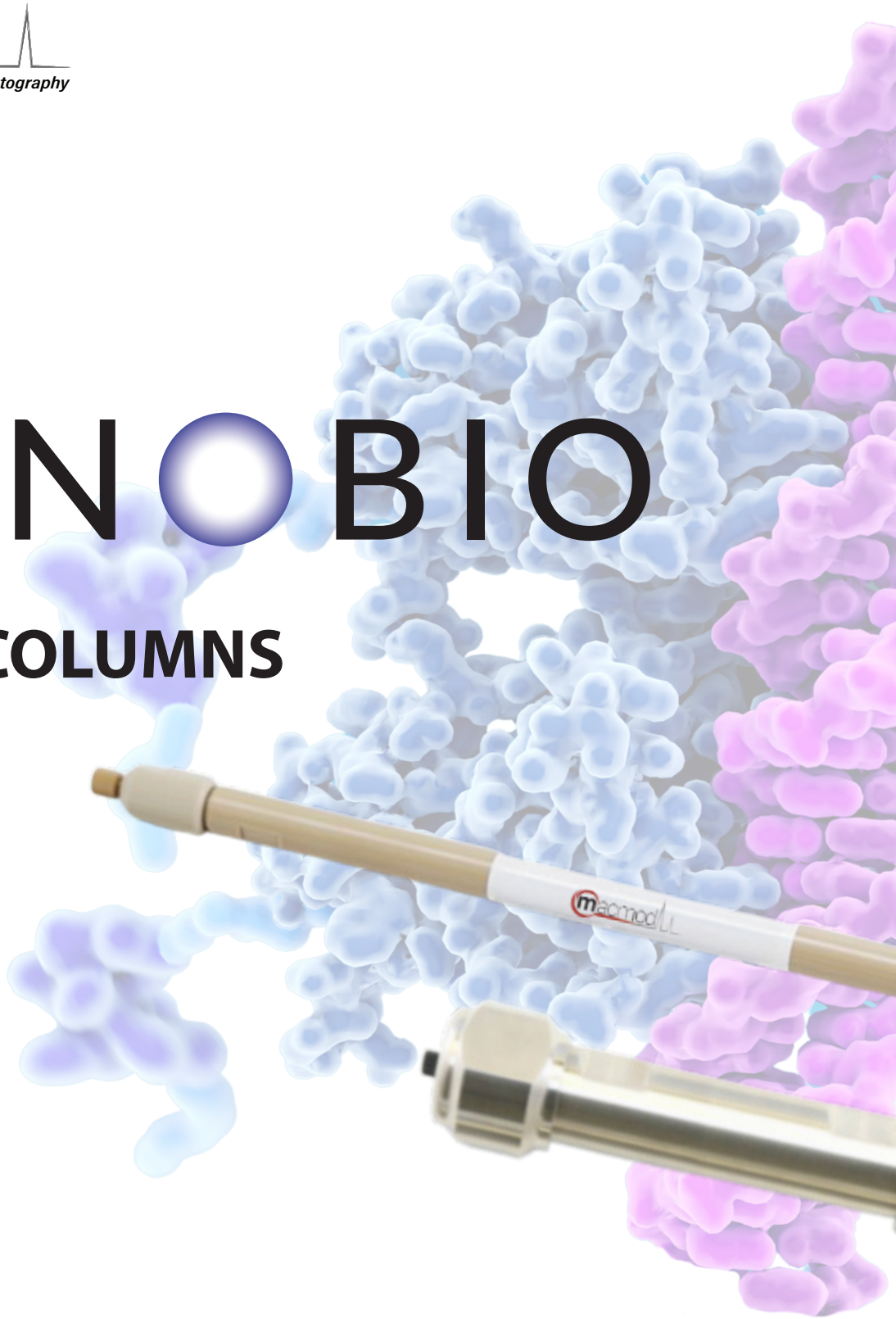
macmod

Innovations in Chromatography

MONOBIO

IEX HPLC COLUMNS



Powered By

MONODISPERSE PARTICLE TECHNOLOGY

TABLE OF CONTENTS

INTRODUCTION TO IEX HPLC

What is IEX Chromatography?

Ion-exchange chromatography is a mode of chromatography where ionic interactions are used to retain charged species. These analytes bind to the stationary phase through ionic attraction and are then reversibly eluted by disrupting this interaction.

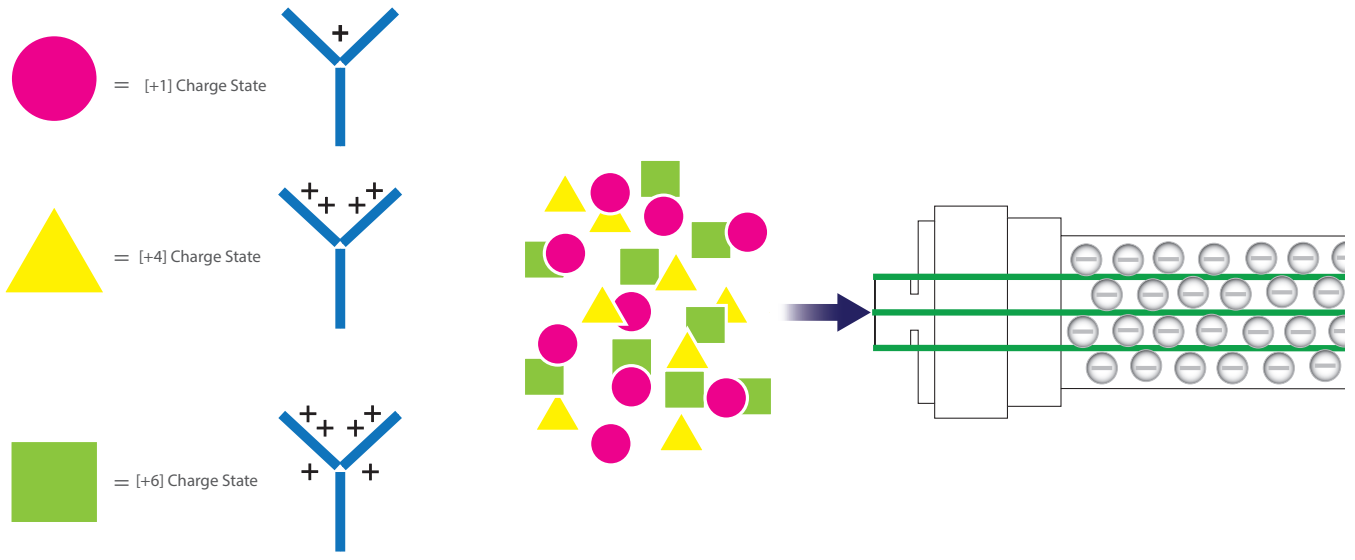
In the case below, we have native monoclonal antibodies that are positively charged due to their amino acid residues. The greater the positive amino acid charge on the monoclonal antibody, the more these analytes are retained on the negatively charged surface.

To elute these molecules from the negatively charged surface, a pH or salt gradient is used. The elution order normally reflects the positive charge on the analyte species (in this case, monoclonal antibodies), with less charged antibodies eluting first and more charged ones eluting later.

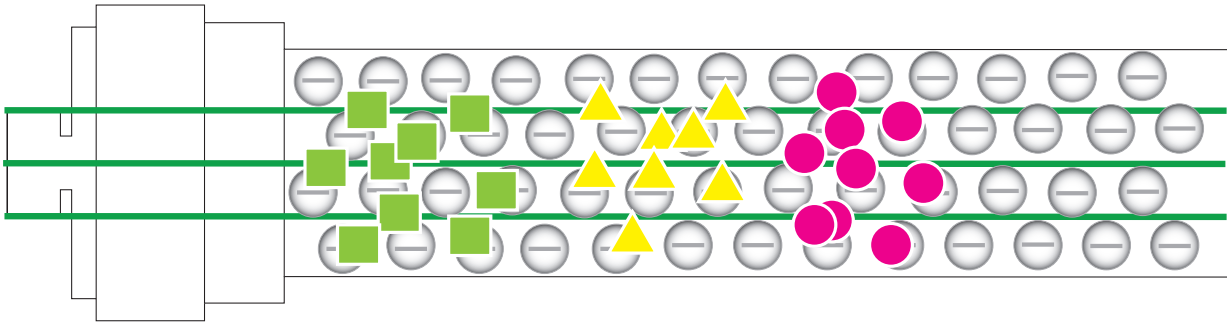
When a negatively charged column retains positively charged species (more basic), this is called CEX (cation-exchange chromatography). Conversely, a positively charged column retaining negatively charged species (more acidic) is called AEX (anion-exchange chromatography). In CEX, the more basic your mAb is, the more likely it will elute later; the more acidic your mAb is, the more likely it will elute earlier. In AEX, the opposite occurs: the more acidic your mAb is, the more likely it will elute later.

Example of mAb Charged Variant Analysis on MONOBIO CEX Column

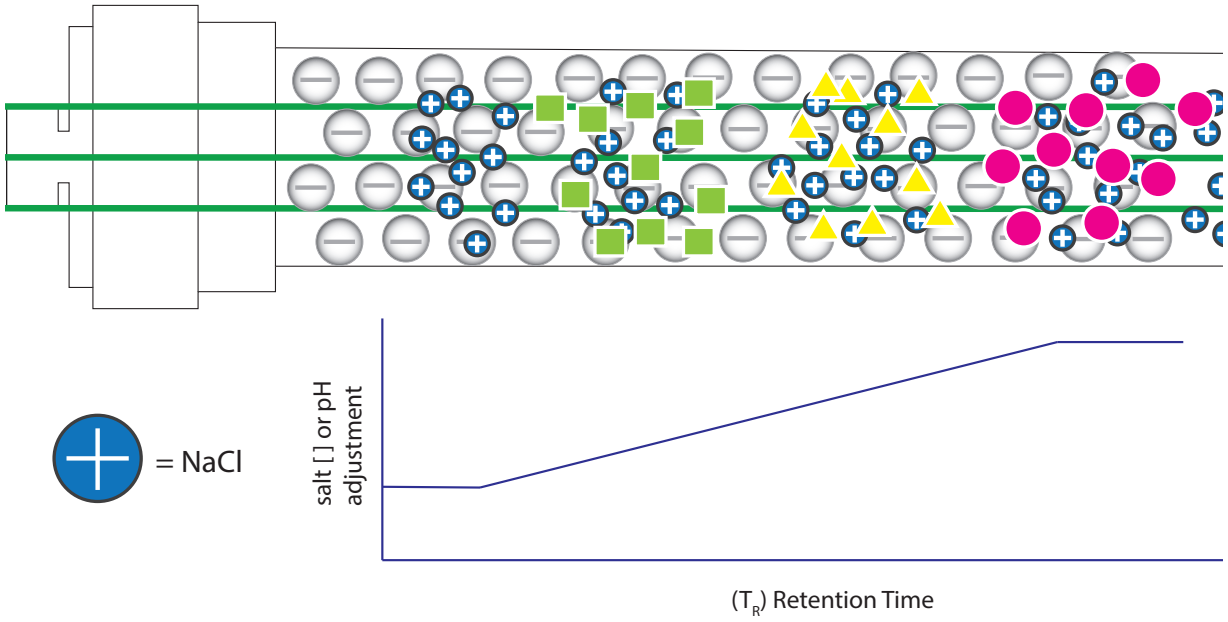
Step 1: Sample injected on column



Step 2: Sample migrates on column dependent on charged surface of monodisperse particle column based on the analytes charged state.



Step 3: A salt or pH gradient is applied to disrupt the ionic attraction on column to migrate differing charged states of the analyte mixture. Once the pH or salt gradient is completed, all analytes should be eluted off of the monodisperse IEX column.



