ThermoFisher SCIENTIFIC

HPLC columns

MAbPac SCX-10 columns

Product manual

thermo scientific

Contents

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Safety and special notices

Make sure you follow the precautionary statements presented in this guide.
The safety and other special notices appear in boxes.

Safety and special notices include the following	
	**Safety** Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury
	**Warning** Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment
	**Caution** Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.
	**Note** Indicates information of general interest
	**Important** Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system
	**Tip** Highlights helpful information that can make a task easier

Introduction

Features of the MAbPac SCX-10 strong cation exchange columns

The Thermo Scientific™ MAbPac™ SCX-10 strong cation exchange columns are designed specifically for high-resolution, high efficiency separation of monoclonal antibodies and their variants. The non-porous pellicular resin provides high resolution, permitting the separation of monoclonal antibody variants that differ by as little as one charge. The MAbPac SCX-10 columns are a new addition to the existing Thermo Scientific™ ProPac™ WCX-10 ion exchange columns providing complimentary selectivity for various mAb separations. The MAbPac SCX-10 columns are available in several particle sizes. 10 µm particle size columns are available in PEEK (polyetheretherketone) while 5 µm columns are available in both PEEK and PEEK-lined stainless steel housings for UHPLC based rapid separation (RS) applications. The MAbPac SCX-10 5 µm shorter columns are recommended for fast separations and are ideal for high throughput needs. Longer columns provide greater resolution.

The MAbPac SCX-10 RS columns are packed in PEEK-lined stainless steel columns for UHPLC operating compatibility. High flow rates can be used to achieve faster mAb analysis. These RS columns are suitable for operation up to 7,000 psi. The RS bioinert column bodies prevent metal interferences which can result in compromised mAb separations. The RS columns are designed for use with bioinert UHPLC systems.

The MAbPac SCX-10 columns are packed with ethylvinylbenzene-divinylbenzene copolymer non-porous beads that are uniformly coated with a proprietary layer of highly hydrophilic material. This layer prevents non-specific interactions between the hydrophobic bead core and the biopolymers/proteins. On the surface of the hydrophilic layer, sulfonic acid functional groups are attached using an ATRP-based grafting approach to control the chain length as well as the density of functional groups.

Operating limits and specifications

MAbPac SCX-10 and MAbPac SCX-10 RS columns are compatible with both aqueous mobile phases and those containing solvents, such as acetonitrile. MAbPac SCX-10 columns should be operated at the recommended flow rates specified for the particle size and column type (see “Column operating conditions” table). It is essential to keep the backpressure below the recommended value. When setting up a system for use with this column, check the special precautions listed in “Operation” section. Column hardware is either PEEK, or PEEK-lined stainless steel. PEEK has excellent resistance to most organic solvents and inorganic solutions.

Note

Although MAbPac SCX columns are compatible with acetonitrile and other solvents, it is recommended to only use solvents during column cleaning procedures to avoid precipitation of biomolecules on the column which can lead to clogging the column with increased back pressures and compromises the performance of the column.

Introduction (continued)

Column operating conditions

Parameter	Column	Recommendation
Flow rate range: Not to exceed the maximum column pressure limit	10 µm, 9 × 250 mm	5 mL/min
	10 µm, 4 × 250 mm	1 mL/min
	10 µm, 2 × 250 mm	0.25 mL/min
	RS, 5 µm, 4.6 × 250 mm	Up to 0.8 mL/min (UHPLC instrumentation required)
	RS, 5 µm, 4.6 × 150 mm	Up to 1.3 mL/min (UHPLC instrumentation required)
	RS, 5 µm, 4.6 × 50 mm	Up to 2.0 mL/min
	RS, 5 µm, 2.1 × 250 mm	Up to 0.28 mL/min
	RS, 5 µm, 2.1 × 150 mm	Up to 0.46 mL/min
	RS, 5 µm, 2.1 × 50 mm	Up to 0.5 mL/min
	5 µm, 4 × 250 mm	0.5 mL/min
	5 µm, 4 × 50 mm	Up to 2 mL/min
Shipping solution	10 µm	10 mM Na_2HPO_4 (pH 6.5) + 162.8 mM NaCl + 0.1% sodium azide
	5 µm	20 mM MES (pH 6.5) + 170 mM NaCl + 0.1% sodium azide
	RS, 5 µm	20 mM MES (pH 6.4) + 160 mM NaCl + 0.1% sodium azide
Short term storage solution (overnight)*	10 µm, 5 µm	Your high salt concentration eluent (pH 5-7.5); CX-1 pH gradient buffer A (pH 5.6; If you are using pH gradient separation. Do not store your column in high pH buffer).
Long term storage solution		20 mM MES (pH 5.5 to 6.5) + 170 mM NaCl + 0.1% sodium azide
Typical buffers		MES, ACES or other Good's buffers or Tris buffers for salt gradient separation; Always maintain a minimum ionic strength of at least 20 mM, to ensure proper operation; For pH gradient separation: CX-1 pHgradient buffers, or other buffer components.
Solvents		50% acetonitrile if needed for cleaning only; Never use H_2O alone for washing the column; Use buffers with minimum ionic strength of 20 mM
Detergent compatibility		Nonionic, anionic or zwitterionic detergents; Do not use cationic detergents. Do not use higher concentration of any detergents.
Temperature range		Ambient to 60 °C
Maximum pressure limit	10 µm PEEK	3,000 psi for all PEEK formats
	5 µm PEEK	5,000 psi for all PEEK formats
	5 µm RS	7,000 psi for RS columns only
pH range		2-12
Capacity**: Determined as "dynamic capacity" for mAb	10 µm, 9 × 250 mm (column volume = 15.7 mL)	Capacity is determined as the "dynamic capacity" 9 × 250 mm = 500 µg mAb 4 × 250 mm = 100 µg mAb
	10 µm, 4 × 250 mm (column volume = 3.14 mL)	
	5 µm, 4 × 50 mm	30 to 40 µg mAb
	5 µm, 4 × 250 mm	150 µg mAb
	RS 5 µm, 4.6 × 250 mm	200 µg mAb
	RS 5 µm, 4.6 × 150 mm	120 µg mAb
	RS 5 µm, 4.6 × 50 mm	40 to 50 µg mAb
	RS 5 µm, 2.1 × 250 mm	40 to 50 µg mAb
	RS 5 µm, 2.1 × 150 mm	24 to 30 µg mAb
	RS 5 µm, 2.1 × 50 mm	8 to 10 µg mAb

* Do not store columns in high/low pH buffers

** Dynamic capacity depends on the mAb/protein. Suggested maximum loading amounts are given for best resolution for a typical mAb. However, verify the capacity with your sample of interest before loading.

Introduction (continued)

Physical characteristics

Parameter	Recommendations
Substrate pore size	Non-porous
Substrate monomers	Ethylvinylbenzene-divinylbenzene
Substrate cross-linking	55%
Mode of interaction	Cation exchange
Functional group	Sulfonic acid; SCX

CX-1 pHgradient buffers

Description	Size	pH	Cat. no.
pH gradient buffer A	125 mL	pH 5.6	083273
	250 mL		085346
	500 mL		302779
	1000 mL		303274
pH gradient buffer B	125 mL	pH 10.2	083275
	250 mL		085348
	500 mL		302780
	1000 mL		303275

Note

For assistance, contact Technical Support or at your nearest Thermo Fisher Scientific office.

Warning

All eluents and samples should be filtered using a 0.2 µm filter unit before use. Unfiltered eluents and samples when employed will result in clogging the column with increased back pressure and leads to compromised columns performance. Also, it is beneficial to include a wash procedure with a high salt (1M NaCl) containing buffer solution to minimize the residue build up on the column during chromatography runs.

System requirements

The MAbPac SCX-10 PEEK columns are designed to be used with a standard bore inert HPLC system having a gradient pump module, injection valve, and a UV detector. The high pressure capable RS version of the MAbPac SCX-10 PEEK lined stainless steel columns are designed to be used with bioinert UHPLC system. A biocompatible Vanquish UHPLC system is recommended for best results. Both PEEK and RS columns can be used with an UHPLC system.

A metal-free system is highly recommended for halide-salt based mobile phases that are predominantly used can cause corrosion of metallic components. Metal leaching from the system on to the column will lead to compromised performance from metal contamination. A metal-free pump is highly recommended. Avoid using stainless steel tubing, ferrules, and bolt assemblies and replace them by PEEK or other equivalent inert alternatives.

