 1) Cytochrome C, 2) Ribonuclease A, 3) Lysozyme  
 Injection: 5 μL (1 mg/mL for each protein)  
 Temperature: 25 °C  
 Detection: UV 280 nm

The uniqueness of non-porous Proteomix SCX, WCX, SAX, and WAX phases offers highest resolution and selectivity for protein separations. Figure 7 shows an elution profile of lysozyme by a Proteomix SCX-NP3 phase (3 μm). Six peaks are well resolved with a short column (4.6 × 50 mm). Such a high selectivity and resolving power is unmatched with any other SCX phases.

Figure 8 is a typical test chromatogram for a 5 μm, Proteomix SAX-NP column for separation of a mixture of ovalbumin and BSA. High resolution and high selectivity of Proteomix SAX-NP phase can well separate the impurities contained in ovalbumin sample, as well as the BSA dimer from BSA.

Fig. 7. The elution profile of lysozyme from its multiple impurities by a Proteomix SCX-NP column (3 μm, 4.6 × 50 mm). The separation conditions are the same as those in Fig. 6.

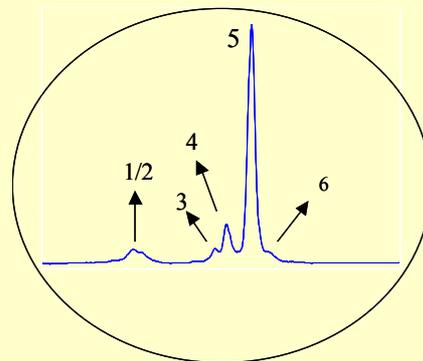
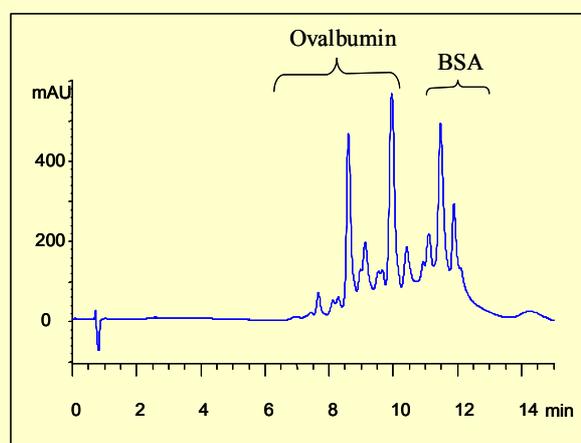
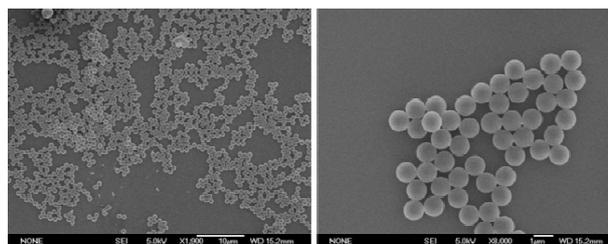


Fig. 8. Elution profile of a mixture of ovalbumin and BSA by Proteomix SAX-NP5 column.



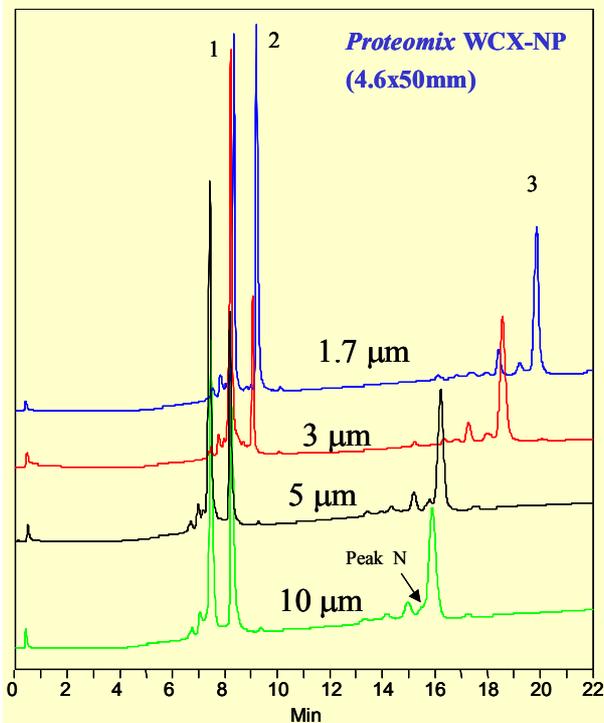
Column: Proteomix SAX-NP5 (5 μm, 4.6x50 mm)  
 Mobile phase: A, 20 mM Tris, pH 8.0  
 B, A + 0.5 M NaCl  
 Gradient: 0-50%B in 15 min  
 Flow rate: 0.5 mL/min  
 Sample: 1) Ovalbumin (10 mg/mL), 2) BSA (5 mg/mL)  
 Injection: 10 μL  
 Temperature: 25 °C  
 Detection: UV 214 nm



### Particle Size Impact on the Separation

The smaller the particle size, the better separation efficiency and higher separation speed. Proteomix ion-exchange resins have a wide range of the particle size selection from 1.0 to 10  $\mu\text{m}$  for analytical and semi-preparative separation applications. Figure 9 is the separation of three proteins by four weak cation exchange resins with the particle size of 1.7, 3, 5, and 10  $\mu\text{m}$ , separately. It is clearly seen that the smaller particle generates higher efficiency and resolution. One of the impurities in lysozyme labeled by Peak N is barely separated with 10  $\mu\text{m}$  WCX resin. For 5  $\mu\text{m}$  WCX resin, it is almost a baseline separation. When the particle size is decreased to 3  $\mu\text{m}$ , it becomes a baseline separation. With the particle size further decreased to 1.7  $\mu\text{m}$ , the impurity is well separated.

Fig. 9. Separation of a protein mixture by Proteomix WCX-NP columns with various particle sizes.