INTRODUCTION TO IEX HPLC

Example Chromatogram: Complex Mixture of Differing mAb Charge States

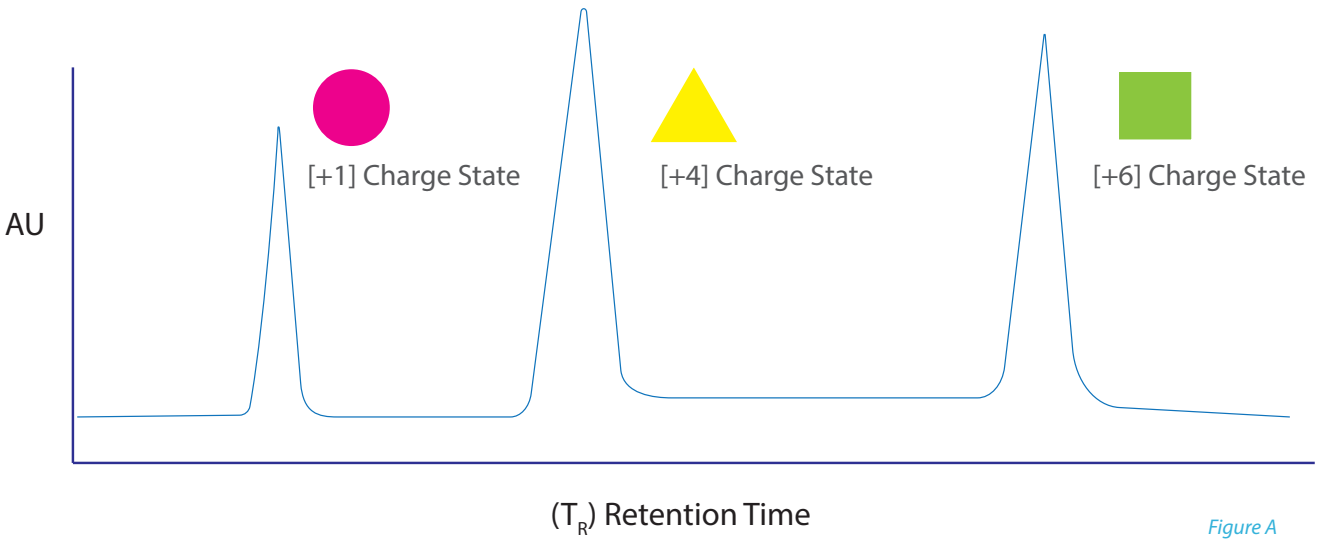


Figure A

Figure A: This chromatogram illustrates the separation of a complex mixture of monoclonal antibody (mAb) charge variants using IEX HPLC. Distinct peaks correspond to differing charge states: [+1], [+4], and [+6], highlighing the resolving power of MONOBIO Monodisperse IEX columns.

INHERENT BENEFITS OF A MONODISPERSE IEX PLATFORM

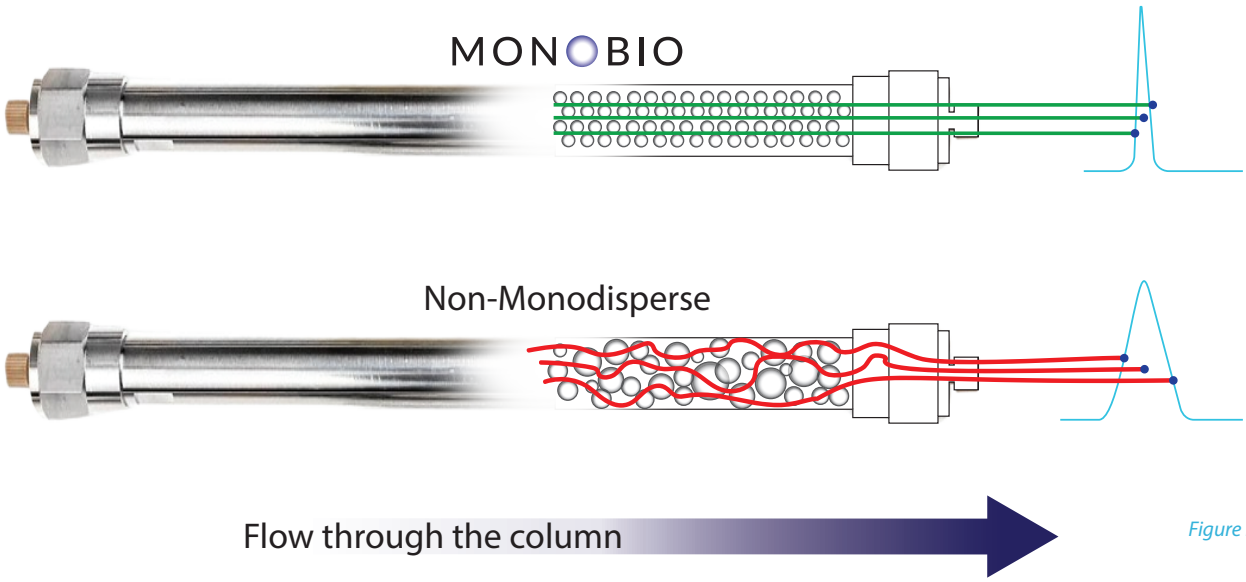


Figure B

Figure B: MONOBIO Monodiseprse IEX columns provide more uniform flow paths, resulting in sharper, more consistent peak profiles. In contrast, non-monodisperse columns introduce irregular flow and peak broadening, reducing resolution and efficiency.

The benefits include:

- Provides high efficiency (N) and excellent mechanical strength, which delivers high R_s
- Advanced column chemistries minimize secondary interactions
- Inherently delivers excellent batch to batch reproducibility and excellent robustness

PRODUCT SPECIFICATIONS

MONOBIO PRO-IEX Columns

Column Type	Pore Size (Å)	Bonded Phase	Particle Composition	Particle Size (µm)	pH Range	Application Areas
Pro-IEX WCX	Nonporous	Carboxylic Acid – Proprietary Hydrophilic Layer	Monodisperse PSDVB Particles	5.0 10.0	2-12	Charged Variants in Antibodies and Proteins
Pro-IEX SCX	Nonporous	Sulfonic Acid – Proprietary Hydrophilic Layer	Monodisperse PSDVB Particles	5.0 10.0	2-12	Charged Variants in Antibodies and Proteins (including mAbs)
Pro-IEX WAX	Nonporous	Tertiary Amine – Proprietary Hydrophilic Layer	Monodisperse PSDVB Particles	5.0 10.0	2-12	Charged Variants in Antibodies and Proteins with low pI
Pro-IEX SAX	Nonporous	Quaternary Ammonium – Proprietary Hydrophilic Layer	Monodisperse PSDVB Particles	5.0 10.0	2-12	Charged Variants in Antibodies and Proteins with l ow pI

MONOBIO DNA-IEX Columns

Column Type	Pore Size (Å)	Bonded Phase	Particle Compostition	Particle Size (µm)	pH Range	Application Areas
DNA-IEX WAX	Nonporous	Tertiary Amine – Proprietary Hydrophilic Layer	Monodisperse PSDVB Particles	5.0 10.0	2-12	Oligonucleotides, DNAs, mRNAs and plasmid topology
DNA-IEX SAX	Nonporous	Quaternary Ammonium – Proprietary Hydrophilic Layer	Monodisperse PSDVB Particles	5.0 10.0	2-12	Oligonucleotides, DNAs, mRNAs and plasmid topology

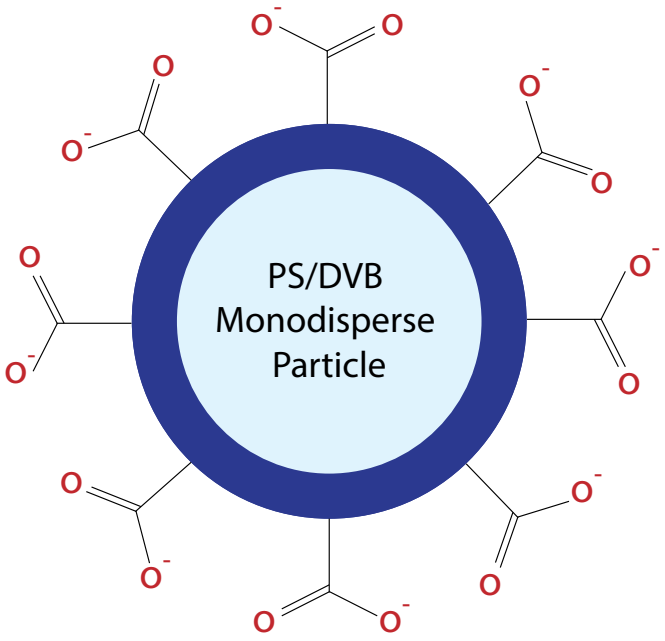
MONOBIO AAV-IEX SAX Columns

Column Type	Pore Size (Å)	Bonded Phase	Particle Compostition	Particle Size (µm)	pH Range	Application Areas
AAV-IEX-SAX	Nonporous	Quaternary Ammonium – Proprietary Hydrophilic Layer	Monodisperse PSDVB Particles	10.0	2-12	Empty/Full ratio analysis of capsid content in different serotypes of AAV

MONOBIO PRO-IEX WCX

MONOBIO Pro-IEX WCX columns are bonded with a carboxylic acid funtional group and treated with a hydrophilic layer on the surface of monodisperse, nonporous, spherical PSDVB particles.

To leverage the benefits of monodispersity and ion-exchange capacity, each column is packed using optimized procedures to deliver maximum efficiency and resolution.



Features

- Innovative monodisperse particle technology
- Optimal selectivity based on carboxylic acid bonded phase technology for separating charged variants in antibodies/proteins
- High column efficiency for better resolving power
- Symmetrical peak shapes and minimal carryover
- Excellent chemical and mechanical stability for long column life
- Superb column-to-column consistency for reproducible results

MONOBIO PRO-IEX WCX

mAb Standard

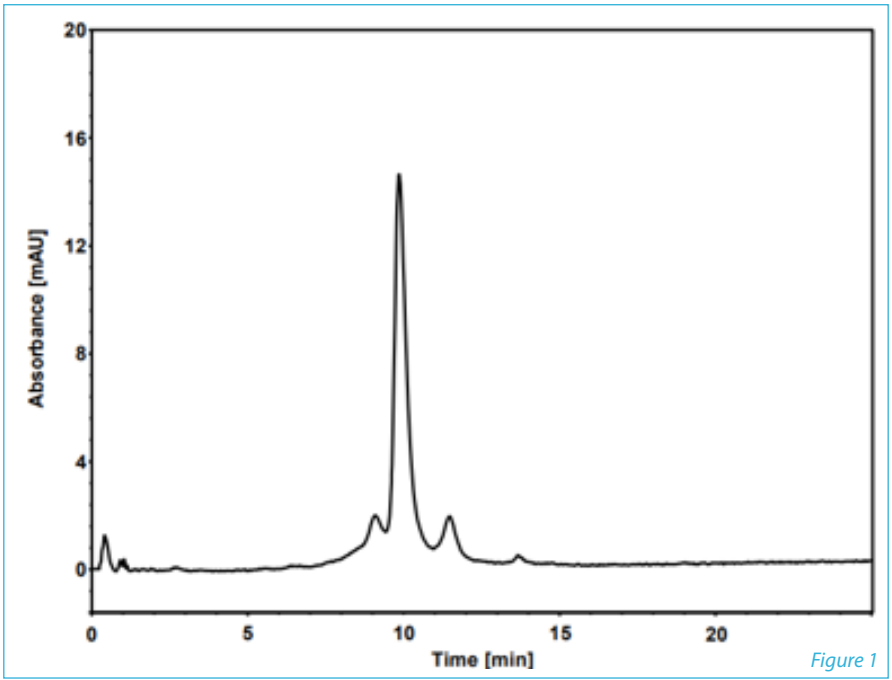


Figure 1

Column: MBIEXWCXN34615
Description: MONOBIO Pro-IEX WCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A.) 20 mM ACES, pH 7.0
B.) 300 mM NaCl in 20 mM ACES, p.H 7.0
Gradient:

t(min)	%A	%B
-20	80	20
0	80	20
5	80	20
25	60	40
25.1	0	100
30	0	100

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 2 µL
Detection: UV 280 nm
Sample: mAb (5.0 mg/mL in mobile phase A)

