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## **New in Bio-separation by Developing High Capacity Sub-2 Micron Non-Porous Ion-Exchange Resins**

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5 Innovation Way, Newark, Delaware 19711**



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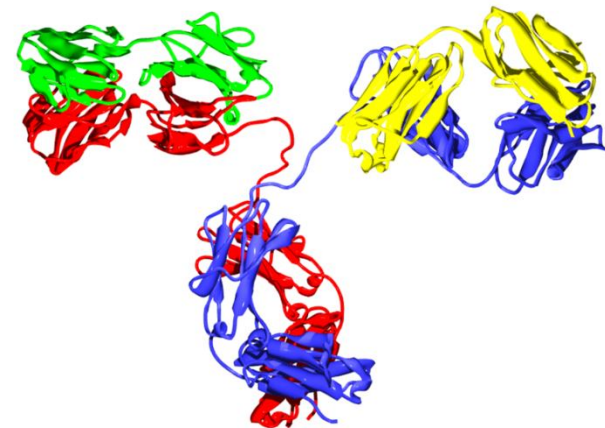
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# Antibodix™ WCX Packings for Monoclonal Antibody Separation

Antibodix™ WCX resins are recently developed non-porous PS/DVB packing materials for ion-exchange chromatography, specifically for monoclonal antibody separation. Their particle sizes are controlled at 1.7, 3, 5 and 10 µm. Those PS/DVB particles are surface modified with a proprietary technology that has negligible non-specific interactions with monoclonal antibodies. The smaller particle size offered higher resolving power for the variants of monoclonal antibodies.

## Applications:

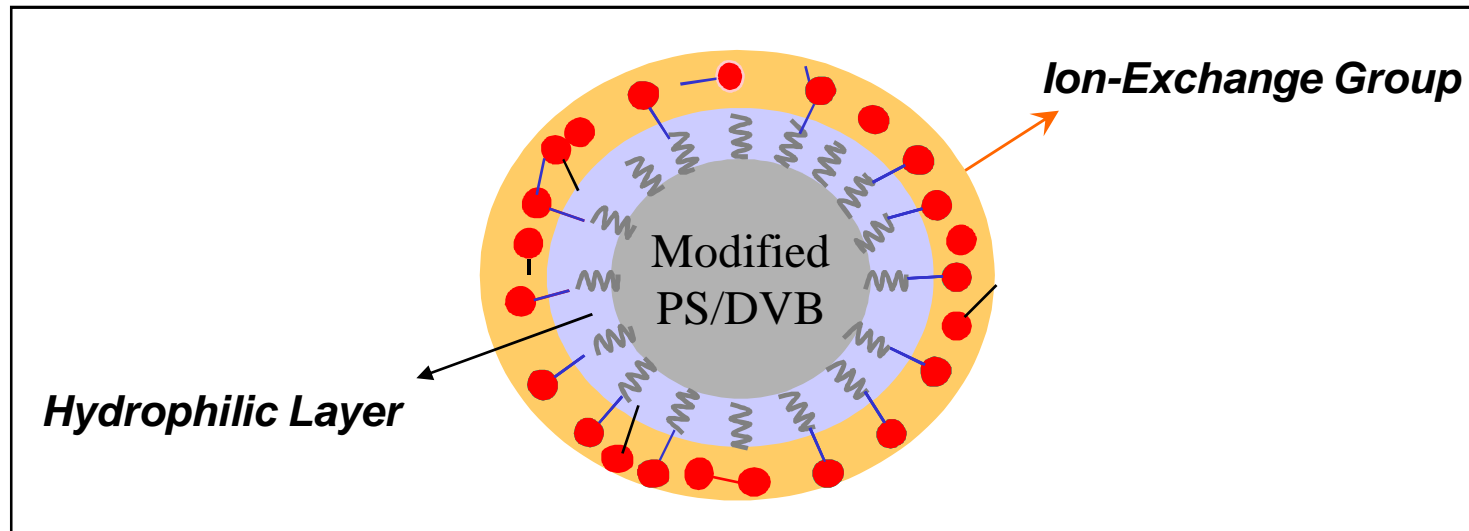
- Monoclonal antibodies (MAb)
- PEGylated Mab
- Peptide modified and other MAb derivatives



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# Antibodix Phase – Chemical Structure



Structure	Function
Highly Cross-linked Non-porous PS/DVB	<ul style="list-style-type: none"> <li>• <i>Rigid support to tolerate high pressure</i></li> <li>• <i>Minimize the diffusion</i></li> </ul>
Hydrophilic Coating	<i>Eliminate non-specific interaction</i>
Ion-exchange Layer	<ul style="list-style-type: none"> <li>• <i>Increased capacity</i></li> <li>• <i>Controllable capacity</i></li> <li>• <i>Tunable hydrophobicity/hydrophilicity</i></li> </ul>

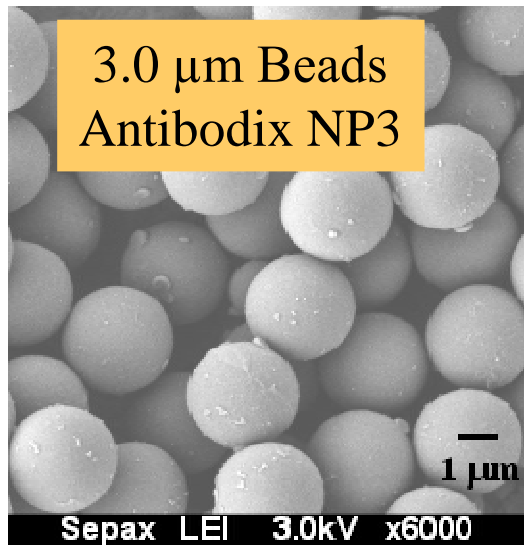
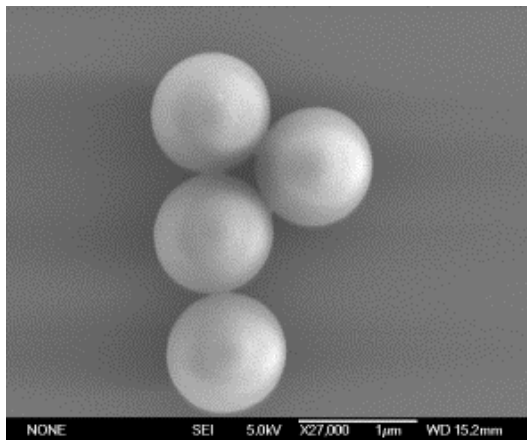


