Analysis of MAb and MAb Fragments on Antibodix™ WCX NP5

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:

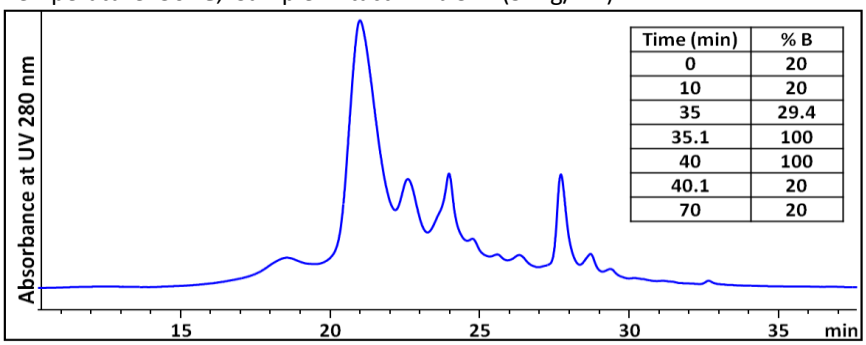
► Antibodix™ WCX NP5 4.6 x 250 mm can successfully separate monoclonal antibody variants under different mobile phase systems such as pH and salt gradients.

► 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 321 using a LiCl gradient

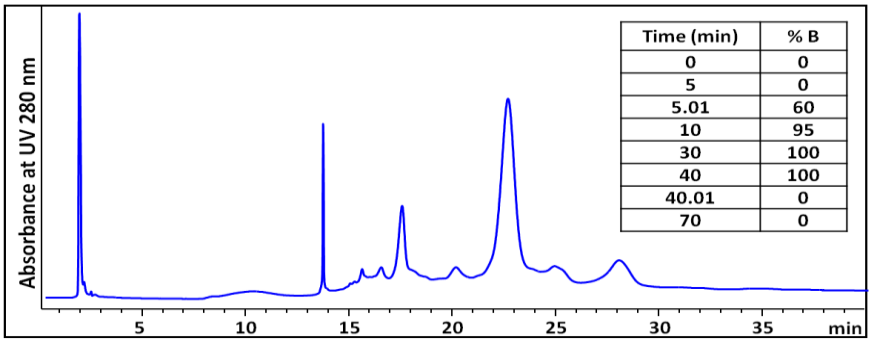
Column: Antibodix™ WCX NP5 (4.6x250 mm)
Mobile phase: A: 20 mM Sodium Acetate pH 5.15, B: A + 1 M LiCl
Flow rate: 0.8 mL/min; Injection volume: 20 µL, Detection: UV280 nm,
Temperature: 30 °C; Sample: Intact MAb 321 (5 mg/mL)



Time (min)	% B
0	20
10	20
35	29.4
35.1	100
40	100
40.1	20
70	20

Analysis of MAb 321 using a NaCl and pH gradient

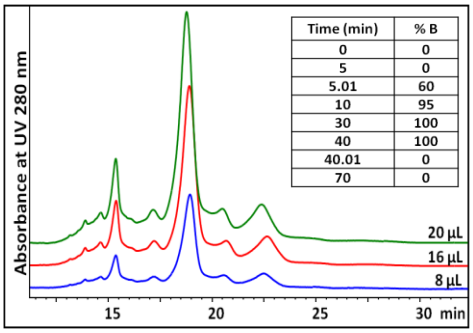
Column: Antibodix™ WCX NP5 (4.6x250 mm)
Mobile phase: A: 20 mM Phosphate buffer pH 5; B: A+10 mM NaCl pH 7.5
Flow rate: 0.8 mL/min; Injection volume: 20 µL; Detection: UV280 nm
Temperature: 30 °C; Sample: Intact MAb 321 (5 mg/mL)



Time (min)	% B
0	0
5	0
5.01	60
10	95
30	100
40	100
40.01	0
70	0

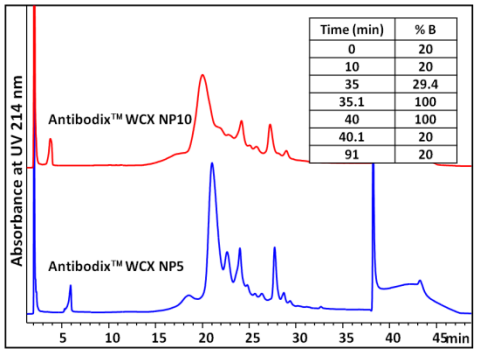
MAb loading test on Antibodix™ WCX NP5

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 °C;
Sample: Intact MAb 321 (5 mg/mL)



