

Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

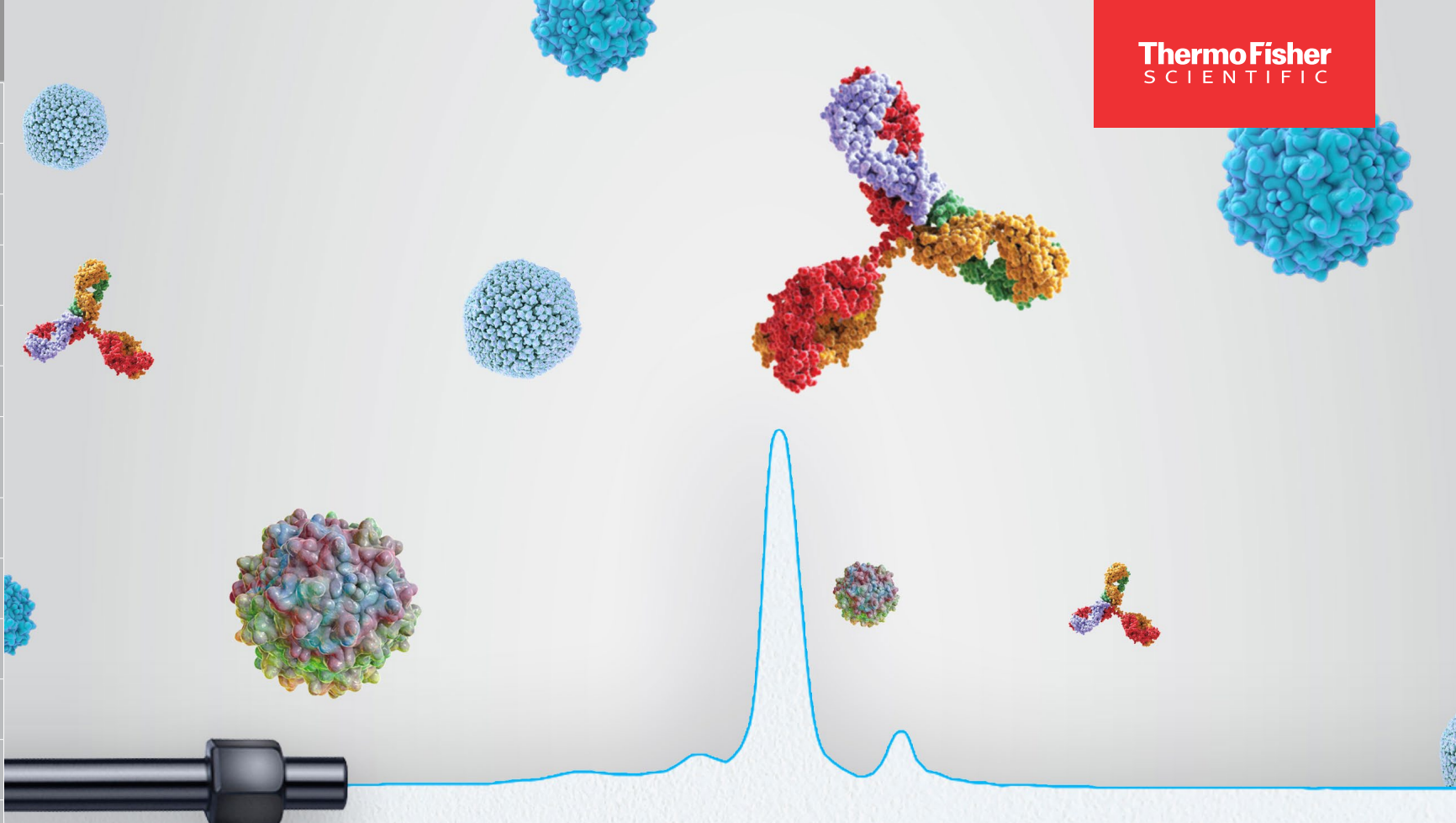
How does ProPac 3R compare?

Related products

CX-1 pH gradient buffers

Quick order guide

ThermoFisher
SCIENTIFIC



LC columns

Robust. Reproducible. Resolution. Every time.

ProPac 3R IEX columns

thermo**scientific**

Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

How does ProPac 3R compare?

Related products

CX-1 pH gradient buffers

Quick order guide

Introducing ProPac 3R columns

Thermo Scientific™ ProPac™ 3R SCX and SAX columns are the newest members of the ProPac family of liquid chromatography products. Designed with bio-inert materials, ProPac 3R columns support the delivery of **reproducible**, high **resolution** and **robust** strong anion and strong cation exchange chromatography separations for protein and monoclonal antibody analysis.

Looking to get reproducible, high resolution, robust data from your method/assay? These two unique ProPac 3R SCX and SAX column chemistries have excellent performance under a broad range of pH, temperature and mobile phase compositions, delivering excellent recovery and low carryover.



Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

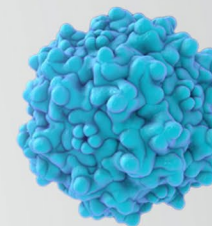
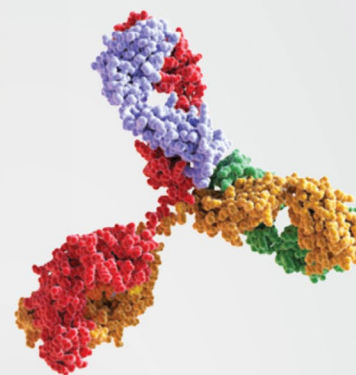
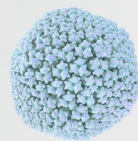
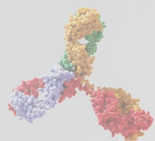
How does ProPac 3R compare?

Related products

CX-1 pH gradient buffers

Quick order guide

ProPac 3R benefits



Robustness

Reproducibility

Resolution

Get the performance you need for your charge variant separations

Achieve analytical flexibility and sensitivity

Breeze through your BioPharma method validation

Releasing drugs to the marketplace in QA/QC laboratories?

