Ion Exchange Chromatography is frequently used for antibody analysis. Antibodies and antibody fragments can all be separated on weak cation exchange columns based on their charge states. Antibodix™ WCX columns are specially designed for high resolution, high efficiency and high recovery separations of antibodies.

In this study, we investigated antibody fragments such as heavy and light chains, Fab/Fc and F(ab’)2 using Antibodix™ WCX separation.

**Highlighted FACTS:**

► Antibodix™ WCX NP5 4.6 x 250 mm can successfully separate monoclonal antibody variants under different mobile phase systems such as pH and salt gradients.

► Monoclonal antibody purity, heterogeneity and stability can be monitored using Antibodix™ WCX NP5.

► The smaller particle size in Antibodix™ WCX NP5 offers superior resolution in comparison to Antibodix™ WCX NP10.

### Analysis of MAb and MAb Fragments on Antibodix™ WCX NP5

**Analysis of MAb 321 using a NaCl and pH gradient**

Column: Antibodix™ WCX NP5 (4.6x250 mm)
Mobile phase: A: 20 mM Phosphate buffer pH 5; B: A+10 mM NaCl pH 7.5
Flow rate: 0.8 mL/min; Injection volume: 20 µL; Detection: UV280 nm, Temperature: 30 ºC; Sample: Intact MAb 321 (5 mg/mL)

![Graph showing absorbance at UV 280 nm](image)

### Analysis of MAb 321 using a LiCl gradient

Column: Antibodix™ WCX NP5 (4.6x250 mm)
Mobile phase: A: 20 mM Sodium Acetate pH 5.15, B: A + 1 M LiCl
Flow rate: 0.8 mL/min; Injection volume: 20 µL; Detection: UV280 nm, Temperature: 30 ºC; Sample: Intact MAb 321 (5 mg/mL)

![Graph showing absorbance at UV 280 nm](image)

### MAb loading test on Antibodix™ WCX NP5

Column: Antibodix™ WCX NP5 (4.6x250 mm)
Mobile phase: A: 20 mM Phosphate buffer pH 5; B: A+10 mM NaCl pH 7.5
Flow rate: 0.8 mL/min; Temperature: 30 ºC; Sample: Intact MAb 321 (5 mg/mL)

![Graph showing absorbance at UV 280 nm](image)

### MAb analysis on Antibodix™ NP5 and NP10

Column: Antibodix™ WCX NP5 and Antibodix™ WCX NP10 (4.6x250 mm)
Mobile phase: A: 20mM Sodium Acetate pH 5.15, B: A + 1M LiCl, Flow rate: 0.8 mL/min; Injection volume: 20 µL, Sample: MAb 321 (5 mg/mL)

![Graph showing absorbance at UV 280 nm](image)

### MAb stability test on Antibodix™ WCX NP5

Column: Antibodix™ WCX NP5
Mobile phase: A: 20 mM Sodium Acetate pH 5.15, B: A + 1 M LiCl, Flow rate: 0.8 mL/min; Injection volume: 20 µL, Sample: MAb 321 (5 mg/mL)

![Graph showing absorbance at UV 280 nm](image)
What is Antibodix™ WCX NP5

Antibodix™ WCX NP5 (Weak Cation Exchange):
Comprised of rigid, spherical, highly cross-linked non-porous poly(styrene divinylbenzene) (PS/DVB) beads. The PS/DVB particle surface is grafted with a hydrophilic, neutral polymer layer which is nanometers thick. The resin surface is covered by a hydrophilic coating which eliminates non-specific bindings with antibody proteins, leading to high efficiency and high recovery separations. On top of the hydrophilic layer, weak cation-exchange functional groups are attached via a proprietary chemistry, resulting in a high capacity ion-exchange layer.

Technical Specifications:

<table>
<thead>
<tr>
<th>Phase</th>
<th>Antibodix™ WCX NP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>Weak cation exchange groups bonded to a hydrophilic film grafted on PS/DVB</td>
</tr>
<tr>
<td>Particle size (µm)</td>
<td>5</td>
</tr>
<tr>
<td>Pore size (Å)</td>
<td>Non-porous</td>
</tr>
<tr>
<td>pH stability</td>
<td>2 – 12</td>
</tr>
<tr>
<td>Backpressure (psi)</td>
<td>~ 2,500</td>
</tr>
<tr>
<td>Maximum backpressure</td>
<td>~ 6,000</td>
</tr>
<tr>
<td>Maximum temperature</td>
<td>~ 80 ºC</td>
</tr>
<tr>
<td>Mobile phase compatibility</td>
<td>Aqueous or a mixture of water and acetonitrile, acetone or methanol</td>
</tr>
</tbody>
</table>

Analysis of Antibody Fragments on Antibodix™ WCX NP5

Papain digested MAb analysis on Antibodix™ NP5 and SDS page gel

Fab/Fc loading test on Antibodix™ NP5 4.6 x 250 mm

Fab/Fc on Antibodix™ NP5

F(α’2)_1 on Antibodix™ NP5

Comparison of Sepax’s Antibodix™ NP5 to a competitor’s column

Column: Antibodix™ WCX NP5 (4.6x250 mm)
Mobile phase:
A: 20 mM Sodium Acetate pH 5.15,
B: A + 1 M LiCl
Flow rate: 0.8 mL/min;
Temperature: 30 ºC;
Sample: Intact MAb 321 (5 mg/mL)
Injection volume: 20 µL

Analysis of MAb and MAb Fragments on Antibodix™ WCX NP5