ION EXCHANGE KIT FOR MAB SEPARATIONS

Antibodix™ WCX NP5
Proteomix® SCX NP5

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Sepax Technologies

Content

Introduction

Both Antibodix™ WCX and Proteomix® SCX phases are comprised of rigid, spherical, highly cross-linked non-porous poly(styrene divinylbenzene) (PS/DVB) beads. The PS/DVB particle surface is grafted with a highly hydrophilic, neutral polymer layer with a thickness in the range of nanometers. The hydrophobic PS/DVB resin surface is totally covered by a hydrophilic coating which eliminates non-specific bindings with antibody proteins, leading to high efficiency and high recovery separations.

Antibodix™ WCX phase

On top of the hydrophilic layer, weak cation-exchange carboxylate (–COOH) functional groups are attached via a proprietary chemistry, resulting in a high capacity ion-exchange layer.

Proteomix® SCX phase

On top of the hydrophilic layer, strong cation-exchange sulfonate ($-SO_3H$) functional groups are attached via a proprietary chemistry, resulting in a high capacity ion-exchange layer.

Stationary Phase Structures

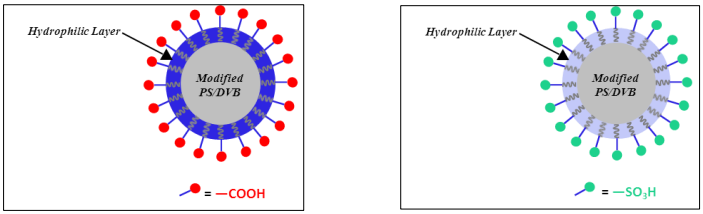


Figure 1. Phase structures for both the surface of Antibodix™ WCX (left) and Proteomix® SCX (right).

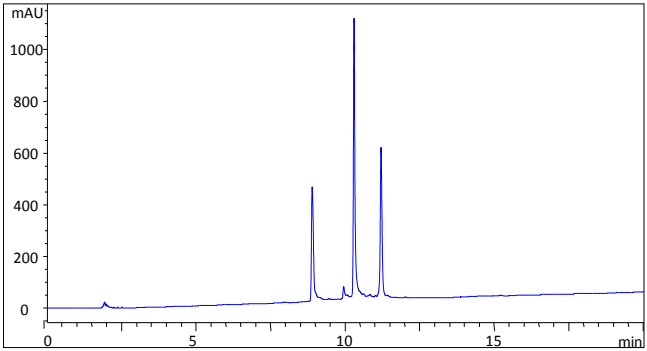
Key features of Antibodix™ and Proteomix® phases

Characteristics	Antibodix™	Proteomix®
Particle size	5 µm	5 µm
Pore size (Å)	Non-porous	Non-porous
Surface structure	Weak cation exchange (—COOH) functional groups	Strong cation exchange (—SO_3H) functional groups

Technical specifications of Antibodix™ WCX NP5 and Proteomix® SCX NP5

Phase	Antibodix™ WCX NP5	Proteomix® SCX NP5
Dimensions	4.6 x 250 mm	4.6 x 250 mm
Material	Carboxylate weak cation exchange groups bonded to a hydrophilic film grafted on PS/DVB.	Sulfonate strong cation exchange groups bonded to a hydrophilic film grafted on PS/DVB.
Particle size	5 µm	5 µm
Pore size (Å)	Non-porous	Non-porous
pH stability	2-12	2-12
Flow rate	0.80 mL/min	0.80 mL/min
Backpressure	< 5,000 psi	< 3,500 psi
Maximum backpressure	~6,000 psi	~ 6,000 psi
Maximum temperature (^{o}C)	~ 80	~ 80
Mobile phase compatibility	Aqueous or a mixture of water and acetonitrile, acetone, or methanol	Aqueous or a mixture of water and acetonitrile, acetone, or methanol