Increase the certainty of quantification of your critical quality attributes (CQAs)

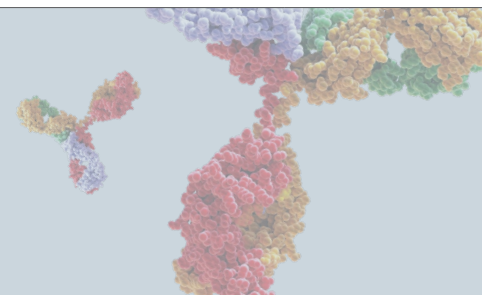
Designed with bio-inert materials to reduce secondary interactions, Thermo Scientific™ ProPac™ 3R columns are made for use with aqueous, high-ionic strength mobile phases and organic solvents used in biopharmaceutical applications.

The high sample loading capacity of ProPac 3R columns enables easy method optimization, as well as excellent performance under a broad range of pH, temperature, mobile phase compositions and offers outstanding MS capabilities.

Outstanding column-to-column reproducibility eliminates doubt in the detection and identification of new acidic or basic variants during late-stage development.

Confidently and consistently ensure reproducible performance for each sample tested.

The monodispersed bead size of ProPac 3R columns enhances resolution, providing consistent separation and improved peak-to-valley resolution of your charge variants.



Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

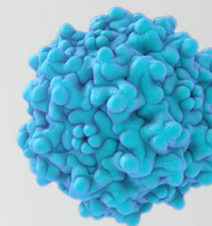
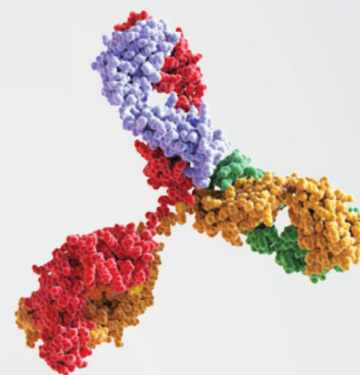
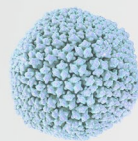
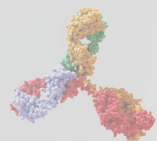
How does ProPac 3R compare?

Related products

CX-1 pH gradient buffers

Quick order guide

Technology and features



ProPac 3R column technology

Thermo Scientific ProPac 3R columns are designed to provide fast, high efficiency, high resolution separations of proteins and glycoproteins based on their accessible surface charge.

The 3 μm , non-porous particle is based on a solvent compatible divinylbenzene polymer resin to provide exceptionally high resolving power. Compared to traditional polydisperse particles (Figure 1 right), the monodisperse particles have a consistent size distribution (Figure 1 left) resulting in improved column packing and lot-to-lot reproducibility.

Column technology and features

- Superior resolution power for proteins, monoclonal antibodies and associated charge variants
- High efficiency with reproducible separations
- High recovery with low carryover
- Wide pH operating range: 2–12
- High temperature stability: up to 60 °C
- High throughput
- SCX compatible with Thermo Scientific™ CX-1 pH gradient buffers

Monodisperse ProPac 3R and polydisperse particles

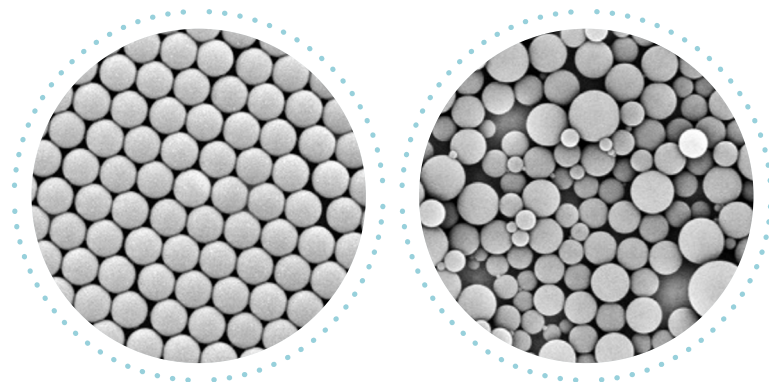
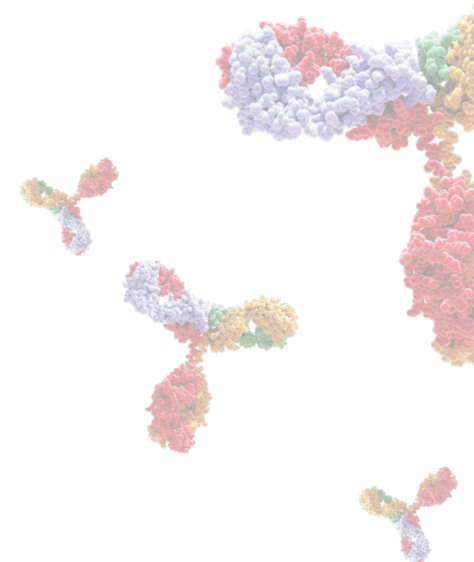


Figure 1: (left) 3 μm monodisperse particles, (right) traditional 3 μm polydisperse particles



Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

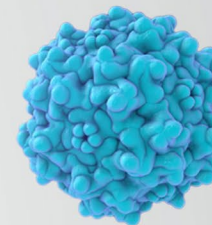
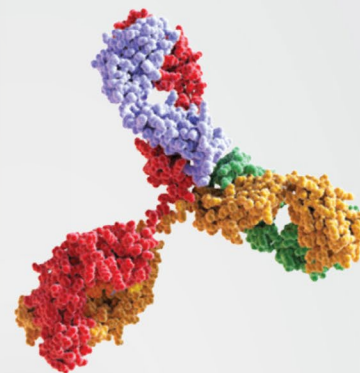
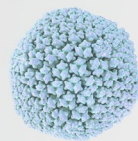
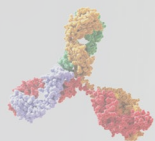
How does ProPac 3R compare?