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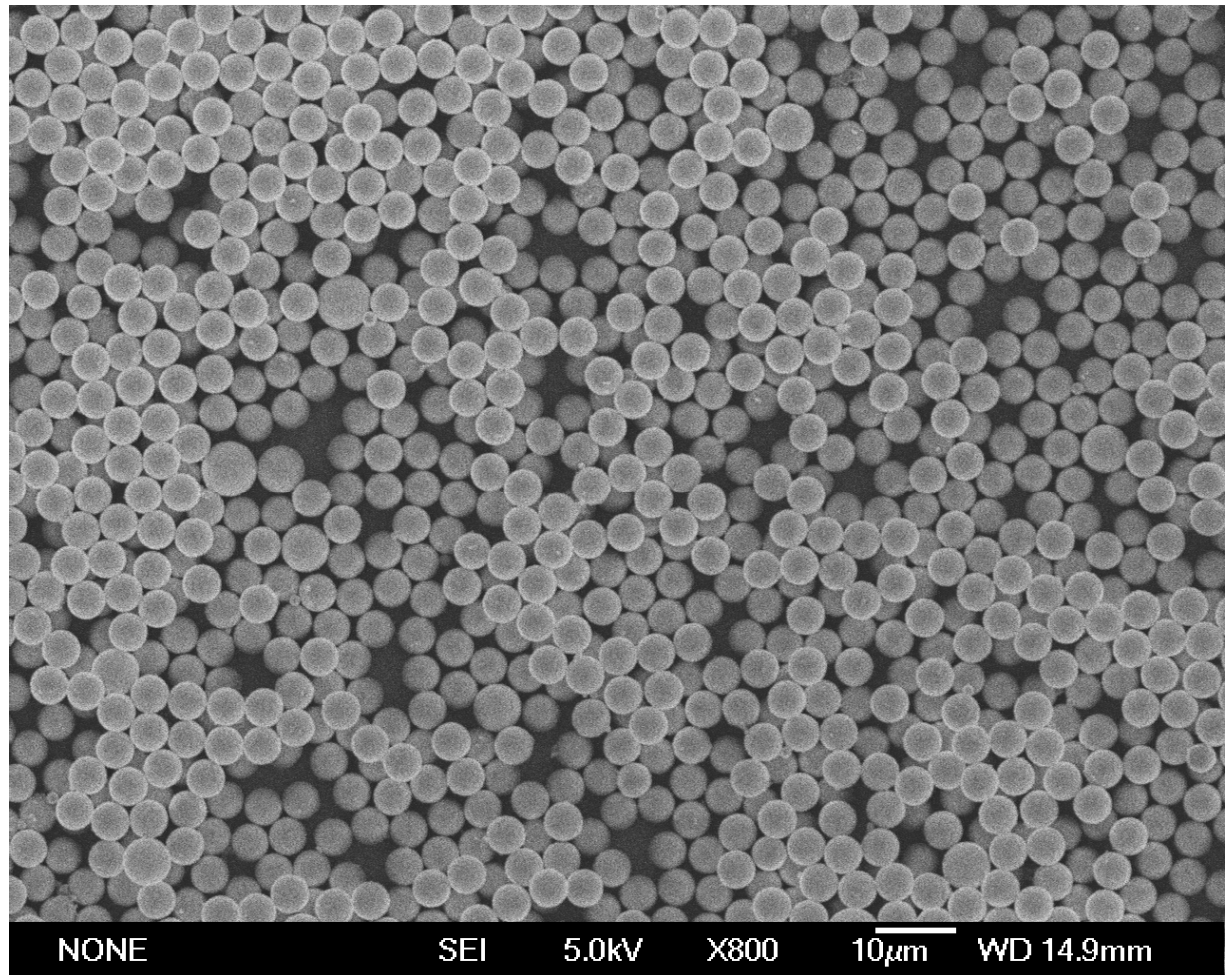
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# Narrow-dispersed Ion-exchange Particles

Sub-2 $\mu\text{m}$  Non-Porous  
PS/DVB Beads



5.0  $\mu\text{m}$  PS/DVB Non-Porous Particles



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# Antibodix™ NP10 Columns - Lot-to-Lot Reproducibility

Columns: Antibodix-NP10 (10  $\mu$ m, 4.6x250 mm)

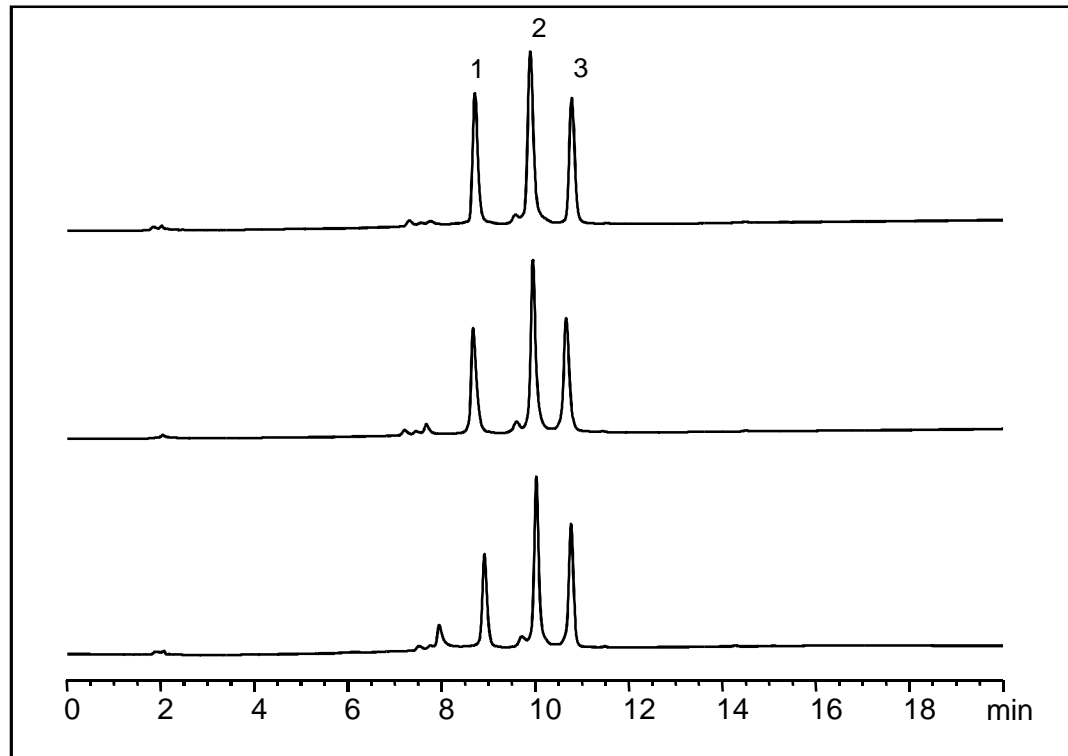
Mobile phase: A, 10 mM phosphate, pH 6.0; B, A + 1.0 M NaCl