The UHPLC MAbPac SCX-10 RS columns, packed in PEEK-lined Stainless Steel (SST), employ end fittings with PEEK inlet and outlet cone details. These parts are not user-replaceable, and may be damaged by attempting to swage SST ferrules into the PEEK cone. For column-to-tubing connections we recommend the use of Thermo Scientific™ Viper™ fingertight fittings or Thermo Scientific™ NanoViper™ fused-silica-lined PEEK tubing equipped with Thermo Scientific™ Viper™ connection fittings. If these are not available, other tubing materials may be connected using SST bolts equipped with captive PEEK ferrules according to the manufacturer's instructions.

Parameter	Recommendations
Typical flow rate	Refer "Column operating conditions" table
Injection volume	1–100 µL; Refer to "Column operating conditions" table for suggested loading amounts
Autosampler	Vanquish UHPLC system
System void volume	Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers
Pumps	SP (single pump) or DP (dual pump)
Detectors	VWD (Variable Wavelength Detector) with a microflow cell

Note

Avoid use of SST ferrules on UHPLC MAbPac SCX-10 RS columns.

System requirements (continued)

System void volume

Tubing between the injection valve and detector should be < 0.0050” ID PEEK tubing. (MP35N Viper Tubing for UHPLC RS system) Minimize the length of all liquid lines, but especially the tubing between the column and the detector. The use of larger diameter and/or longer tubing may decrease peak efficiency and peak resolution.

Mobile phase limitations

The MAbPac SCX-10 and MAbPac SCX-10 RS columns are compatible with typical mobile phases, such as sodium or potassium chloride salts in phosphate, MES or acetate buffers, up to the limit of their solubility. Use of organic solvents in the mobile phase is usually unnecessary. However, they are recommended to be used only for cleaning the column, if necessary. If you choose to use one, test the solubility limit of the mobile phase in the presence of the chosen organic solvent. Some combinations of salts and organic solvents are not miscible. Use of cationic detergents should be completely avoided. Minimize the concentration of non-ionic detergents as they can influence the chromatography.

Warning

Cationic detergents irreversibly bind to the MAbPac SCX-10 and MAbPac SCX-10 RS columns and their use should be avoided. Non-ionic detergents at high concentrations can also influence the binding of the analyte to the stationary phase, and should be minimized.

Chemical purity requirements

Reliable, reproducible results require mobile phases that are free from impurities and prepared consistently.

Deionized water

The deionized water used to prepare your mobile phase should be Type I reagent grade water with a specific resistance of 18.2 megohm-cm. The water should be free from ionized impurities, organics, microorganisms and particulate matter. UV treatment in the water purification unit is recommended. Follow the manufacturer’s instructions regarding the replacement of ion exchange and adsorbent cartridges. All filters used for water purification must be free from UV-absorbing components. Contaminated water in the mobile phase causes high background signals, gradient artifacts, and even sample degradation.

Warning

Never wash the column in water alone. Always use a buffer with minimum ionic strength of 20 mM.

Operations

Mobile phase selection

Monoclonal antibody samples and variants are eluted by using a gradient of either increasing ionic strength or by pH titration.

Salt based gradient elution

The mobile phase for the MAbPac SCX-10, or MAbPac SCX-10 RS, columns consists of a buffer component and a salt component. The buffer selected depends upon the pI of the proteins to be separated, and should provide minimal UV interference at the wavelength to be monitored. Although phosphate buffers were used earlier for various applications, usage of MES containing buffers or other Good's buffers are becoming increasingly popular and are recommended for mAb separations at the pH range 5.5-6.5. Good's buffers can buffer the stationary phase of the column effectively that could result in improved separation and resolution.

Minimum concentration of 20 mM NaCl, or equivalent ionic strength in buffers

Thermo Fisher Scientific recommends a minimum concentration of 20 mM NaCl or equivalent ionic strength in buffers. Failure to maintain a minimum ionic strength in buffers will result in alteration of the stationary phase conformation resulting in an increase in the column backpressure beyond the maximum recommended value. If this occurs, remove the column from the system, flush the buffer from the system and replenish with buffer B containing your high salt concentration. Pump buffer B through the column at a low flow rate (0.1-0.2 mL/min), until the backpressure returns to the original pressure.

pH gradient elution

A pH gradient can also be used in monoclonal antibody variant separations. The isoelectric point (pI) is the pH at which a particular protein carries no net charge and can no longer bind to the charged surface and therefore gets eluted. pH gradients are becoming increasingly popular to ease the method development process.[1-2]

We provide a cation-exchange pH gradient buffer system which meets the fast and robust platform method requirement.[3] This buffer system consists of a low-pH buffer A at pH 5.6 and a high-pH buffer B at pH 10.2. A linear pH gradient from pH 5.6 to pH 10.2 is generated over time by running a linear pump gradient from 100% buffer A to 100% buffer B.

Mobile phase constituent	Recommendations
Buffer	Salt gradients: MES, ACES, TRIS or other Good's buffers; pH gradients: buffers with various pka values to cover pH range from 5 to 11, CX-1 pH gradient buffers
Salt	Sodium or potassium salts of chloride, acetate
pH modifier	Phosphoric acid, HCl, or NaOH
Column cleaning/ pretreatment	10 to 100 mM sodium hydroxide at room temperature
Solvent	Up to 50% acetonitrile (be careful to use minimal salt content in buffers)
Detergent	Limited concentration of non-ionic, anionic, or zwitterionic detergents only if required; otherwise, avoid using detergents
Anti-microbial	0.1% sodium azide

Warning

Do not operate the MAbPac SCX-10 or MAbPac SCX-10 RS column in the absence of a minimal ionic strength of at least 20 mM. If the ionic strength is too low, the structure of the stationary phase will be affected, causing a significant increase in backpressure. This effect can be reversed by pumping a buffer containing high salt solution (500 mM NaCl) through the column at a low flow rate (0.1-0.2 mL/min) until the backpressure is reduced. It is recommended that at least 20 mM be present in the mobile phase at all times.

Operations (continued)

Mobile phase preparation

Adjusting the pH of the mobile phase

The mobile phase should contain all the electrolytes before adjusting the pH. It is important to prepare buffers gravimetrically (by weight) when possible and without need to adjust the pH each time. Slight pH variations can lead to substantial differences in the reproducibility of retention times and resolution. If a pH meter is used in order to make sure that the pH reading is correct, the pH meter should be calibrated at least once a day choosing at least two standards, one below and one above the desired pH. Stirring as well as temperature correction should be employed.

Note

pH measurements of buffers containing Tris should not be performed with a Ross electrode as this electrode produces erroneous results with amine containing solutions).

Filtering the mobile phase

To extend the lifetime of your column as well as your HPLC pump, all the eluents must be filtered using a 0.2 µm membrane filter to remove insoluble contaminants from the eluents. This is especially essential when using 3 and 5 µm columns.

Degassing the mobile phase

Before using buffers, they must be degassed. The degassing can be done either using the Thermo Scientific™ pump degas function as described in the manual, or by using a vacuum pump. Vacuum degas the solvent by placing the mobile phase reservoir in a sonicator and drawing a vacuum on the filled reservoir with a vacuum pump for 5-10 minutes while sonicating.

Validating column performance

Thermo Fisher Scientific recommends that you perform an efficiency test on your MAbPac SCX-10 or MAbPac SCX-10 RS column before your first use. The purpose of column performance validation is to make sure that no damage has been done to the column during shipping. Test the column using the conditions described on the Quality Assurance Report enclosed in the column box, Repeat the test periodically to track the column performance over time. Note that slight variations may be obtained on two different HPLC systems due to system electronics, plumbing, operating environment, reagent quality, column conditioning, and operator technique. Each column is tested and shipped with a Quality Assurance Report.

Procedure for validating column performance

1. Connect the column to the LC system.
2. Purge the column with the mobile phase listed on the QA report for 20 to 40 column volumes.
3. Inject the test mix shown in the QA report and collect the data.
4. Compare your result with the QA report provided in the column box.
5. If the chromatograms look similar, you can use the column for your application work.

Operations (continued)

Equilibrating the column

Equilibrate the MAbPac SCX-10 or MAbPac SCX-10 RS column after installing it for the first time. Always re-equilibrate the column prior to use following periods of storage.

Purge the column of shipping or storage solvent until the baseline is stable. Equilibrate the column with at least 5-10 column volumes of mobile phase A, or until a stable baseline is achieved.

