

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, bio-surfaces 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 μm to 100 μm and pore size from non-porous to 2000 \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[®], SRT[®]-C

Nanofilm[®]

Zenix[™], Zenix[™]-C

Ion-exchange

Proteomix[®]

Glycomix[™]

Antibody Separation

Antibodix[™]

Carbohydrate Separation

Carbomix[®]

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 μm . The PS/DVB resin surface is grafted with a highly hydrophilic, neutral polymer thin layer with the thickness in the range of a 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.

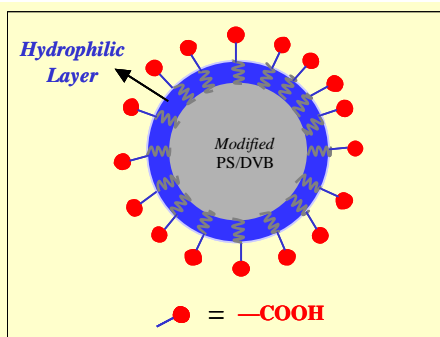
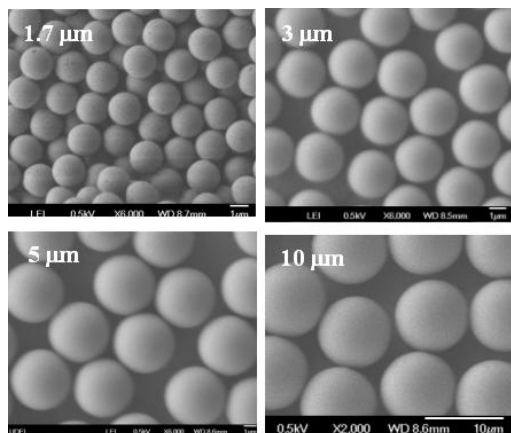


Figure 1. Schematic illustration of the chemical structure of Antibodix NP phases.



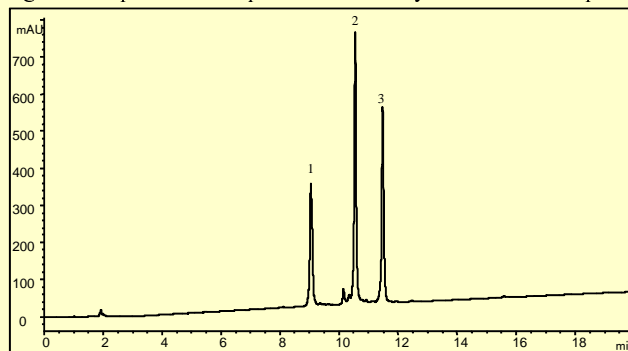
Highlights of Antibodix NP Resins

- High separation efficiency and resolution
- Particle size selection of 1.7, 3, 5 and 10 μm
- Mono-dispersed particles
- Medium capacity
- High pressure tolerance: 4,000, 6,000, 8,000 and 12,000 psi for 10, 5, 3 and 1.7 μm resins, respectively
- Wide pH range: 2-12
- High resolving power for slightly differed structures of monoclonal antibodies
- 1.7 and 3 μm 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 particles, a hydrophilic surface, and a uniform layer of ion-exchange functional groups, which enables high efficiency separations. Figure 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.

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



Column: Antibodix-NP5 (5 μm , 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: 0.8 mL/min
Sample: 1) Aprotinin, 2) Lysozyme, 3)
 Ribonuclease A
Injection: 5 μL (1 mg/mL for each protein)
Temperature: 25 $^{\circ}\text{C}$
Detection: UV 214 nm