IgG1

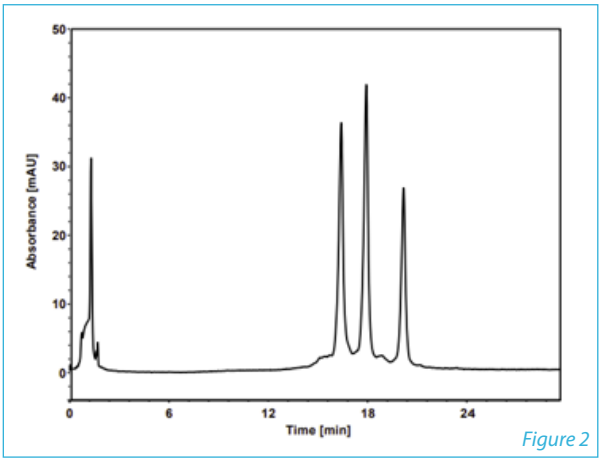


Figure 2

Column: MBIEXWCXN34625
Description: MONOBIO Pro-IEX WCX NP 10 µm, 4.6 x 250 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM MES, pH 6.1
B) 200 mM NaCl in 20 mM MES, pH 6.1
Gradient:

t(min)	%A	%B
-15	83	17
0	83	17
5	83	17
30	50	50
30.1	0	100
35	0	100

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 20 µL
Detection: UV 280 nm
Sample: IgG1 (~2 mg/mL in H_2O)

IgG1

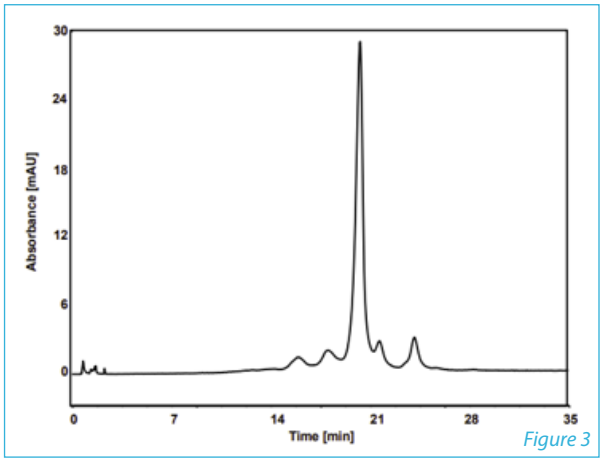


Figure 3

Column: MBIEXWCXN34625
Description: MONOBIO Pro-IEX WCX NP 10 µm, 4.6 x 250 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM MES, pH 6.5
B) 150 mM NaCl in 20 mM MES, pH 6.5
Gradient:

t(min)	%A	%B
-15	95	5
0	95	5
0.1	95	5
40	80	20
40.1	0	100
43	0	100

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 10 µL
Detection: UV 280 nm
Sample: IgG1 (~2.5 mg/mL in mobile phase A)

Figures 1-3: The MONOBIO Pro-IEX WCX columns were used to determine charge variants of monoclonal antibodies of different subclasses, including a mAb standard and two IgG1 samples.

MONOBIO PRO-IEX WCX

IgG2

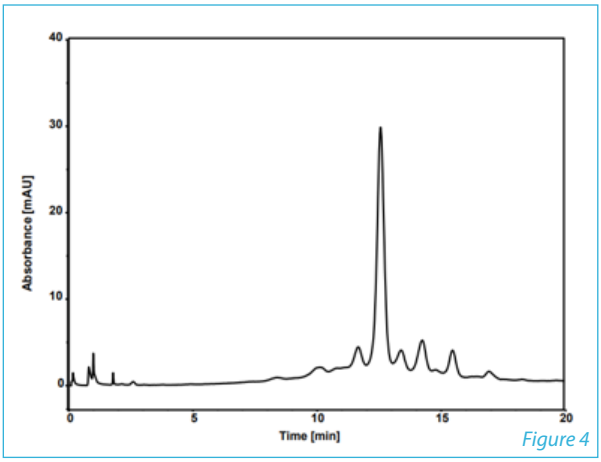


Figure 4

Column: MBIEXWCXN34615
Description: MONOBIO Pro-IEX WCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM phosphate buffer, pH 7.0
B) 300 mM NaCl in 20 mM phosphate buffer, pH 7.0
Gradient:

t(min)	%A	%B
-15	95	5
0	95	5
0.1	95	5
20	80	20
20.1	0	100
23	0	100

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 25 µL
Detection: UV 280 nm
Sample: IgG2 (1 mg/mL in H_2O)

IgG2

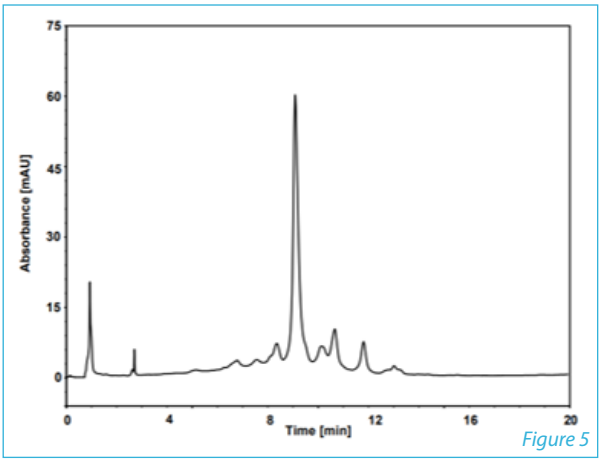


Figure 5

Column: MBIEXWCXN24615
Description: MONOBIO Pro-IEX WCX NP 5 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM phosphate buffer, pH 6.5
B) 300 mM NaCl in 20 mM phosphate buffer, pH 6.5
Gradient:

t(min)	%A	%B
-15	85	15
0	85	15
0.1	85	15
20	70	30
20.1	0	100
23	0	100

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 25 µL
Detection: UV 280 nm
Sample: IgG2 (1 mg/mL in mobile phase A)

IgG4

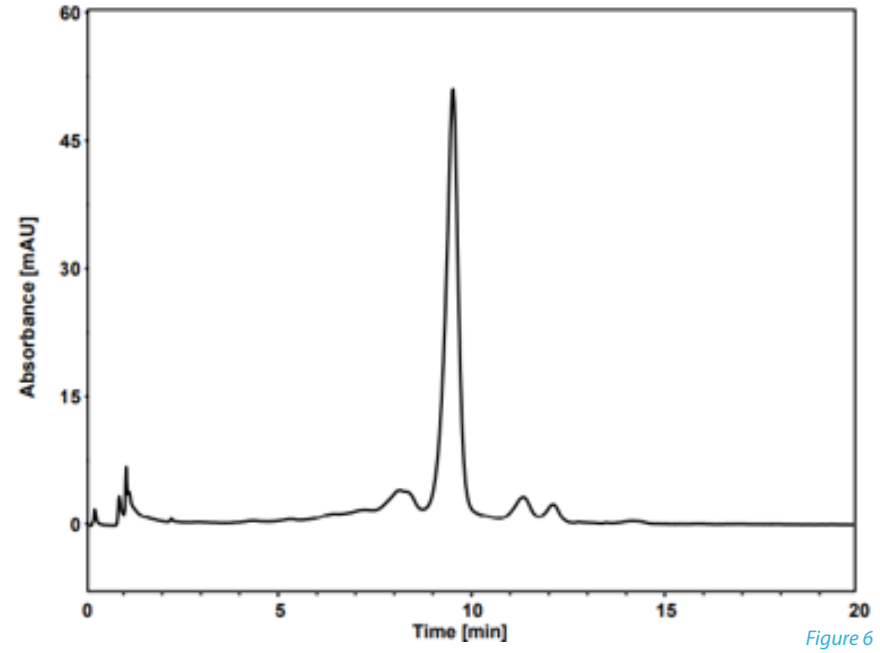


Figure 6

Column:	MBIEXWCXN34615
Description:	MONOBIO Pro-IEX WCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase:	A) 20 mM phosphate buffer, pH 6.8 B) 300 mM NaCl in 20 mM phosphate buffer, pH 7.0
Gradient:	

t(min)	%A	%B
-15	95	5
0	95	5
0.1	95	5
20	80	20
20.1	0	100
23	0	100

Flow Rate:	1.0 mL/min
Temperature:	30°C
Injection:	25 µL
Detection:	UV 280 nm
Sample:	IgG4 (1 mg/mL in H_2O)

Figures 4-6: The MONOBIO Pro-IEX WCX columns were used to determine charge variants of monoclonal antibodies of different subclasses (IgG2 and IgG4).

MONOBIO PRO-IEX WCX

Fab and Fab-PEG

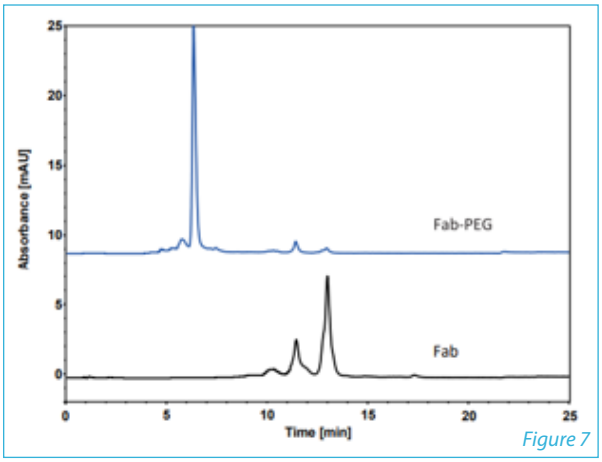


Figure 7

Column: MBIEXWCXN34615
Description: MONOBIO Pro-IEX WCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM MES, pH 5.5
B) 300 mM NaCl in 20 mM MES, pH 5.5
Gradient:

t(min)	%A	%B
-10	100	0
0	100	0
20	60	40
20.1	0	100
25	0	100

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 2 µL
Detection: UV 280 nm
Sample: Fab-PEG (3mg/mL in 50 mM sodium acetate solution)
Fab (5 mg/mL in 50 mM phosphate buffer)

Bispecific Antibody

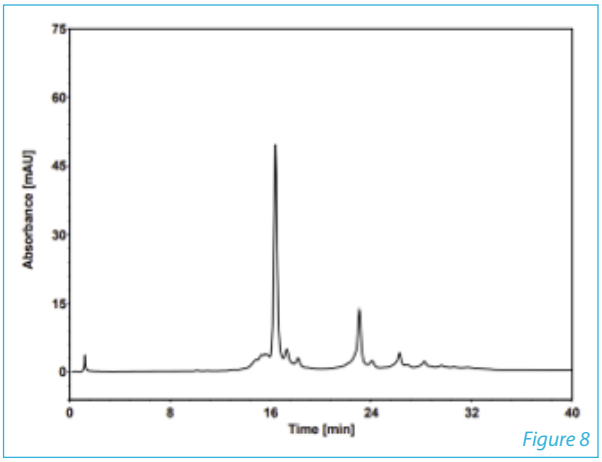


Figure 8

Column: MBIEXWCXN34615
Description: MONOBIO Pro-IEX WCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM phosphate buffer, pH 6.5
B) 300 mM NaCl in 20 mM phosphate buffer, pH 6.5
Gradient:

t(min)	%A	%B
0	100	0
40	65	35
40.1	0	100
45	0	100
45.1	100	0
60	100	0

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 20 µL
Detection: UV 280 nm
Sample: Bispecific antibody (~5.0 mg/mL, pI 8.5)

Bispecific Antibody

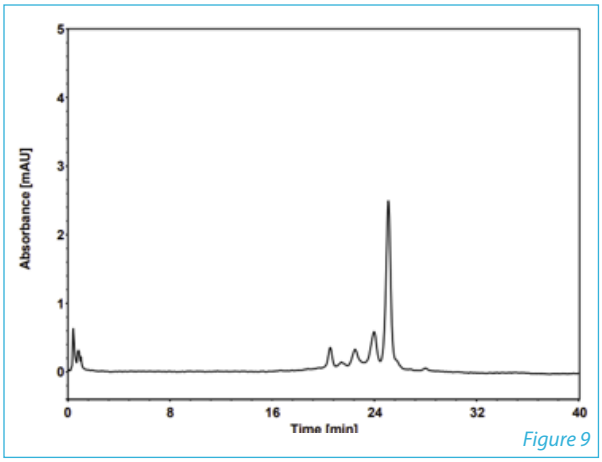


Figure 9

Column: MBIEXWCXN34615
Description: MONOBIO Pro-IEX WCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM phosphate buffer, pH 6.5
B) 300 mM NaCl in 20 mM phosphate buffer, pH 6.5
Gradient:

t(min)	%A	%B
0	100	0
0.1	100	0
40	70	30
40.1	0	100
43	0	100

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 3 µL
Detection: UV 280 nm
Sample: Bispecific antibody (1.72 mg/mL, pl 8.15)