Caring for the MAbPac SCX-10 and MAbPac SCX-10 RS column

To ensure the high performance of the column, the following guidelines should be followed.

1. Protect the column from contamination using a guard column, if available.
2. Make sure that solvents are miscible when changing mobile phases.
3. Always degas and filter mobile phases through a 0.2 µm membrane filter.
4. When switching to a new mobile phase, the column should be equilibrated with at least 30 column volumes before injecting the sample.
5. The recommended pH range is from pH 2 to 12. However, it is preferred that the column be used between pH 3 and pH 11 to achieve longer lifetime.
6. The column can be stored in mobile phase for short-term storage (e.g. overnight). However, it is highly recommended that the column be stored in storage buffer containing 0.1% sodium azide (more than 3 days) to prevent from bacterial growth.

Warning

Never wash the MAbPac SCX-10 or MAbPac SCX-10 RS column with H_2O. Always maintain minimum ionic strength 20 mM buffer (MES or sodium phosphate, or equivalent) in the eluents.

Example applications: MAbPac SCX-10 PEEK columns

MAbPac SCX-10 columns are available in PEEK and PEEK-lined stainless steel inert housings. PEEK columns are available in two different particle sizes of 10 µm and 5 µm. Depending on the resolution desired and the time allotted for specific applications, 10 µm or 5 µm columns may be chosen. For standard HPLC based applications, PEEK columns are suitable and are recommended. Although the following application examples are shown mainly with 10 µm column, 5 µm columns may be used to achieve better resolution and faster separation.

1. Monoclonal antibody analysis by salt gradients; heterogeneity characterization of acidic and basic variants.
2. Monoclonal antibody analysis by pH gradients; heterogeneity characterization of acidic and basic variants.
3. Examples of analysis of mAb C-terminal lysine truncation variants.
4. Examples of analysis of mAb Fab and Fc variants after carboxy peptidase and papain treatments.
5. Other mAb applications involving charged variants.

Appropriate flow rate and gradient conditions should be established and applied to achieve the best resolution for any mAb separation. The same gradient method that was developed for 10 µm or 5 µm columns may not be ideal when other particle size columns are used due to differences in their column volumes and capacities. Therefore, it is essential to reestablish and optimize these parameters to obtain the best results.

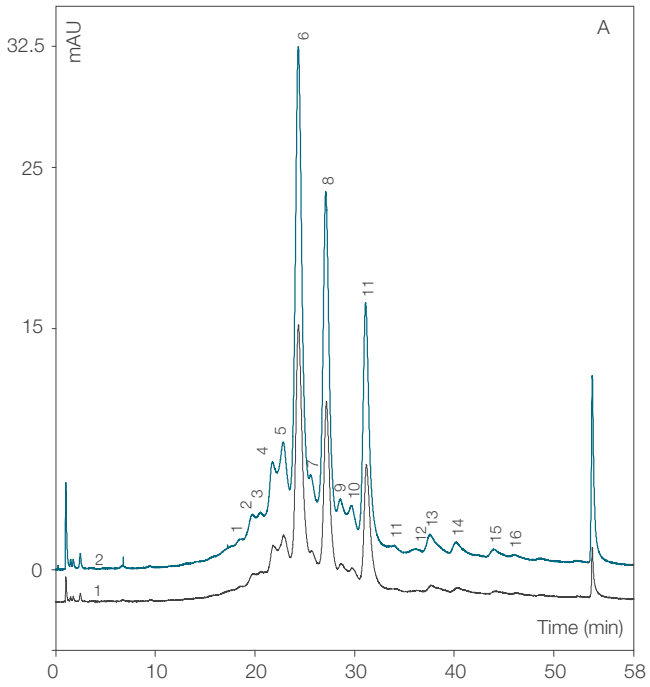
The MAbPac SCX-10 columns are a complimentary addition to the existing ProPac WCX-10 columns providing orthogonal selectivity for mAb heterogeneity characterization. Shorter 5 µm particle size columns are designed to provide fast, high throughput separations of monoclonal antibody variants while maintaining reasonably high resolution. The MAbPac SCX-10 5 µm longer columns take advantage of the length as well as smaller particle size for producing very-high resolution separations. Some typical application examples are listed below.

Separation of acidic and basic variants of monoclonal antibodies

Monoclonal antibodies (mAbs) are developed by pharmaceutical and biotechnology companies for various therapeutic applications. The mAbs undergo several post-translational modifications including oxidations, deamidations, truncations, as well as glycan modifications.[4-13] Manufacturing of mAbs and subsequent stability testing procedures involve routine analysis and monitoring of the impurities resulting from asparagines deamidation, aspartic acid isomerization, disulfide interchange, peptide bond cleavage, oxidation and others. The MAbPac SCX-10 column can be used to characterize mAb heterogeneity. Different mAbs separation using salt gradient is shown in Figure 1A, 1B and 1C.

Example applications: MAbPac SCX-10 PEEK columns (continued)

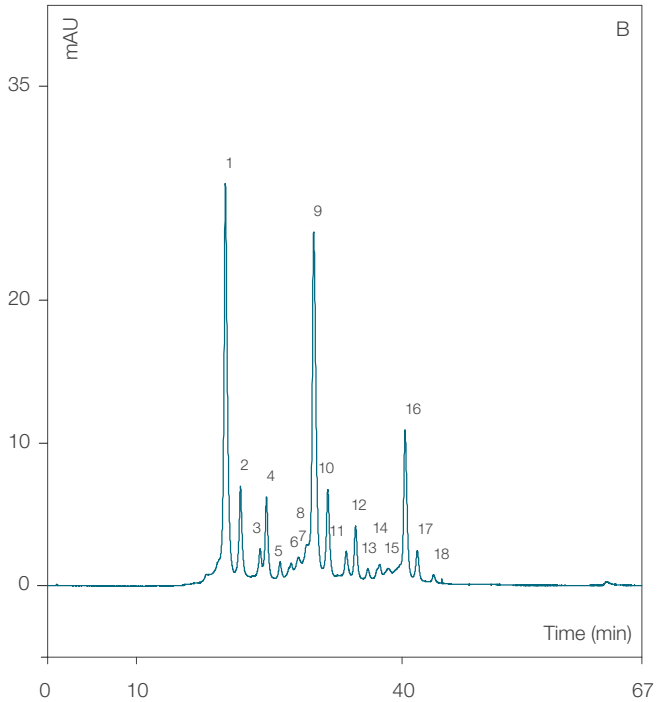
Separation of monoclonal antibody variants using MES eluents



Column	MAbPac SCX-10, 10 µm, 4 × 250 mm
Cat. no.	074625
Mobile phase	A: 20 mM MES, pH 5.6 + 60 mM NaCl B: 20 mM MES, pH 5.6 + 300 mM NaCl
Flow rate	1.0 mL/min
Temp	30 °C
Detection	280 nm
Sample	mAb, 5 mg/mL 1: 25 µg 2: 50 µg
Gradient	15 - 36.44 % B in 50 min Peaks 1-5: acidic variants

Figure 1A. Separation of monoclonal antibody variants using MES eluents on a MAbPac SCX-10, 10 µm column with 25 µg (back) and 50 µg (blue) mass loading

Separation of monoclonal antibody variants using MES eluents

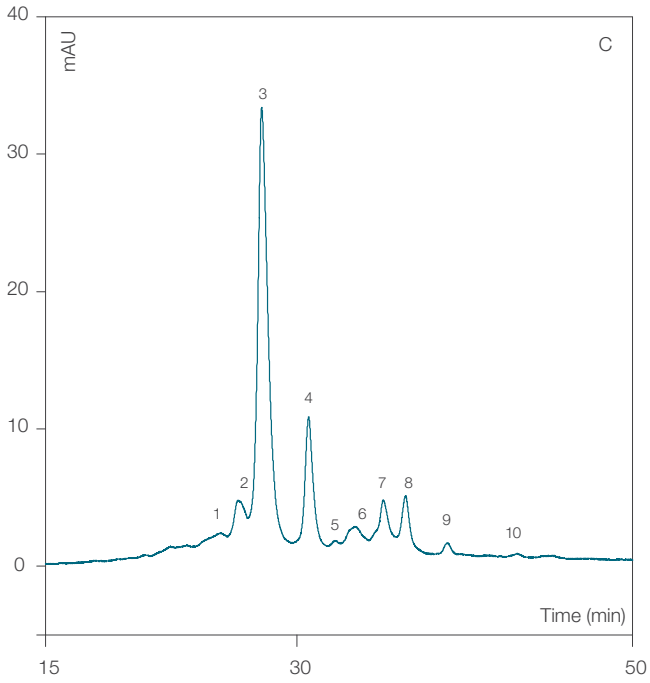


Column	MAbPac SCX-10, 10 µm, 4 × 250 mm
Cat. no.	074625
Mobile phase	A: 20 mM MES + 60 mM NaCl pH 5.6 B: 20 mM MES + 300 mM NaCl, pH 5.6
Flow rate	1.0 mL/min
Temp	30 °C
Detection	280 nm
Sample	mAb, 5 mg/mL 1: 25 µg 2: 50 µg
Gradient	15 - 36.44 % B in 50 min Peaks 1-5: acidic variants