Lot-to-Lot Reproducibility

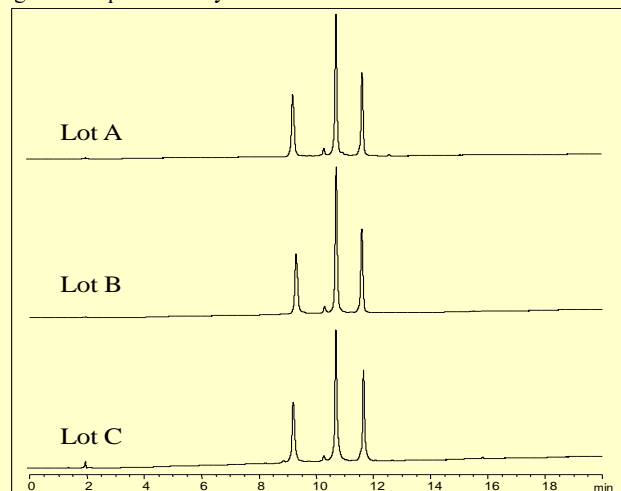
With well-controlled resin production and surface chemistry, manufacturing of Antibodix NP resins is highly reproducible. The typical variation of the retention time is less than 5% from batch to batch. One example is shown in Figure 3 for the production of three lots of Antibodix NP5 resins.

Chemical 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.



Figure 3. Reproducibility of three lots of Antibodix NP10 columns



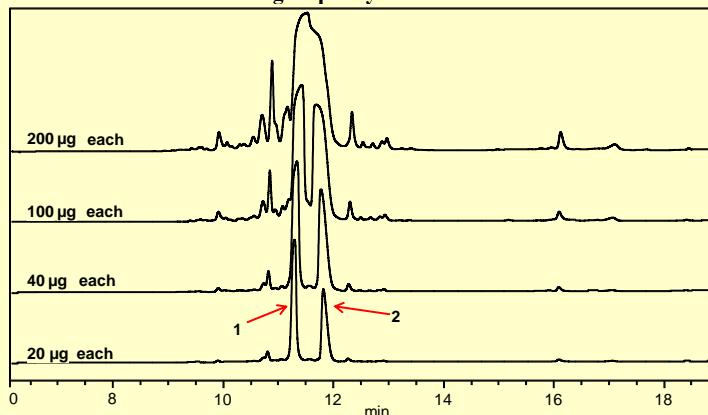
Column: Antibodix-NP5 (5 μ m, 4.6x250 mm)
 Mobile phase: A, 10 mM phosphate, pH 6.0
 B, A + 1.0 M NaCl
 Gradient: 10-100%B in 25 min
 Flow rate: 0.8 mL/min
 Sample: Aprotinin, Lysozyme, and Ribonuclease A
 Injection: 5 μ L (1 mg/mL for each protein)
 Temperature: 25 $^{\circ}$ C
 Detection: UV 214 nm

Technical Specifications

Products	Particle size (μ m)	Pressure limit (psi)	pH range	Temperature limit ($^{\circ}$ C)	DBC* (mg/mL)
Antibodix-NP1.7	1.7	12,000	2-12	80	26.53 \pm 0.41
Antibodix-NP3	3	8,000	2-12	80	19.50 \pm 0.74
Antibodix-NP5	5	6,000	2-12	80	13.41 \pm 0.17
Antibodix-NP10	10	4,000	2-12	80	8.41 \pm 0.40

Dynamic binding capacity tests conditions: Sample: 3.0 mg/mL Lysozyme in 20 mM sodium phosphate buffer, pH 6.0; Flow rate: 0.5 mL/min (0.25 mL/min for 1.7 μ m); Detection: UV 254 nm. Test on 5 different resin lots

Antibodix WCX NP5 Loading Capacity Test



Columns: Antibodix WCX NP5 (5 μ m, 4.6x250 mm)
 Mobile phase: A, 20 mM phosphate, pH 6
 B, A + 1 M NaCl
 Gradient: 0-100%B in 25 minutes
 Flow rate: 0.8 mL/min
 Sample: 1. Lysozyme
 2. Ribonuclease A
 Detection: UV 214 nm

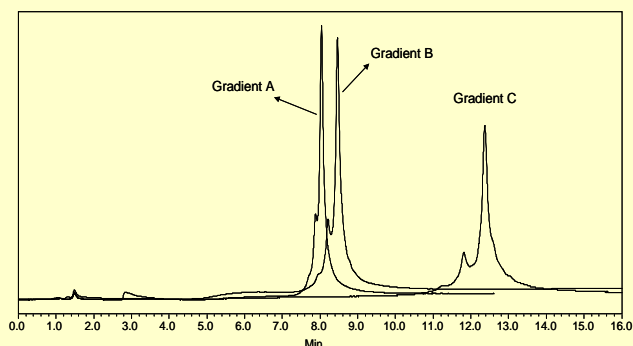
Applications

Separation & Analysis
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.