Bispecific Antibody

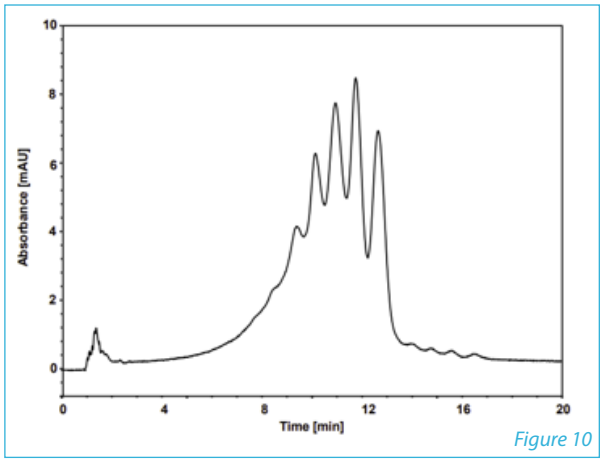


Figure 10

Column: MBIEXWCXN34615
Description: MONOBIO Pro-IEX WCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM phosphate buffer, pH 6.5
B) 300 mM NaCl in 20 mM phosphate buffer, pH 6.5
Gradient:

t(min)	%A	%B
-15	90	10
0	90	10
0.1	90	10
20	75	25
20.1	0	100
23	0	100

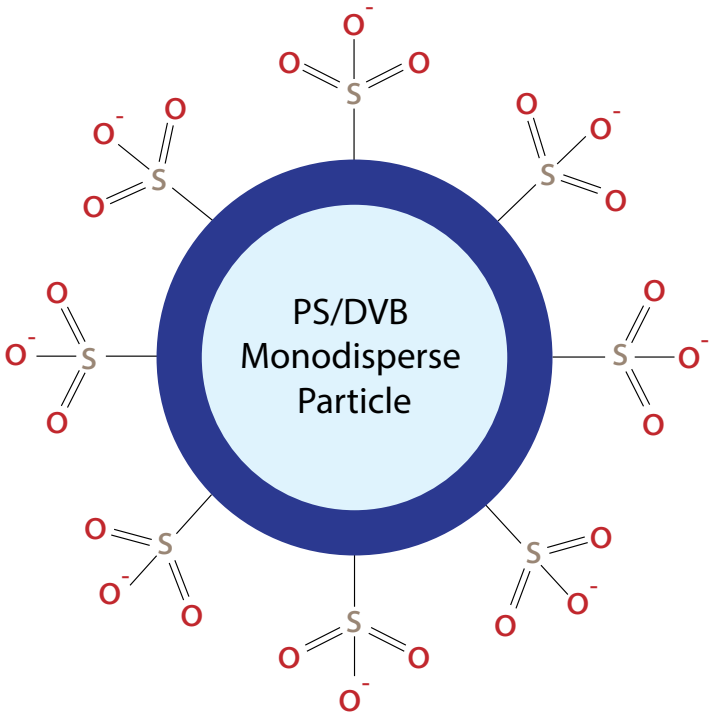
Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 5 µL
Detection: UV 280 nm
Sample: Bispecific antibody (~5.0 mg/mL in mobile phase A)

Figures 7–10: The MONOBIO Pro-IEX WCX columns were used to analyze charge variants of different monoclonal antibody types: Fab (fragment antigen-binding) and Fab-PEG (pegylated fragment antigen-binding) in the same separation, and three distinct bispecific antibody types in separate separations.

MONOBIO PRO-IEX SCX

MONOBIO Pro-IEX WCX columns are bonded with a proprietary sulfonic acid functional group and treated with a hydrophilic layer on the surface of monodispersed, nonporous, spherical PS/DVB particles.

To leverage the benefits of monodispersity and ion-exchange capacity, each column is packed using optimized procedures to deliver maximum efficiency and resolution.



Features

- Innovative monodisperse particle technology
- Optimal selectivity based on sulfonic acid bonded phase technology for separating charged variants in antibodies/proteins
- High column efficiency for better resolving power
- Symmetrical peak shapes and minimal carryover
- Excellent chemical and mechanical stability for long column life
- Superb column-to-column consistency for reproducible results

IgG2

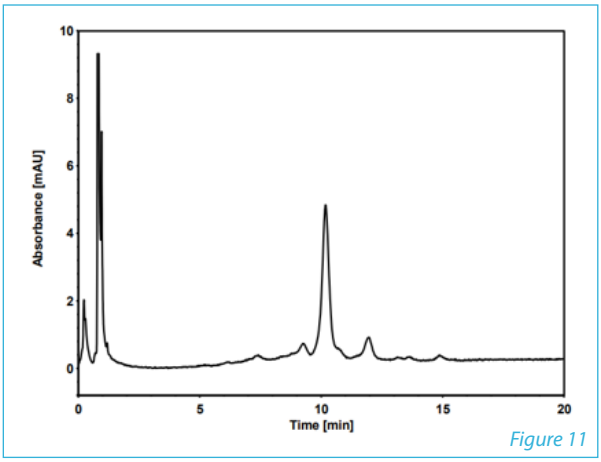


Figure 11

Column: MBIEXSCXN34615
Description: MONOBIO Pro-IEX SCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM MES, pH 6.5
B) 300 mM NaCl in 20 mM MES, pH 6.5
Gradient:

t(min)	%A	%B
-15	82	18
0	82	18
20	70	30
20.1	0	100
23	0	100

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 10 µL
Detection: UV 280 nm
Sample: IgG2 (1 mg/mL in H_2O)

IgG4

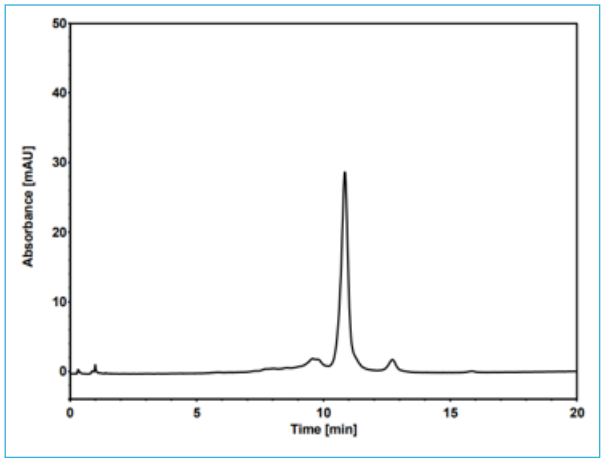


Figure 12

Column: MBIEXSCXN34615
Description: MONOBIO Pro-IEX SCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM MES, pH 6.5
B) 300 mM NaCl in 20 mM MES, pH 6.5
Gradient:

t(min)	%A	%B
-15	93	7
0	93	7
20	79	21
20.1	0	100
23	0	100

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 10 µL
Detection: UV 280 nm
Sample: IgG4 (1 mg/mL in H_2O)

MONOBIO PRO-IEX SCX

Bispecific Antibody

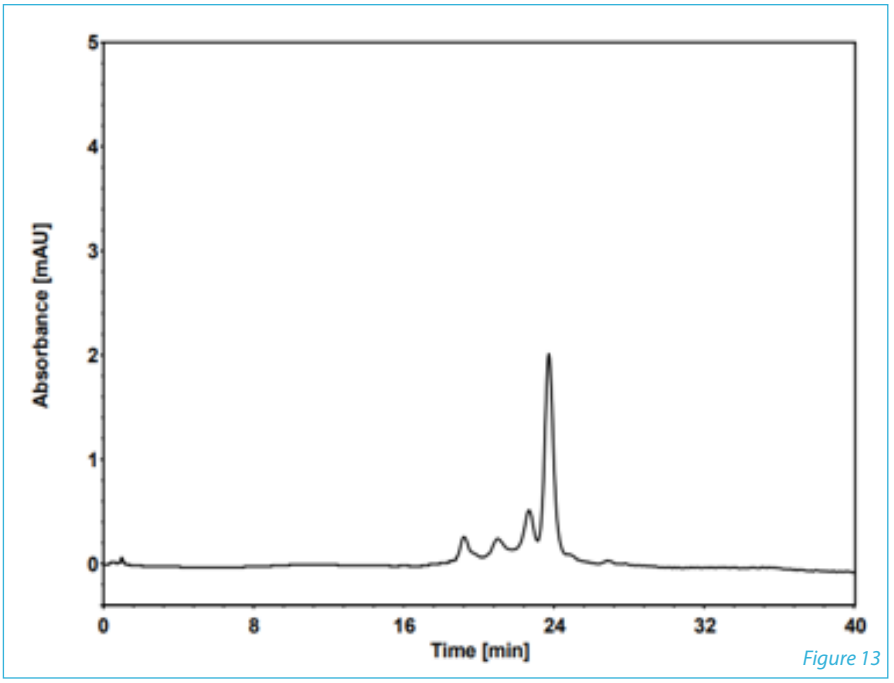


Figure 13

Column: MBIEXSCXN34615
Description: MONOBIO Pro-IEX SCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM phosphate buffer, pH 6.5
B) 300 mM NaCl in 20 mM phosphate buffer, pH 6.5
Gradient:

t(min)	%A	%B
0	100	0
0.1	100	0
40	70	30
40.1	0	100
43	0	100

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 3 µL
Detection: UV 280 nm
Sample: Bispecific antibody (1.72 mg/mL, pI 8.15)

Figures 11-13: The MONOBIO Pro-IEX SCX columns were used to determine charge variants of monoclonal antibodies of different subclasses (IgG2 and IgG4) and a different type (a bispecific antibody).

Analytical Scale: ADC Analysis

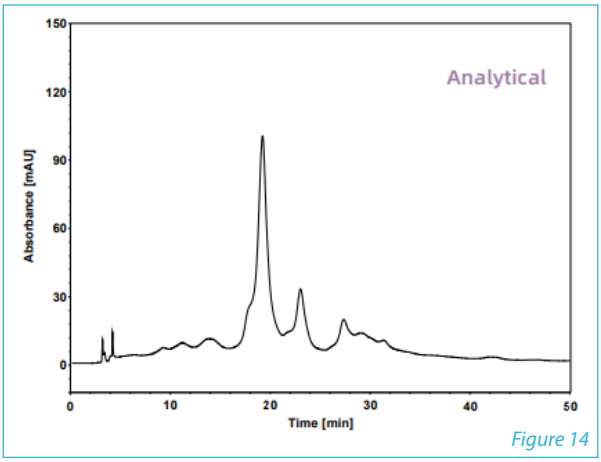


Figure 14

Column: MBIEXSCXN34625
Description: MONOBIO Pro-IEX SCX NP 10 µm, 4.6 x 250 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM phosphate buffer, pH 6.5
B) 300 mM NaCl in 20 mM phosphate buffer, pH 6.5

Gradient:

t(min)	%A	%B
0	100	0
2	100	0
52	94.2	5.8
52.1	0	100
57	0	100
57.1	100	0
77	100	0

Flow Rate: 0.5 mL/min
Temperature: 25°C
Injection: 10 µL
Detection: UV 280 nm
Sample: ADC (30 mg/mL, pI 7.4)

Preparative Scale: ADC Purifiation

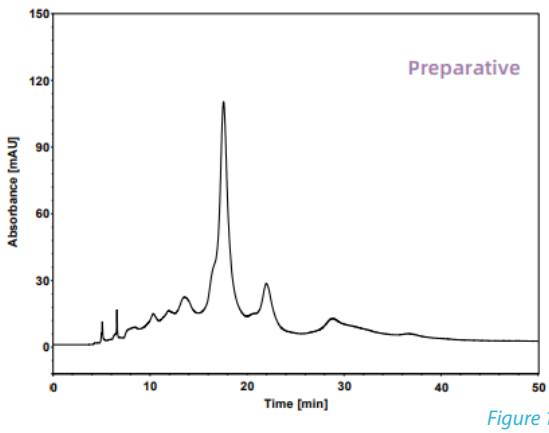


Figure 15

Column: MBIEXSCXN32125
Description: MONOBIO Pro-IEX SCX NP 10 µm, 21.2 x 250 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM phosphate buffer, pH 6.5
B) 300 mM NaCl in 20 mM phosphate buffer, pH 6.5

Gradient:

t(min)	%A	%B
0	100	0
2	100	0
52	92.7	7.3
52.1	0	100
57	0	100
57.1	100	0
77	100	0

Flow Rate: 6.0 mL/min
Temperature: 25°C
Injection: 50 µL
Detection: UV 280 nm
Sample: ADC (30 mg/mL, pI 7.4)

Figures 14 and 15: The MONOBIO Pro-IEX SCX columns were used to determine charge variants of ADCs (antibody drug conjugates) in order to isolate them via preparative chromatography. The method scaled the column from an analytical size (10 µm, 4.6 x 250 mm, PEEK hardware) to a preparative size (10 µm, 21.2 x 250 mm, stainless steel hardware). This represents an exciting application for further verification of charge variant species within the ADC platform.