Figure 1B. Separation of monoclonal antibody variants using MES eluents on a MAbPac SCX-10, 10 µm column

Example applications: MAbPac SCX-10 PEEK columns (continued)

Separation of monoclonal antibody variants using Tris eluents



Column	MAbPac SCX-10, 10 µm, 4 × 250 mm
Cat. no.	074625
Mobile phase	A: 20 mM Tris pH 7.3 B: 500 mM NaCl in eluent A
Flow rate	1.0 mL/min
Inj. volume	10 µL
Temp	30 °C
Detection	280 nm
Sample	mAb, 5 mg/mL
Gradient	5–15 % B in 60 min

Figure 1C. Separation of monoclonal antibody variants using Tris-based eluents on a MAbPac SCX-10, 10 µm column

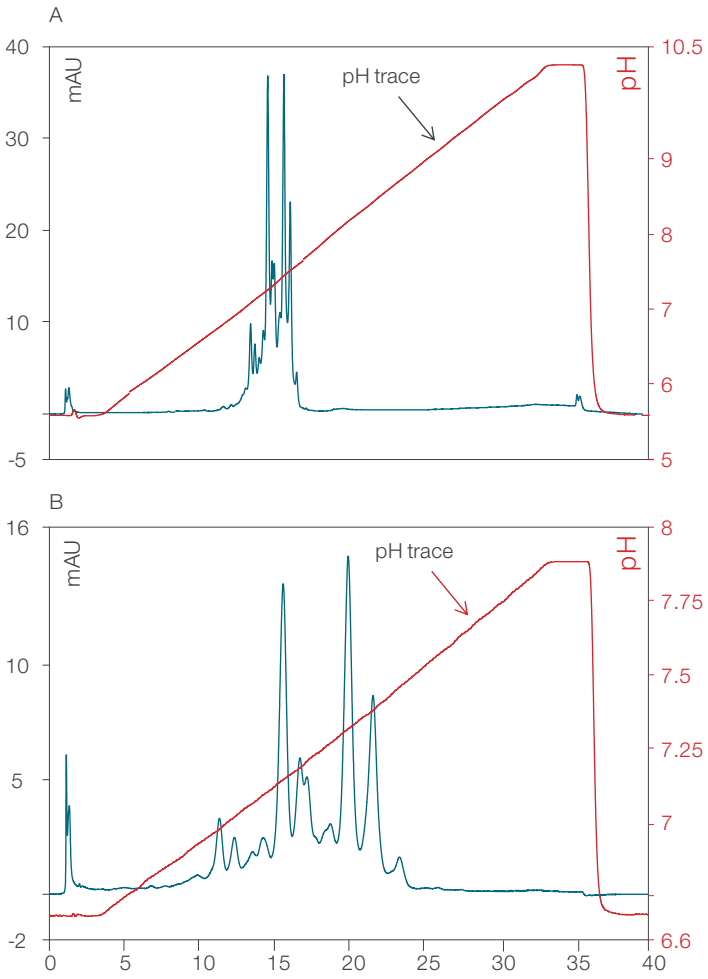
pH gradient based mAb separation: platform-based method development

Separation of mAbs based on pH gradients is becoming increasingly popular due to the reduced method development time.[1, 2] Most mAbs have pI values in the range of 6 to 10. The pH gradient separation method can serve as a platform for charge variant separation. Using a full range of pH gradient from pH 5.6 to
pH 10.2, the pH elution range of mAb is established in the initial run with a pH gradient slope of 0.153 pH unit/min. Further optimization of separation can simply be achieved by running a shallower pH gradient over a narrower pH range.

For pH gradient based separation Thermo Scientific™ CX-1 pH gradient buffers are recommended. CX-1 pH gradient buffer buffers are designed to generate a linear pH gradient on MAbPac columns. They consist of buffer A and buffer B which are titrated to pH 5.6 and pH 10.2 respectively.

Therefore, running a gradient from 100% A to 100% B will generate a linear pH gradient from pH 5.6 to 10.2. If the pI of the mAb is not known, a broad range of pH gradient (0-100% B; Figure 2A) may be employed to obtain a specific pH range of interest. Once you know where it is eluting and the pI values of the mAb of your interest, you can run a shallow pH gradient that covers the pI of the mAb. An example of pH gradient based mAb separation with MAbPac SCX-10 4.0 × 250 mm is shown in Figure 2. In the initial run, 0-100% B pH gradient was chosen. Further optimization of the separation was achieved by simply running a shallower pH gradient (25-50% B; Figure 2B).

Example applications: MAbPac SCX-10 PEEK columns (continued)



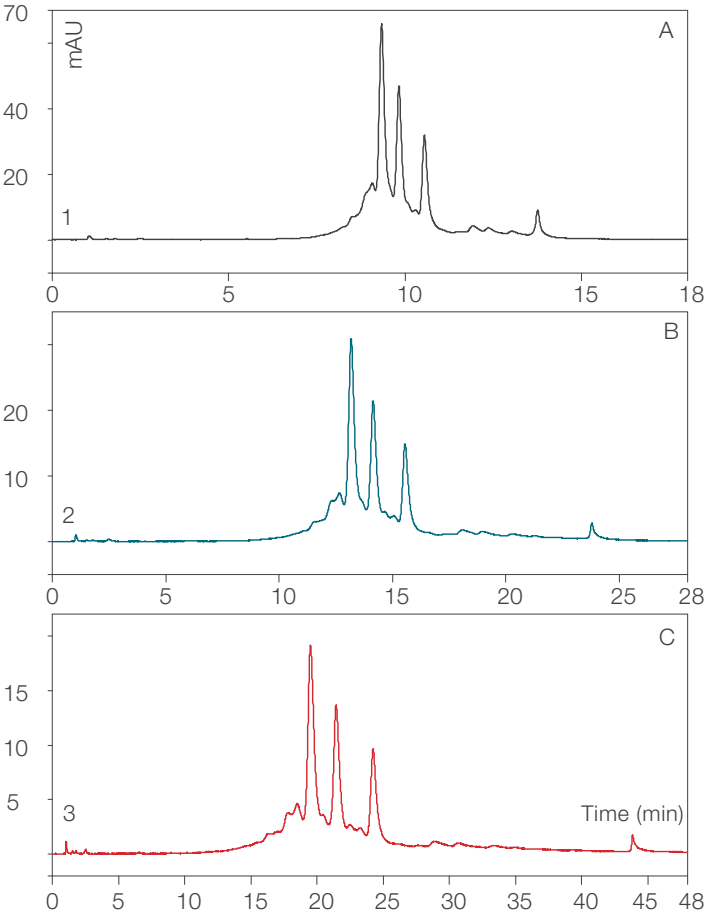
Column	MAbPac SCX-10, 10 µm, 4 × 250 mm
Cat. no.	074625
Mobile phase	A: 1X CX-1, pH gradient buffer A (pH 5.6) B: 1X CX-1, pH gradient buffer B (pH 10.2)
Flow rate	1.0 mL/min
Inj. volume	10 µL
Temp	30 °C
Sample	mAb, 5 mg/mL
Gradient	A: 0-100% B in 30 minutes B: 25-50% B in 30 minutes

Figure 2. Separation of monoclonal antibody variants using pH gradient on a MAbPac SCX-10, 10 µm, 4 × 250 mm column

Example applications: MAbPac SCX-10 PEEK columns (continued)

Influence of different gradients and flow rates

Separation of a mAb using different gradient conditions (Figure 3) and various flow rates (Figure 4) on a MAbPac SCX-10, 10 µm column is shown below. Steep gradients can be used if the desired resolution is met for faster analysis time. Different flow rates and gradients may be attempted to finalize the most appropriate flow rate and gradient for the specific analyte.