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



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# Antibodix™ NP10 Columns - Lot-to-Lot Reproducibility

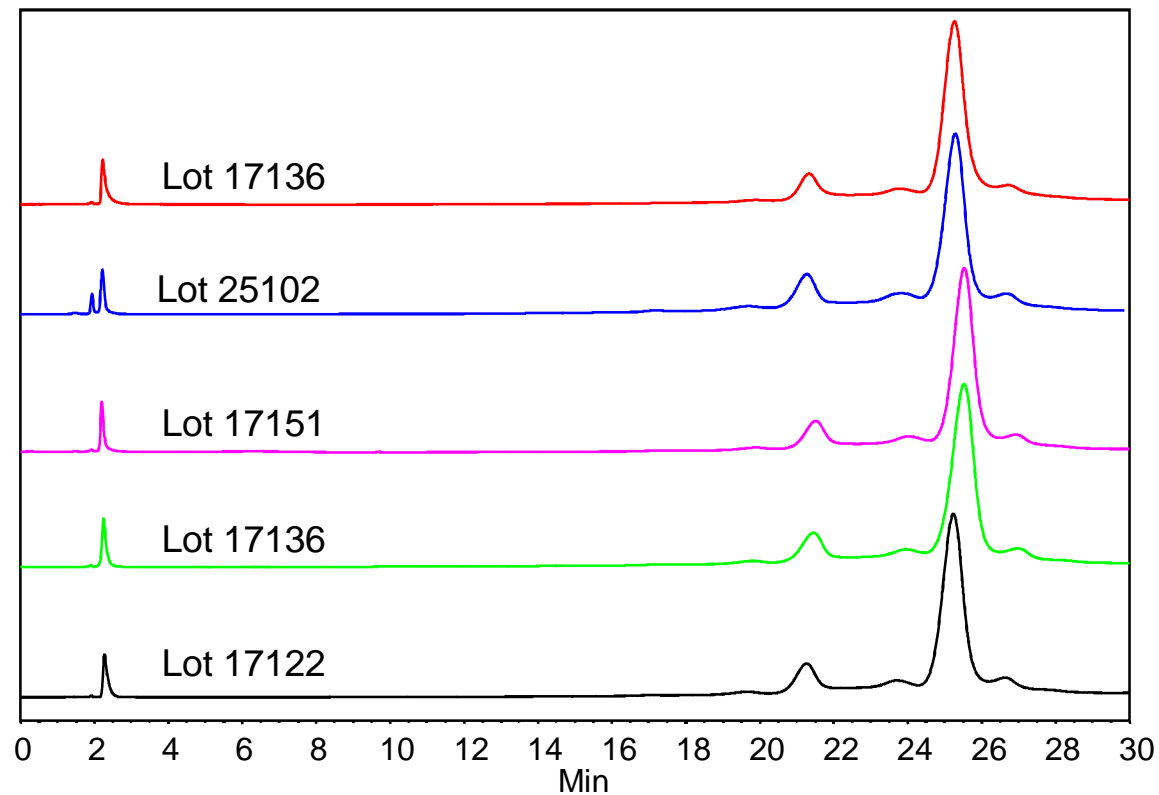
Column: Antibodix NP10 (10 $\mu$ m, 4.6x250mm)

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; Temperature: Ambient

Sample: Monoclonal antibody (5 mg/mL); Injection: 5  $\mu$ L



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# Comparison of WCX Columns for Antibody Separation

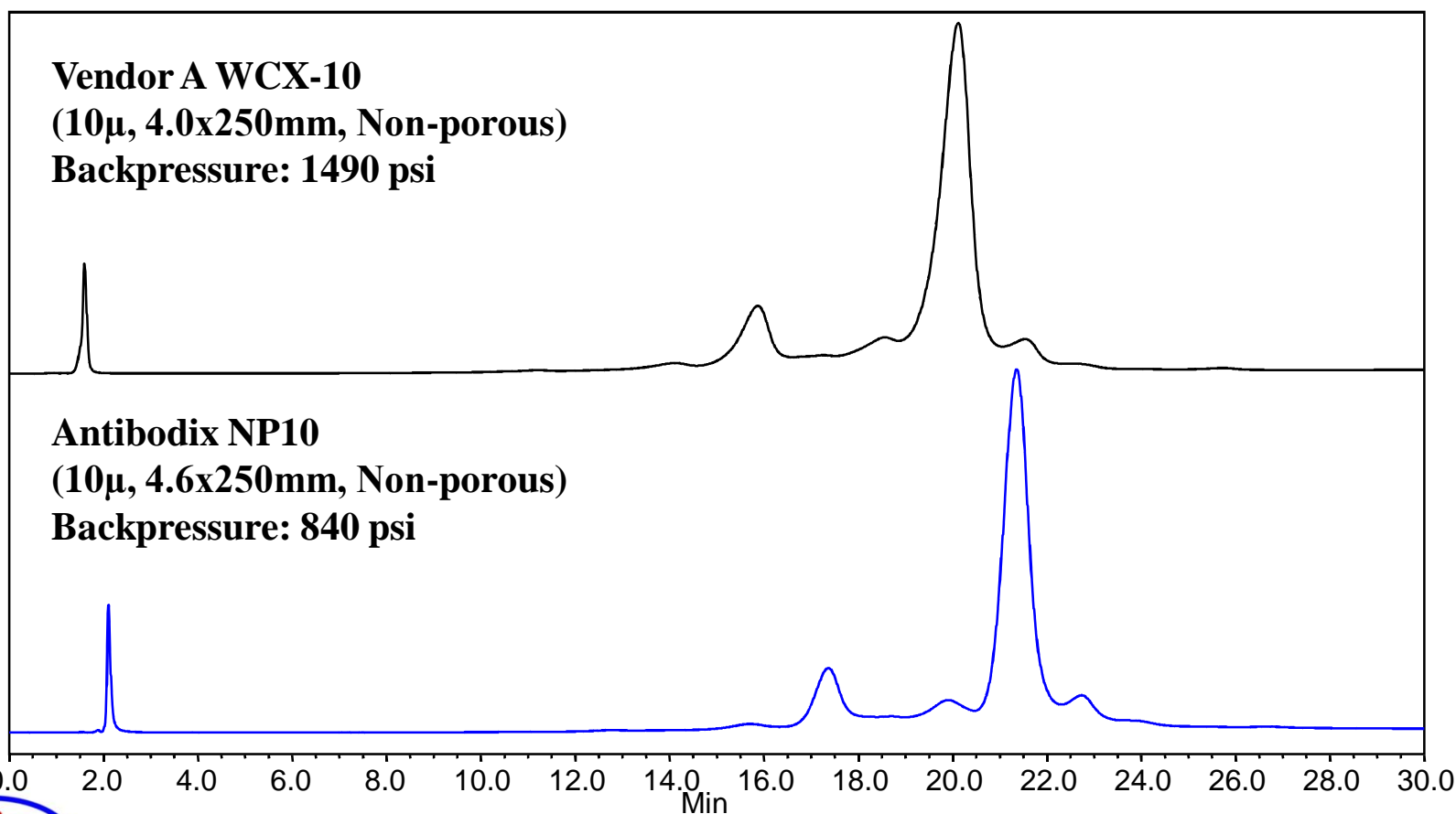
Mobile phase: A, 10 mM Phosphate, pH 7.5; B, A + 100 mM NaCl