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

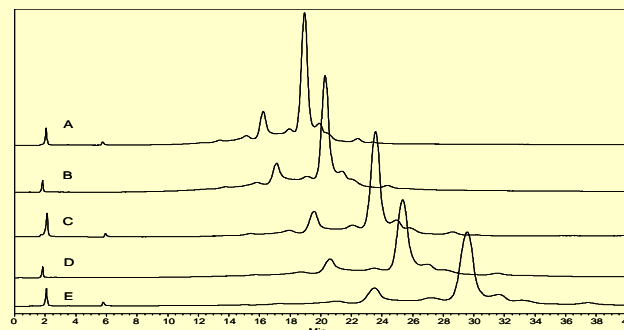


Columns: Antibodix-NP10 (10 μ m, 4.6x250 mm)
 Mobile phase: A, 50 mM phosphate, pH 6.0
 B, A + 0.25 M NaCl
 Gradient: A) 0-100%B in 30 min
 B) 0-100%B in 45 min
 C) 0-30%B in 30 min
 Flow rate: 0.8 mL/min
 Sample: MAb-X22
 Injection: 10 μ L (1.5 mg/mL)
 Temperature: 25 $^{\circ}$ C
 Detection: UV 214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

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

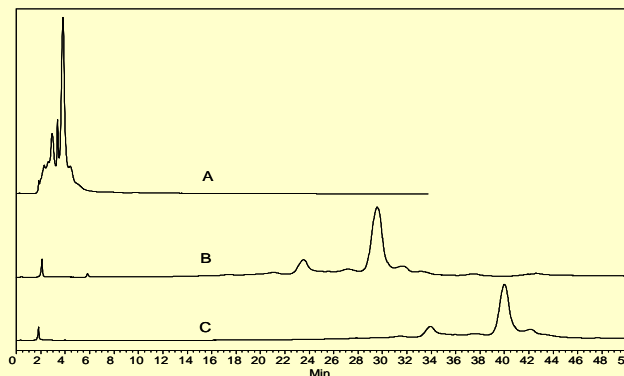
The initial salt concentration has great impact on the resolution of MAb samples. Figure 6 shows the separations of MAb-X22 sample with initial salt concentrations at 5, 10 and 20 mM phosphate at pH 7.5. 20 mM phosphate salt resulted in poor resolution, indicating that the initial salt concentration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

Figure 5. Separation of MAb-X22 with optimized conditions



Columns: Antibodix-NP10 (10 μ m, 4.6x250 mm)
 Mobile phase: A, 10 mM phosphate, pH 7.5
 B, A + 0.1 M 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 μ L (1.5 mg/mL)
 Temperature: 25 $^{\circ}$ C
 Detection: UV 214 nm

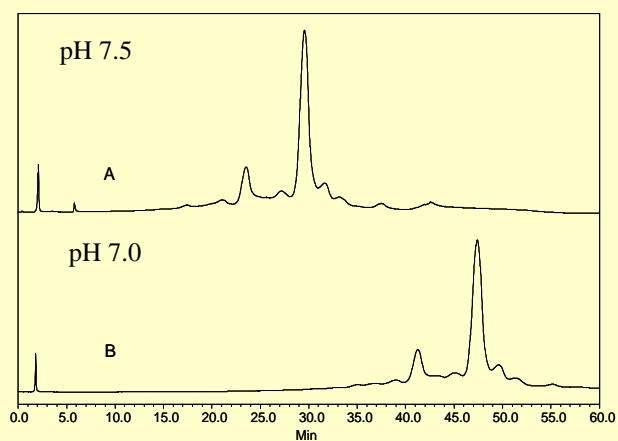
Figure 6. The Impact of initial salt on the separation of MAb-X22