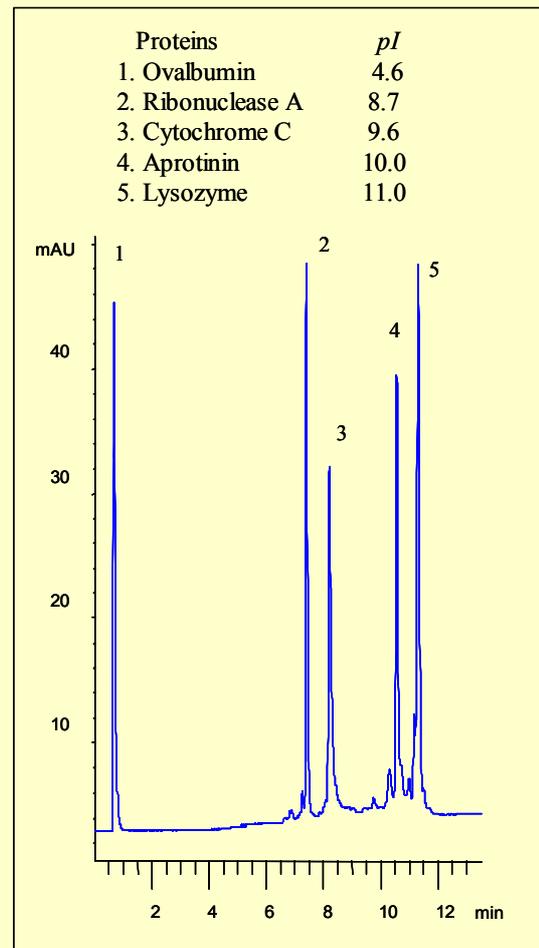


Column: Proteomix WCX-NP (4.6x50 mm)  
 Mobile phase: A, 10 mM phosphate, pH 6.0  
 B, A + 1.0 M NaCl  
 Gradient: 0-100%B in 20 min  
 Flow rate: 1.0 mL/min, 0.75 mL/min for WCX-NP1.7  
 Sample: 1) Cytochrome C, 2) Ribonuclease A,  
 3) Lysozyme  
 Injection: 5  $\mu\text{L}$  (1 mg/mL for each protein)  
 Temperature: 25  $^{\circ}\text{C}$   
 Detection: UV 280 nm

### Charge Dependent Separation

Figure 10 is a typical test chromatogram for a Proteomix SCX-NP column (3  $\mu\text{m}$ ). Extremely high efficiency separation was achieved for 5 proteins. Those proteins were eluted based on their charge status (pI) from low to high: ovalbumin, ribonuclease A, cytochrome C, aprotinin, and lysozyme. At the separation conditions (pH 6.0), ovalbumin is negatively charged and eluted at the void volume, indicating no non-specific bindings.

Fig. 10. Separation of a protein mixture with various pI by a Proteomix SCX column.

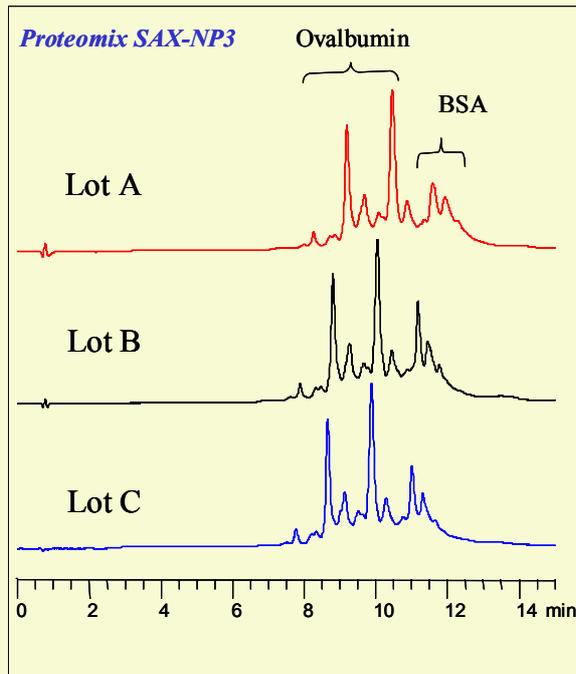


Column: Proteomix SCX-NP3 (3  $\mu\text{m}$ , 4.6x50 mm)  
 Mobile phase: A, 10 mM phosphate, pH 6.0  
 B, A + 1.0 M NaCl  
 Gradient: 0-100%B in 15 min  
 Flow rate: 0.5 mL/min  
 Injection: 5  $\mu\text{L}$  (1 mg/mL for each protein)  
 Temperature: 25  $^{\circ}\text{C}$   
 Detection: UV 280 nm

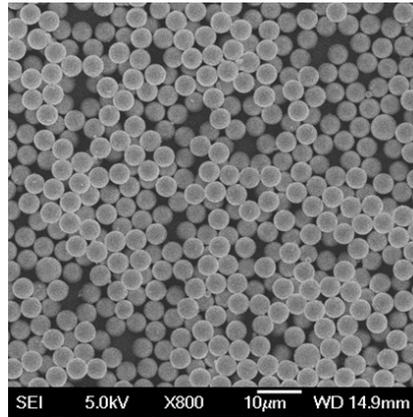
## High Lot-to-Lot Reproducibility

With well-controlled resin production and the surface chemistry, the manufacturing of the Proteomix ion-exchangers is highly reproducible. The typical variation of the retention time is less than 6% from batch to batch. One example is shown in Figure 11 for the production of three lots of 3  $\mu\text{m}$  Proteomix SAX-NP resins.

Fig. 11. The reproducibility of three lots of Proteomix SAX resins.



Column: Proteomix SAX-NP3 (3  $\mu\text{m}$ , 4.6x50 mm)  
Mobile phase: A, 20 mM Tris, pH 8.0  
B, A + 0.3 M NaCl  
Gradient: 0-100%B in 15 min  
Flow rate: 0.5 mL/min  
Sample: 1) Ovalbumin (10 mg/mL), 2) BSA (5 mg/mL)  
Injection: 10  $\mu\text{L}$   
Temperature: 25  $^{\circ}\text{C}$   
Detection: UV 214 nm



Mono-dispersed Proteomix ion-exchange particles enable uniform column packing bed, which improves separation efficiency.

## Column Dimension Availability

The column dimensions of the Proteomix SCX, WCX, SAX, and WAX products are 0.75, 2.1, 3.0, 4.6, 7.8, 10, and 21.2 mm I.D., and 2, 3, 5, 10, 15, 25, and 30 cm length. Sepax also offers custom-made columns.