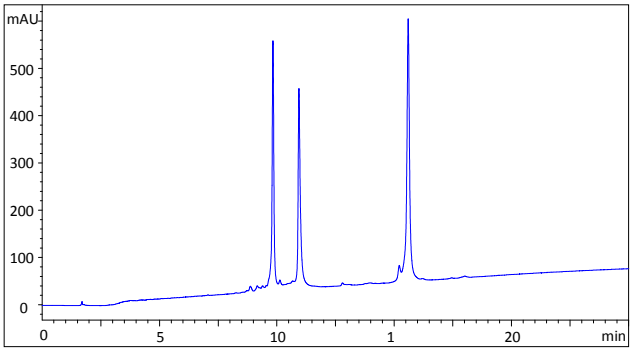
Quality Control Test for Antibodix™ WCX NP5 4.6 x 250 mm



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
Aprotinin	8.91	1820	87584	1.30	—
Lysozyme	10.29	3811	234544	1.34	13.43
Ribonuclease A	11.20	2277	192966	1.12	9.72

Figure 2. A standard quality control test on an Antibodix™ WCX NP5 4.6 x 250 mm. Mobile phases were A: 10 mM sodium phosphate buffer, pH 6.0 and B: A + 1.0 M NaCl. Flow rate was 0.8 mL/min and the gradient went from 10-100% B in 25 minutes. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of Aprotinin, Lysozyme and Ribonuclease A (1 mg/mL each).

Quality Control Test for Proteomix® SCX NP5 4.6 x 250 mm



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
Ribonuclease A	9.83	2926	101936	0.91	—
Cytochrome C	10.94	2825	85018	1.44	8.10
Lysozyme	15.60	4743	109745	0.93	27.47

Figure 3. A standard quality control test on a Proteomix® SCX NP5 4.6 x 250 mm. Mobile phases were A: 20 mM sodium phosphate buffer, pH 6.0 and B: A + 1.0 M NaCl. Flow rate was 1.0 mL/min and the gradient went from 0-75% B in 25 minutes. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of Cytochrome C, Lysozyme and Ribonuclease A (1 mg/mL each).

Antibodix™ WCX NP5 4.6 x 250 mm
Monoclonal Antibody and Fragments
Separation Applications

MAb321 separation on Antibodix™ WCX NP5 with a LiCl salt gradient

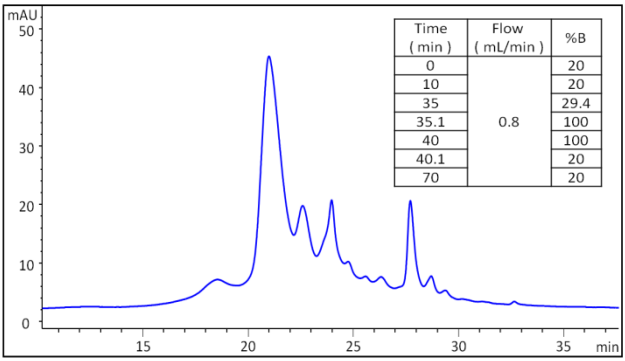


Figure 4. Monoclonal Antibody separation on an Antibodix™ WCX NP5 4.6 x 250 mm column, using a lithium chloride salt gradient. The mobile phases were A: 20 mM sodium acetate pH 5.15 and B: A + 1 M LiCl. Flow rate was 0.8 mL/min with UV 280 nm detection. The column temperature was set at 30 ˚C and 20 µL of a 5 mg/mL MAb sample was injected for analysis.

