

# Antibody Separation and Analysis



*Sepax Technologies*

## Antibodix™

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

# Antibodix™ NP Phases

## General Description

Antibodix NP columns are specially designed for high resolution, high efficiency and high recovery separations of antibodies. The packing support is composed of a rigid, spherical, highly cross-linked poly(styrene divinylbenzene) (PS/DVB) non-porous bead. The non-porous resin has particle size of 1.7, 3, 5 and 10  $\mu\text{m}$ . The PS/DVB resin surface is grafted with a highly hydrophilic, neutral polymer thin layer with the thickness in the range of nanometer. On the top of the hydrophilic layer, weak cation-exchange functional groups are attached via a proprietary chemistry, resulting in high capacity ion-exchange layer.

## Chemical Structure of Antibodix Resins

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

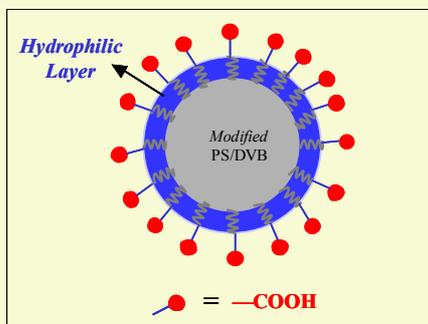
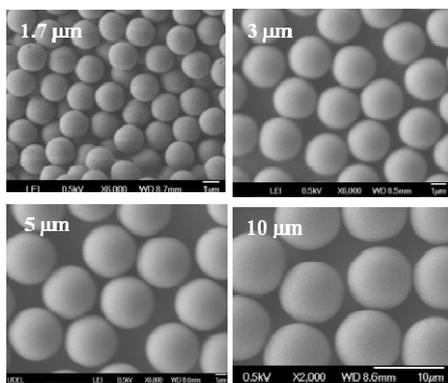


Fig. 1. Schematic illustration of the chemical structure of Antibodix NP phases.



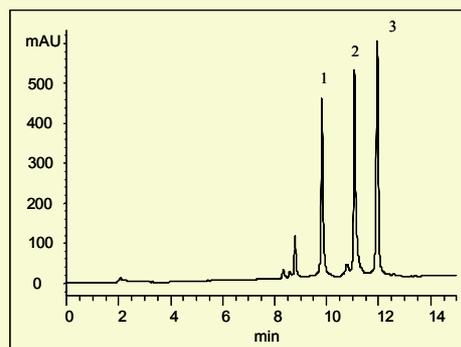
## Highlights of Antibodix NP Resins

- High separation efficiency and resolution
- Particle size selection of 1.7, 3, 5 and 10  $\mu\text{m}$
- Mono-dispersed particles
- Medium capacity
- High pressure tolerance: 4,000, 6,000, 8,000 and 10,000 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 monoclonal antibodies
- 1.7 and 3  $\mu\text{m}$  particles are best suitable for high efficiency separation of proteins and MABs
- Suitable for both analytical and scale-up separations of monoclonal antibodies and other proteins

## High Separation Efficiency

Antibodix NP resins have three unique features: non-porous particle, hydrophilic surface and a uniform layer of ion-exchange functional groups, which enables high efficiency separations. Fig. 2 is an example for separation of three proteins: ribonuclease A, cytochrome C, and lysozyme by Antibodix NP5 column. The average efficiency of three proteins reaches 132,000 of plates.

