

Antibody Separation and Analysis



Sepax Technologies

Antibodix™

Better Surface Chemistry for Better Separation

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 nanometer. On the top of the hydrophilic layer, weak anion-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.

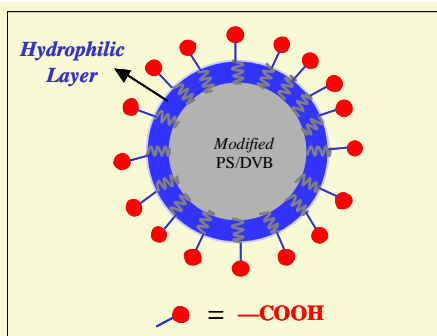
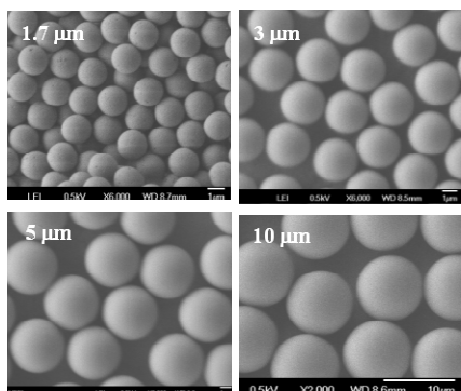


Fig. 1. Schematic illustration of the chemical structure of Antibodix NP phases.



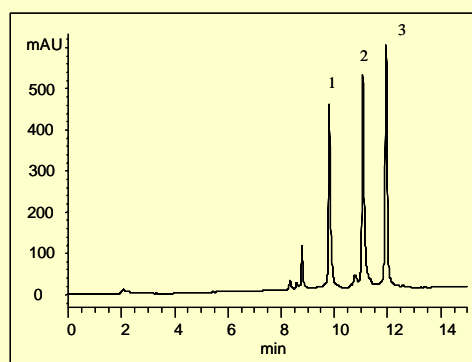
Highlights of Antibodix NP Resins

- High separation efficiency and resolution
- Particle size selection of 1.7, 3, 5 and 10 μm
- 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 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 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 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: 0.7 mL/min
Sample: 1) Cytochrome C, 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 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.

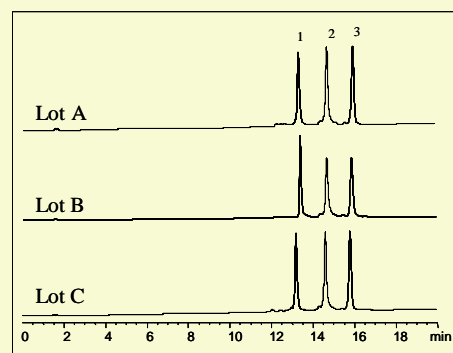
Column Dimension Availability

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 (µm)	Pressure limit (psi)	pH range	Temperature limit (°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

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



Columns: Antibodix-NP10 (10 µm, 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 µL (1 mg/mL for each protein)
 Temperature: 25 °C
 Detection: UV 214 nm

Product Information of Antibodix NP columns

Phase	ID x Length (mm)	P/N	Note	Phase	ID x Length (mm)	P/N	Note
Antibodix NP1.7 (1.7 µm)	7.8 x 75	602NP2-7807	*	Antibodix NP3 (3 µm)	7.8 x 100	602NP3-7810	*
	7.8 x 50	602NP2-7805	*		7.8 x 50	602NP3-7805	*
	4.6 x 150	602NP2-4615			4.6 x 150	602NP3-4615	
	4.6 x 100	602NP2-4610			4.6 x 100	602NP3-4610	
	4.6 x 50	602NP2-4605			4.6 x 50	602NP3-4605	
	4.0 x 10 (Guard)	602NP2-4001			4.0 x 10 (Guard)	602NP3-4001	
	3.0 x 100	602NP2-3010			3.0 x 100	602NP3-3010	
	3.0 x 50	602NP2-3005			3.0 x 50	602NP3-3005	
	2.1 x 50	602NP2-2105			2.1 x 100	602NP3-2110	
	2.1 x 30	602NP2-2103			2.1 x 50	602NP3-2105	
2.0 x 10 (Guard)	602NP2-2001		2.0 x 10 (Guard)	602NP3-2001			
Antibodix NP5 (5 µm)	10 x 250	602NP5-10025		Antibodix NP10 (10 µm)	10 x 250	602NP10-10025	
	7.8 x 150	602NP5-7815	*		7.8 x 250	602NP10-7825	
	7.8 x 100	602NP5-7810	*		7.8 x 150	602NP10-7815	*
	7.8 x 50	602NP5-7805			7.8 x 50	602NP10-7805	
	4.6 x 250	602NP5-4625			4.6 x 250	602NP10-4625	*
	4.6 x 150	602NP5-4615			4.6 x 150	602NP10-4615	
	4.6 x 100	602NP5-4610			4.6 x 100	602NP10-4610	
	4.6 x 50	602NP5-4605			4.6 x 50	602NP10-4605	
	4.0 x 10 (Guard)	602NP5-4001			4.0 x 10 (Guard)	602NP10-4001	
	2.1 x 150	602NP5-2115			2.1 x 150	602NP10-2115	
	2.1 x 100	602NP5-2110			2.1 x 100	602NP10-2110	
	2.1 x 50	602NP5-2105			2.1 x 50	602NP10-2105	
	2.0 x 10 (Guard)	602NP5-2001			2.0 x 10 (Guard)	602NP10-2001	
<i>Preparative Columns</i>				<i>Preparative Columns</i>			
21.2 x 250	602NP5-21225		21.2 x 250	602NP10-21225			
21.2 x 150	602NP5-21215		21.2 x 150	602NP10-21215			

* Most suitable column dimensions for separation of monoclonal antibodies



Sepax Technologies

Sepax Technologies, Inc.
5 Innovation Way, Newark, Delaware 19711, USA
Tel: (302) 366-1101
Fax: (302) 366-1151
www.sepax-tech.com