## Features

- ✧ Uniform polymer beads as the packing support
- ✧ Proprietary surface chemistry specially designed for elimination of non-specific bindings
- ✧ Unprecedented separation efficiency, selectivity and resolving power
- ✧ Complete selection for analytical, semi-preparative and preparative separations
- ✧ Wide particle size selection
- ✧ High stability
- ✧ High recovery
- ✧ Well suited for UPLC system

## Technical Specifications

Products	Pore Size	Particle Size (µm)	Dynamic binding capacity	pH range
Proteomix SCX-NP1.7, NP3, NP5 & NP10	Non-porous	1.7, 3, 5, 10	~60, 54, 38, and 20 mg/mL	2-12
Proteomix WCX-NP1.7, NP3, NP5 & NP10	Non-porous	1.7, 3, 5, 10	~25, 19, 15 and 10 mg/mL	2-12
Proteomix SAX-NP1.7, NP3, NP5 & NP10	Non-porous	1.7, 3, 5, 10	~43, 35, 28, and 17 mg/mL	2-12
Proteomix WAX-NP1.7, NP3, NP5 & NP10	Non-porous	1.7, 3, 5, 10	~35, 26, 18, and 12 mg/mL	2-12

## Applications

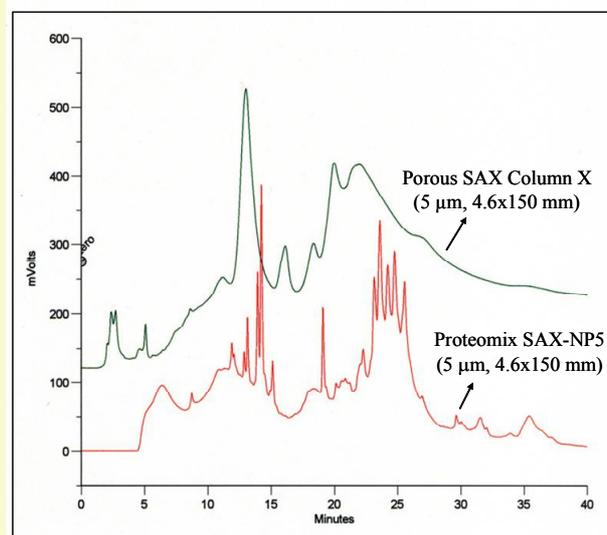
Separation and Analysis
<i>Proteins</i>
<i>Cell lysates</i>
<i>Nucleic acids</i>
<i>Peptides</i>
<i>Carbohydrates and glycans</i>
<i>Nucleotides</i>
<i>Nanoparticles</i>
<i>Nanotubes</i>

Proteomix ion-exchange phases have wide applications for separation, identification and purification of proteins, protein variants, peptide fragments, phosphorylated, sialylated, and other derivatized proteins. They are excellent for monitoring enzymatic reactions and protein-protein interactions. With extremely high separation efficiency, Proteomix phases are well designed for proteomics applications and 2D or multi-dimensional separations. One example is the high resolution of cell lysates by Proteomix SAX-NP phases. Proteomix SAX and WAX phases are suitable for separation of oligonucleotides. Porous Proteomix ion-exchange phases provide high resolution and high capacity separation for surface charged nanoparticles, nanorods and nanotubes.

### Separation of Horse Serum, a Protein Mixture

Figure 12 shows the separation of a horse serum by using Proteomix SAX-NP5 and a commercial porous SAX column. Non-porous Proteomix SAX-NP5 has much high resolution than the porous SAX column.

Fig. 12. The separation of a horse serum (20 µL, 2x diluted) from BioWhittaker, a Cambrex company (Walkersville, MD). (Courtesy of Miyako Kawakatsu, M&S Instruments, Inc.)

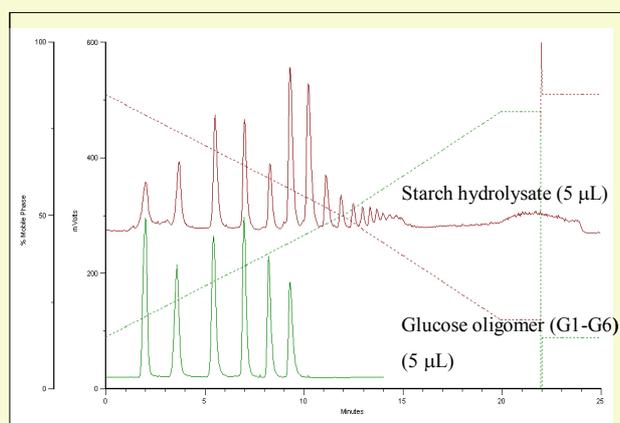


Mobile phase: (A) 25 mM Tris acetate, pH 7.3  
 (B) A + 0.8 M sodium acetate  
 Gradient: 0-50%-70%B (0-25-28 min)  
 Flow rate: 0.5 mL/min for SAX-NP5  
 1.0 mL/min for Porous SAX  
 Detection: UV 280 nm

## Separation of Carbohydrates and Glycans

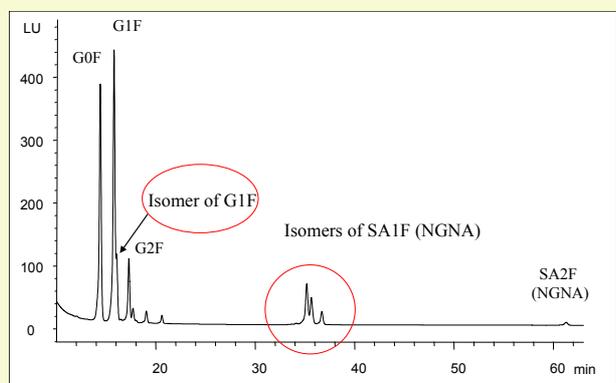
Figure 13 shows the separation of glucose oligomers and corn starch hydrolysate. ELSD is utilized to detect carbohydrate molecules. For ELSD detection, bleeding is a big problem for most columns, especially silica based amino columns. However, Proteomix SAX columns not only solved the bleeding problem, but also achieved excellent separation efficiency and resolution. Figure 14 is another example of high resolution separation of glycans and their isomers by non-porous Proteomix SAX column.

Fig. 13. Separation of glucose oligomers and corn starch hydrolysate by Proteomix SAX-NP5 phase. (Courtesy of Miyako Kawakatsu, M&S Instruments, Inc.)