MAb321 separation on Antibodix™ WCX NP5 with a NaCl salt and pH gradient

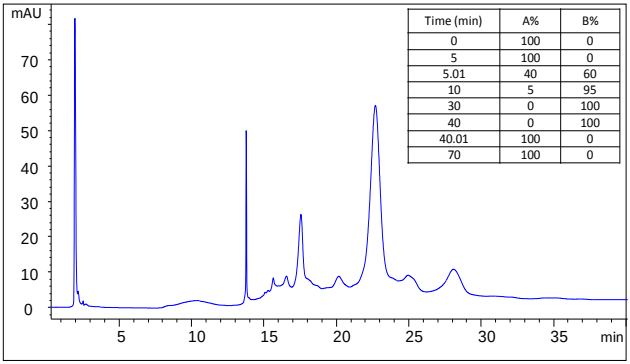


Figure 5. Monoclonal Antibody separation on an Antibodix™ WCX NP5 4.6 x 250 mm column, using a sodium chloride salt and pH gradient. The mobile phases were A: 20 mM Phosphate buffer, pH 5 and B: A + 10 mM NaCl, pH 7.5. Flow rate was 0.8 mL/min with UV 280 nm detection. The column temperature was set at 30 ˚C and 20 µL of a 5 mg/mL MAb sample was injected for analysis.

MAb321 stability test on Antibodix™ WCX NP5

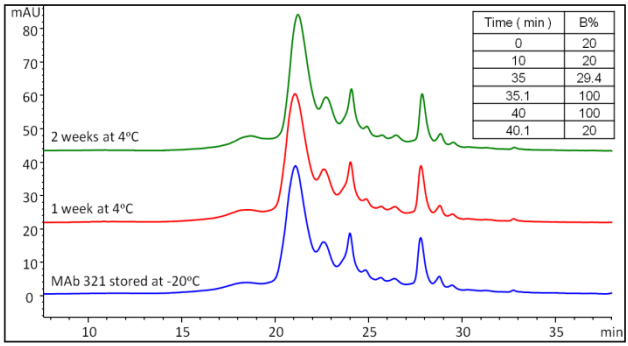


Figure 6. Monoclonal Antibody stability test on an Antibodix™ WCX NP5 4.6 x 250 mm column. The mobile phases were A: 20 mM sodium acetate, pH 5.15 and B: A + 1 M LiCl. Flow rate was 0.8 mL/min with detection at UV 280 nm. 20 µL of each sample was injected and the column temperature was 30 ℃. Samples were 5.0 mg/mL MAb in complex Tris buffer (sample freshly defrosted from -20 °C, one week old and two weeks old at 4 °C).

Papain digested MAb321 separation on Antibodix™ WCX NP5

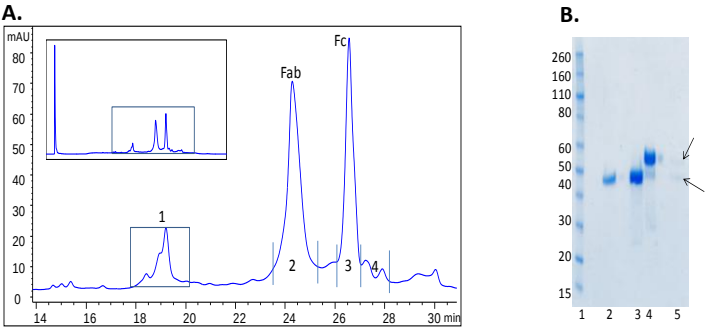


Figure 7. Panel A shows papain digested MAb separation on an Antibodix™ WCX NP5 4.6 x 250 mm column. The mobile phases were A: 20 mM acetic acid + 50 mM NaCl, pH 3.5 and B: 20 mM sodium succinate + 50 mM NaCl, pH 6.0. The gradient was: 5 min 30% B, 0.8 mL/min; 25 min 85%-100% B, 0.65 mL/min. 100 µg of digested MAb was injected for analysis. The papain digestion was 2 mM EDTA, 5 mM Cysteine and 100 mM Tris-HCl at pH 7.6 for 3.5 hour at 37 °C, with MAb at 1 mg/mL and protein : papain = 100 : 1. Detection at UV 280 nm. Panel B shows the 4-12% Bis-Tris gel of Fab and Fc fractions. Lane 1: Markers; Lane 2: Peak 1/Fab; Lane 3: Peak 2/Fab; Lane 4: Peak 3/Fc; Lane 5: Peak 4/Fc.