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

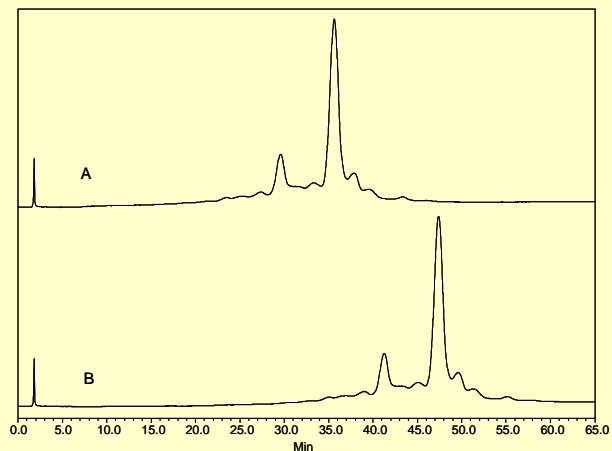
Figure 7 shows the impact of pH on the separation of MABs. When pH decreased from 7.5 to 7.0, the resolution of the basic compound from the main component had some improvement with the compromise of longer retention time.

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



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

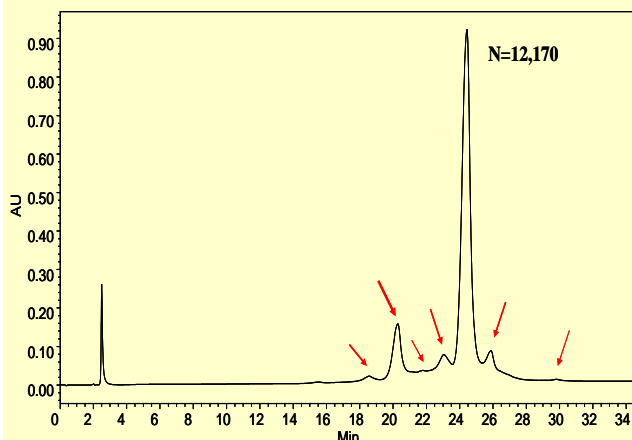
Figure 8. Separation of MAb-X22 with various gradients at pH 7



Columns: Antibodix-NP10 (10 μ m, 4.6x250 mm)
 Mobile phase: A, 10 mM phosphate, pH 7.0
 B, A + 0.1 M 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 μ L (1.5 mg/mL)
 Temperature: 25 $^{\circ}$ C
 Detection: UV 214 nm

Figure 9 presents a separation profile of the monoclonal antibody. The main peak has a plate number of 12,170.

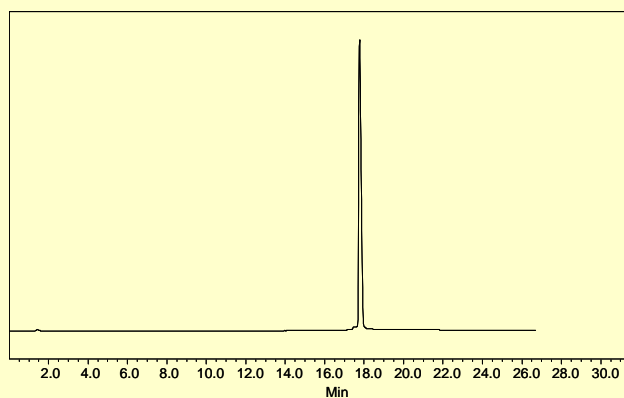
Figure 9. Separation of a monoclonal antibody