Related products

CX-1 pH gradient buffers

Quick order guide

ProPac family



ProPac ion-exchange chromatography family

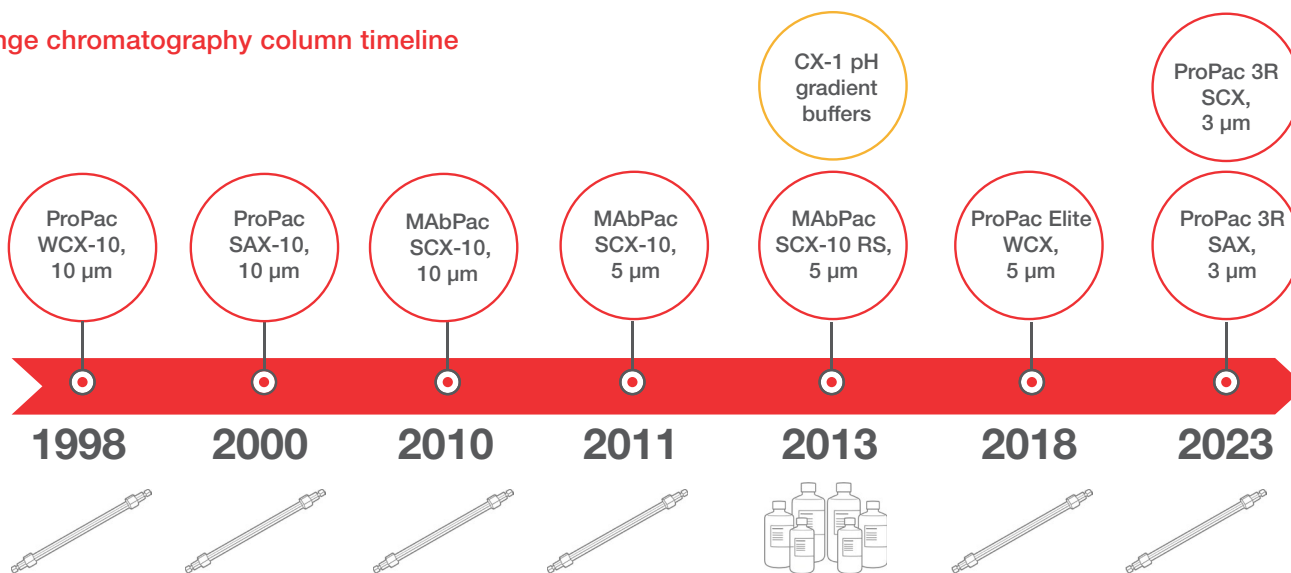
Our Thermo Scientific™ ProPac™ ion-exchange products can be used to help ensure the selection of stable and efficacious drug candidates, from discovery through to manufacturing.

Building on the proven capabilities of the ProPac family of columns, the Thermo Scientific™ ProPac™ WCX-10 column is the industry's GOLD standard for charge variant analysis. Along with our Thermo Scientific™ ProPac™ Elite WCX column, these columns are used extensively in leading BioPharma labs for the separation and quantitation of charge variants of mAbs and other proteins that can arise during cellular production, downstream purification, storage and shipping.

Ion-exchange chromatography column timeline

- 25-year history of innovation in ion-exchange columns for protein analysis
- Weak and strong cation and anion exchange technologies
- Gold-standard technology for variant analysis

Ion-exchange chromatography column timeline



Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

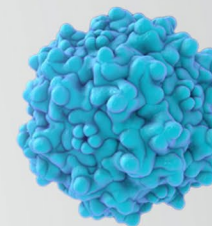
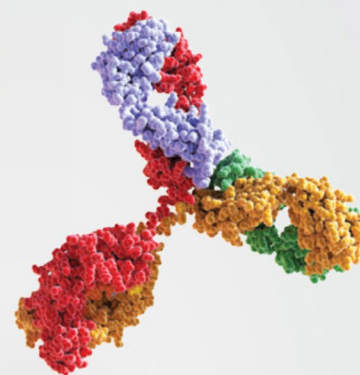
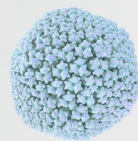
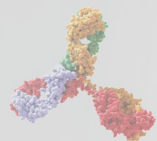
How does ProPac 3R compare?

Related products

CX-1 pH gradient buffers

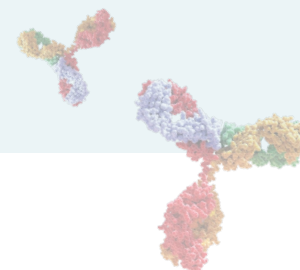
Quick order guide

ProPac family continued



ProPac 3R columns: the best ion-exchange chromatography columns from Thermo Scientific

	ProPac 3R SAX column	ProPac SAX-10 column	ProPac 3R SCX column	MAbPac SCX-10 column	ProPac WCX-10 column	ProPac Elite WCX column
Chemistry	Strong anion-exchange		Strong cation-exchange		Weak cation-exchange	
Particle	Monodisperse	Polydisperse	Monodisperse	Polydisperse	Polydisperse	Polydisperse
Particle type	Non-porous	Non-porous	Non-porous	Non-porous	Non-porous	Non-porous
Particle size	3 μm	10 μm	3 μm	5 μm , 10 μm	10 μm	5 μm



Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

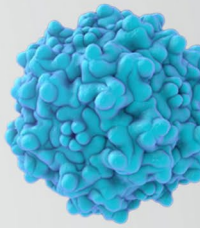
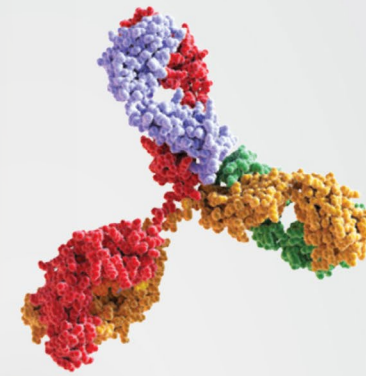
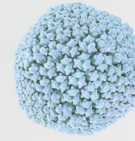
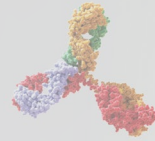
How does ProPac 3R compare?