Gradient: 15-55% B (30 min)

Flow rate: 0.8 mL/min; Detection: UV 214 nm

Sample: Monoclonal antibody (5 mg/mL); Injection: 5  $\mu$ L

Temperature: Ambient



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# Antibodix NP1.7 Column for MAb Separation

Column: Antibodix NP1.7 (**1.7 $\mu$ m, 4.6x50mm**)

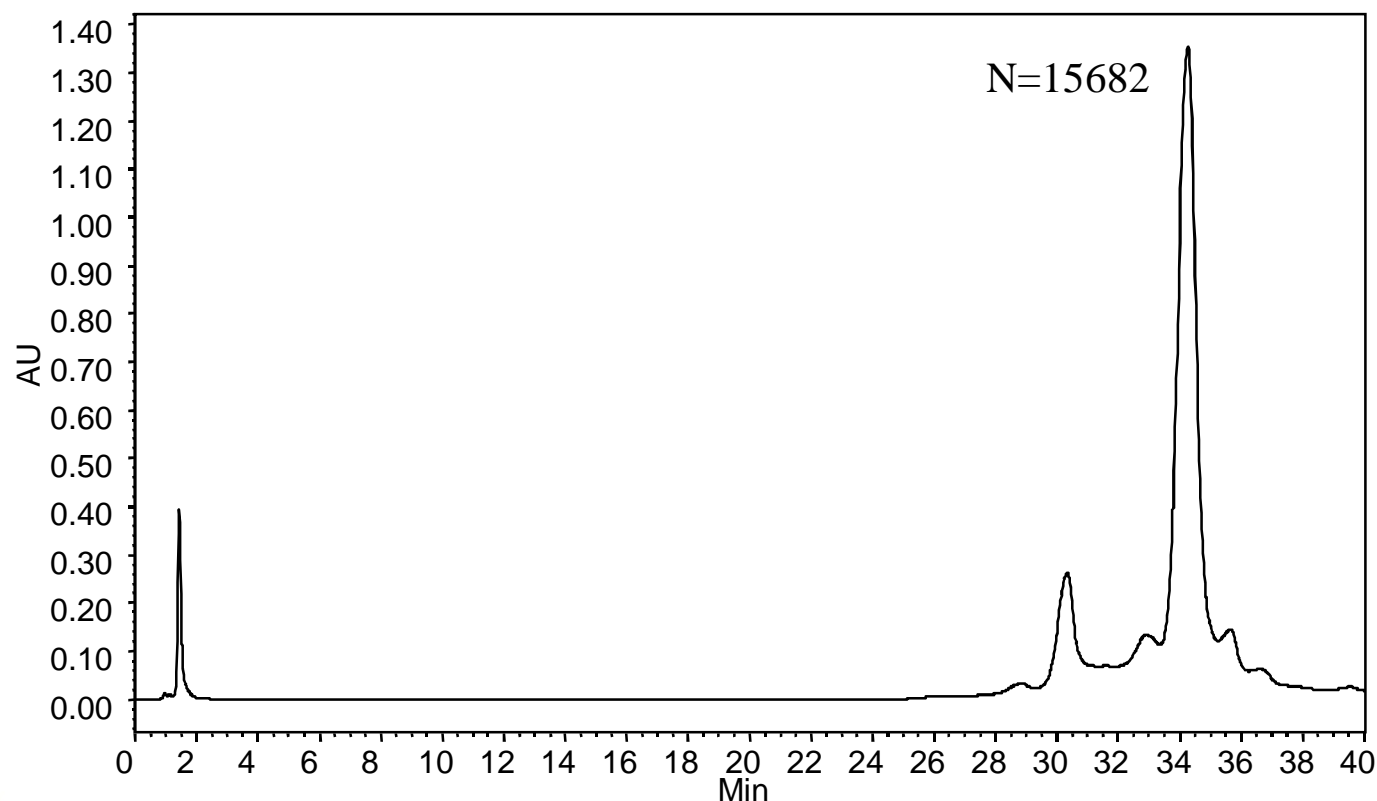
Mobile phase: A, 10 mM Phosphate, pH 7.5; B, A + 100 mM NaCl

Gradient: 15-100% B (60 min)