Fig. 2. Separation of a protein mixture by Antibodix NP5 phase

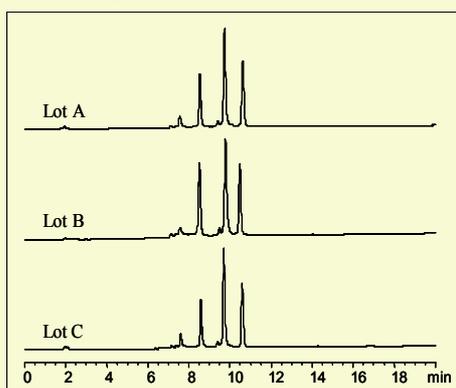


Column: Antibodix-NP5 (5  $\mu\text{m}$ , 4.6x250 mm)  
Mobile phase: A, 10 mM phosphate, pH 6.0  
B, A + 1.0 M NaCl  
Gradient: 10-100%B in 25 min  
Flow rate: 0.7 mL/min  
Sample: 1) Cytochrome C, 2) Lysozyme, 3) Ribonuclease A  
Injection: 5  $\mu\text{L}$  (1 mg/mL for each protein)  
Temperature: 25  $^{\circ}\text{C}$   
Detection: UV 214 nm

### Lot-to-Lot Reproducibility

With well-controlled resin production and the surface chemistry, manufacturing of Antibodix NP resins is highly reproducible. The typical variation of the retention time is less than 5% from batch to batch. One example is shown in Fig. 3 for the production of three lots of Antibodix NP10 resins.

Fig. 3. Reproducibility of three lots of Antibodix NP10 columns



Columns: Antibodix-NP10 (10  $\mu$ m, 4.6x250 mm)  
 Mobile phase: A, 10 mM phosphate, pH 6.0  
                   B, A + 1.0 M NaCl  
 Gradient: 0-100%B in 42 min  
 Flow rate: 1.0 mL/min  
 Sample: 1) Cytochrome C, 2) Lysozyme, 3) Ribonuclease A  
 Injection: 5  $\mu$ L (1 mg/mL for each protein)  
 Temperature: 25  $^{\circ}$ C  
 Detection: UV 214 nm

### Column Dimension Availability

The column dimensions of Antibodix NP 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. We also offer custom-made columns.

### Technical Specifications

Products	Particle size ( $\mu$ m)	Pressure limit (psi)	pH range	Temperature limit ( $^{\circ}$ C)
Antibodix-NP1.7	1.7	10,000	2-12	80
Antibodix-NP3	3	8,000	2-12	80
Antibodix-NP5	5	6,000	2-12	80
Antibodix-NP10	10	4,000	2-12	80

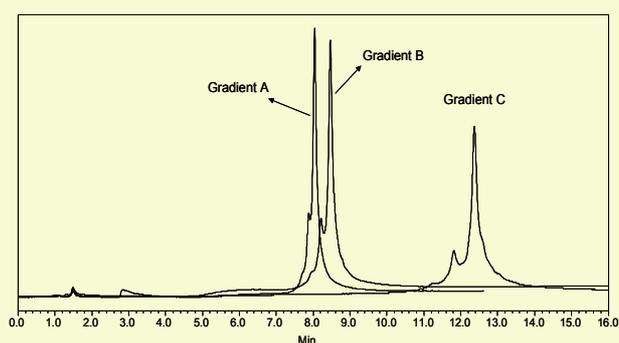
### Applications

Separation and analysis of monoclonal antibodies (MAb), MAb derivatives, modified MAb molecules, other proteins and peptides.

### Separation method development of a commercial monoclonal antibody sample

For a commercial monoclonal antibody sample, the separation conditions are critical for achieving optimized resolution. The key parameters of the separation conditions include salt concentration, pH and salt gradient. Figure 4 shows the separation of a commercial MAb sample, MAb-X22 with the mobile phase of 50 mM phosphate buffer, pH 6.0 at various gradients. Apparently the resolution is poor under those separation conditions.