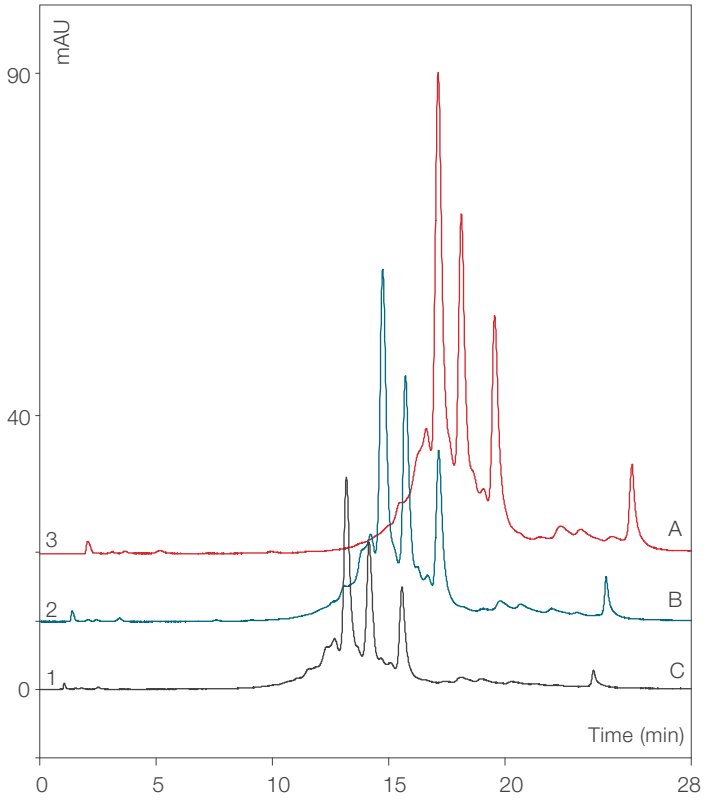
Column	MAbPac SCX-10, 10 µm, 4 × 250 mm
Cat. no.	074625
Mobile phase	A: 20 mM MES, pH 5.6 + 60 mM NaCl B: 20 mM MES, pH 5.6 + 300 mM NaCl
Flow rate	1.0 mL/min
Inj. volume	10 µL
Detection	280 nm
Temp	30 °C
Sample	mAb, 5 mg/mL
Gradient	A: 15 – 40 % B in 10 min B: 15 – 40 % B in 20 min C: 15 – 40 % B in 40 min

Please note different time scales (X-axis)

Figure 3. Chromatograms obtained for mAb sample using different gradient conditions on a MAbPac SCX-10, 10 µm, column

Example applications: MAbPac SCX-10 PEEK columns (continued)

mAb separation on MAbPac SCX-10 10 µm column using different flow rates



Column	MAbPac SCX-10, 10 µm, 4 × 250 mm
Cat. no.	074625
Mobile phase	A: 20 mM MES, pH 5.6 + 60 mM NaCl6 B: 20 mM MES, pH 5.6 + 300 mM NaCl
Flow rate	A: 0.5 mL/min B: 0.75 mL/min C: 1.0 mL/min
Inj. volume	10 µL
Detection	280 nm
Temp	30 °C
Sample	mAb, 5 mg/mL
Gradient	15 – 40 % B in 20 min

Figure 4. mAb separation using different flow rates

Monitoring processing of C-Terminal lysine residues of proteins

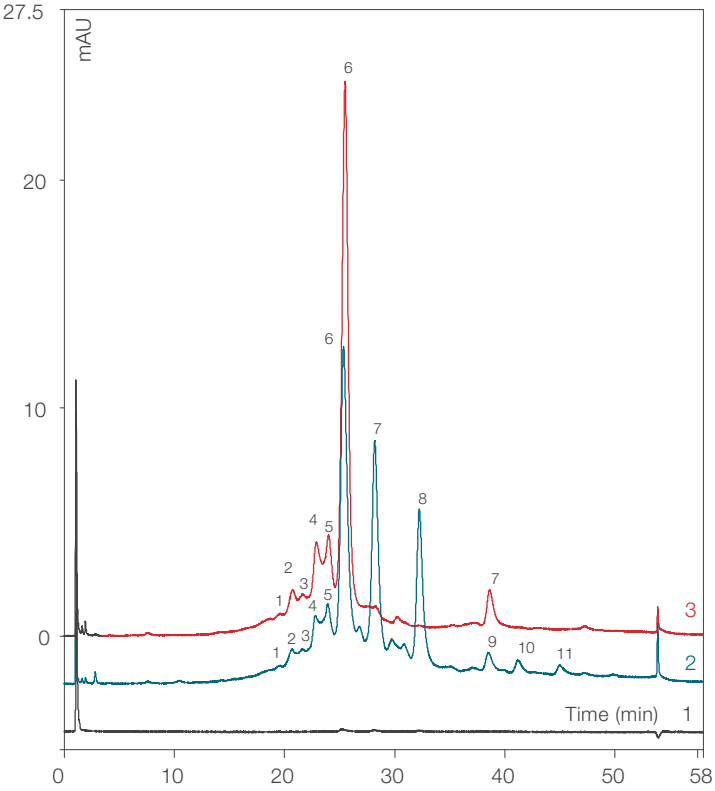
Processing of C-terminal lysine and arginine residues of proteins isolated from mammalian cell culture has been described.[13] As a result of processing techniques, the presence of C-terminal Lys or Arg residues, which could be expected based on gene sequence information, are often absent in proteins isolated from mammalian cell culture. This discrepancy, which is common in plasma derived proteins, may result from the activity of one or more basic carboxypeptidases. Charge heterogeneity can result if the processing is incomplete. The resulting charge heterogeneity of the variant forms can be identified by cation exchange chromatography. C terminal processing of lysine residues from heavy chains of monoclonal antibodies from a variety of sources has been reported.[14-18]

In this example the MAbPac SCX-10 column was used to separate variants of a humanized IgG, suspected of having lysine residue variation at the C-terminal of the heavy chains. As shown in Figure 5, a shallow NaCl gradient resolves three variant forms differing by the presence of lysine at the C-terminal of the heavy chains (with either 0, 1, or 2 lysine residues).

To verify that the reason for the different retention times of the three peaks was the different content of heavy chain C terminal lysine, the IgG preparation was treated with carboxypeptidase B, an exopeptidase that specifically cleaves C terminal lysine residues. This treatment of the IgG preparation resulted in the quantitative disappearance of peaks 7 and 8 (containing 1 and 2 terminal lysine residues, respectively, on their heavy chains). The decreased peak areas in peaks 7 and 8 were accompanied by a corresponding quantitative increase in peak area 6 (0s Lysine present). Similarly, another minor variant with lysine truncations shown as Peaks 9, 10 and 11 collapsed to peak 7 after carboxypeptidase treatment.

Example applications: MAbPac SCX-10 PEEK columns (continued)