Fab/Fc different loadings on Antibodix™ WCX NP5

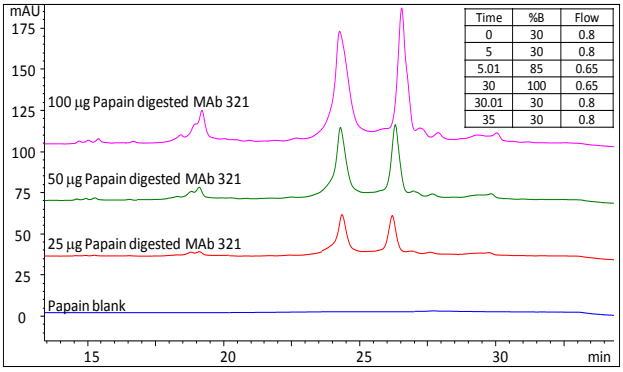


Figure 8. Papain digested MAb separation on an Antibodix™ WCX NP5 4.6 x 250 mm column. The mobile phases were A: 20 mM acetic acid + 50 mM NaCl, pH 3.5 and B: 20 mM sodium succinate + 50 mM NaCl, pH 6.0. The papain digestion was 2 mM EDTA, 5 mM Cysteine and 100 mM Tris-HCl at pH 7.6 for 3.5 hour at 37 °C, with MAb at 1 mg/mL and protein : papain = 100 : 1. Detection at UV 280 nm.

Fab/Fc separation on Antibodix™ WCX NP5

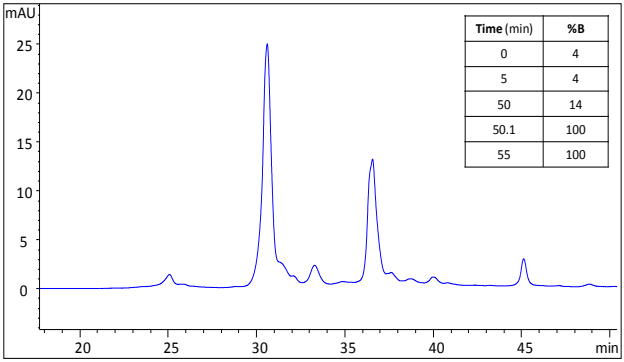


Figure 9. Papain digested MAb separation on an Antibodix™ WCX NP5 4.6 x 250 mm column. The mobile phases were A: 20 mM Phosphate Buffer, pH 5.5 and B: A + 1 M NaCl. 25 µg of sample was injected for analysis. The papain digestion was 2 mM EDTA, 5 mM Cysteine and 100 mM Tris-HCl at pH 7.6 for 3.5 hour at 37 °C, with MAb at 1 mg/mL and protein : papain = 100 : 1. Flow rate was 0.8 mL/min with UV 280 nm detection.

F(ab')$_2$ separation on Antibodix™ WCX NP5

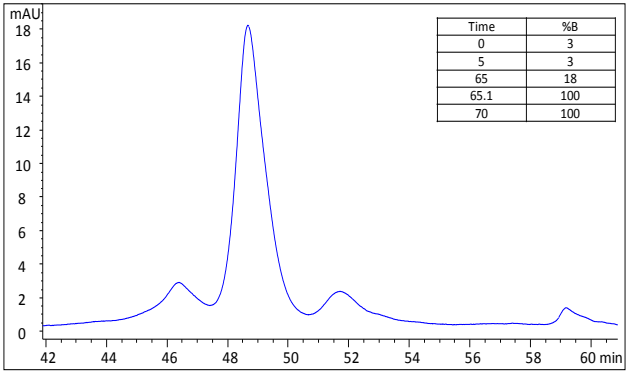


Figure 10. Pepsin digested MAb separation on an Antibodix™ WCX NP5 4.6 x 250 mm column. The mobile phases were A: 20 mM Phosphate Buffer pH 5.5 and B: A+ 1 M NaCl. 50 µg of sample was injected for analysis. The pepsin digestion used 1 mg/mL MAb with a ratio of MAb : pepsin= 40 : 1 in 20 mM sodium acetate, pH 4.0 for 15.5 hours at 37 ^{o}C; then quenched with 25 µL of 2 M Tris. Flow rate was 0.8 mL/min with UV 280 nm detection.