Fig. 4. Separation of MAb-X22 with non-optimized conditions

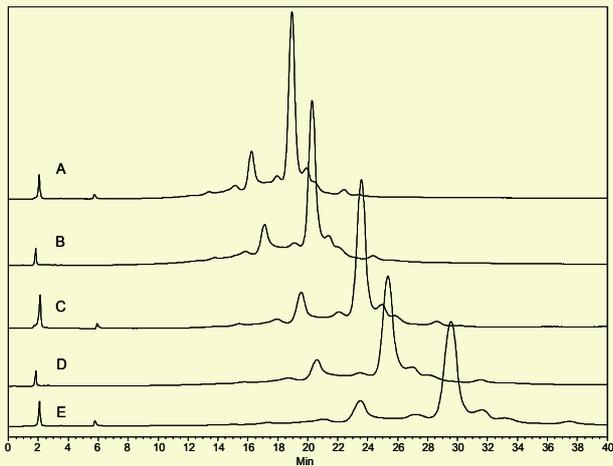


Columns: Antibodix-NP10 (10  $\mu$ m, 4.6x250 mm)  
 Mobile phase: A, 50 mM phosphate, pH 6.0  
                   B, A + 0.25 M NaCl  
 Gradient: A) 0-100%B in 30 min  
               B) 0-100%B in 45 min  
               C) 0-30%B in 30 min  
 Flow rate: 0.8 mL/min  
 Sample: MAb-X22  
 Injection: 10  $\mu$ L (1.5 mg/mL)  
 Temperature: 25  $^{\circ}$ C  
 Detection: UV 214 nm

After we optimized the separation conditions, the resolution of MAb-X22 is much better than that in Fig. 4, as shown in Fig. 5. Further on, we investigated the impact of gradient from shallow to deeper (25% to 60%B for 30 min). The trend is that more resolution with shallower gradient. However, the retention time increases when the gradient becomes shallower.

The initial salt concentration has great impact on the resolution of MAb samples. Fig. 6 shows the separations of MAb-X22 sample with initial salt concentrations at 5, 10 and 20 mM phosphate at pH 7.5. 20mM phosphate salt resulted in poor resolution, indicating that the initial salt concentration is very sensitive for resolving the fine structures of the MAb samples.

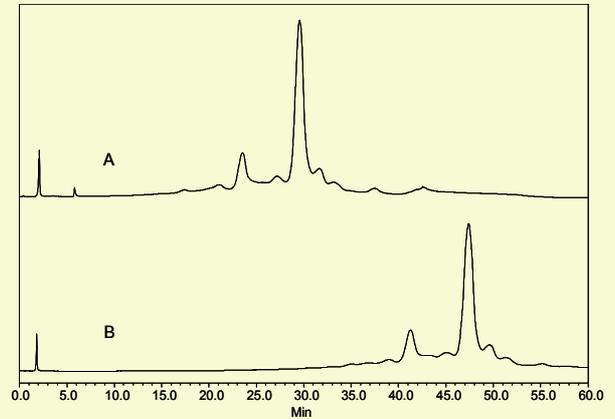
Fig. 5. Separation of MAb-X22 with optimized conditions



Columns: Antibodix-NP10 (10  $\mu$ m, 4.6x250 mm)  
 Mobile phase: A, 10 mM phosphate, pH 7.5  
 B, A + 0.1M NaCl  
 Gradient: A) 15-75%B in 30 min  
 B) 15-65%B in 30 min  
 C) 15-55%B in 30 min  
 D) 15-47.5%B in 30 min  
 E) 15-40%B in 30 min and 40%B after 30min  
 Flow rate: 0.8 mL/min  
 Sample: MAb-X22  
 Injection: 10  $\mu$ L (1.5 mg/mL)  
 Temperature: 25  $^{\circ}$ C  
 Detection: UV 214 nm

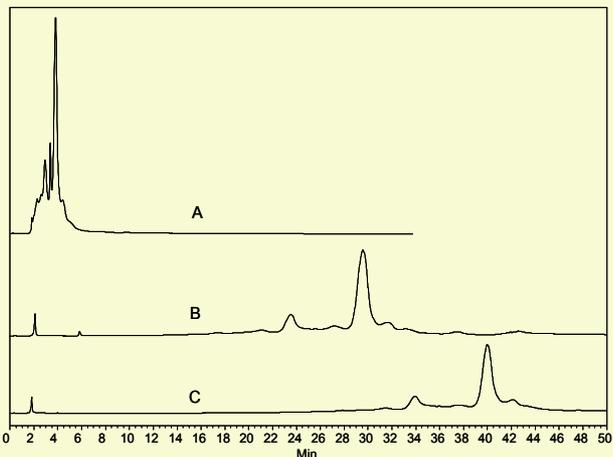
Fig. 7 shows the impact of pH on the separation of MAbs. When pH decreased from 7.5 to 7.0, the resolution of the basic compound from the main component had some improvement with the compromise of longer retention time.