Column: Proteomix SAX-NP5 (5 µm, 4.6x150 mm)  
 Mobile phase: A, 0.05% (25% NH<sub>4</sub>OH) in CH<sub>3</sub>CN  
 B, 0.05% (25% NH<sub>4</sub>OH) in H<sub>2</sub>O  
 Gradient: 15-80%B (20 min)  
 Flow rate: 0.7 mL/min  
 Sample: Corn starch hydrolysate  
 Temperature: 35 °C  
 Detection: ELSD

Fig. 14. Separation of 2-AA (anthranilic acid) labeled N-linked oligosaccharides profiling of an IgG1 sample.



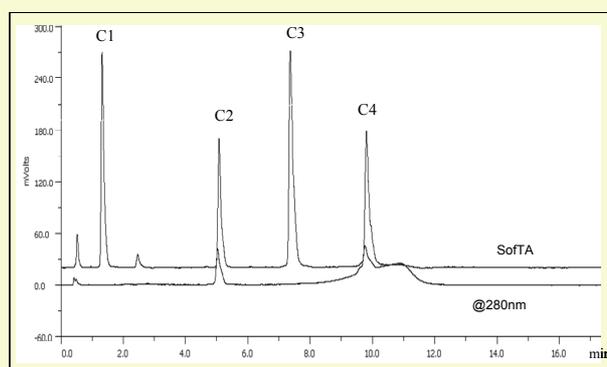
Column: Proteomix SAX-NP5 (5 µm, 4.6x150 mm)  
 Mobile phase: A, 2.5% (v/v) acetic acid, 0.5% TEA in H<sub>2</sub>O

B, 0.5% acetic acid in ACN  
 Gradient: 0-100%B (60 min)  
 Flow rate: 0.3 mL/min  
 Detection: Fluorescence Ex/Em=360/425nm  
 Sample: G0F: asialo, agalacto, core-fucosylated biantennary glycan; G1F: asialo, mono-galacto, core-fucosylated biantennary glycan; G2F: asialo, di-galacto, core-fucosylated biantennary glycan; SA1F: mono-sialylated, galactosylated, core-fucosylated biantennary glycan; SA2F: di-sialylated, galactosylated, core-fucosylated biantennary glycan; NGNA: N-glycolylneuraminic acid.

## Separation of Peptides in Volatile Buffer

The peptides with different hydrophobicity can be resolved by reversed phases, such as C18. However, peptides with different charges may not be well separated on reversed phase mode if their hydrophobic properties are close. As an alternative method, ion exchange chromatography is recommended. With the net charge from +1 to +4, four peptides (C1, C2, C3 and C4 with their sequences listed in Figure 15) were well separated by Proteomix SCX-NP phase in a volatile buffer of ammonium acetate and acetonitrile, which makes it compatible for LC/MS application.

Fig. 15. Cation exchange separation of 4 peptides C1, C2, C3 and C4 (0.1 mg/mL). (Courtesy of Miyako Kawakatsu, M&S Instruments, Inc.)



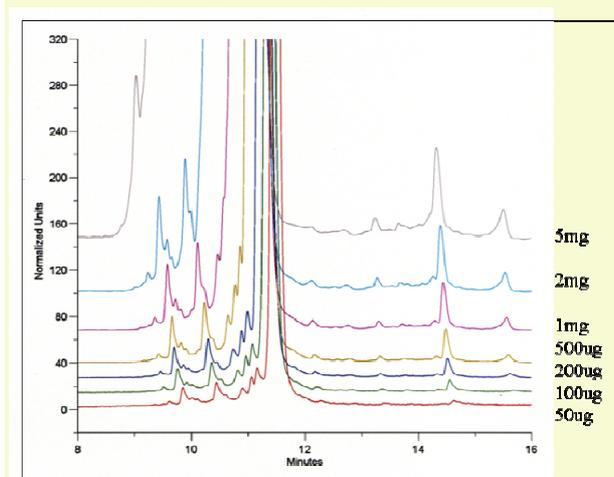
Column: Proteomix SCX-NP3 (3 µm, 4.6x50 mm)  
 Mobile phase: A, 5 mM CH<sub>3</sub>COONH<sub>4</sub>  
 B, 500 mM CH<sub>3</sub>COONH<sub>4</sub>:CH<sub>3</sub>CN=4:1(v/v)  
 Gradient: 0-50%B (20 min)  
 Flow rate: 0.6 mL/min  
 Temperature: 25 °C  
 Detection: ELSD  
 Injection: 5 µL (0.1 mg/mL)  
 Sample: Peptides C1, C2, C3 and C4

Peptide	Sequence	Net Charge
C1	Ac-Gly-Gly-Gly-Leu-Gly-Gly-Ala-Gly-Gly-Leu-Lys-amide	□1
C2	Ac-Lys-Tyr-Gly-Leu-Gly-Gly-Ala-Gly-Gly-Leu-Lys-amide	□2
C3	Ac-Gly-Gly-Ala-Leu-Lys-Ala-Leu-Lys-Gly-Leu-Lys-amide	□3
C4	Ac-Lys-Tyr-Ala-Leu-Lys-Ala-Leu-Lys-Gly-Leu-Lys-amide	□4

## High Protein Loading Separation

Ribonuclease A was separated by a non-porous SCX column. When the injection amount of Ribonuclease A was increased from 50  $\mu$ g to 5 mg, the separation resolution had negligible change and each of the impurities was well separated, as shown in Figure 16. The combination of high loading capacity and high resolution separation enables non-porous Proteomix ion-exchange columns very well suited for both analytical and preparative separations of biological molecules.

Fig. 16. Elution profiles of RNaseA by Proteomix SCX-NP3 column with various loadings. (Courtesy of Miyako Kawakatsu, M&S Instruments, Inc.)



Column: Proteomix SCX-NP3 (3  $\mu$ m, 4.6x50 mm)  
Eluent: 10 mM PBS, pH 6.0  
Gradient: 0-1.0 M NaCl (20 min)  
Flow rate: 1.0 mL/min  
Detection: 280 nm;  
Temperature: 25  $^{\circ}$ C  
Injection: 100  $\mu$ L  
Sample: Ribonuclease A (from Bovine Pancreas for Genomics, Wako Pure Chemical industries, Ltd.)

## Separation of Oligonucleotides

High resolution separation is critical for DNA analysis and purification. The non-porous Proteomix SAX resins offer high separation efficiency with high capacity. Figure 17 shows the separation of a 50-mer oligonucleotide synthesized by ABI synthesizer. The largest peak is the modified 50-mer oligo and the others are failures or the impurities. As a comparison, the same sample was separated with a commercial porous SAX column. The results showed similar retention time for the 50-mer oligonucleotide from both non-porous and porous SAX columns, which indicated that their capacities are close to each other. However, the non-porous SAX generated much higher efficiency and resolution. Figure 18 is another example of high resolution separation of oligonucleotides of

Mw 12196 from its degraded fragments. At least 35 species of those degraded oligo fragments were well resolved within 22 minutes.