Related products

CX-1 pH gradient buffers

Quick order guide

Ion-exchange column selection guide



Protein isoelectric point (pI)

<7

>7

ProPac 3R SAX column

ProPac 3R SCX column

MABPac SCX-10 column

ProPac WCX-10 column

ProPac Elite WCX column

- Works well with salt and pH gradient buffers
- Best choice for proteins with acidic pI
- Analyze full/empty AAV capsid ratios

- Highest resolution with excellent reproducibility
- Works well with CX-1 buffers

- Alternative selectivity to WCX, scalable from short methods over comprehensive analysis to semi-prep formats
- Works well with CX-1 buffers

- Industry GOLD standard – widely used and published

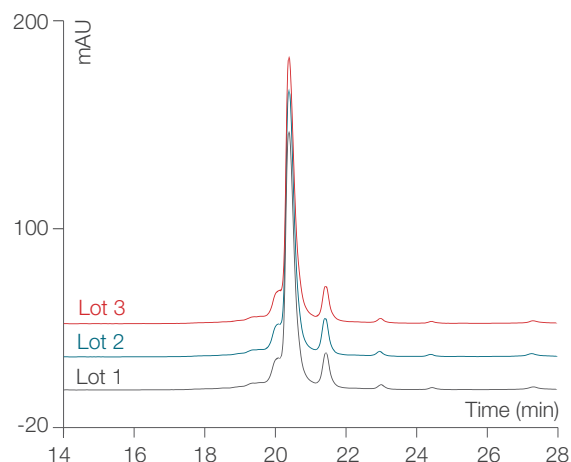
- Improved resolution, speed and reproducibility over ProPac WCX-10 column
- Works well with CX-1 buffers

Reproducible variant separation

A hydrophilic coating paired with precisely controlled grafting of functional groups builds on the monodisperse particle platform to provide the ProPac 3R columns with excellent lot-to-lot reproducibility.

From one lot to another, expect consistent variant separation profiles and resolution to give you confidence in detecting and quantifying the composition of your molecule.

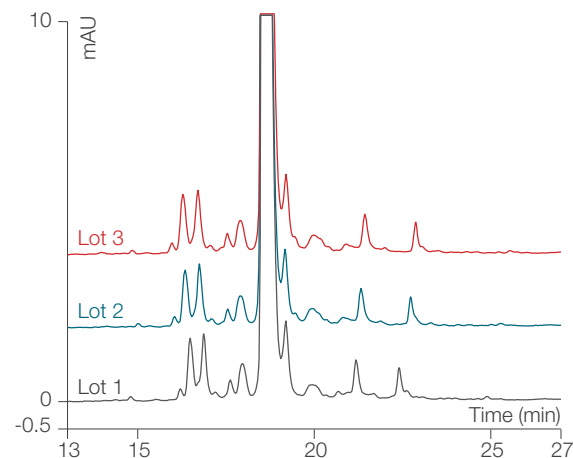
Lot-to-lot reproducibility of NISTmAb salt gradient separation on a ProPac 3R SCX column



Column	ProPac 3R SCX column, 3 μ m	
Format	4 x 100 mm	
Mobile phase	A: 20 mM MES, pH 6.5 B: 20 mM MES, pH 6.5 + 0.5 M NaCl	
Flow rate	0.3 mL/min	
Injection	2 μ L	
Temp	30 $^{\circ}$ C	
Detection	UV, 280 nm	
Sample	NISTmAb - 10 mg/mL	
Gradient	%A	%B
Time (min)	0.0	10
	30.0	30
	30.1	80
	33.0	80
	33.1	10
	40.0	10

Figure 2: Comparison of NISTmAb separation on the 3 different lots of ProPac 3R SCX media. Retention is normalized for comparison of variant separation

Lot-to-lot reproducibility of protein G salt gradient separation on a ProPac 3R SAX column



Column	ProPac 3R SAX column, 3 μ m	
Format	4 x 100 mm	
Mobile phase	A: 20 mM Tris, pH 8.0 B: 20 mM Tris + 500 mM NaCl, pH 8.0	
Flow rate	0.5 mL/min	
Injection	1 μ L	
Temp	30 $^{\circ}$ C	
Detection	UV, 280 nm	
Sample	Protein G - 5 mg/mL	
Gradient	%A	%B
Time (min)	0.0	12
	1.0	12
	31.0	42
	31.1	100
	33.0	100
	33.1	12
	45.0	12

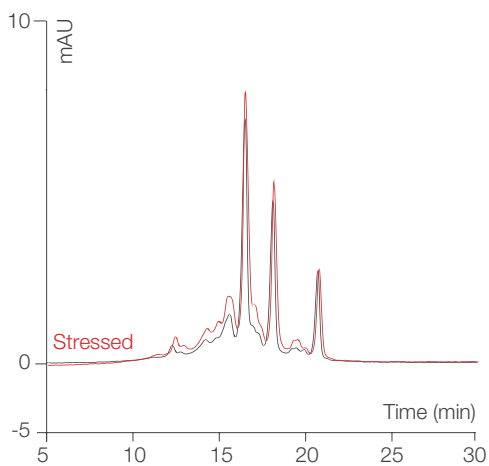
Figure 3: Comparison of Protein G separation on the 3 different lots of ProPac 3R SAX media. Retention is normalized for comparison of variant separation

High resolution variant separation

The exceptional performance of the ProPac 3R columns provides the separation power needed to resolve proteins and their associated variants for easy detection and quantification. Thermal stress of proteins can alter the structure of monoclonal antibodies via asparagine deamidation resulting in an increase in acid variants

as shown in Figure 4 for infliximab. Thermal stress of protein G results in the increase and generation of new variants/impurities which can be resolved using the ProPac 3R SAX columns to differentiate modified proteins with complex profiles from their native state.