Flow rate: 0.3 mL/min; Detection: UV 214 nm

Sample: Monoclonal antibody (5 mg/mL); Injection: 5  $\mu$ L

Temperature: Ambient

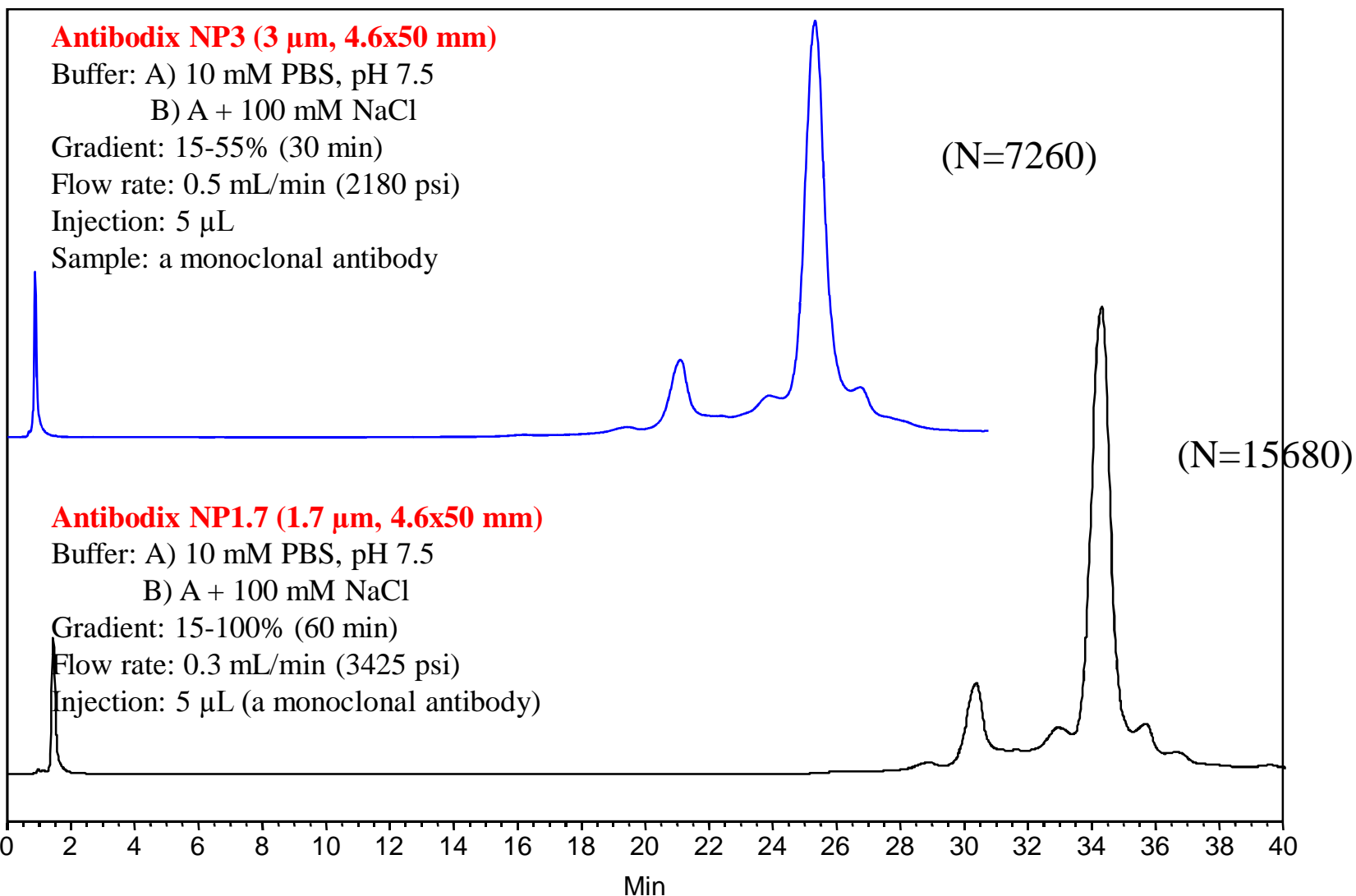


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# Particle Size Impact on Antibody Separation



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# Column Dimension Impact on Antibody Separation

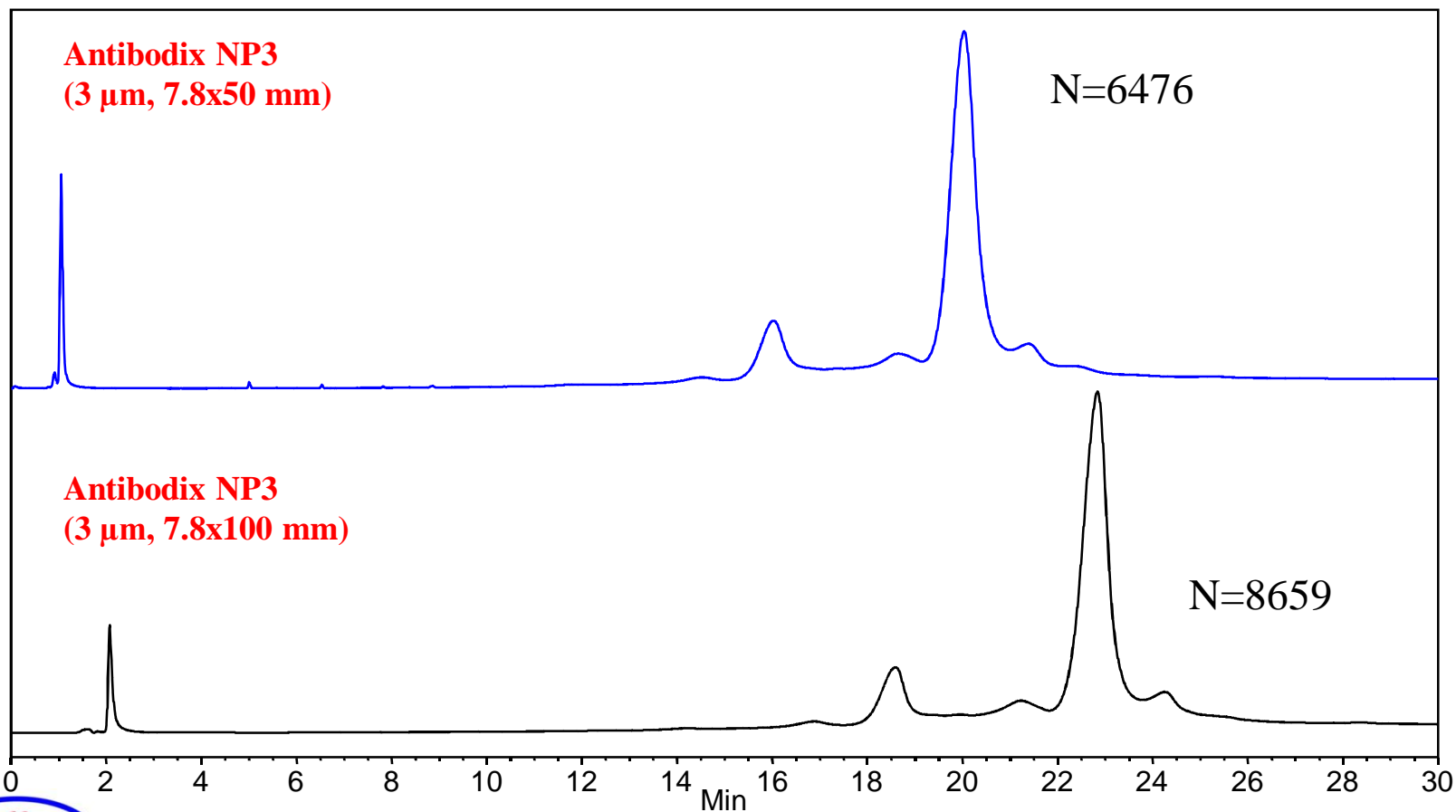
Buffer: A) 10 mM PBS, pH 7.5; B) A + 100 mM NaCl

Gradient: 15-95% (60 min)