Column: Antibodix NP5 (5 μ m, 4.6x250mm)
 Mobile Phase: A, 10 mM Phosphate, pH 7.5;
 B, A + 100 mM NaCl
 Gradient: 15-55% B (30 min); Flow rate: 0.8 mL/min
 Detection: UV 214 nm
 Sample: Monoclonal antibody (2.5 mg/mL);
 Injection: 10 μ L

Quality control of a peptide drug molecule

Figure 10. Analysis of a commercial peptide drug molecule



Columns: Antibodix-NP10 (10 μ 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 μ L (2.0 mg/mL)
 Temperature: 25 $^{\circ}$ C
 Detection: UV 214 nm

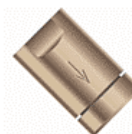
Product Information for Antibodix NP Phase

Phase	ID x Length (mm)	P/N	Column Material	Phase	ID x Length (mm)	P/N	Column Material
Antibodix NP1.7 (1.7 µm)	7.8 x 75	602NP2-7807	SS*	Antibodix NP3 (3 µm)	7.8 x 75	602NP3-7875	SS
	7.8 x 50	602NP2-7805	SS		7.8 x 50	602NP3-7805	SS
	4.6 x 100	602NP2-4610	SS/PEEK		4.6 x 150	602NP3-4615	SS/PEEK
	4.6 x 50	602NP2-4605	SS/PEEK		4.6 x 50	602NP3-4605	SS/PEEK
	4.0 x 10 (Guard)	602NP2-4001C	SS/PEEK		4.0 x 10 (Guard)	602NP3-4001C	SS/PEEK
	2.1 x 50	602NP2-2105	SS/PEEK		2.1 x 50	602NP3-2105	SS/PEEK
	2.0 x 10 (Guard)	602NP2-2001C	SS/PEEK		2.0 x 10 (Guard)	602NP3-2001C	SS/PEEK
	4.6 x 50	602NP2P-4605	SS/PEEK		4.6 x 50	602NP3P-4605	SS/PEEK
	Precolumn Filter**	102000-P356	SS/PEEK		Precolumn Filter	102000-P356	SS/PEEK
Antibodix NP5 (5 µm)	10.0x250	602NP5-10025	SS	Antibodix NP10 (10 µm)	10.0x250	602NP10-10025	SS
	7.8 x 150	602NP5-7815	SS		7.8 x 150	602NP10-7815	SS
	7.8 x 75	602NP5-7807	SS		7.8 x 75	602NP10-7807	SS
	4.6 x 250	602NP5-4625	SS/PEEK		4.6 x 250	602NP10-4625	SS/PEEK
	4.6 x 50	602NP5-4605	SS/PEEK		4.6 x 50	602NP10-4605	SS/PEEK
	4.0 x 10 (Guard)	602NP5-4001C	SS/PEEK		4.0 x 10 (Guard)	602NP10-4001C	SS/PEEK
	2.1 x 150	602NP5-2115	SS/PEEK		2.1 x 250	602NP10-2115	SS/PEEK
	2.1 x 50	602NP5-2105	SS/PEEK		2.1 x 50	602NP10-2105	SS/PEEK
	2.0 x 10 (Guard)	602NP5-2001C	SS/PEEK		2.0 x 10 (Guard)	602NP10-2001C	SS/PEEK
	4.6 x 250	602NP5P-4625	SS/PEEK		4.6 x 250	602NP10P-4625	SS/PEEK
	4.6 x 50	602NP5P-4605	SS/PEEK		4.6 x 50	602NP10P-4605	SS/PEEK
	Precolumn Filter	102000-P355	SS/PEEK		Precolumn Filter	102000-P355	SS/PEEK
	Semi-prep and preparative columns				Semi-prep and preparative columns		
	21.2 x 250	602NP5-21225	SS		21.2 x 250	602NP10-21225	SS
	21.2 x 150	602NP5-21215	SS		21.2 x 150	602NP10-21215	SS

* SS means Stainless steel.

** Precolumn Filters comes with 0.5 µm PEEK frit for 102000-P356 and 2.0 µm PEEK frit for 102000-P355.

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



Precolumn Filter