Proteomix® SCX NP5 4.6 x 250 mm
Monoclonal Antibody Analysis
Applications

Intact MAb 321 analysis on Proteomix® SCX NP5

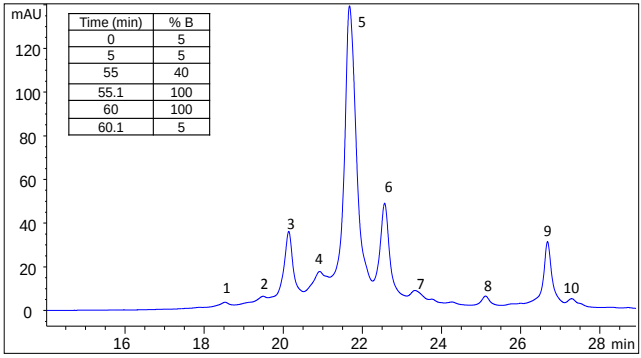


Figure 11. Intact MAb 321 analysis on Proteomix® SCX NP5 4.6 x 250 mm. Mobile phase was A: 2.4 mM Tris, 1.5 mM Imidazole, 11.6 mM piperazine pH 6.0 and B: A + 0.5 M NaCl at pH 10.5. Flow rate was 0.8 mL/min. Column temperature was 30˚C and UV detection was set at 280 nm. 100 µg of intact MAb 321 was injected.

Intact MAb 321 loading test on Proteomix® SCX NP5

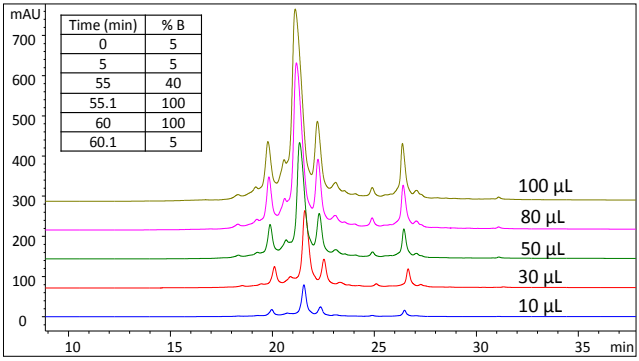


Figure 12. Intact MAb 321 analysis on Proteomix® SCX NP5 4.6 x 250 mm. Mobile phase was A: 2.4 mM Tris, 1.5 mM Imidazole, 11.6 mM piperazine pH 6.0 and B: A + 0.5 M NaCl at pH 10.5. Flow rate was 0.8 mL/min. Column temperature was 30˚C and UV detection was set at 280 nm. The intact MAb 321 was at a concentration of 5 mg/mL.

Intact MAb 202 analysis on Proteomix® SCX NP5

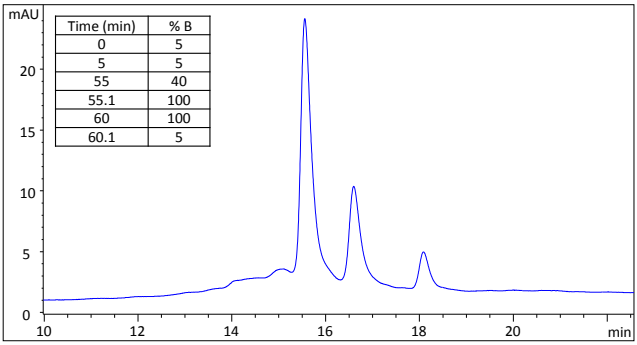


Figure 13. Intact MAb 202 analysis on Proteomix® SCX NP5 4.6 x 250 mm. Mobile phase was A: 2.4 mM Tris, 1.5 mM Imidazole, 11.6 mM piperazine pH 6.0 and B: A + 0.5 M NaCl at pH 10.5. Flow rate was 0.8 mL/min. Column temperature was 30˚C and UV detection was set at 280 nm. 10 µg of intact MAb 202 was injected.