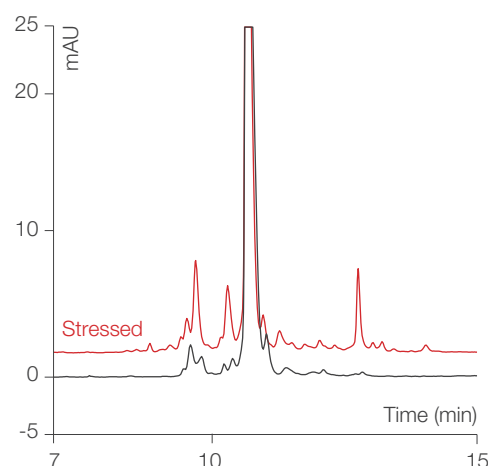
Salt gradient analysis of infliximab using a ProPac 3R SCX column



Column	ProPac 3R SCX column, 3 µm	
Format	4 × 100 mm	
Mobile phase	A: 20 mM MES, pH 6.5 B: 20 mM MES, pH 6.5 + 0.5 M NaCl	
Flow rate	0.3 mL/min	
Injection	2 µL	
Temp	30 °C	
Detection	UV, 280 nm	
Sample	Infliximab – 5 mg/mL	
Gradient	%A	%B
Time (min)	0.0	93
	30.0	78
	30.1	20
	33.0	20
	33.1	93
	40.0	93

Figure 4: Separation comparison of infliximab samples thermally stressed at 40 °C for 72 hours (red) and untreated (black)

Salt gradient analysis of protein G using a ProPac 3R SAX column



Column	ProPac 3R SAX column, 3 µm	
Format	4 × 100 mm	
Mobile phase	A: 20 mM Tris, pH 8.0 B: 20 mM Tris + 500 mM NaCl, pH 8.0	
Flow rate	0.5 mL/min	
Injection	1 µL	
Temp	30 °C	
Detection	UV, 280 nm	
Sample	Protein G – 5 mg/mL	
Gradient	%A	%B
Time (min)	0.0	88
	1.0	88
	16.0	58
	16.1	0
	18.0	0
	18.1	88
	30.0	88

Figure 5: Separation comparison of protein G samples thermally stressed at 40 °C for 72 hours (red) and untreated (black)

Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

How does ProPac 3R compare?

Related products

CX-1 pH gradient buffers

Quick order guide

Robust variant separation

The ProPac 3R columns provide excellent separation over hundreds of runs. Expect consistent, high resolution performance whether you are analyzing larger molecules such as 150 kDa mAbs on the SCX

column or smaller biomolecules such as Protein G (21.6 kDa) with highly complex variant profiles on the SAX column.

Run-to-run reproducibility of NISTmAb salt gradient analysis on a ProPac 3R SCX column

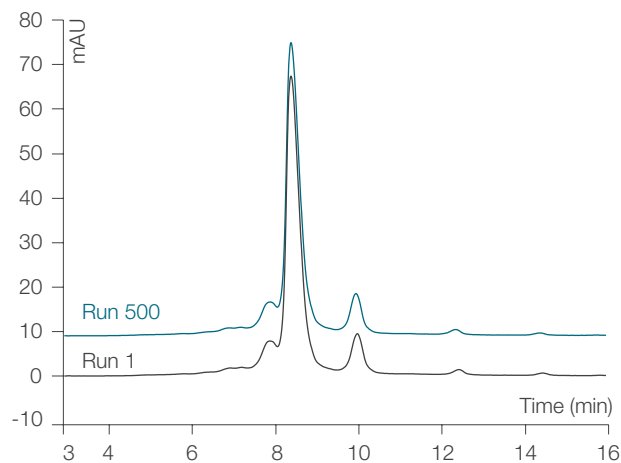


Figure 6: Reproducibility of NISTmAb separation over 500 runs on a 4 x 100 mm ProPac 3R SCX column. Retention time normalized for comparison of variant separation.

Run-to-run reproducibility of protein G salt gradient analysis on a ProPac 3R SAX column

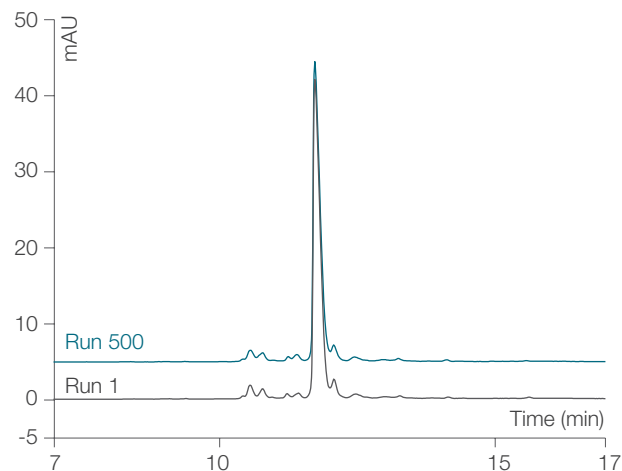
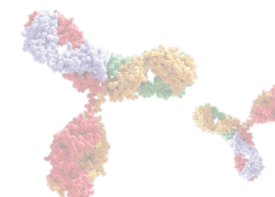


Figure 7: Reproducibility of Protein G separation over 500 runs on a 4 x 100 mm ProPac 3R SAX column. Retention time is normalized for comparison of variant separation.