Fig. 17. Separation a 50-mer oligonucleotide from its impurities by non-porous Proteomix SAX and porous SAX columns.

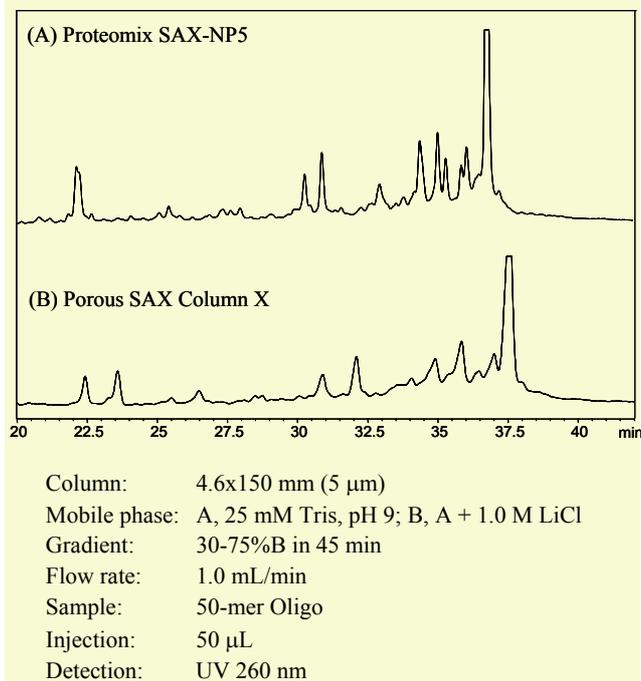
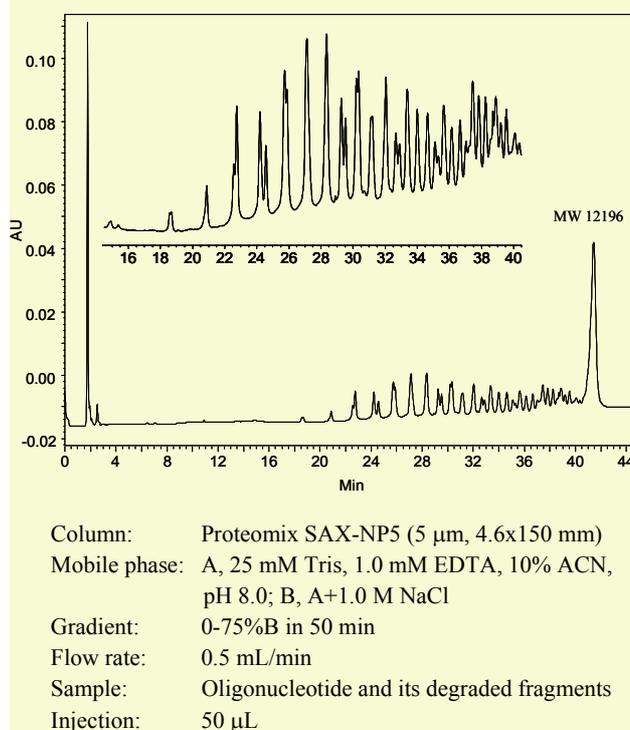


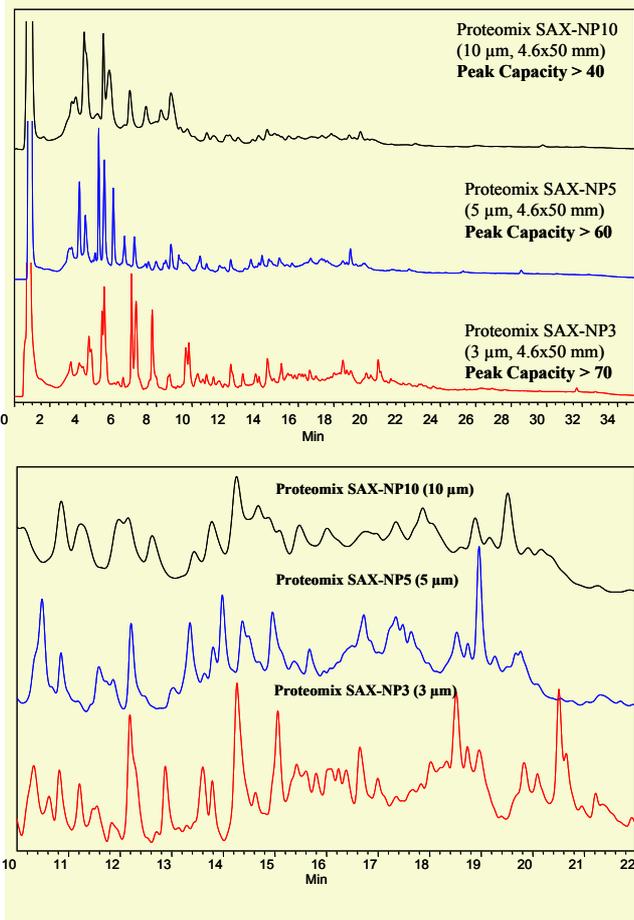
Fig. 18. Separation of an oligonucleotide (MW 12196) and its degraded fragments.



## Separation and Analysis of Cell Lysates

The key issue for proteome studies is to separate and identify a large number of biological species in a cell, such as proteins, nucleotides, peptides and others. The demand for separation is unprecedented. With the uniqueness of high resolution and high capacity, the non-porous Proteomix ion-exchange resins are very much suitable for separating cell lysates. Figure 19(a) showed the separation profiles of *E. coli* lysate with 3, 5, and 10  $\mu\text{m}$  non-porous Proteomix SAX particles. The minimum number of resolved peaks increased from 40 to 60 to 75 when the particle size decreased from 10 to 5 to 3  $\mu\text{m}$ . To better view the separation performance, the elution profiles in the range of 10-22 minutes were shown in Figure 19(b). At least 45, 38, 27 peaks were resolved at the retention time of 10-22 minutes for 3, 5, and 10  $\mu\text{m}$  SAX columns, respectively. Figure 20 showed various sample loading for a 3  $\mu\text{m}$ , 4.6x50 mm non-porous SAX column. When the amount of *E. coli* lysate increased from 25  $\mu\text{g}$  to 50  $\mu\text{g}$  to 125  $\mu\text{g}$ , the separation efficiency and resolution remained consistent.