Analysis of mAb lysine truncation cariants on the MAbPac SCX-10, 10 µm, column



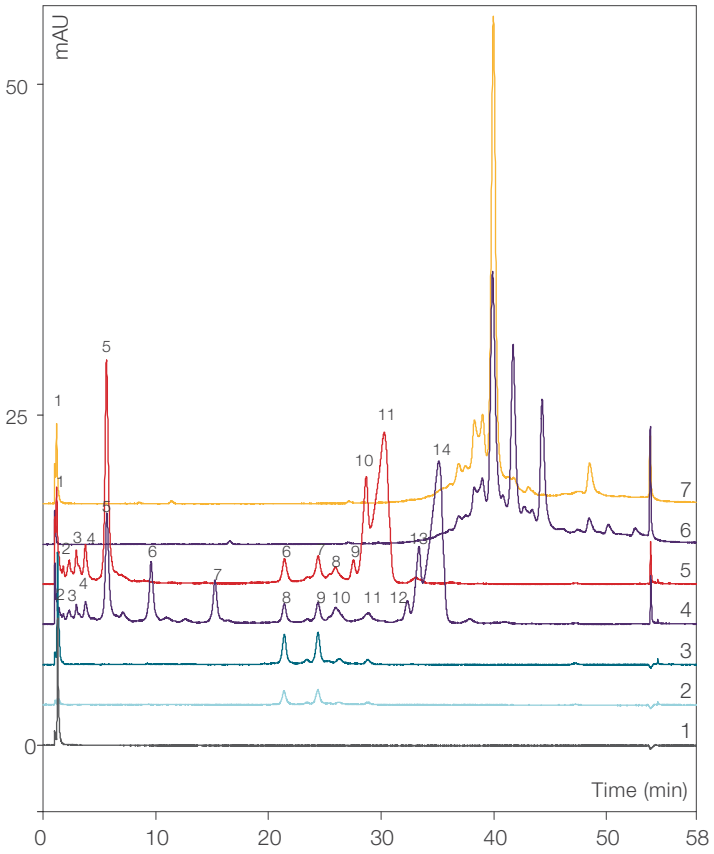
Column	MAbPac SCX-10, 10 µm, 4 × 250 mm	
Cat. no.	074625	
Mobile phase	A: 20 mM MES, pH 5.6 + 60 mM NaCl B: 20 mM MES, pH 5.6 + 300 mM NaCl	
Flow rate	1 mL/min	
Inj. volume	5 µL	
Detection	280 nm	
Total volume	100 µL	
Temp	30 °C	
Sample	1. Carboxypeptidase blank 50 µg/100 uL (No mAb) 2. mAb 900 ug in 100 µL (no carboxypeptidase) 3. mAb 900 ug in 100 µL + carboxypeptidase 50 µg	
Gradient	36 – 44 % B in 50 min	
Peaks 1-5: acidic variants	**Sample 2:** Peaks 6, 7, 8: C-terminal Lys truncation variants of main peak; Peaks 9, 10, 11: C-terminal Lys truncation variants of a minor variant peak	**Sample 3:** Peak 6 is resulting from 6, 7, 8 peaks after CPB treatment; Peak 7 is resulting from peaks 9, 10 and 11 after CPB treatment

Figure 5. Characterization ofllLysine truncation variants: mAb sample +/- treatment with carboxypeptidase B for 3 hrs at 37 °C

Example applications: MAbPac SCX-10 PEEK columns (continued)

Analysis of mAb Fab and Fc fragments after carboxypeptidase and papain treatments

Figure 6 shows the analysis of mAb after papain treatment alone (Sample 4) or, papain and carboxypeptidase treatments together (Sample 5) on a MAbPac SCX-10, 10 µm column. Other appropriate control conditions are used. Lysine truncations are located on the C terminus of the heavy chain. Carboxypeptidase removes C-terminal lysine/Arg residues. Papain is a cysteine protease and cleaves antibodies into Fab and Fc fragments.



Column	MAbPac SCX-10, 10 µm, 4 × 250 mm
Cat. no.	074625
Mobile phase	A: 20 mM MES, pH 5.6 + 60 mM NaCl B: 20 mM MES, pH 5.6 + 300 mM NaCl
Flow rate	1 mL/min
Inj. volume	5 µL
Detection	280 nm
Total volume	100 µL
Temp	30 °C
Total volume	300 µL
Sample	1. Carboxypeptidase blank 10 uL (50 µg; no mAb) 2. Papain blank 10 µL (100 µg; no mAb) 3. Carboxypeptidase + papain blank (1 and 2) 4. mAb 3mg/300 µL + papain10 µL 5. mAb 3mg/300 µL + papain10 µL + carboxypeptidase 10µL 6. mAb 3mg/300 µL 7. mAb 3mg/300 µL + carboxypeptidase 10 µL
Gradient	1 – 35 % B in 50 min
Peaks	**Sample 4 (papain treated):** Peaks 1-4: acidic variants Peaks 5, 6, 7: C-terminal Lys truncation variants of papain treated sample Peaks 12-14: Fab peaks **Sample 5 (papain and carboxypeptidase treated):** Peaks 1-4: acidic variants Peak 5 is resulting from 5, 6, 7 peaks (from sample 4) after papain and carboxypeptidase treatments together Peaks 9-11: Fab peaks

Figure 6. mAb sample +/- treatment with carboxypeptidase and/or papain for 3 hrs at 37° C

Example applications: MAbPac SCX-10 PEEK columns (continued)

Monoclonal antibody analysis using MAbPac SCX-10, 5 µm small particle columns

MAbPac SCX-10 small particle columns are available in PEEK and PEEK-lined stainless steel (RS series of columns) housings. While PEEK columns are available in two different particle sizes of 10 µm and 5 µm, PEEK-lined stainless steel columns are currently available in 5 µm particle sizes. Depending on the resolution desired and the time allotted for specific applications, 10 µm or 5 µm columns may be used. For HPLC based applications, PEEK columns are suitable and are recommended. For UHPLC applications, PEEK-lined stainless steel RS columns are recommended as they are compatible with high pressure and can withstand pressures up to 7,000 psi. Higher flow rates may be used with RS columns for faster run time and high throughput analysis. High pressure capable RS version of the MAbPac SCX-10 PEEK lined stainless steel columns are designed to be used with bioinert UHPLC system.

MAbPac SCX-10 5 µm columns are recommended for high resolution and high-throughput separations of mAbs for characterization of mAb heterogeneity. The main advantage being the run time is reduced when 5 µm, shorter columns are used as compared to a longer MAbPac SCX-10 10 µm, 4 × 250 mm columns. Since the column length is short, chromatography runs can be completed at a faster rate, therefore increasing the throughput. Longer MAbPac SCX-10 5 µm 4.6 × 250 mm columns are designed for ultra-high-resolution analysis.

Warning

All eluents, samples should be filtered using a 0.2 µm filter unit before use. Failure to do so will result in clogging the column with increased back pressure; this will lead to compromised column performance.

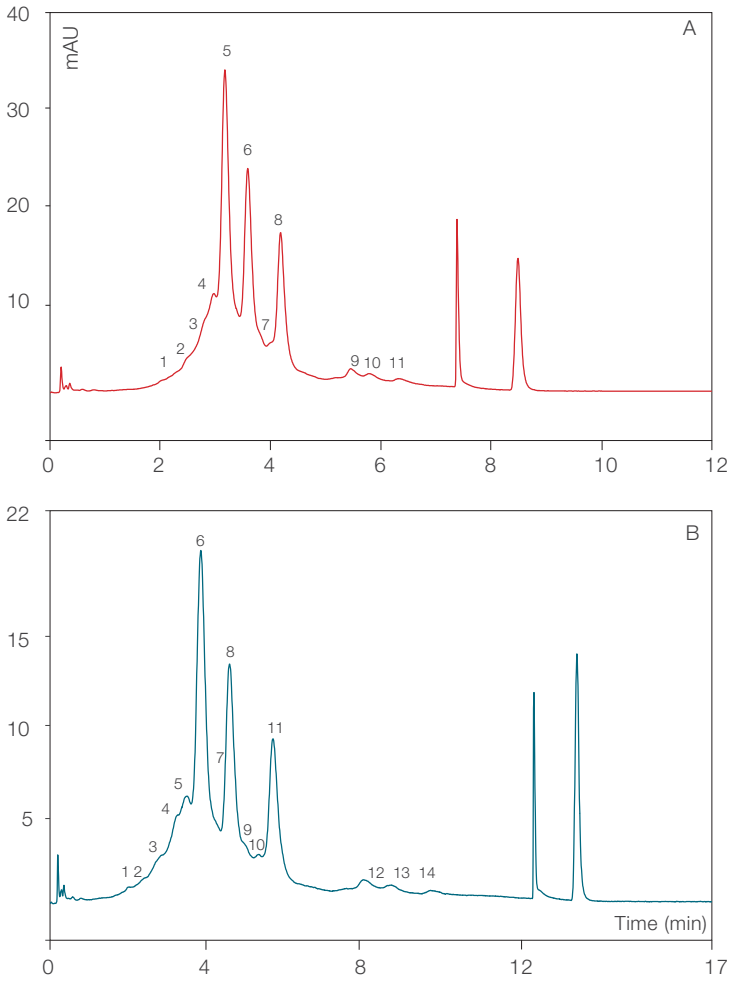
Note

Include a wash procedure using a buffer with high salt solution (For eg. 1M NaCl) to minimize the sample build up on the column during chromatography runs.