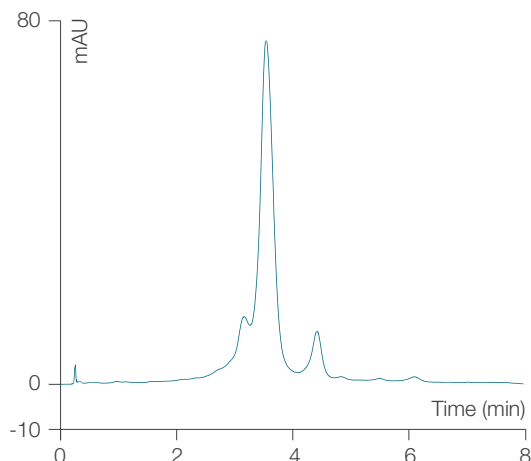


Robust variant separation continued

The design of the ProPac 3R columns enables their use for a variety of samples and separation methods. Combine the Thermo Scientific CX-1 pH gradient buffers with the SCX column for straightforward, method development of mAb variant separations.

Leverage the pH stability and capacity of our SAX column to analyze AAV (Adeno-Associated Virus) particles for empty/full capsid ratios and sample artifacts.

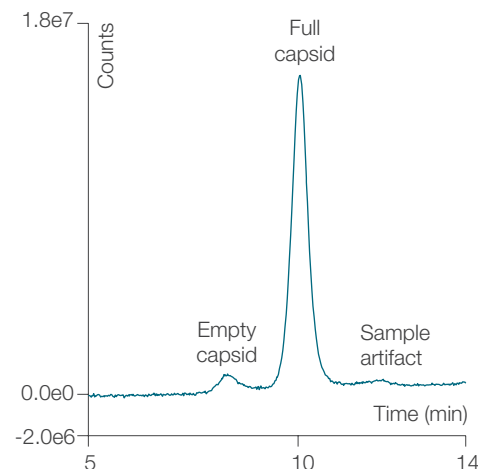
Analysis of NISTmAb using CX-1 pH gradient buffers on a ProPac 3R SCX column



Column	ProPac 3R SCX column, 3 μ m	
Format	2 x 50 mm	
Mobile phase	A: 1X, CX-1 buffer A pH 5.6 B: 1X, CX-1 buffer B pH 10.2	
Flow rate	0.3 mL/min	
Injection	1 μ L	
Temp	30 $^{\circ}$ C	
Detection	UV, 280 nm	
Sample	NISTmAb – 10 mg/mL	
Gradient	%A	%B
Time (min)		
-0.2	50	50
0.0	50	50
5.0	40	60
6.0	40	60
6.1	100	0
7.0	100	0
7.1	50	50
14.0	50	50

Figure 8: Zoomed in chromatogram of NISTmAb pH gradient analysis using 5-minute gradient

Salt gradient analysis of AAV empty and full capsids on a ProPac 3R SAX column



Column	ProPac 3R SAX column, 3 μ m		
Format	2 x 50 mm		
Mobile phase	A: Water B: 1 M Tetramethylammonium chloride C: 200 mM Bis-Tris propane, pH 9.0		
Flow rate	0.2 mL/min		
Injection	0.2 μ L		
Temp	20 $^{\circ}$ C		
Detection	FLD (Ex: 280 nm, Em: 330 nm)		
Sample	AAV6 (2×10^{13} vg/mL)		
Gradient	%A	%B	%C
Time (min)			
0.0	78	12	10
1.0	78	12	10
11.0	58	32	10
11.1	0	90	10
13.0	0	90	10
13.1	78	12	10
25.0	78	12	10

Figure 9: Separation of empty and full AAV6 capsids on a 2 x 50 mm ProPac 3R SAX column

Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

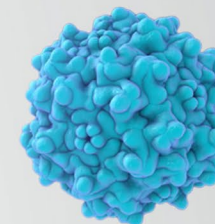
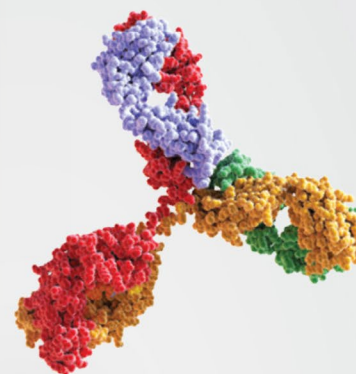
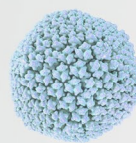
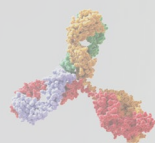
How does ProPac 3R compare?

Related products

CX-1 pH gradient buffers

Quick order guide

ProPac 3R family technology highlights



ProPac 3R SCX column

- ProPac 3R SCX column is a strong cation exchange column with a unique monodisperse resin containing a hydrophilic layer and sulfonic acid groups designed to achieve high-efficiency protein separations

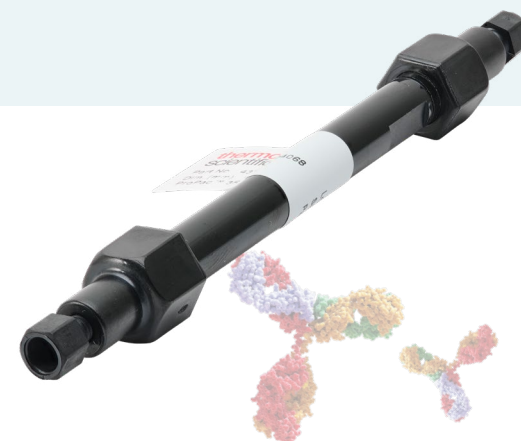
- The sulfonate functionality grafted to the hydrophilic layer introduces permanently charged anionic sites that provide the strong cation exchange character required for promoting protein binding when using a low ionic strength mobile phase at an appropriate pH (e.g., 20 mM MES, pH 6.5)

ProPac 3R SAX column

- ProPac 3R SAX column is a strong anion exchange column with a unique monodisperse resin containing a hydrophilic layer and quaternary ammonium groups designed to achieve high-efficiency protein separations

- The quaternary ammonium functionality grafted to the hydrophilic layer introduces permanently charged cationic sites to provide the strong anion exchange character required for promoting protein binding when using a low ionic strength mobile phase at an appropriate pH (e.g., 20 mM Tris, pH 8)

Combine **ProPac 3R SCX columns** with our proprietary **CX-1 buffer** formulations to enable fast, robust and reproducible pH gradients that are simple to optimize and easily automated - without the need for time-consuming mobile phase adjustments.



Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

How does ProPac 3R compare?

Related products

CX-1 pH gradient buffers

Quick order guide

How does ProPac 3R compare?

Building on the technologies that made our previous columns so great, the ProPac 3R columns are our highest resolution ion-exchange columns available. Resolve peaks better and see more than before as the ProPac 3R columns increase detection sensitivity and separation of both acidic and basic peaks relative to the MAbPac SCX-10 columns.

The ProPac 3R SCX also provides improved acidic peak detection to a leading competitor's 3 μ m product. The same holds true for the ProPac 3R SAX column which provides far superior peak resolution and detection compared to the ProPac SAX-10 column, which has been a leading technology since its release in 2000.

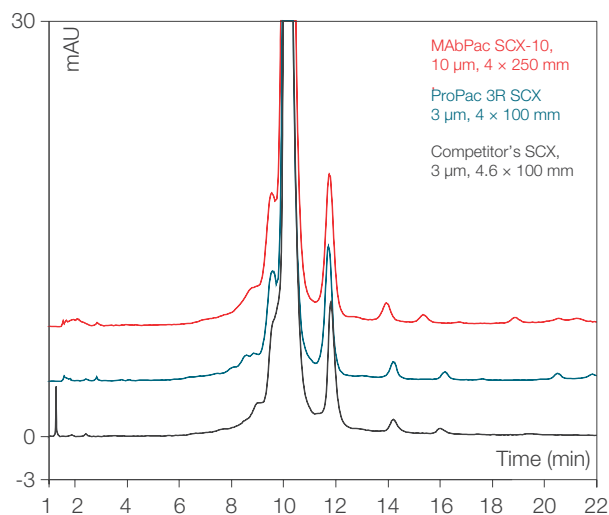


Figure 10: Comparison of NISTmAb separation on MAbPac SCX-10, 10 μ m 4 x 250 mm, ProPac 3R SCX, 3 μ m 4 x 100 mm; and a competitor's SCX 3 μ m 4.6 x 100 mm column using a high-resolution 25-minute gradient method.

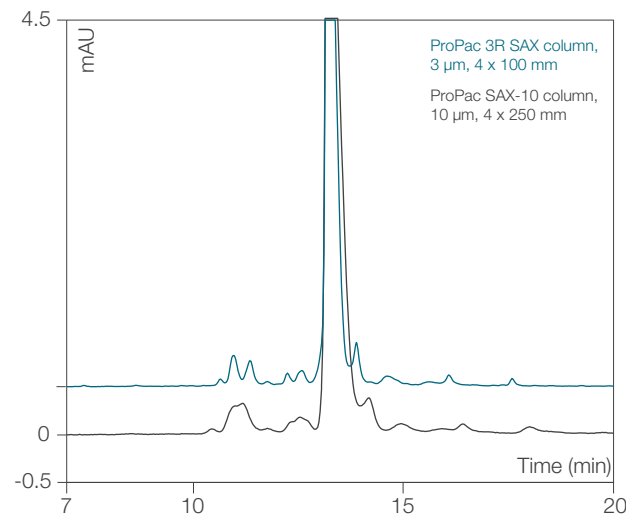
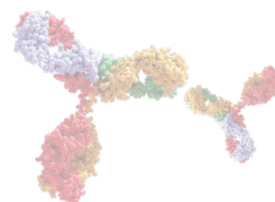


Figure 11: Comparison of Protien G separation on ProPac 3R SAX, 3 μ m and a ProPac SAX 10 μ m column using high-resolution 30-minute gradient method.



Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

How does ProPac 3R compare?

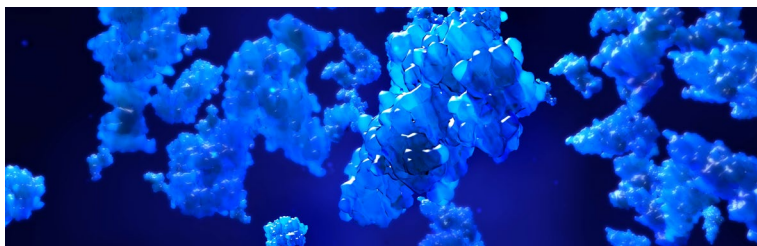
Related products

CX-1 pH gradient buffers

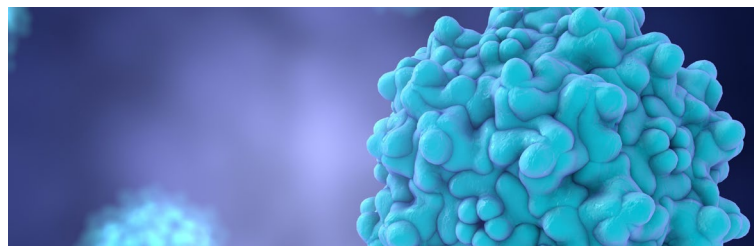
Quick order guide

How does ProPac 3R compare?

[Salt gradient analysis of Protein G using a 3 \$\mu\$ m monodisperse SAX chromatography column](#)



[Salt gradient separation and analysis of adeno-associated virus samples using a 3 \$\mu\$ m monodisperse strong anion exchange chromatography column](#)



[Salt gradient analysis of monoclonal antibodies using a 3 \$\mu\$ m monodisperse SCX chromatography column](#)



[Method development for pH gradient analysis of monoclonal antibodies using a 3 \$\mu\$ m monodisperse particle strong cation exchange chromatography column](#)



Contents

Introducing ProPac 3R columns

ProPac 3R benefits

Technology and features

ProPac 3R column technology

Column technology and features

ProPac family

ProPac family ion-exchange chromatography

Ion exchange chromatography

How does ProPac 3R compare?