MONOBIO PRO-IEX SCX

Fusion Protein

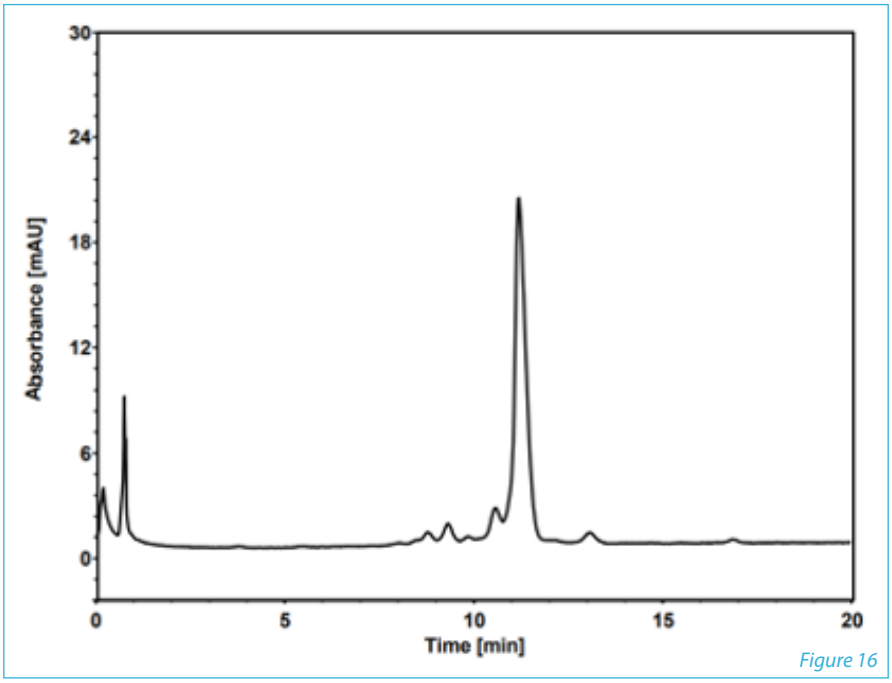


Figure 16

Column: MBIEXSCXN34615
Description: MONOBIO Pro-IEX SCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM MES, pH 6.0
B) 300 mM NaCl in 20 mM MES, pH 6.0

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 10 µL
Detection: UV 280 nm
Sample: Fusion protein (2.5 mg/mL in mobile phase A)

Gradient:

t(min)	%A	%B
-15	72	28
0	72	28
25	45	55
25.1	0	100
28	0	100

Fab and Fab-PEG

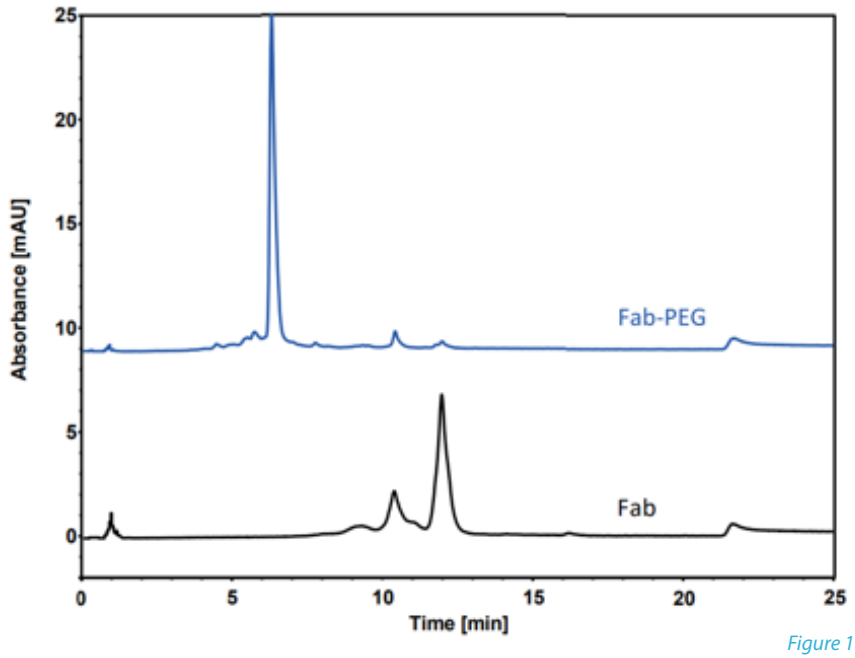


Figure 17

Column: MBIEXSCXN34615
Description: MONOBIO Pro-IEX SCX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM MES, pH 5.5
B) 300 mM NaCl in 20 MES, pH 5.5

Gradient:

t(min)	%A	%B
-10	100	0
0	100	0
20	60	40
20.1	0	100
25	0	100

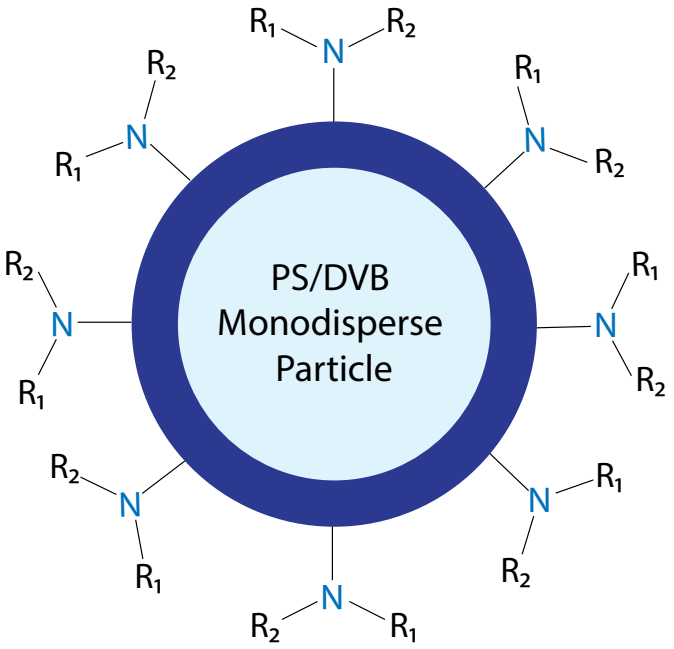
Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 2 µL
Detection: UV 280 nm
Sample: Fab-PEG (3 mg/mL in 50 mM sodium acetate solution)
Fab (5 mg/mL in 50 mM phosphate buffer)

Figures 16 and 17: The MONOBIO Pro-IEX SCX columns were used to determine charge variants in a fusion protein, as well as a Fab alongside its pegylated form, Fab-PEG.

MONOBIO PRO-IEX WAX

MONOBIO Pro-IEX WAX columns are bonded with a proprietary tertiary amine phase chemistry and then treated with a hydrophilic surface modification on the monodisperse, nonporous, spherical PS/DVB particles. These columns are designed for separating charged variants in proteins with low isoelectric points (pI).

To leverage the benefits of monodispersity and ion-exchange capacity, each column is packed using optimized procedures to deliver maximum efficiency and resolution.



Features

- Innovative monodisperse particle technology
- Optimal selectivity based on tertiary amine bonded phase technology for separating charged variants in antibodies/proteins
- High column efficiency for better resolving power
- Symmetrical peak shapes and minimal carryover
- Excellent chemical and mechanical stability for long column life
- Superb column-to-column consistency for reproducible results

Ovalbumin

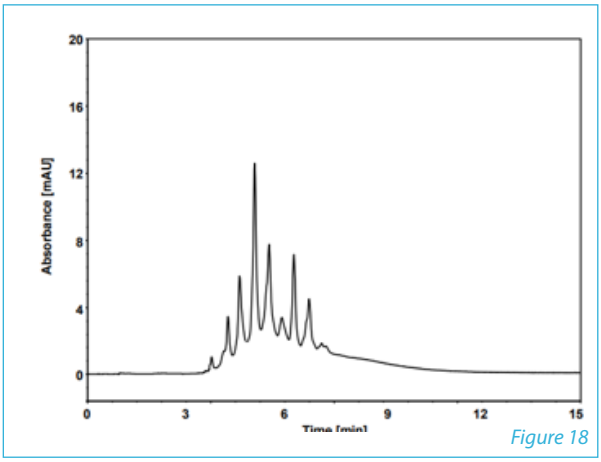


Figure 18

Column: MBIEXWAXN24615
Description: MONOBIO Pro-IEX WAX NP 5 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM Tris, pH 8.0
B) 500 mM NaCl in 20 mM Tris, pH 8.0
Gradient:

t(min)	%A	%B
0	100	0
0.1	100	0
15	50	50
15.1	99	1
20	99	1
20.1	100	0
30	100	0

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 10 µL
Detection: UV 280 nm
Sample: Ovalbumin (5 mg/mL)

Recombinant Protein

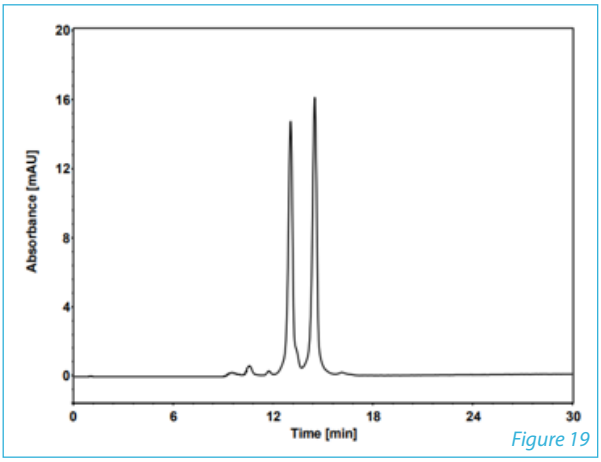


Figure 19

Column: MBIEXWAXN34615
Description: MONOBIO Pro-IEX WAX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM Tris, pH 7.0
B) 250 mM NaCl in 20 mM Tris, pH 7.0
Gradient:

t(min)	%A	%B
0	100	0
0.5	100	0
30	60	40
30.1	100	0
40	100	0

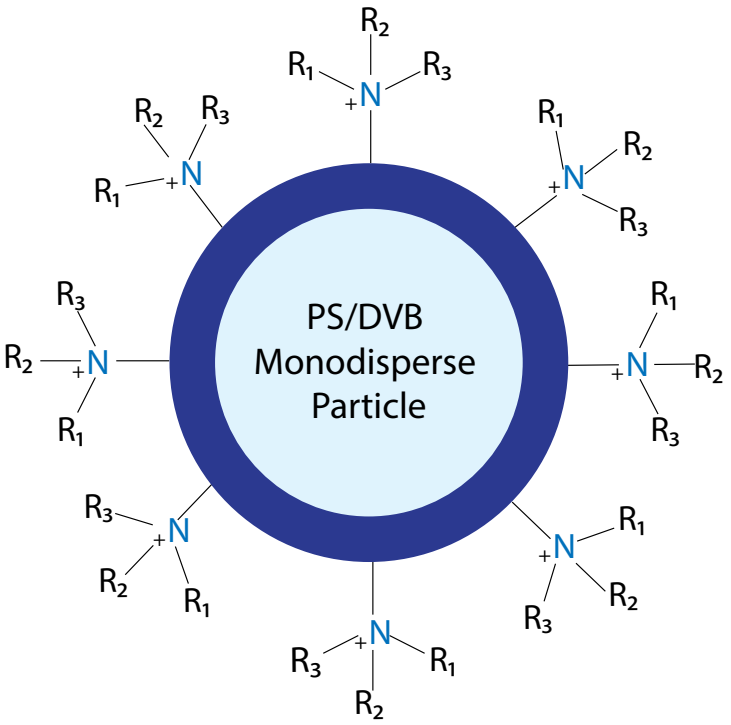
Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 5 µL
Detection: UV 280 nm
Sample: Recombinant protein (2 mg/mL, pI 4~5)

Figures 18 and 19: The MONOBIO Pro-IEX WAX columns were used to determine charge variants of different analyte types including an ovalbumin and a recombinant protein.