Flow rate: 1.0 mL/min

Detection: UV 214 nm; Temperature: Ambient Sample:

a monoclonal antibody; Injection: 10  $\mu$ L



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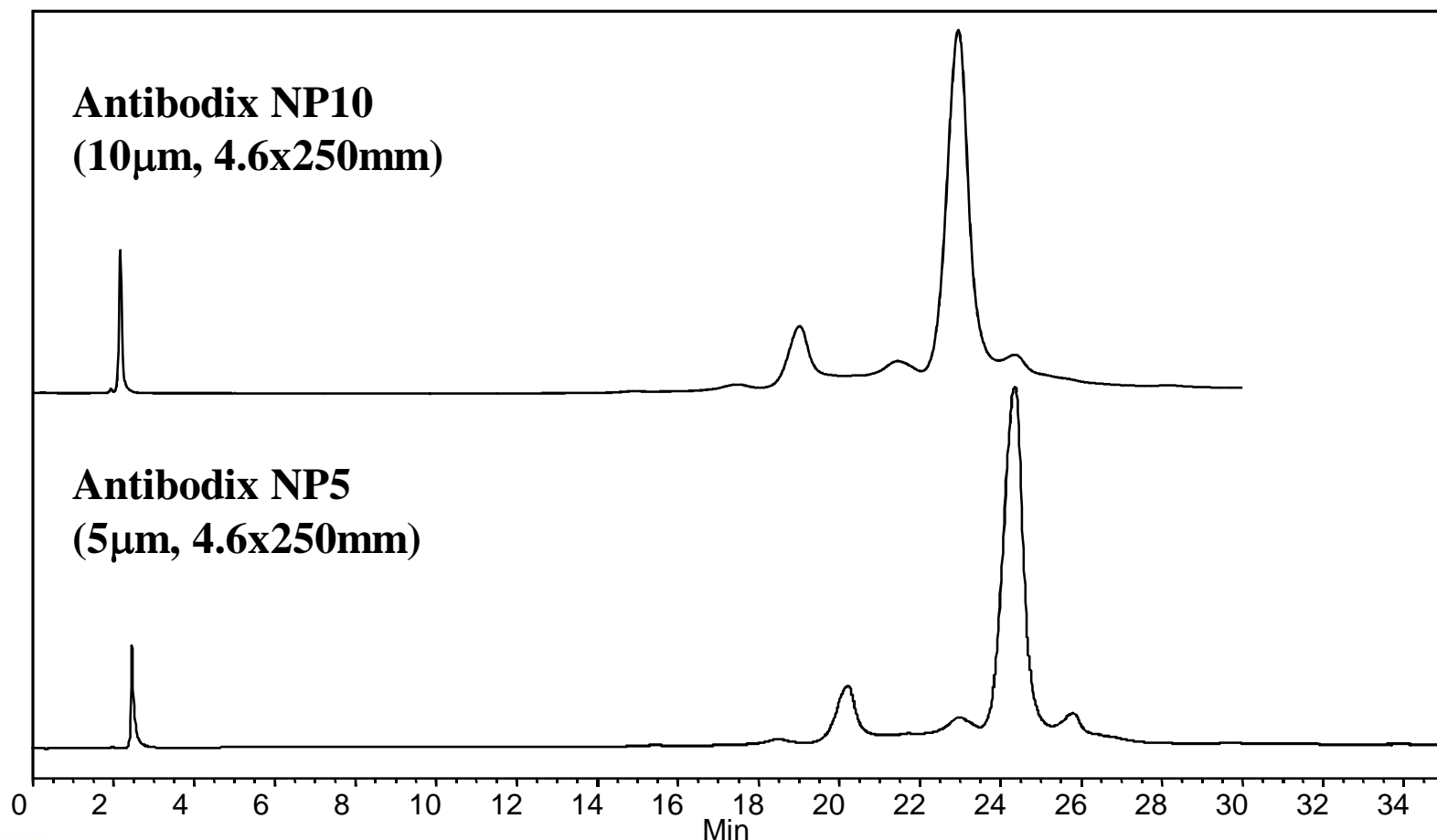
# Comparison of 5 & 10 $\mu\text{m}$ Antibodix for MAb Separation

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; Temperature: Ambient

Sample: Monoclonal antibody (5 mg/mL); Injection: 5  $\mu\text{L}$



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# Separation of a Monoclonal Antibody Sample

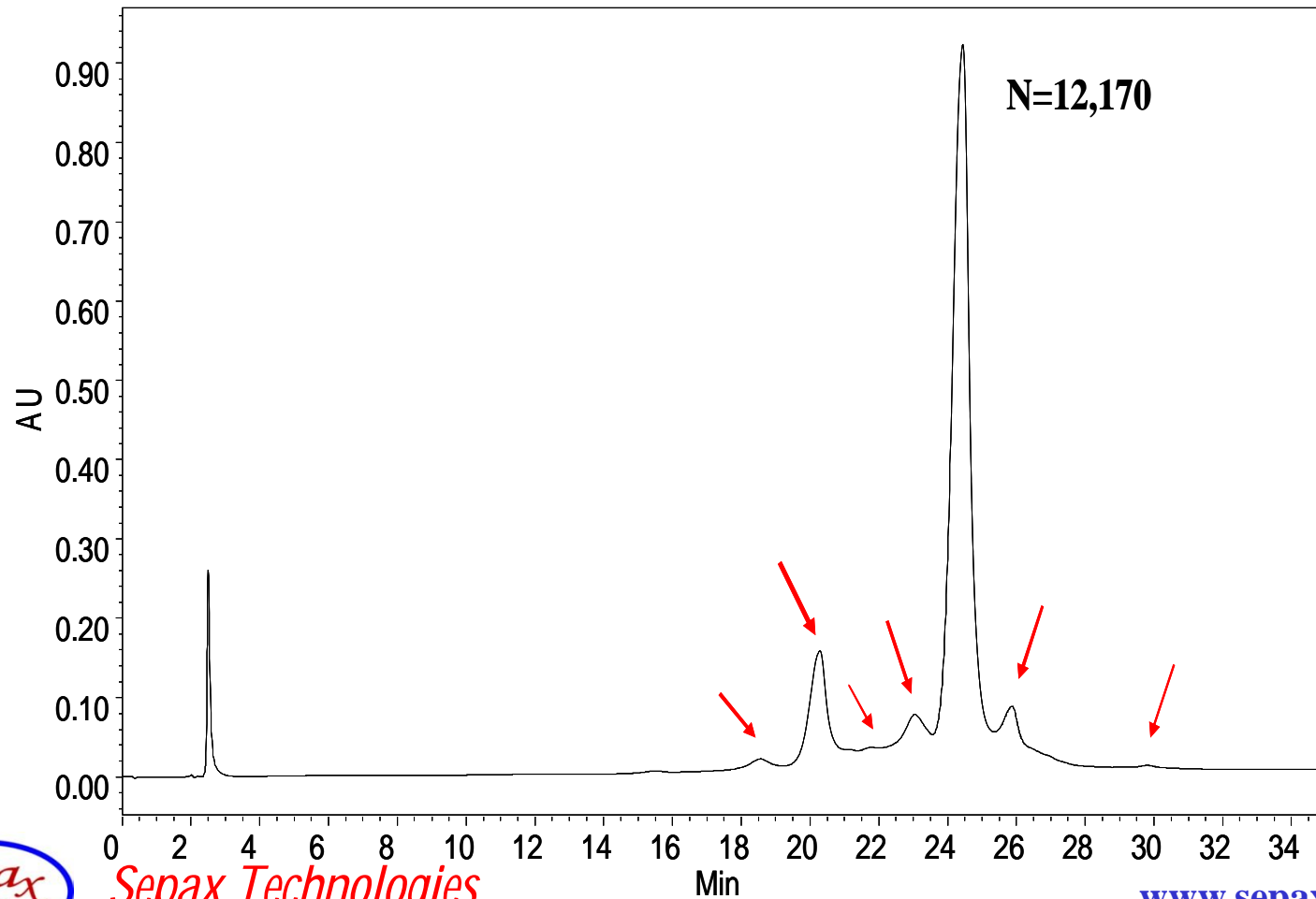
Column: Antibodix NP5 (5 $\mu$ m, 4.6x250mm)