Salt gradient elution: MES based eluents; mAb separation using 5 µm columns

Separation of mAbs on a MAbPac SCX-10 5 µm columns 4 × 50 mm and 4 × 150 mm columns are shown in Figures 7 and 8 respectively. A shallow 10-minute gradient resulted in a better separation with higher resolution as expected (Figure 7). A 5 µm 50 mm column can be used for high throughput separations. Characterization of mAb variants on MAbPac SCX-10 5 µm, 4 × 150 mm column resulted in a high-resolution separation of variants with narrow peak widths. It should be noted that the flow rate of 1 mL/min could be used with this dimension for faster separations (Figure 9). Characterization of mAb variants on MAbPac SCX-10, 5 µm, 4 × 250 mm column resulted in a very high-resolution separation of variants with narrow peak widths (Figure 9). However, flow rates could not be exceeded more than 0.7 mL/min due to back pressure limitations. The high-pressure limit on this PEEK column dictated the maximum flow limit. To overcome this bottleneck, and to achieve mAb/protein separations at much higher flow rates, bioinert PEEK lined stainless steel RS column hardware that was suitable for operation up to 7,000 psi was developed and used.

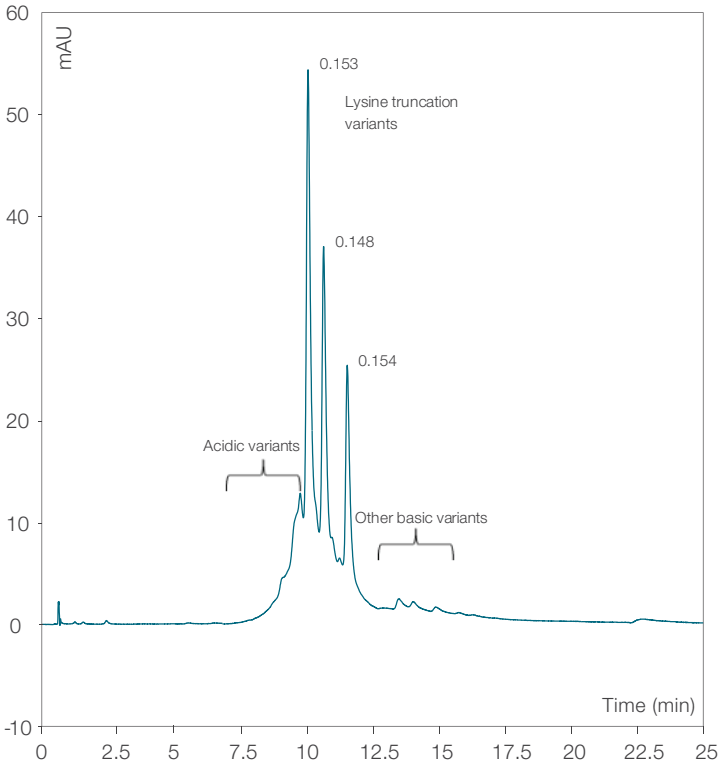
Example applications: MAbPac SCX-10 PEEK columns (continued)



Column	MAbPac SCX-10, 10 µm, 4 × 50 mm	
Cat. no.	078656	
Mobile phase	A: 20 mM MES + 60 mM NaCl, pH 5.6 B: 20 mM MES + 300 mM NaCl, pH 5.6 C: 20 mM MES + 1 M NaCl, pH 5.6 (Wash 1 min before equilibration)	
Flow rate	1.5 mL/min	
Inj. volume	10 µL	
Detection	UV at 280 nm	
Total volume	100 µL	
Temp	30 °C	
Equilibration	~5 min	
Sample	mAb, 5 mg/mL	
Gradient	A: 20–35% B in 5 min B: 20–35% B in 10 min	
Peaks	**A: 5 min gradient** 1–4: acidic variants 5, 6, 8: C-Terminal lysine variants 9-11: basic variants	**B: 10 min gradient** 1–5: acidic variants 6, 8, 11: C-Terminal lysine variants 12–14: basic variants

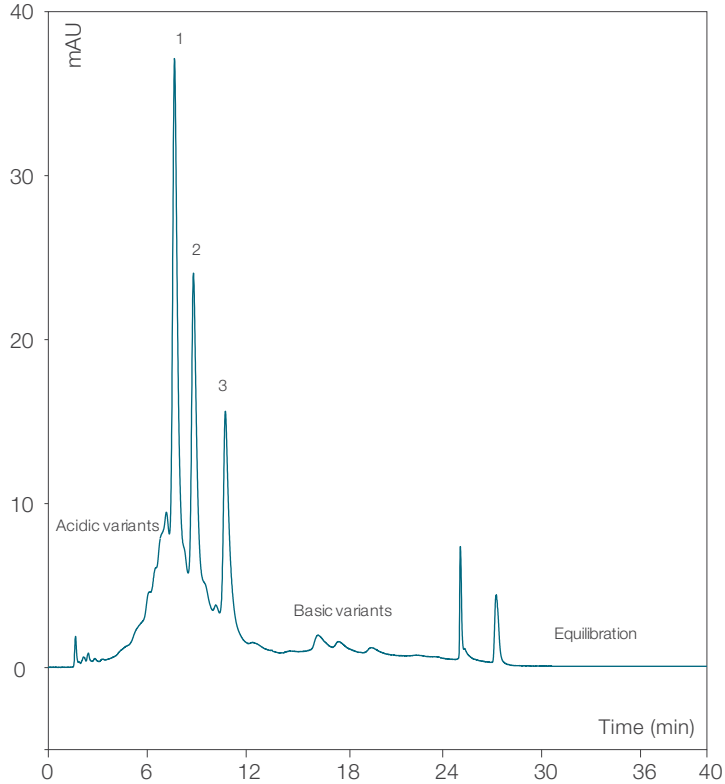
Figure 7. Separation of acidic and basic variants of a mAb using two different salt gradients on MAbPac SCX-10, 5 µm 4 × 50 mm column

Example applications: MAbPac SCX-10 PEEK columns (continued)



Column	MAbPac SCX-10, 5 µm, 4 × 150 mm
Cat. no.	085198
Mobile phase	A: 20 mM MES (pH 5.6) B: 20 mM MES (pH 5.6) + 300 mM NaCl
Flow rate	1 mL/min
Inj. volume	9 µL
Detection	280 nm
Temp	30 °C
Sample	mAb, 5 mg/mL
Gradient	30-60 % B in 20 min
Peaks	Peak width at half height (minutes) is shown for lysine truncation peaks

Figure 8. Separation of acidic and basic variants of a mAb using MAbPac SCX-10, 5 µm 4 × 150 mm column



Column	MAbPac SCX-10, 5 µm, 4 × 250 mm
Cat. no.	078655
Mobile phase	A: 20 mM MES, pH 5.6 + 60 mM NaCl B: 20 mM MES, pH 5.6 + 300 mM NaCl C: 20 mM MES, pH 5.6 + 1M NaCl (wash 2 min before equilibration)
Flow rate	0.7 mL/min
Inj. volume	10 µL
Detection	UV at 280 nm
Temp	30 °C
Sample	mAb, 5 mg/mL
Gradient	25–40% B in 20 min
Equilibration	15 min
Peaks	1, 2, 3: C-terminal lysine truncation variants

Figure 9. Separation of acidic and basic variants of a mAb using a salt gradient on MAbPac SCX-10, 5 µm 4 × 250 mm column

Example applications: MAbPac SCX-10 PEEK columns (continued)

Figure 10 shows the comparison of fast mAb variant analysis on MAbPac SCX-10, 5 µm and 10 µm columns. As expected, mAb separation on a 5 µm column provides superior performance over the 10 µm column of the same length. Both acidic and basic variants resolved better with the small particle column. Peaks 1, 2 and 3 are identified as mAb C-terminal lysine truncations peaks. Their peak width at half height measurements in minutes are shown. For 5 µm column, these are: Peak 1: 0,124, peak 2: 0.118 and peak 3: 0.127. In comparison, for 10 µm column for the same peaks, they are noted as 0.171, 0.164 and 0.167, respectively. When a 5 µm column is used, the resolution of peak 1 and 2 are 1.88 and 2.83. These values are lower for 10 µm column (Peak 1: 1.53, Peak 2: 2.11). The mAb variant separation data clearly demonstrates that both peak efficiency and resolution are superior for small particle size columns as compared with the large particle columns.