Fig. 19(a) (Top). Separation of *E. coli* lysate by 3, 5 and 10  $\mu\text{m}$  Proteomix SAX particles. (b) (Bottom). Expanded region of retention time from 10 to 22 min.



Column: Proteomix SAX-NP (4.6x50 mm)  
 Mobile phase: A, 20 mM Tris, pH 9.0; B, A + 0.5 M NaCl  
 Gradient: 0-100%B in 30 min  
 Flow rate: 0.5 mL/min  
 Sample: *E. coli* lysate (2.5 mg/mL)  
 Injection: 10  $\mu\text{L}$   
 Temperature: 25  $^{\circ}\text{C}$   
 Detection: UV 280 nm

Fig. 20. Elution profile of *E. coli* Lysate by non-porous Proteomix SAX column with various loadings. The separation conditions are the same as those in Fig. 19.

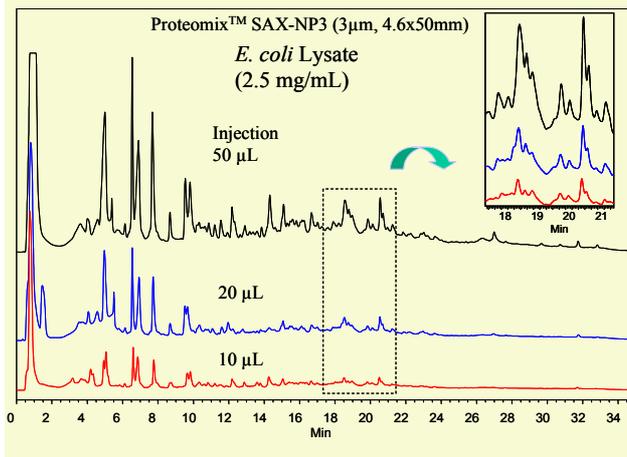
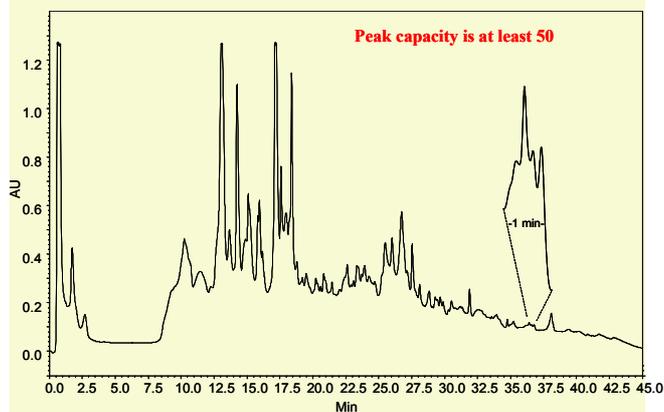


Fig. 21. High peak capacity separation of yeast lysate by non-porous Proteomix SAX column. (Courtesy of Prof. Fred Regnier, Purdue University)



Column: Proteomix SAX-NP3 (3  $\mu\text{m}$ , 4.6x50 mm)  
 Mobile phase: A, 20 mM Tris, pH 9.0; B, A + 0.5 M NaCl  
 Gradient: 0-100%B in 30 min  
 Flow rate: 0.5 mL/min  
 Sample: Yeast lysate (2.5 mg/mL)  
 Injection: 40  $\mu\text{L}$   
 Temperature: 25  $^{\circ}\text{C}$   
 Detection: UV 280 nm

## Ordering Information of Proteomix Ion-Exchange Columns

### Proteomix SCX-NP Columns

Phase	ID x Length (mm)	P/N	Phase	ID x Length (mm)	P/N
1.7 $\mu$ m <i>Proteomix</i> SCX-NP1.7	7.8 x 50	SEP401NP2-7805	3 $\mu$ m <i>Proteomix</i> SCX-NP3	7.8 x 50	SEP401NP3-7805
	4.6 x 150	SEP401NP2-4615		4.6 x 150	SEP401NP3-4615
	4.6 x 100	SEP401NP2-4610		4.6 x 100	SEP401NP3-4610
	4.6 x 50	SEP401NP2-4605		4.6 x 50	SEP401NP3-4605
	4.6 x 30	SEP401NP2-4603		4.6 x 30	SEP401NP3-4603
	4.0 x 10 (Guard)	SEP401NP2-4001		4.0 x 10 (Guard)	SEP401NP3-4001
	3.0 x 100	SEP401NP2-3010		3.0 x 100	SEP401NP3-3010
	3.0 x 50	SEP401NP2-3005		3.0 x 50	SEP401NP3-3005
	3.0 x 30	SEP401NP2-3003		3.0 x 30	SEP401NP3-3003
	2.1 x 100	SEP401NP2-2110		2.1 x 100	SEP401NP3-2110
	2.1 x 50	SEP401NP2-2105		2.1 x 50	SEP401NP3-2105
	2.1 x 30	SEP401NP2-2103		2.1 x 30	SEP401NP3-2103
	2.0 x 10 (Guard)	SEP401NP2-2001		2.0 x 10 (Guard)	SEP401NP3-2001
	Precolumn Filter (PEEK) <sup>*</sup>	SEP102000-P356		Precolumn Filter (PEEK)	SEP102000-P356
5 $\mu$ m <i>Proteomix</i> SCX-NP5	10 x 250	SEP401NP5-10025	10 $\mu$ m <i>Proteomix</i> SCX-NP10	10 x 250	SEP401NP10-10025
	7.8 x 250	SEP401NP5-7825		7.8 x 250	SEP401NP10-7825
	7.8 x 100	SEP401NP5-7810		7.8 x 150	SEP401NP10-7815
	7.8 x 50	SEP401NP5-7805		7.8 x 50	SEP401NP10-7805
	4.6 x 250	SEP401NP5-4625		4.6 x 250	SEP401NP10-4625
	4.6 x 150	SEP401NP5-4615		4.6 x 150	SEP401NP10-4615
	4.6 x 100	SEP401NP5-4610		4.6 x 100	SEP401NP10-4610
	4.6 x 50	SEP401NP5-4605		4.6 x 50	SEP401NP10-4605
	4.6 x 30	SEP401NP5-4603		4.6 x 30	SEP401NP10-4603
	4.0 x 10 (Guard)	SEP401NP5-4001		4.0 x 10 (Guard)	SEP401NP10-4001
	2.1 x 150	SEP401NP5-2115		2.1 x 150	SEP401NP10-2115
	2.1 x 100	SEP401NP5-2110		2.1 x 100	SEP401NP10-2110
	2.1 x 50	SEP401NP5-2105		2.1 x 50	SEP401NP10-2105
	2.1 x 30	SEP401NP5-2103		2.1 x 30	SEP401NP10-2103
	2.0 x 10 (Guard)	SEP401NP5-2001		2.0 x 10 (Guard)	SEP401NP10-2001
	Precolumn Filter (PEEK)	SEP102000-P355		Precolumn Filter (PEEK)	SEP102000-P355
	<i>Preparative Columns</i>			<i>Preparative Columns</i>	
21.2 x 250	SEP401NP5-21225	21.2 x 250	SEP401NP10-21225		
21.2 x 150	SEP401NP5-21215	21.2 x 150	SEP401NP10-21215		