Buffer: 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  $\mu$ L



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# Separation of MAb-X22 with optimizing conditions

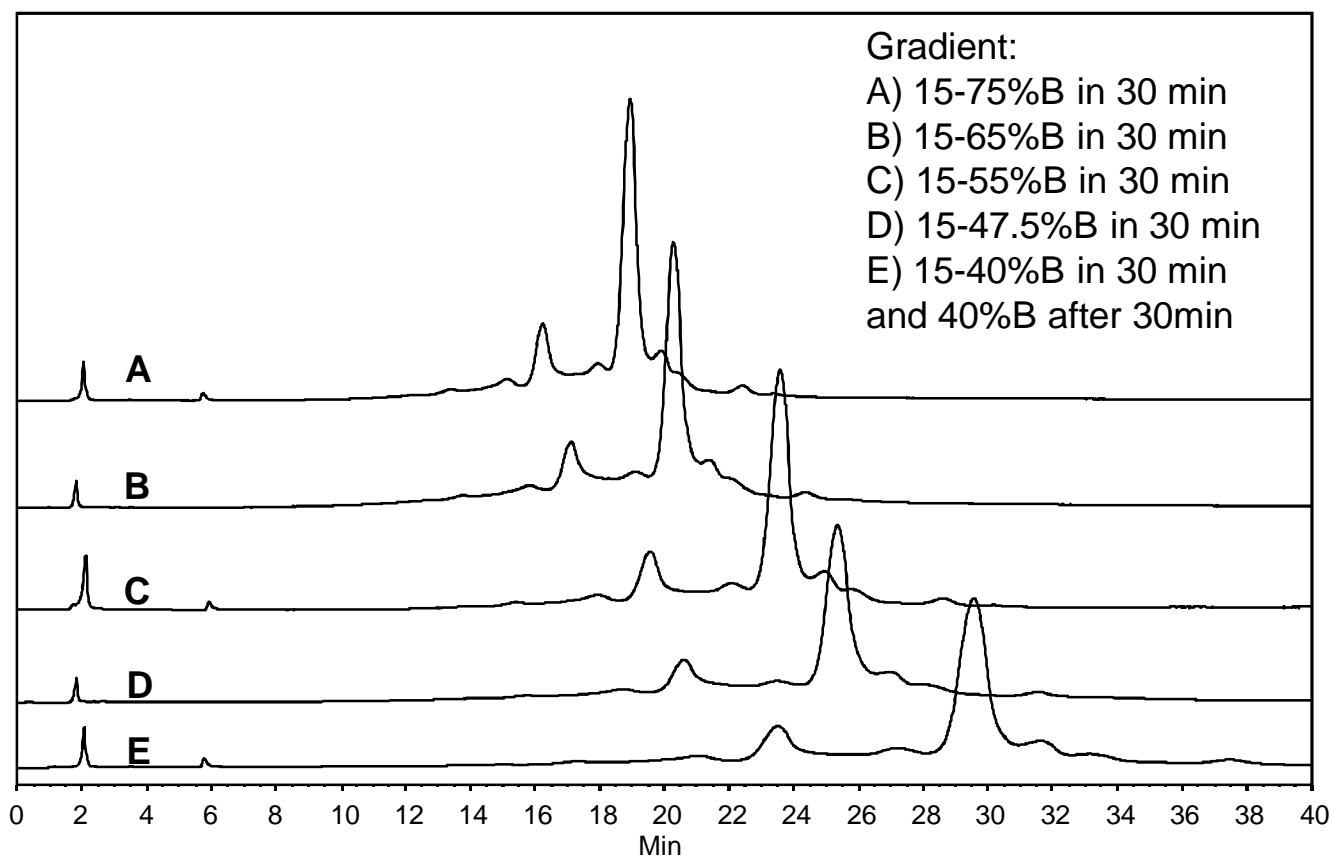
Columns: Antibodix-NP10 (10  $\mu\text{m}$ , 4.6x250 mm)

Mobile phase: A, 10 mM phosphate, pH 7.5; B, A + 0.1M NaCl

Flow rate: 0.8 mL/min

Sample: MAb-X22; Injection: 10  $\mu\text{L}$  (1.5 mg/mL)

Temperature: 25  $^{\circ}\text{C}$ ; Detection: UV 214 nm



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# The Impact of initial salt on the separation of MAb-X22