Fig. 7. The impact of mobile phase pH on MAb-X22 separation



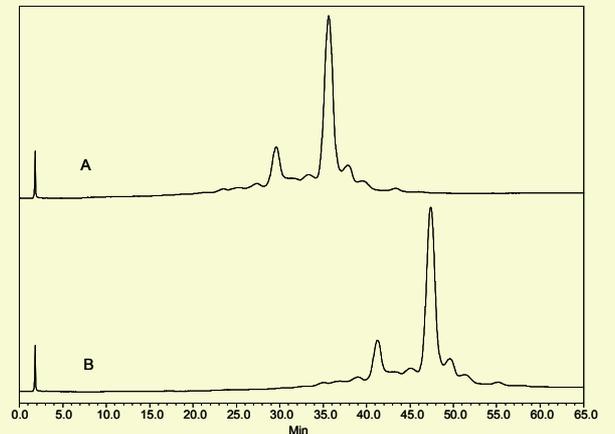
Columns: Antibodix-NP10 (10  $\mu$ m, 4.6x250 mm)  
 Mobile phase: A, 10 mM phosphate  
 B, A + 0.1M NaCl  
 pH: A) pH 7.5; B) pH 7.0  
 Gradient: 15-65%B in 60 min  
 Flow rate: 0.8 mL/min  
 Sample: MAb-X22  
 Injection: 10  $\mu$ L (1.5 mg/mL)  
 Temperature: 25  $^{\circ}$ C  
 Detection: UV 214 nm

Fig. 6. The Impact of initial salt on the separation of MAb-X22



Columns: Antibodix-NP10 (10  $\mu$ m, 4.6x250 mm)  
 Mobile phase: A, Phosphate buffer, pH 7.5  
 B, A + 0.1M NaCl  
 Initial salt: A/B/C=20/10/5mM phosphate  
 Gradient: 15-65%B in 60 min  
 Flow rate: 0.8 mL/min  
 Sample: MAb-X22  
 Injection: 10  $\mu$ L (1.5 mg/mL)  
 Temperature: 25  $^{\circ}$ C  
 Detection: UV 214 nm

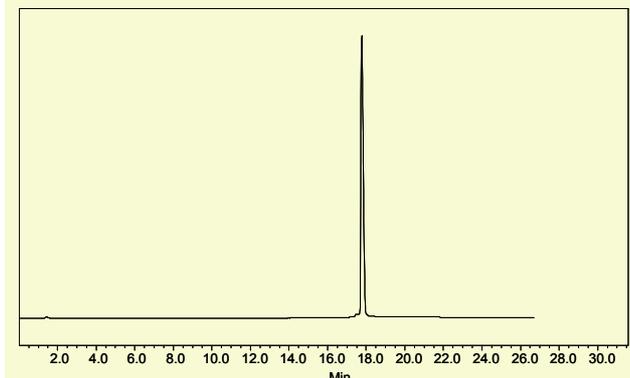
Fig. 8. Separation of MAb-X22 with various gradients at pH 7



Columns: Antibodix-NP10 (10  $\mu$ m, 4.6x250 mm)  
 Mobile phase: A, 10 mM phosphate, pH 7.0  
 B, A + 0.1M NaCl  
 Gradient: A) 25-75%B in 60 min  
 B) 15-65%B in 60 min  
 Flow rate: 0.8 mL/min  
 Sample: MAb-X22  
 Injection: 10  $\mu$ L (1.5 mg/mL)  
 Temperature: 25  $^{\circ}$ C  
 Detection: UV 214 nm