Time (min)	% B
0	0
5	0
5.01	60
10	95
30	100
40	100
40.01	0
70	0

MAb analysis on Antibodix™ NP5 and NP10

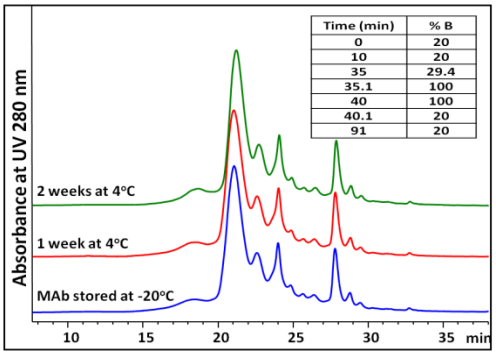


Time (min)	% B
0	20
10	20
35	29.4
35.1	100
40	100
40.1	20
91	20

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)

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: Intact MAb 321 (5 mg/mL)



Time (min)	% B
0	20
10	20
35	29.4
35.1	100
40	100
40.1	20
91	20

Analysis of MAb and MAb Fragments on Antibodix™ WCX NP5

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:

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

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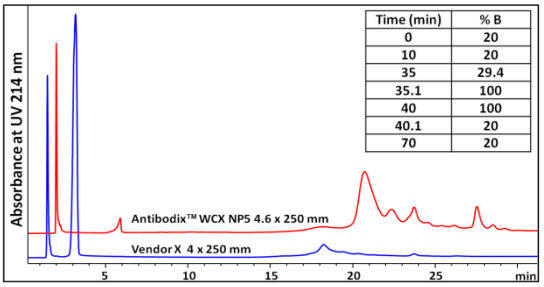
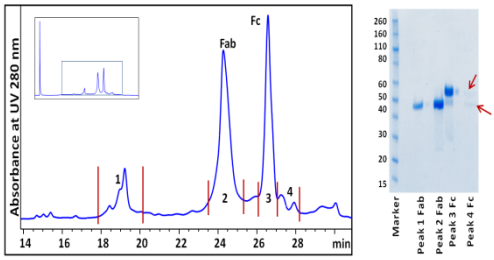
Comparison of Sepax's Antibodix™ NP5 to a competitor's column



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 °C;
Sample: Intact MAb 321 (5 mg/mL)
Injection volume: 20 µL

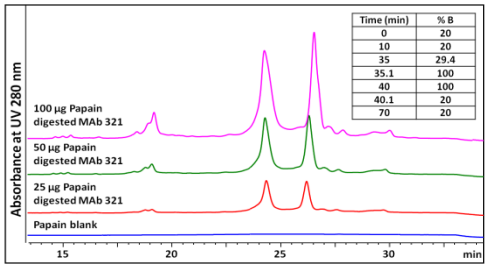
Analysis of Antibody Fragments on Antibodix™ WCX NP5

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



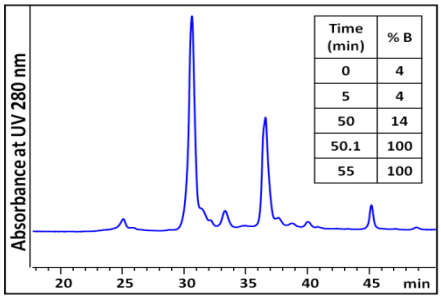
Column:
Antibodix™ WCX NP5 (4.6x250 mm)
Mobile phase:
A: 20mM acetic acid+50mM NaCl pH3.5
B: 20mM sodium succinate+50mM NaCl pH6
Sample: 100µg Papain digest MAb321
Gradient: 5 min 30% B, 0.8 mL/min;
25 min 85%-100% B, 0.65mL/min

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



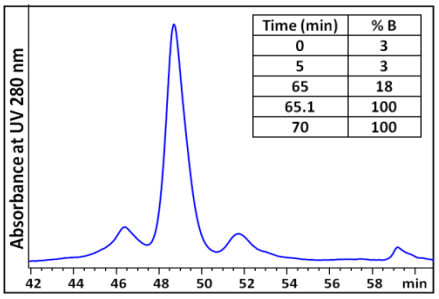
Column:
Antibodix™ WCX NP5 (4.6x250 mm)
Mobile phase:
A: 20mM acetic acid+50mM NaCl pH3.5
B: 20mM sodium succinate+50mM NaCl pH6
Sample: Papain digest MAb321
Gradient: 5 min 30% B, 0.8 mL/min;
25 min 85%-100% B, 0.65mL/min

Fab/Fc on Antibodix™ NP5



Column:
Antibodix™ WCX NP5 (4.6x250 mm)
Mobile phase:
A: 20 mM Phosphate Buffer pH 5.5
B: A + 1 M NaCl
Flow rate: 0.8 mL/min;
Detection: UV 280 nm
Sample: 25 µg Papain digested MAb

F(ab')$_2$ on Antibodix™ NP5



Column:
Antibodix™ WCX NP5 (4.6x250 mm)
Mobile phase:
A: 20 mM phosphate buffer, pH 5.5,
B: A+ 1 M NaCl
Flow rate: 0.8 mL/min;
Detection: UV 280 nm
Sample: 50 µg Pepsin digested MAb