Gradient modification for the analysis of intact MAb 202 on Proteomix® SCX NP5

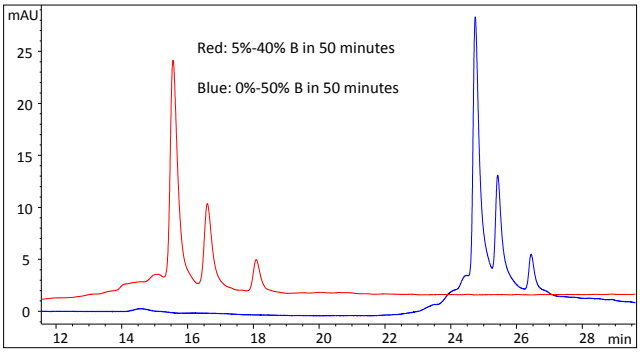


Figure 14. Intact MAb 202 analysis on Proteomix® SCX NP5 4.6 x 250 mm and Antibodix™ WCX NP5 4.6 x 250 mm. Mobile phase was A: 2.4 mM Tris, 1.5 mM Imidazole, 11.6 mM piperazine pH 6.0 and B: A + 0.5 M NaCl at pH 10.5. Flow rate was 0.8 mL/min. Column temperature was 30˚C and UV detection was set at 280 nm. 10 µg of intact MAb 202 was injected.

Monoclonal Antibody Analysis Comparisons on Antibodix™ WCX NP5 and Proteomix® SCX NP5

pH gradient for intact MAb 321 analysis on Antibodix™ WCX NP5 and Proteomix® SCX NP5

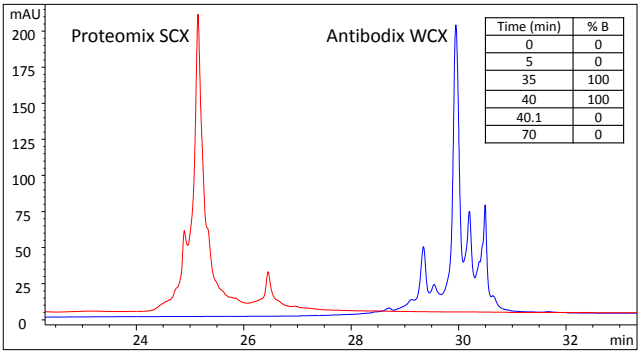


Figure 15. Intact MAb 321 analysis on Proteomix® SCX NP5 4.6 x 250 mm and Antibodix™ WCX NP5 4.6 x 250 mm. Mobile phase was A: 2.4 mM Tris, 1.5 mM Imidazole, 11.6 mM piperazine pH 6.0 and B: A at pH 10.5. Flow rate was 0.8 mL/min. Column temperature was 30˚C and UV detection was set at 280 nm. 50 µg of intact MAb 321 was injected.

Salt and pH gradient for intact MAb 321 analysis on Antibodix™ WCX NP5 and Proteomix® SCX NP5

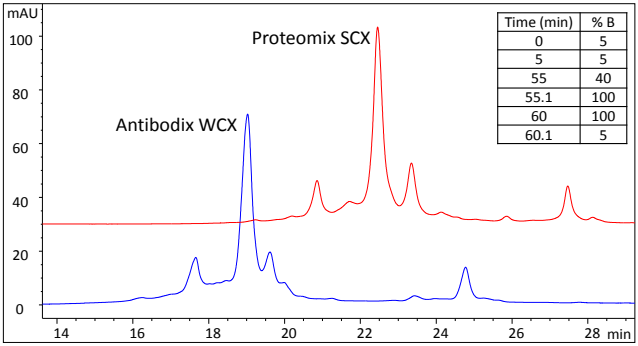


Figure 16. Intact MAb 321 analysis on Proteomix® SCX NP5 4.6 x 250 mm and Antibodix™ WCX NP5 4.6 x 250 mm. Mobile phase was A: 2.4 mM Tris, 1.5 mM Imidazole, 11.6 mM piperazine pH 6.0 and B: A + 0.5 M NaCl at pH 10.5. Flow rate was 0.8 mL/min. Column temperature was 30˚C and UV detection was set at 280 nm. 50 µg of intact MAb 321 was injected.