MONOBIO PRO-IEX SAX

MONOBIO Pro-IEX SAX columns are bonded with a quaternary ammonium functional phase chemistry and treated with a hydrophilic layer on the surface of monodispersed, nonporous, spherical PS/DVB particles.

To leverage the benefits of monodispersity and ion-exchange capacity, each column is packed using optimized procedures to deliver maximum efficiency and resolution.



Features

- Innovative monodisperse particle technology
- Optimal selectivity based on quaternary ammonium bonded phase technology for separating charged variants in antibodies/proteins
- High column efficiency for better resolving power
- Symmetrical peak shapes and minimal carryover
- Excellent chemical and mechanical stability for long column life
- Superb column-to-column consistency for reproducible results

Ovalbumin

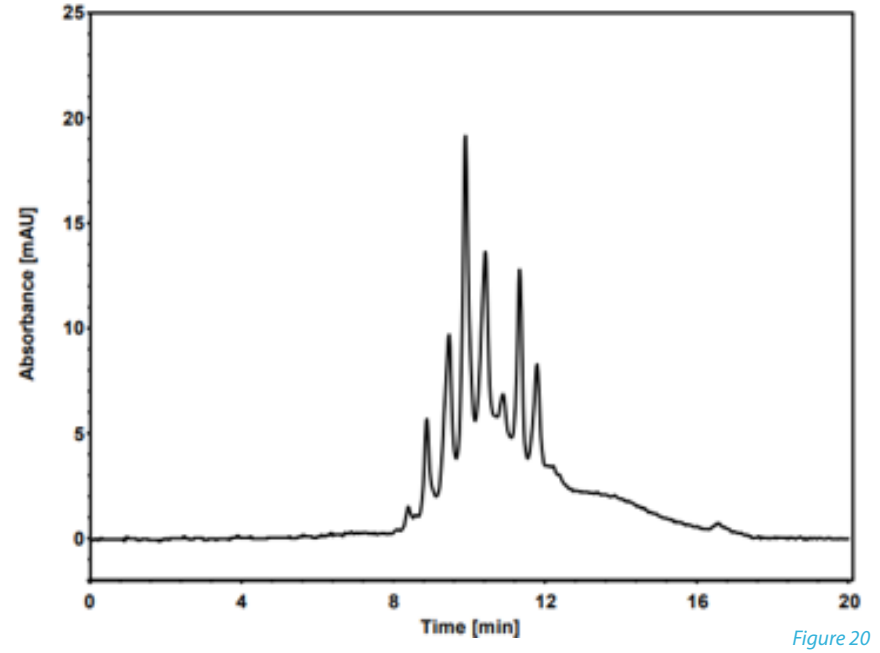


Figure 20

Column: MBIEXSAXN34615
Description: MONOBIO Pro-IEX SAX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM Tris, pH 8.5
B) 500 mM NaCl in 20 mM Tris, pH 8.5
Gradient:

t(min)	%A	%B
-15	100	0
0	100	0
15	50	50
15.1	0	100
20	0	100

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 10 µL
Detection: UV 280 nm
Sample: Ovalbumin (5 mg/mL in H_2O)

MONOBIO PRO-IEX SAX

Recombinant Fusion Protein

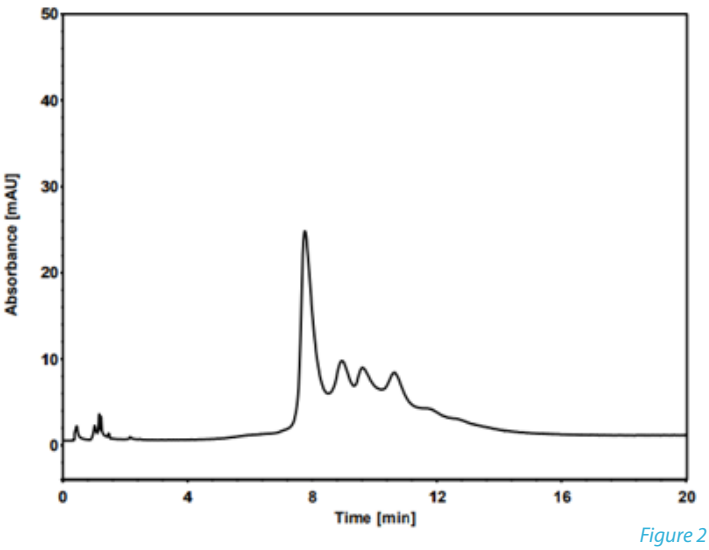


Figure 21

Column: MBIEXSAXN34615
Description: MONOBIO Pro-IEX SAX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM MES, pH 6.5
B) 300 mM NaCl in 20 mM MES, pH 6.5

Gradient:

t(min)	%A	%B
-15	70	30
0	70	30
20	40	60
20.1	0	100
23	0	100

Flow Rate: 0.8 mL/min
Temperature: 20°C
Injection: 10 µL
Detection: UV 280 nm
Sample: Recombinant fusion protein (1 mg/mL in H_2O)

Figures 20 and 21: The MONOBIO Pro-IEX SAX columns were used to determine charge variants of an ovalbumin and a recombinant fusion protein.

BSA

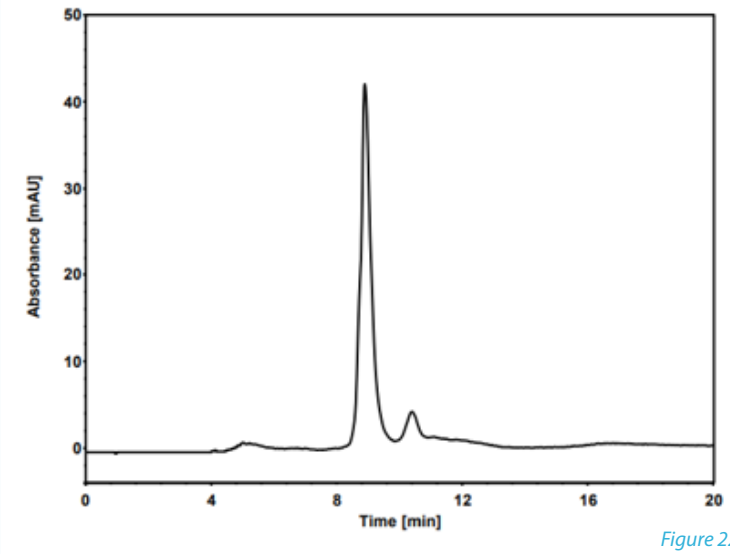


Figure 22

Column:	MBIEXSAXN34615	Flow Rate:	1.0 mL/min
Description:	MONOBIO Pro-IEX SAX NP 10 µm, 4.6 x 150 mm Monodisperse HPLC Column	Temperature:	30°C
		Injection:	10 µL
Mobile Phase:	A) 20 mM Tris, pH 8.5	Detection:	UV 280 nm
	B) 500 mM NaCl in 20 mM Tris, pH 8.5	Sample:	BSA (5 mg/mL in H_2O)

Gradient:

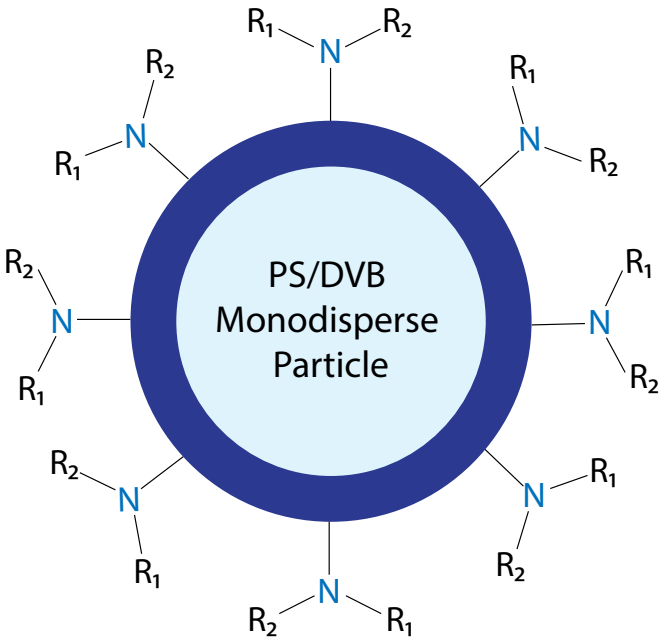
t(min)	%A	%B
-15	100	0
0	100	0
15	0	100
20	0	100
20.1	100	0

Figure 22: The MONOBIO Pro-IEX SAX column was used to characterize the charge variants of BSA (bovine serum albumin).

MONOBIO DNA-IEX WAX

MONOBIO DNA-IEX WAX columns are treated with a tertiary amine bonded phase and treated with a hydrophilic layer on the surface of monodispersed, nonporous, spherical PS/DVB particles. These columns are designed for separating oligonucleotides, DNAs, mRNAs, and plasmids to explore their topology based on charge states.

To leverage the benefits of monodispersity and ion-exchange capacity, each column is packed using optimized procedures to deliver maximum efficiency and resolution.



Features

- Innovative monodisperse particle technology
- Optimal selectivity based on tertiary amine bonded phase technology for separating charged variants in antibodies/proteins
- High column efficiency for better resolving power
- Symmetrical peak shapes and minimal carryover
- Excellent chemical and mechanical stability for long column life
- Superb column-to-column consistency for reproducible results

Plasmid

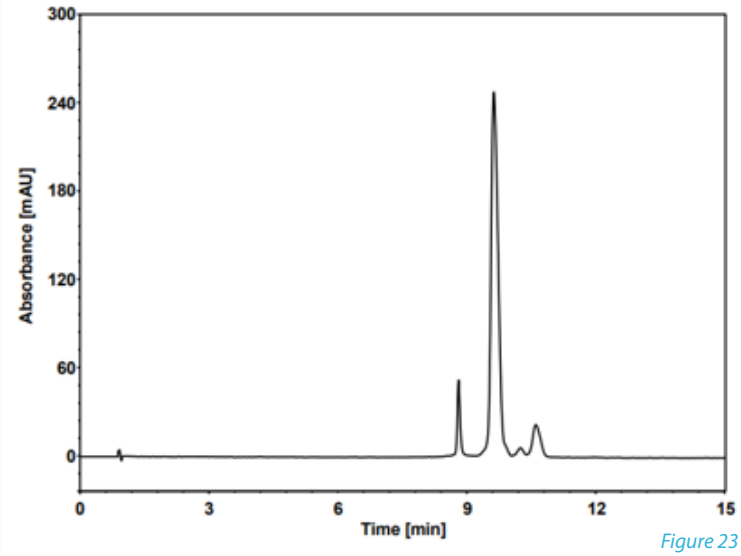


Figure 23

Column:	MBIEXWAXN24615D	Flow Rate:	1.0 mL/min
Description:	MONOBIO DNA-IEX WAX NP 5 µm, 4.6 x 150 mm Monodisperse HPLC Column	Temperature:	20°C
		Injection:	3 µL
Mobile Phase:	A) 100 mM NaCl in 300 mM GuHCl and 100 mM Tris, pH 8.0	Detection:	UV 260 nm
		Sample:	Plasmid (~8000 base pairs)
	B) 700 mM NaCl in 300 mM GuHCl and 100 mM Tris, pH 8.0		

Gradient:

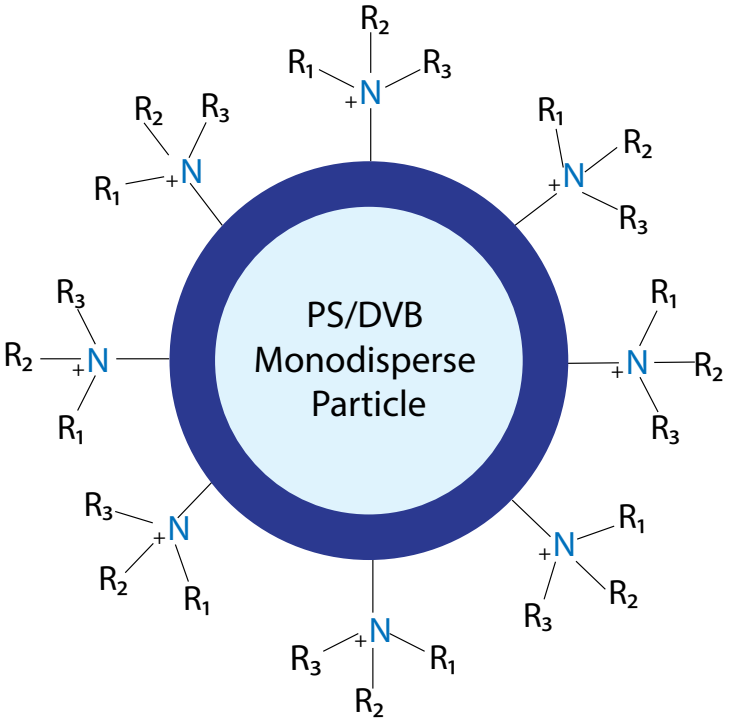
t(min)	%A	%B
0	75	25
15	0	100
20	0	100
20.1	75	25
30	75	25

Figure 23: The MONOBIO DNA-IEX WAX column is used to determine plasmid topology differences based on different charge states.