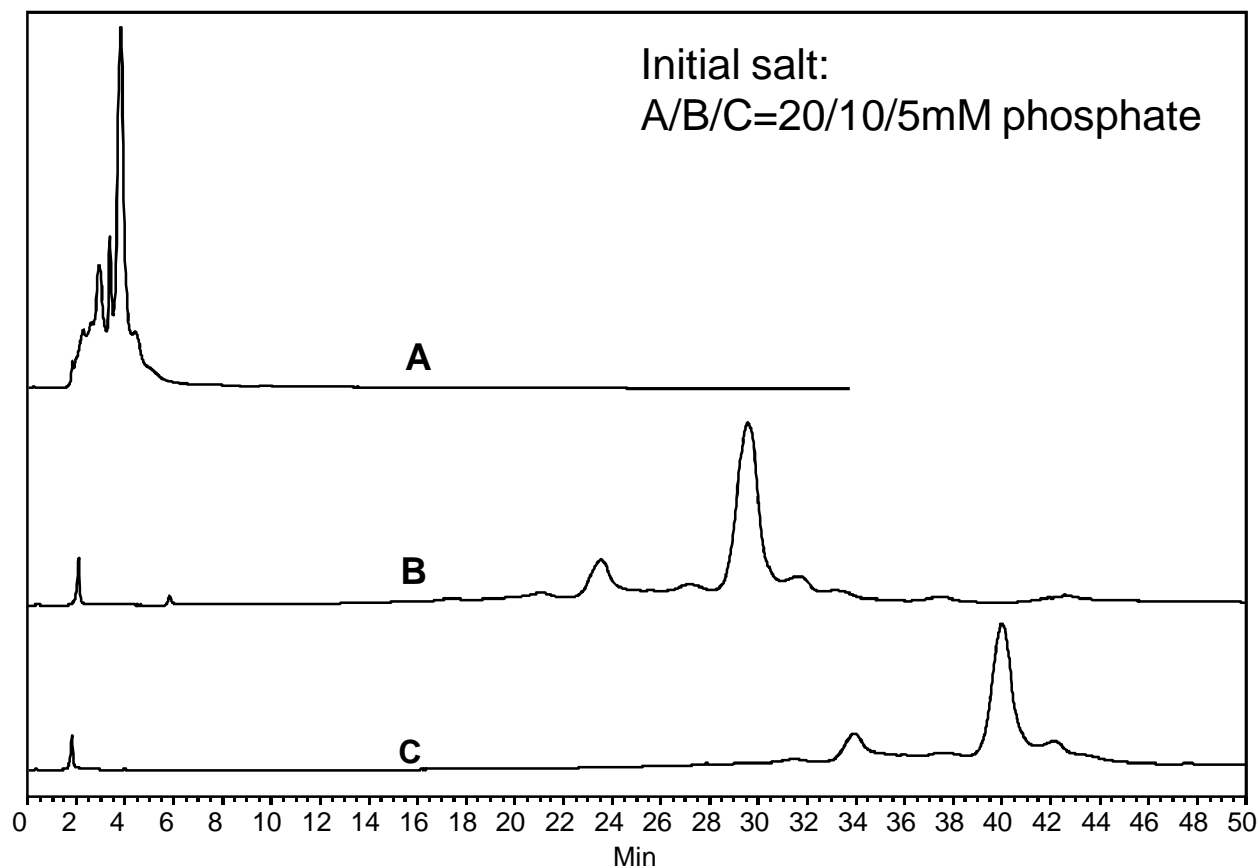
Columns: Antibodix-NP10 (10  $\mu$ m, 4.6x250 mm)

Mobile phase: A, Phosphate buffer, pH 7.5; B, A + 0.1M NaCl

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

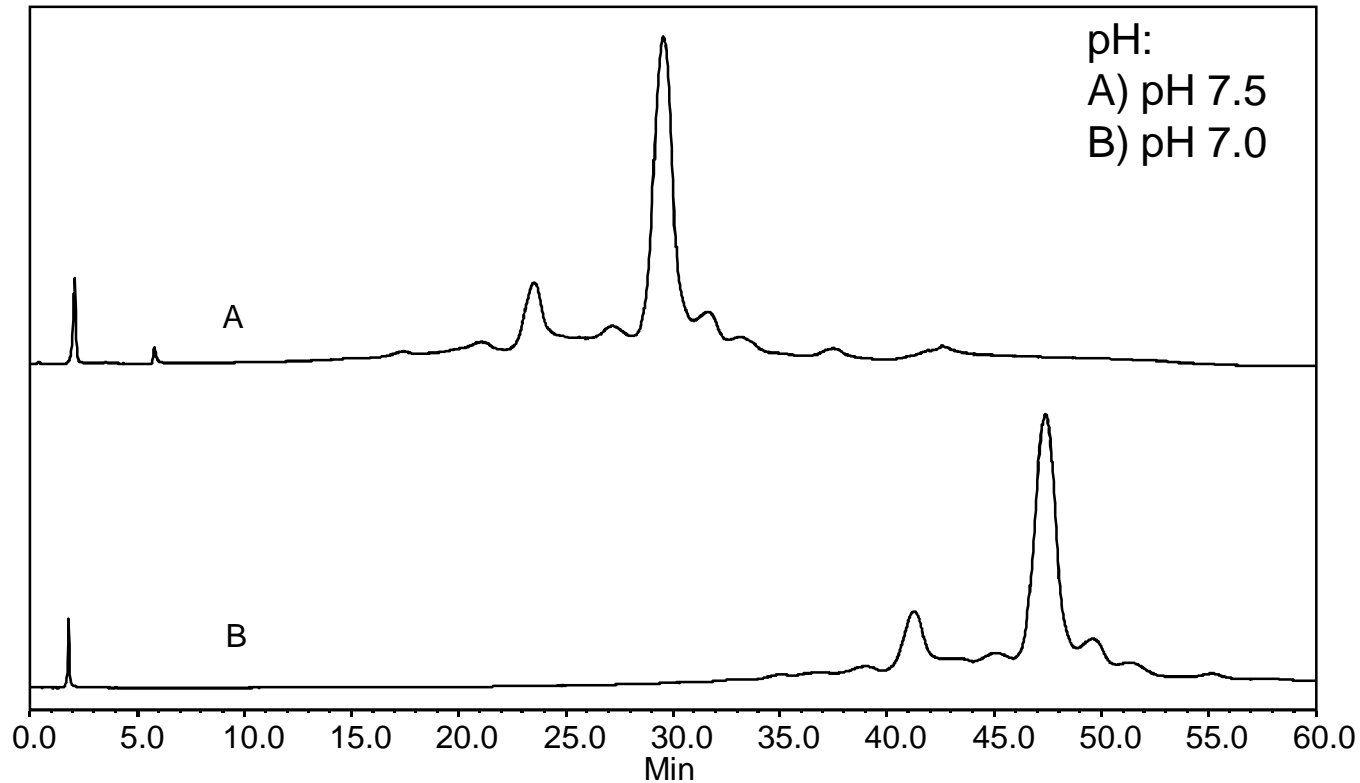


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# The impact of mobile phase pH on MAb-X22 separation

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



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## Antibodix WCX-NP Columns - Summary

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

- Based on uniform, non-porous PS/DVB based ion-exchange particles, Antibodix columns have high resolution and high efficiency with reasonable high capacity.
- Particle size selection: 1.7, 3, 5 and 10 µm. Smaller particle size, higher separation efficiency
- The chemistry are specifically designed for separation of monoclonal antibodies
- The resolution and selectivity of monoclonal antibodies depend on the separation conditions



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