Intact MAb 321 analysis on Antibodix™ WCX NP5 and Proteomix® SCX NP5

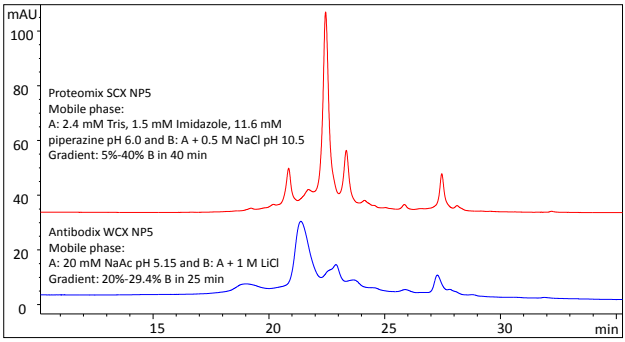


Figure 17. Intact MAb 321 analysis on Proteomix® SCX NP5 4.6 x 250 mm and Antibodix™ WCX NP5 4.6 x 250 mm. Mobile phase for each column was indicated on the chromatogram. Flow rate was 0.8 mL/min. Column temperature was 30˚C and UV detection was set at 280 nm. 50 µg of intact MAb 321 was injected.

Intact MAb 202 analysis on Antibodix™ WCX NP5 and Proteomix® SCX NP5

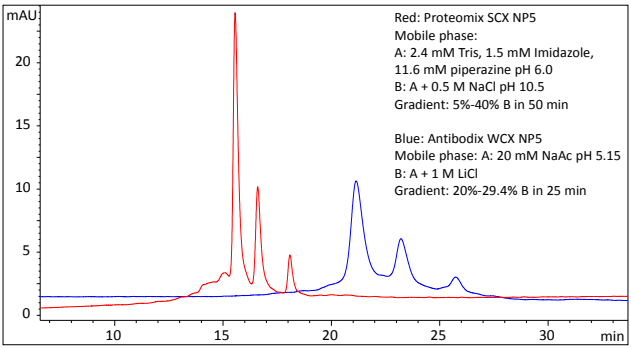


Figure 18. Intact MAb 202 analysis on Proteomix® SCX NP5 4.6 x 250 mm and Antibodix™ WCX NP5 4.6 x 250 mm. Flow rate was 0.8 mL/min. Column temperature was 30˚C and UV detection was set at 280 nm. 10 µg of intact MAb 202 was injected for each.

Column Installation and Operation for Antibodix™ WCX NP5 4.6 x 250 mm and Proteomix® SCX NP5 4.6 x 250 mm columns

1. Filter all samples and mobile phases through 0.45 µm or 0.2 µm filters before use.
2. Attach the column to your HPLC system following the flow direction as marked.
3. New columns are shipped in 20 mM sodium phosphate buffer, pH 6.0. Run 10-20 column volumes of 20 mM sodium phosphate buffer at pH 6.0 to activate the column. Equilibrate the column with desired mobile phase until detection signal reaches baseline.
4. Inject desired amount of sample and run the column with desired flow rate.
5. Store columns in 20 mM sodium phosphate buffer, pH 6.0 for long term storage.

Note: Solvent compatibility

Antibodix™ and Proteomix® columns are compatible with aqueous mobile phases or a mixture of organic and water, such as methanol or acetonitrile and water. Typical eluants contain sodium, potassium salts of phosphate, chloride, acetate, or Tris. Always use an inline degasser or degas the mobile phase prior to use. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum. Antibodix™ and Proteomix® columns are compatible with nonionic and zwitterionic detergents. Antibodix™ and Proteomix® columns are incompatible with cationic detergents.