### Proteomix WCX-NP Columns

Phase	ID x Length (mm)	P/N	Phase	ID x Length (mm)	P/N
1.7 $\mu$ m <i>Proteomix</i> WCX-NP1.7	7.8 x 50	SEP402NP2-7805	3 $\mu$ m <i>Proteomix</i> WCX-NP3	7.8 x 50	SEP402NP3-7805
	4.6 x 150	SEP402NP2-4615		4.6 x 150	SEP402NP3-4615
	4.6 x 100	SEP402NP2-4610		4.6 x 100	SEP402NP3-4610
	4.6 x 50	SEP402NP2-4605		4.6 x 50	SEP402NP3-4605
	4.6 x 30	SEP402NP2-4603		4.6 x 30	SEP402NP3-4603
	4.0 x 10 (Guard)	SEP402NP2-4001		4.0 x 10 (Guard)	SEP402NP3-4001
	3.0 x 100	SEP402NP2-3010		3.0 x 100	SEP402NP3-3010
	3.0 x 50	SEP402NP2-3005		3.0 x 50	SEP402NP3-3005
	3.0 x 30	SEP402NP2-3003		3.0 x 30	SEP402NP3-3003
	2.1 x 100	SEP402NP2-2110		2.1 x 100	SEP402NP3-2110
	2.1 x 50	SEP402NP2-2105		2.1 x 50	SEP402NP3-2105
	2.1 x 30	SEP402NP2-2103		2.1 x 30	SEP402NP3-2103
	2.0 x 10 (Guard)	SEP402NP2-2001		2.0 x 10 (Guard)	SEP402NP3-2001
	Precolumn Filter (PEEK)	SEP102000-P356		Precolumn Filter (PEEK)	SEP102000-P356

Phase	ID x Length (mm)	P/N	Phase	ID x Length (mm)	P/N
5 $\mu$ m <i>Proteomix</i> WCX-NP5	10 x 250	SEP402NP5-10025	10 $\mu$ m <i>Proteomix</i> WCX-NP10	10 x 250	SEP402NP10-10025
	7.8 x 250	SEP402NP5-7825		7.8 x 250	SEP402NP10-7825
	7.8 x 100	SEP402NP5-7810		7.8 x 150	SEP402NP10-7815
	7.8 x 50	SEP402NP5-7805		7.8 x 50	SEP402NP10-7805
	4.6 x 250	SEP402NP5-4625		4.6 x 250	SEP402NP10-4625
	4.6 x 150	SEP402NP5-4615		4.6 x 150	SEP402NP10-4615
	4.6 x 100	SEP402NP5-4610		4.6 x 100	SEP402NP10-4610
	4.6 x 50	SEP402NP5-4605		4.6 x 50	SEP402NP10-4605
	4.6 x 30	SEP402NP5-4603		4.6 x 30	SEP402NP10-4603
	4.0 x 10 (Guard)	SEP402NP5-4001		4.0 x 10 (Guard)	SEP402NP10-4001
	2.1 x 150	SEP402NP5-2115		2.1 x 150	SEP402NP10-2115
	2.1 x 100	SEP402NP5-2110		2.1 x 100	SEP402NP10-2110
	2.1 x 50	SEP402NP5-2105		2.1 x 50	SEP402NP10-2105
	2.1 x 30	SEP402NP5-2103		2.1 x 30	SEP402NP10-2103
	2.0 x 10 (Guard)	SEP402NP5-2001		2.0 x 10 (Guard)	SEP402NP10-2001
	Precolumn Filter (PEEK)	SEP102000-P355		Precolumn Filter (PEEK)	SEP102000-P355
	<i>Preparative Columns</i>			<i>Preparative Columns</i>	
250 x 21.2	SEP402NP5-21225	21.2 x 250	SEP402NP10-21225		
150 x 21.2	SEP402NP5-21215	21.2 x 150	SEP402NP10-21215		