MONOBIO DNA-IEX SAX

MONOBIO DNA-IEX SAX columns are treated with a proprietary quaternary ammonium bonded phase and treated with a proprietary hydrophilic layer on the surface of monodispersed, nonporous, spherical PS/DVB particles. These columns are designed for separating oligonucleotides, DNAs, mRNAs, and plasmids to explore their topology based on charge states.

To leverage the benefits of monodispersity and ion-exchange capacity, each column is packed using optimized procedures to deliver maximum efficiency and resolution.



Features

- Innovative monodisperse particle technology
- Optimal selectivity based on quaternary ammonium bonded phase technology for separating charged variants in antibodies/proteins
- High column efficiency for better resolving power
- Symmetrical peak shapes and minimal carryover
- Excellent chemical and mechanical stability for long column life
- Superb column-to-column consistency for reproducible results

Plasmid

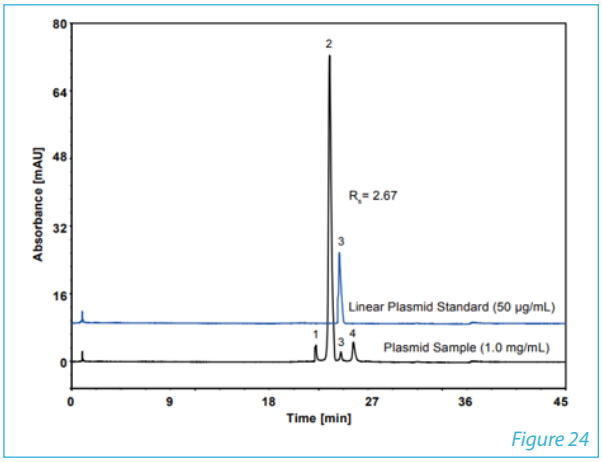


Figure 24

Column: MBIEXSAXN24615D
Description: MONOBIO DNA-IEX SAX NP 5 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM Tris, pH 8.5
B) 1 M NaCl in 20 mM Tris, pH 8.5
Gradient:

t(min)	%A	%B
0	50	50
30	15	85
30.1	0	100
35	0	100
35.1	50	50
45	50	50

Flow Rate: 1.0 mL/min
Temperature: 30°C
Injection: 3 µL
Detection: UV 260 nm
Sample: 1. Open-circular plasmid
2. Supercoiled plasmid
3. Linear plasmid
4. Unknown peak

Plasmid

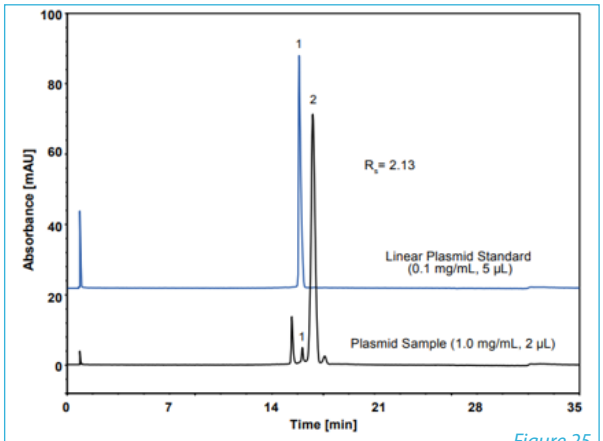


Figure 25

Column: MBIEXSAXN24615D
Description: MONOBIO DNA-IEX SAX NP 5 µm, 4.6 x 150 mm Monodisperse HPLC Column
Mobile Phase: A) 20 mM Tris, pH 8.5
B) 1 M NaCl in 20 mM Tris, pH 8.5
Gradient:

t(min)	%A	%B
-12	40	60
0	40	60
25	15	85
25.1	0	100
30	40	60
30.1	40	60
35	40	60

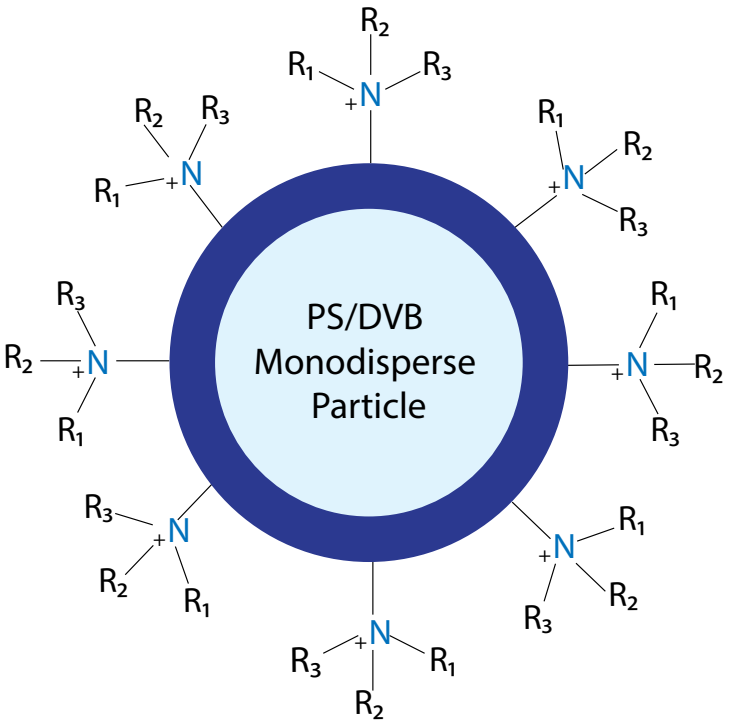
Flow Rate: 1.0 mL/min
Termperature: 30°C
Injection: 3 µL
Detection: UV 260 nm
Sample: 1. Linear plasmid
2. Supercoiled plasmid

Figures 24 and 25: The MONOBIO DNA-IEX SAX columns were used to determine plasmid topology differences based on charge, separating open-circular, linear, and supercoiled forms across two different plasmid types.

MONOBIO AAV-IEX SAX

MONOBIO DNA-IEX SAX columns are treated with a proprietary quaternary amine bonded phase with hydrophilic proprietary layer on the surface of monodispersed, nonporous, spherical PS/DVB particles. These columns are designed for separating oligonucleotides, DNAs, mRNAs, and plasmids to explore their topology based on charge states.

To leverage the benefits of monodispersity and ion-exchange capacity, each column is packed using optimized procedures to deliver maximum efficiency and resolution.



Features

- Innovative monodisperse particle technology
- Optimal selectivity based on quaternary amine bonded phase technology for oligonucleotides, DNAs, mRNAs, and Plasmid topology
- High column efficiency for better resolving power
- Symmetrical peak shapes and minimal carryover
- Excellent chemical and mechanical stability for long column life
- Superb column-to-column consistency for reproducible results

AAV5

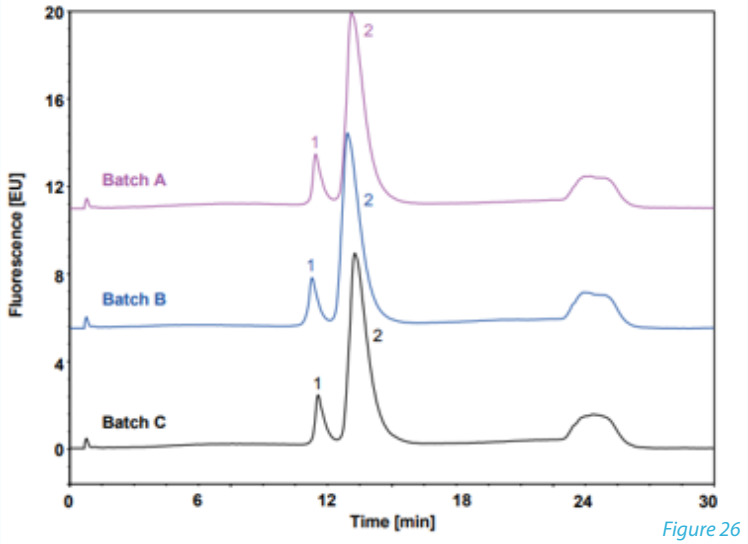


Figure 26

Column: MBIEXSAXN34605A
Description: MONOBIO AAV-IEX SAX NP 10 µm, 4.6 x 50 mm Monodisperse HPLC Column
Mobile Phase: A) H_2O
B) 1 M TMAC
C) 200 mM BTP, pH 8.5
Gradient:

t(min)	%A	%B	%C
0	85	5	10
0.5	85	5	10
20.5	55	35	10
20.6	0	90	10
22.5	0	90	10
22.6	85	5	10
30	85	5	10

Flow Rate: 0.5 mL/min
Temperature: 30°C
Injection: 5 µL
Detection: FLD (Ex 280 nm, Em 350 nm)
Sample: AAV5 GFP (1.0E+12 vg/mL)
1) Empty
2) Full

Figure 26: Demonstrated here is the separation of empty and full capsids of AAV5 using three batches of MONOBIO AAV-IEX columns. The excellent RSD (relative standard deviation) in retention times for both capsid types demonstrates the inherent benefit of monodisperse particles in providing batch-to-batch consistency. Resolution RSD was also highly reproducible at approximately 1.0%.