## Quality control of a peptide drug molecule

Fig. 9. Analysis of a commercial peptide drug molecule



Columns: Antibodix-NP10 (10  $\mu$ m, 4.6x250 mm)  
 Mobile phase: A, 3.0 mM phosphate, pH=5.0  
 B, 20 mM phosphate, pH=7.5  
 Gradient: 0-50%B in 20 min  
 Flow rate: 1.0 mL/min  
 Sample: A peptide from a drug company (MW~4,000)  
 Injection: 5  $\mu$ L (2.0 mg/mL)  
 Temperature: 25  $^{\circ}$ C  
 Detection: UV 214 nm

## Product Information of Antibodix NP columns

Phase	ID x Length (mm)	P/N	Column Material	Phase	ID x Length (mm)	P/N	Column Material		
Antibodix NP1.7 (1.7 $\mu$ m)	7.8 x 75	SEP602NP2-7807	SS	Antibodix NP3 (3 $\mu$ m)	7.8 x 75	SEP602NP3-7875	SS		
	7.8 x 50	SEP602NP2-7805	SS		7.8 x 50	SEP602NP3-7805	SS		
	4.6 x 100	SEP602NP2-4610	SS		4.6 x 150	SEP602NP3-4615	SS		
	4.6 x 50	SEP602NP2-4605	SS		4.6 x 50	SEP602NP3-4605	SS		
	4.0 x 10 (Guard)	SEP602NP2-4001C	SS		4.0 x 10 (Guard)	SEP602NP3-4001C	SS		
	3.0 x 50	SEP602NP2-3005	SS		3.0 x 50	SEP602NP3-3005	SS		
	2.1 x 50	SEP602NP2-2105	SS		2.1 x 50	SEP602NP3-2105	SS		
	2.0 x 10 (Guard)	SEP602NP2-2001C	SS		2.0 x 10 (Guard)	SEP602NP3-2001C	SS		
	4.6 x 50	SEP602NP2P-4605	PEEK		4.6 x 50	SEP602NP3P-4605	PEEK		
	Precolumn Filter**	SEP102000-P356	PEEK		Precolumn Filter	SEP102000-P356	PEEK		
Antibodix NP5 (5 $\mu$ m)	10.0x250	SEP602NP5-10025	SS	Antibodix NP10 (10 $\mu$ m)	10.0x250	SEP602NP10-10025	SS		
	7.8 x 150	SEP602NP5-7815	SS		7.8 x 150	SEP602NP10-7815	SS		
	7.8 x 75	SEP602NP5-7807	SS		7.8 x 75	SEP602NP10-7807	SS		
	4.6 x 250	SEP602NP5-4625	SS		4.6 x 250	SEP602NP10-4625	SS		
	4.6 x 50	SEP602NP5-4605	SS		4.6 x 50	SEP602NP10-4605	SS		
	4.0 x 10 (Guard)	SEP602NP5-4001C	SS		4.0 x 10 (Guard)	SEP602NP10-4001C	SS		
	2.1 x 150	SEP602NP5-2115	SS		2.1 x 250	SEP602NP10-2115	SS		
	2.1 x 50	SEP602NP5-2105	SS		2.1 x 50	SEP602NP10-2105	SS		
	2.0 x 10 (Guard)	SEP602NP5-2001C	SS		2.0 x 10 (Guard)	SEP602NP10-2001C	SS		
	4.6 x 250	SEP602NP5P-4625	PEEK		4.6 x 250	SEP602NP10P-4625	PEEK		
	4.6 x 50	SEP602NP5P-4605	PEEK		4.6 x 50	SEP602NP10P-4605	PEEK		
	Precolumn Filter	SEP102000-P355	PEEK		Precolumn Filter	SEP102000-P355	PEEK		
	<b>Semi-prep and preparative columns</b>				<b>Semi-prep and preparative columns</b>				
	21.2 x 250	SEP602NP5-21225	SS		21.2 x 250	SEP602NP10-21225	SS		
21.2 x 150	SEP602NP5-21215	SS	21.2 x 150	SEP602NP10-21215	SS				

\* SS means Stainless steel.

\*\* 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

## *Ordering Information*

### *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)

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