### *Proteomix SAX-NP Columns*

Phase	ID x Length (mm)	P/N	Phase	ID x Length (mm)	P/N
1.7 $\mu$ m <i>Proteomix</i> SAX-NP1.7	7.8 x 50	SEP403NP2-7805	3 $\mu$ m <i>Proteomix</i> SAX-NP3	7.8 x 50	SEP403NP3-7805
	4.6 x 150	SEP403NP2-4615		4.6 x 150	SEP403NP3-4615
	4.6 x 100	SEP403NP2-4610		4.6 x 100	SEP403NP3-4610
	4.6 x 50	SEP403NP2-4605		4.6 x 50	SEP403NP3-4605
	4.6 x 30	SEP403NP2-4603		4.6 x 30	SEP403NP3-4603
	4.0 x 10 (Guard)	SEP403NP2-4001		4.0 x 10 (Guard)	SEP403NP3-4001
	3.0 x 100	Sep403NP2-3010		3.0 x 100	SEP403NP3-3010
	3.0 x 50	Sep403NP2-3005		3.0 x 50	SEP403NP3-3005
	3.0 x 30	SEP403NP2-3003		3.0 x 30	SEP403NP3-3003
	2.1 x 100	SEP403NP2-2110		2.1 x 100	SEP403NP3-2110
	2.1 x 50	SEP403NP2-2105		2.1 x 50	SEP403NP3-2105
	2.1 x 30	SEP403NP2-2103		2.1 x 30	SEP403NP3-2103
	2.0 x 10 (Guard)	SEP403NP2-2001		2.0 x 10 (Guard)	SEP403NP3-2001
	Precolumn Filter (PEEK)	SEP102000-P356		Precolumn Filter (PEEK)	SEP102000-P356
5 $\mu$ m <i>Proteomix</i> SAX-NP5	10 x 250	SEP403NP5-10025	10 $\mu$ m <i>Proteomix</i> SAX-NP10	10 x 250	SEP403NP10-10025
	7.8 x 250	SEP403NP5-7825		7.8 x 250	Sep403NP10-7825
	7.8 x 100	SEP403NP5-7810		7.8 x 150	Sep403NP10-7815
	7.8 x 50	SEP403NP5-7805		7.8 x 50	SEP403NP10-7805
	4.6 x 250	SEP403NP5-4625		4.6 x 250	SEP403NP10-4625
	4.6 x 150	SEP403NP5-4615		4.6 x 150	SEP403NP10-4615
	4.6 x 100	SEP403NP5-4610		4.6 x 100	SEP403NP10-4610
	4.6 x 50	SEP403NP5-4605		4.6 x 50	SEP403NP10-4605
	4.6 x 30	SEP403NP5-4603		4.6 x 30	SEP403NP10-4603
	4.0 x 10 (Guard)	SEP403NP5-4001		4.0 x 10 (Guard)	SEP403NP10-4001
	2.1 x 150	SEP403NP5-2115		2.1 x 150	SEP403NP10-2115
	2.1 x 100	SEP403NP5-2110		2.1 x 100	SEP403NP10-2110
	2.1 x 50	SEP403NP5-2105		2.1 x 50	SEP403NP10-2105
	2.1 x 30	SEP403NP5-2103		2.1 x 30	SEP403NP10-2103
	2.0 x 10 (Guard)	SEP403NP5-2001		2.0 x 10 (Guard)	SEP403NP10-2001
	Precolumn Filter (PEEK)	SEP102000-P355		Precolumn Filter (PEEK)	SEP102000-P355
	<i>Preparative Columns</i>			<i>Preparative Columns</i>	
21.2 x 250	SEP403NP5-21225	21.2 x 250	SEP403NP10-21225		
21.2 x 150	SEP403NP5-21215	21.2 x 150	SEP403NP10-21215		

## Proteomix WAX-NP Columns

Phase	ID x Length (mm)	P/N	Phase	ID x Length (mm)	P/N
1.7 $\mu$ m <i>Proteomix</i> WAX-NP1.7	7.8 x 50	SEP404NP2-7805	3 $\mu$ m <i>Proteomix</i> WAX-NP3	7.8 x 50	SEP404NP3-7805
	4.6 x 150	SEP404NP2-4615		4.6 x 150	SEP404NP3-4615
	4.6 x 100	SEP404NP2-4610		4.6 x 100	SEP404NP3-4610
	4.6 x 50	SEP404NP2-4605		4.6 x 50	SEP404NP3-4605
	4.6 x 30	SEP404NP2-4603		4.6 x 30	SEP404NP3-4603
	4.0 x 10 (Guard)	SEP404NP2-4001		4.0 x 10 (Guard)	SEP404NP3-4001
	3.0 x 100	SEP404NP2-3010		3.0 x 100	SEP404NP3-3010
	3.0 x 50	SEP404NP2-3005		3.0 x 50	SEP404NP3-3005
	3.0 x 30	SEP404NP2-3003		3.0 x 30	SEP404NP3-3003
	2.1 x 100	SEP404NP2-2110		2.1 x 100	SEP404NP3-2110
	2.1 x 50	SEP404NP2-2105		2.1 x 50	SEP404NP3-2105
	2.1 x 30	SEP404NP2-2103		2.1 x 30	SEP404NP3-2103
	2.0 x 10 (Guard)	SEP404NP2-2001		2.0 x 10 (Guard)	SEP404NP3-2001
	Precolumn Filter (PEEK)	SEP102000-P356		Precolumn Filter (PEEK)	SEP102000-P356
5 $\mu$ m <i>Proteomix</i> WAX-NP5	10 x 250	SEP404NP5-10025	10 $\mu$ m <i>Proteomix</i> WAX-NP10	10 x 250	SEP404NP10-10025
	7.8 x 250	SEP404NP5-7825		7.8 x 250	SEP404NP10-7825
	7.8 x 100	SEP404NP5-7810		7.8 x 150	SEP404NP10-7815
	7.8 x 50	SEP404NP5-7805		7.8 x 50	SEP404NP10-7805
	4.6 x 250	SEP404NP5-4625		4.6 x 250	SEP404NP10-4625
	4.6 x 150	SEP404NP5-4615		4.6 x 150	SEP404NP10-4615
	4.6 x 100	SEP404NP5-4610		4.6 x 100	SEP404NP10-4610
	4.6 x 50	SEP404NP5-4605		4.6 x 50	SEP404NP10-4605
	4.6 x 30	SEP404NP5-4603		4.6 x 30	SEP404NP10-4603
	4.0 x 10 (Guard)	SEP404NP5-4001		4.0 x 10 (Guard)	SEP404NP10-4001
	2.1 x 150	SEP404NP5-2115		2.1 x 150	SEP404NP10-2115
	2.1 x 100	SEP404NP5-2110		2.1 x 100	SEP404NP10-2110
	2.1 x 50	SEP404NP5-2105		2.1 x 50	SEP404NP10-2105
	2.1 x 30	SEP404NP5-2103		2.1 x 30	SEP404NP10-2103
	2.0 x 10 (Guard)	SEP404NP5-2001		2.0 x 10 (Guard)	SEP404NP10-2001
	Precolumn Filter (PEEK)	SEP102000-P355		Precolumn Filter (PEEK)	SEP102000-P355
	<i>Preparative Columns</i>			<i>Preparative Columns</i>	
21.2 x 250	SEP404NP5-21225	21.2 x 250	SEP404NP10-21225		
21.2 x 150	SEP404NP5-21215	21.2 x 150	SEP404NP10-21215		

\* Precolumn Filters comes with 0.5  $\mu$ m PEEK frit for SEP102000-P356 and 2.0  $\mu$ m PEEK frit for SEP102000-P355.

\*\*Other column dimensions and custom-made column dimensions are available.



Precolumn Filter