MONOBIO AAV-IEX SAX

AAV5
Linear Gradient vs. Step Gradient

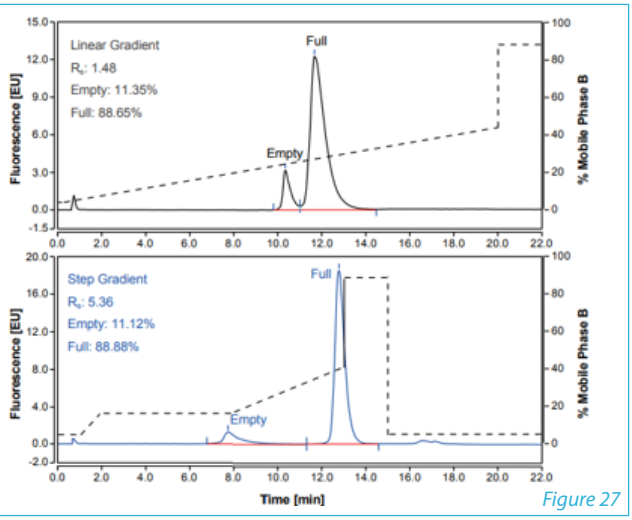


Figure 27

Column: MBIEXSAXN34605A
Description: MONOBIO AAV-IEX SAX NP 10 µm, 4.6 x 50 mm Monodisperse HPLC Column
Mobile Phase: A) H_2O
B) 1 M TMAC
C) 200 mM BTP, pH 8.5
Flow Rate: 0.5 mL/min
Temperature: 30°C
Injection: 5 µL
Detection: FLD (Ex 280 nm, Em 350 nm)
Sample: AAV5 GFP (1.0E+12 vg/mL)

Linear Gradient:

t(min)	%A	%B	%C
0	85	5	10
0.1	85	5	10
20	45	45	10
20.1	0	90	10
22	0	90	10
22.1	85	5	10
30	85	5	10

Step Gradient:

t(min)	%A	%B	%C
0	85	5	10
1	85	5	10
2	79	17	10
8	79	17	10
13	50	40	10
13.1	0	90	10
15	0	90	10
15.1	85	5	10
25	85	5	10

AAV2
Linear Gradient vs. Step Gradient

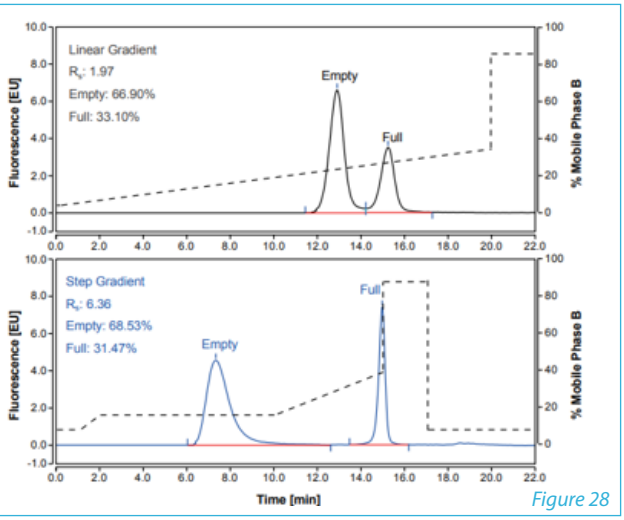


Figure 28

Column: MBIEXSAXN34605A
Description: MONOBIO AAV-IEX SAX NP 10 µm, 4.6 x 50 mm Monodisperse HPLC Column
Mobile Phase: A) H_2O
B) 1 M TMAC
C) 200 mM BTP, pH 9.5
D) $MgCl_2$
Flow Rate: 0.5 mL/min
Temperature: 30°C
Injection: 1 µL
Detection: FLD (Ex 280 nm, Em 350 nm)
Sample: AAV2 GFP (1.0E+12 vg/mL)

Linear Gradient:

t(min)	%A	%B	%C	%D
0	81	5	10	4
0.1	81	5	10	4
20	51	35	10	4
20.1	0	86	10	4
22	0	86	10	4
22.1	81	5	10	4
30	81	5	10	4

Step Gradient:

t(min)	%A	%B	%C	%D
0	78	8	10	4
1	78	8	10	4
2	68	18	10	4
10	68	18	10	4
15	46	40	10	4
15.1	0	86	10	4
17	0	86	10	4
17.1	78	8	10	4
25	78	8	10	4

MONOBIO AAV-IEX SAX

AAV6
Linear Gradient vs. Step Gradient

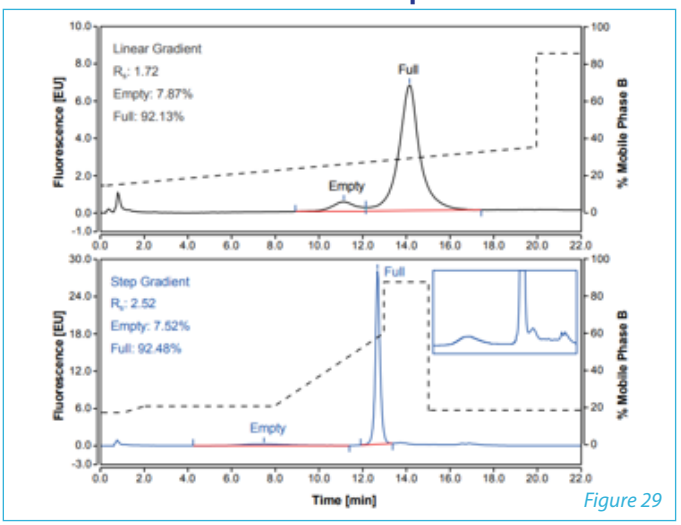


Figure 29

Column: MBIEXSAXN34605A
Description: MONOBIO DNA-IEX SAX NP 10 µm, 4.6 x 50 mm Monodisperse HPLC Column
Mobile Phase: A) H_2O
B) 1 M TMAC
C) 200 mM BTP, pH 9.0
D) 25 mM $MgCl_2$
Flow Rate: 0.5 mL/min
Temperature: 30°C
Injection: 5 µL
Detection: FLD (Ex 280 nm, Em 350 nm)
Sample: AAV6-GFP (1.0E+12 vg/mL)

Linear Gradient:

t(min)	%A	%B	%C	%D
0	71	15	10	4
0.1	71	15	10	4
20	61	25	10	4
20.1	0	86	10	4
22	0	86	10	4
22.1	71	15	10	4
30	71	15	10	4

Step Gradient:

t(min)	%A	%B	%C	%D
0	68	18	10	4
1	68	18	10	4
2	65	21	10	4
8	65	21	10	4
13	26	60	10	4
13.1	0	86	10	4
15	0	86	10	4
15.1	68	18	10	4
25	68	18	10	4

AAV8
Linear Gradient vs. Step Gradient

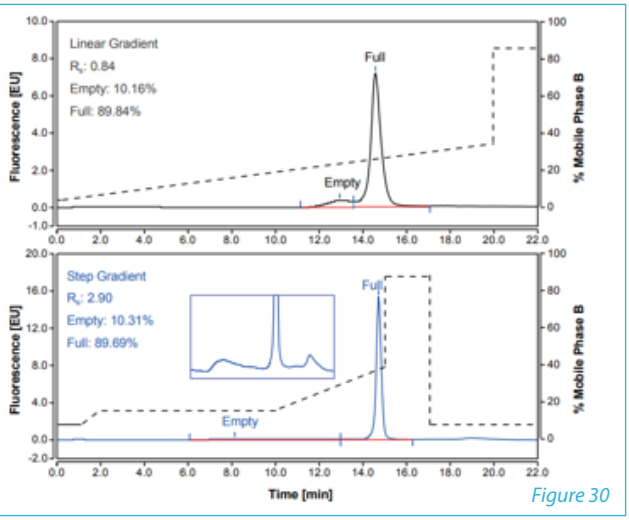


Figure 30

Column:	MBIEXSAXN34605A
Description:	MONOBIO AAV-IEX SAX NP 10 um, 4.6 x 50 mm Monodisperse HPLC Column
Mobile Phase:	A) H_2O
	B) 1 M TMAC
	C) 200 mM BTP, pH 8.5
	D) 25 mM $MgCl_2$
Flow Rate:	0.5 mL/min
Temperature:	30°C
Injection:	5 µL
Detection:	FLD (Ex 280 nm, Em 350 nm)
Sample:	AAV8-GFP (1.0E+12 vg/mL)

Linear Gradient:

t(min)	%A	%B	%C	%D
0	81	5	10	4
0.1	81	5	10	4
20	51	35	10	4
20.1	0	86	10	4
22	0	86	10	4
22.1	81	5	10	4
30	81	5	10	4

Step Gradient:

t(min)	%A	%B	%C	%D
0	78	8	10	4
1	78	8	10	4
2	71	15	10	4
10	71	15	10	4
15	46	40	10	4
15.1	0	86	10	4
17	0	86	10	4
17.1	78	8	10	4
25	78	8	10	4

Figure 27-30: Demonstrated here is the separation of empty and full capsids of AAV5, AAV2, AAV6, and AAV8 using MONOBIO AAV-IEX columns. A linear gradient and a step gradient were compared to highlight the improved resolution of empty and full capsids when using the step gradient approach, which retains the full capsid longer on column.

MONOBIO PRO-IEX PART NUMBER TABLES

MONOBIO Pro-IEX WCX Columns

Pore Size (Å)	Particle Size (µm)	Column ID (mm)	Column Length (mm)	Part Number
Nonporous	5.0	4.6	50	MBIEXWCXN24605
Nonporous	5.0	4.6	100	MBIEXWCXN24610
Nonporous	5.0	4.6	150	MBIEXWCXN24615
Nonporous	5.0	4.6	250	MBIEXWCXN24625
Nonporous	10.0	4.6	50	MBIEXWCXN34605
Nonporous	10.0	4.6	150	MBIEXWCXN34615
Nonporous	10.0	4.6	250	MBIEXWCXN34625

MONOBIO Pro-IEX SCX Columns

Pore Size (Å)	Particle Size (µm)	Column ID (mm)	Column Length (mm)	Part Number
Nonporous	5.0	4.6	50	MBIEXSCXN24605
Nonporous	5.0	4.6	100	MBIEXSCXN24610
Nonporous	5.0	4.6	150	MBIEXSCXN24615
Nonporous	5.0	4.6	250	MBIEXSCXN24625
Nonporous	10.0	4.6	50	MBIEXSCXN34605
Nonporous	10.0	4.6	150	MBIEXSCXN34615
Nonporous	10.0	4.6	250	MBIEXSCXN34625

MONOBIO Pro-IEX WAX Columns

Pore Size (Å)	Particle Size (µm)	Column ID (mm)	Column Length (mm)	Part Number
Nonporous	5.0	4.6	50	MBIEXWAXN24605
Nonporous	5.0	4.6	100	MBIEXWAXN24610
Nonporous	5.0	4.6	150	MBIEXWAXN24615
Nonporous	5.0	4.6	250	MBIEXWAXN24625
Nonporous	10.0	4.6	50	MBIEXWAXN34605
Nonporous	10.0	4.6	150	MBIEXWAXN34615
Nonporous	10.0	4.6	250	MBIEXWAXN34625

MONOBIO Pro-IEX SAX Columns

Pore Size (Å)	Particle Size (µm)	Column ID (mm)	Column Length (mm)	Part Number
Nonporous	5.0	4.6	50	MBIEXSAXN24605
Nonporous	5.0	4.6	100	MBIEXSAXN24610
Nonporous	5.0	4.6	150	MBIEXSAXN24615
Nonporous	5.0	4.6	250	MBIEXSAXN24625
Nonporous	10.0	4.6	50	MBIEXSAXN34605
Nonporous	10.0	4.6	150	MBIEXSAXN34615
Nonporous	10.0	4.6	250	MBIEXSAXN34625

MONOBIO PART NUMBER TABLES

MONOBIO DNA-IEX WAX Columns

Pore Size (Å)	Particle Size (µm)	Column ID (mm)	Column Length (mm)	Part Number
Nonporous	5.0	4.6	50	MBIEXWAXN24605D
Nonporous	5.0	4.6	150	MBIEXWAXN24615D
Nonporous	5.0	4.6	250	MBIEXWAXN24625D
Nonporous	10.0	4.6	50	MBIEXWAXN34605D
Nonporous	10.0	4.6	150	MBIEXWAXN34615D
NonPorous	10.0	4.6	250	MBIEXWAXN34625D

MONOBIO DNA-IEX SAX Columns

Pore Size (Å)	Particle Size (µm)	Column ID (mm)	Column Length (mm)	Part Number
Nonporous	5.0	4.6	50	MBIEXSAXN24605D
Nonporous	5.0	4.6	150	MBIEXSAXN24615D
Nonporous	5.0	4.6	250	MBIEXSAXN24625D
Nonporous	10.0	4.6	50	MBIEXSAXN34605D
Nonporous	10.0	4.6	150	MBIEXSAXN34615D
Nonporous	10.0	4.6	250	MBIEXSAXN34625D

MONOBIO AAV-IEX SAX Columns

Pore Size (Å)	Particle Size (µm)	Column ID (mm)	Column Length (mm)	Part Number
Nonporous	10.0	4.6	50	MBIEXSAXN34605A

CONTACT US

Ordering: info@mac-mod.com

Technical Support: technical@mac-mod.com

(610) 358-9696

103 Commons Court
Chadds Ford, PA 19317

www.mac-mod.com/brands/monobio/iex/