Ion-exchange column selection guide

Reproducible variant separation

High resolution variant separation

Robust variant separation

ProPac 3R family technology highlights

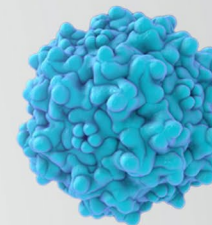
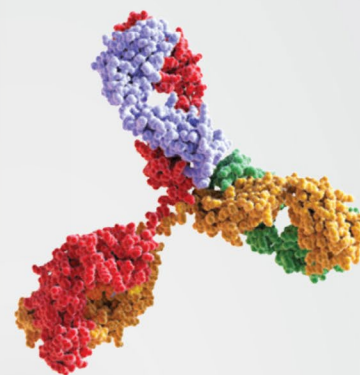
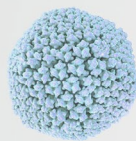
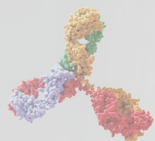
How does ProPac 3R compare?

Related products

CX-1 pH gradient buffers

Quick order guide

Related products for enhanced reproducibility



CX-1 pH gradient buffers

Obtain fast and highly robust, reproducible HPLC gradients using Thermo Scientific CX-1 pH gradient buffers. Simple to optimize and easily automated, these buffers are applicable for cation-exchange based separations of the majority of monoclonal antibody (mAb) biotherapeutic drugs.

- CX-1 pH gradient buffers are ready to use with our ProPac cation-exchange columns without the need to formulate mobile phases
- The CX-1 buffers save time in method development, facilitate method transfer to QA/QC for a wide range of mAb charge variants through a generic HPLC-based approach to mAb characterization, and do not require time-consuming mobile phase adjustments

- pH buffer solutions can be used to generate highly reproducible, linear pH gradients in cation exchange chromatography
 - Robust, reproducible gradients
 - Simple method optimization
 - Easily automated
 - Applicable to the majority of mAbs

Unlike traditional cation exchange chromatography using salt gradients, it is possible to predict the pH and the expected retention of the charge variants and use a narrow pH range to get a higher resolution separation.

Find out more

thermo scientific PRODUCT BROCHURE

CX-1 pH Gradient Buffers

Simple method development for charge variant characterization

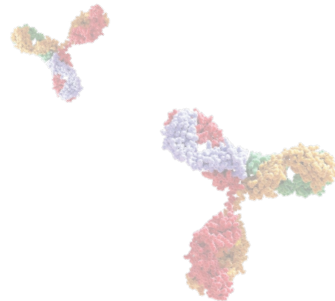
Benefits

- Accelerates buffer formulation development and reduces development time
- Simple to optimize and easily automated
- Ready to use with existing CEX methods and systems, without the need for complex mobile phase formulations
- Available in the majority of mAbs

Features

- CX-1 buffers are prepared using high-purity, high-purity reagents, including monovalent sodium, sodium chloride, and sodium acetate
- Available in a range of concentrations and pH values
- Available in a range of volumes

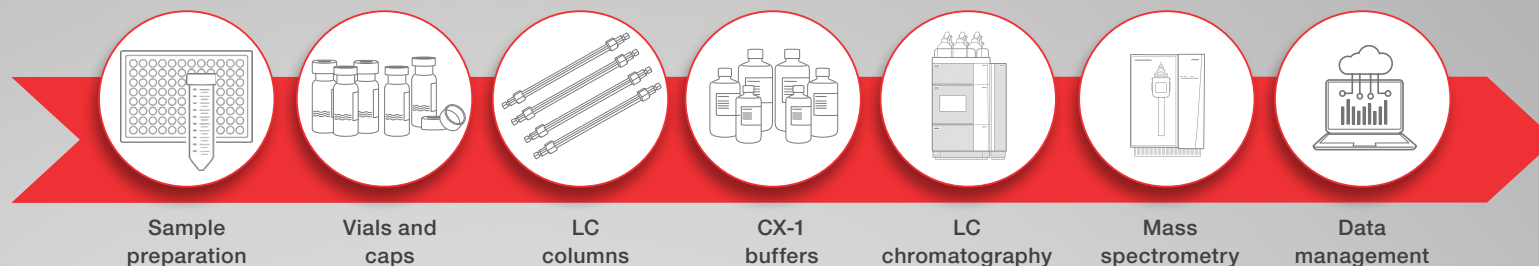
Thermo Scientific CX-1 pH gradient buffers are ready to use with our ProPac cation-exchange columns without the need to formulate mobile phases. The CX-1 buffers save time in method development, facilitate method transfer to QA/QC for a wide range of mAb charge variants through a generic HPLC-based approach to mAb characterization, and do not require time-consuming mobile phase adjustments.



Quick order guide

Related workflow products

Click for more information



Sample preparation

Vials and caps

LC columns

CX-1 buffers

LC chromatography

Mass spectrometry

Data management

Ordering information

Description	Dimensions	Particle size	Cat. no
ProPac 3R SCX columns	2 mm x 50 mm	3 µm	43103-052068
	2 mm x 100 mm	3 µm	43103-102068
	4 mm x 50 mm	3 µm	43103-054068
	4 mm x 100 mm	3 µm	43103-104068
ProPac 3R SAX columns	2 mm x 50 mm	3 µm	43203-052068
	2 mm x 100 mm	3 µm	43203-102068
	4 mm x 50 mm	3 µm	43203-054068
	4 mm x 100 mm	3 µm	43203-104068



Learn more at thermofisher.com/propac

For Research Use Only. Not for use in diagnostic procedures. © 2023 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details. **EB001998-EN 0323**