Ion exchange chromatography operates under the consideration that for every protein there is a unique relationship between net surface charge and pH. A protein with no net charge at a pH equal to its pI will exhibit no interactions with a charged stationary phase. At a pH below its pI, a protein will behind to a negatively charged stationary phase; a cation exchanger. When using a cation exchanger it is a good idea to begin with a starting buffer which is 0.5-1 pH unit less than the sample's pI and an elution buffer which is either at a higher pH or at a higher salt concentration.

Troubleshooting for Proteomix® and Antibodix™

It is the user's responsibility to determine the optimum sample loading and running conditions to best utilize Antibodix™ WCX NP5 and Proteomix® SCX NP5 columns. The following information is provided for reference to troubleshoot your experiments.

High back pressure

A sudden increase in backpressure suggests that the column inlet frit might be blocked. In this case it is recommended that the column be flushed in reverse flow with an appropriate solvent. To prevent the clogging, remove the particulates from samples and mobile phases with filtration.

Poor resolution

1. Column may be overloaded. Reduce sample injection.
2. Try using different mobile phases in order to optimize you running conditions. Vary buffers, concentrations and pHs.

Peak tailing

This indicates that a different starting mobile phase condition should be used. To promote sample binding to the column try starting conditions at different pHs and at different salt concentrations.

No protein binding

Column equilibration may not be enough, try to extend the column equilibration. Mobile phase pH and ionic strength may be incorrect. Prepare new solutions or change starting salt concentrations. If the protein is still not binding, decrease the starting buffer pH.

Protein eluting late in the gradient

Protein binding is too strong. Increase the ionic strength of the gradient. Also pH can be adjusted higher.

Column cleaning and regeneration

Antibodix™ WCX and Proteomix® SCX columns may be contaminated by strongly adsorbed samples, which results in decreasing column performance. It is usually indicated by an increase in backpressure and a broader peak. When this happens, the general procedure for column cleaning is as follows:

1. Disconnect the column from the detector.
2. Clean your column in the reverse flow direction.
3. Run the column at less than 50% of the maximum recommended flow rate. Monitor the backpressure.
4. 10-15 column volumes of cleaning solution are sufficient. Run 3-5 column volumes of Nanopure water between each solution.

In general, the recommended cleaning solution is 50 mM phosphate buffer with 1.0 M NaCl at pH 10.

Note: Separations on exchange columns are sensitive to the pH changes in the mobile phases. In order to have good reproducibility of the separations, make sure the pHs of the same buffer in different lots are the same. pH meters need to be calibrated correctly each time for buffer making.

Column Protection

In addition to filtering the sample and the mobile phase, the best way to protect the separation column is to install a guard column or a pre-column filter in front of it. In most cases a pre-column filter helps to remove the residual particulates that are in the sample, the mobile phase, or leached from the HPLC system, such as pump and injector seals. However, a guard column is highly recommended because it is more effective in trapping highly adsorptive sample components and residual particulates in the sample, the mobile phase or from the HPLC system.

Ordering Information

Ion Exchange Kit (P/N MABIEXKIT-0000) Includes:

Antibodix™ WCX NP5

P/N	ID x Length (mm)	Column Material
602NP5-4625	4.6 x 250	Stainless Steel

Proteomix® SCX NP5

P/N	ID x Length (mm)	Column Material
401NP5-4625	4.6 x 250	Stainless Steel

Other Available Dimensions:

Antibodix™ WCX NP5

P/N	ID x Length (mm)	Column Material
602NP5-4605	4.6 x 50 (Guard)	Stainless Steel
602NP5P-4605	4.6 x 50 (Guard)	PEEK
602NP5P-4625	4.6 x 250	PEEK

Proteomix® SCX NP5

P/N	ID x Length (mm)	Pore Size (Å)
401NP5-4605	4.6 x 50 (Guard)	Stainless Steel
401NP5P-4605	4.6 x 50 (Guard)	PEEK
401NP5P-4625	4.6 x 250	PEEK