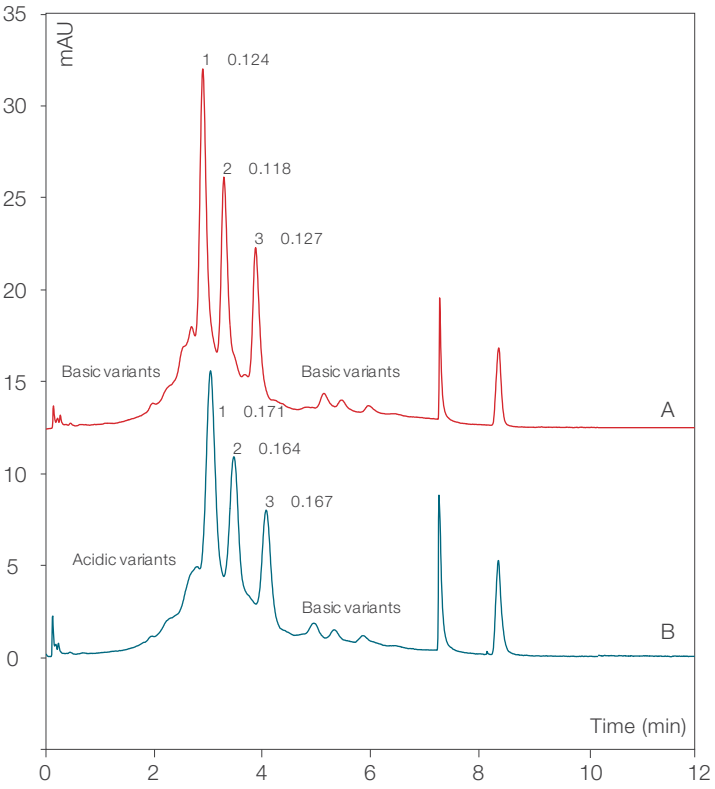


Figure 10. Comparison of separation of mAb on MAbPac SCX-10, 5 µm, 4 × 50 column with a MAbPac SCX-10, 10 µm, 4 × 50 mm column

Column	A: MAbPac SCX-10, 5 µm, 4 × 50 mm B: MAbPac SCX-10, 10 µm, 4 × 50 mm
Cat. no.	A: 078656 B: 074631
Mobile phase	A: 20 mM MES, pH 5.6 + 60 mM NaCl B: 20 mM MES, pH 5.6 + 300 mM NaCl C: 20 mM MES, pH 5.6 + 1 M NaCl (Wash 1 min before equilibration)
Flow rate	2 mL/min
Inj. volume	5 µL
Detection	UV at 280 nm
Temp	30 °C
Equilibration	4 min
Sample	mAb, 5 mg/mL
Gradient	20–35% B in 5 min
Peaks	1, 2, 3 : C-terminal lysine truncation variants; Peak width at half height is shown next to the peak number

Example applications: MAbPac SCX-10 RS, small particle columns

UHPLC based mAb separation: salt gradients

High resolution analysis of mAb charge variants is achieved using UHPLC based separations. Bioinert PEEK-lined stainless steel column housings are used for this rapid separation (RS), small particle column. These columns take advantage of smaller resin size as well as longer column length to maximize the resolution of mAb variant separation, and are suitable for operation up to the higher pressure limit of 7,000 psi. High pressure capable RS version of the MAbPac SCX-10 PEEK lined stainless steel columns are designed to be used with bioinert UHPLC system. A biocompatible Vanquish UHPLC system is recommended for best results.

Figure 11 shows an example of mAb separation comparing MAbPac SCX-10 RS, 5 µm, 4.6 x 150 mm with 4.6 × 250 mm columns at 1.3 mL flow rate (Panels A, C). Both columns resolved acidic and basic variants from the main lysine truncation peaks. Resolution values of lysine truncation peaks are shown. Comparison of different flow rates was made using MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm column (Figure 11; Panel B). Higher pressure compatibility of the column hardware allows the use of high flow rates (2 mL/min) while maintaining decent resolution. Ruggedness of MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm column was evaluated at 2 mL/min flow rate (Figure 12) and resolution data obtained for the lysine truncation peaks is shown.

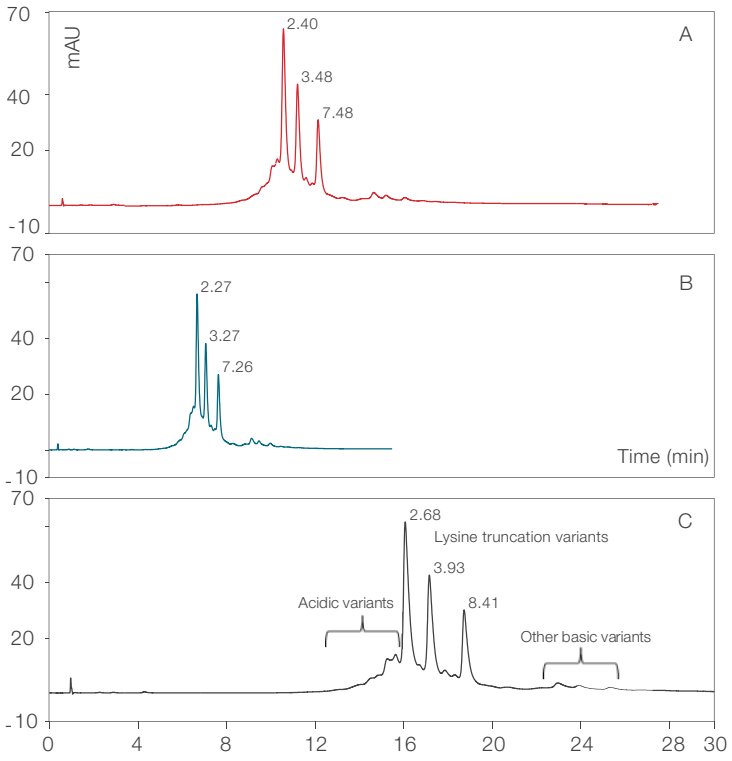
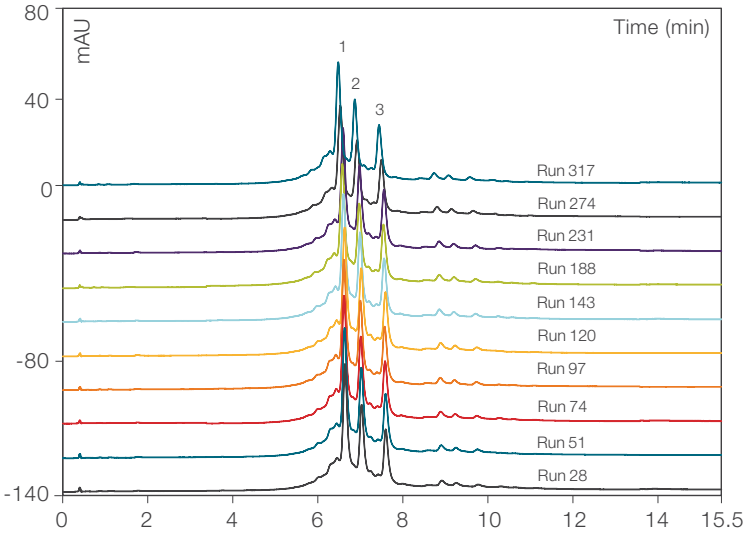


Figure 11. mAb separation on MAbPac SCX-10 RS, 5 µm columns
Comparison of different flow rates and different lengths of RS columns

Column	A, B: MAbPac SCX-10 RS, 5 µm 4.6 × 150 mm C: MAbPac SCX-10 RS, 5 µm 4.6 × 250 mm
Cat. no.	A, B: 085209 C: 082673
Mobile phase	A: 20 mM MES, pH 5.6 B: 20 mM MES, pH 5.6 + 300 mM NaCl
Flow rate	A: 1.3 mL/min B: 2 mL/min C: 1.3 mL/min
Inj. volume	A, B: 12 µL C: 20 µL
Sample	A: mAb 5 mg/mL
Gradient	A: 30-60% B in 20 min B: 30-60% B in 12 min

Resolution values are shown for lysine truncation variants

Example applications: MAbPac SCX-10 RS, small particle columns (continued)



Column	MAbPac SCX-10 RS, 5 µm 4.6 × 150 mm
Cat. no.	085209
Mobile phase	A: 20 mM MES, pH 5.6 B: 20 mM MES, pH 5.6 + 300 mM NaCl
Flow rate	2 mL/min
Inj. volume	12 µL
Detection	UV at 280 nm
Equilibration	8 min
Sample	mAb, 5 mg/mL
Gradient	30-60% B in 12 min
Peaks	1, 2, 3: C-terminal lysine truncation variants mAb sample was injected intermittently approximately every 20 runs

Figure 12. Ruggedness testing of MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm column at flow rate of 2 mL/min. Lysine truncation peaks are identified as 1, 2, 3 peaks and their peak width half height data (in minutes) is given below.

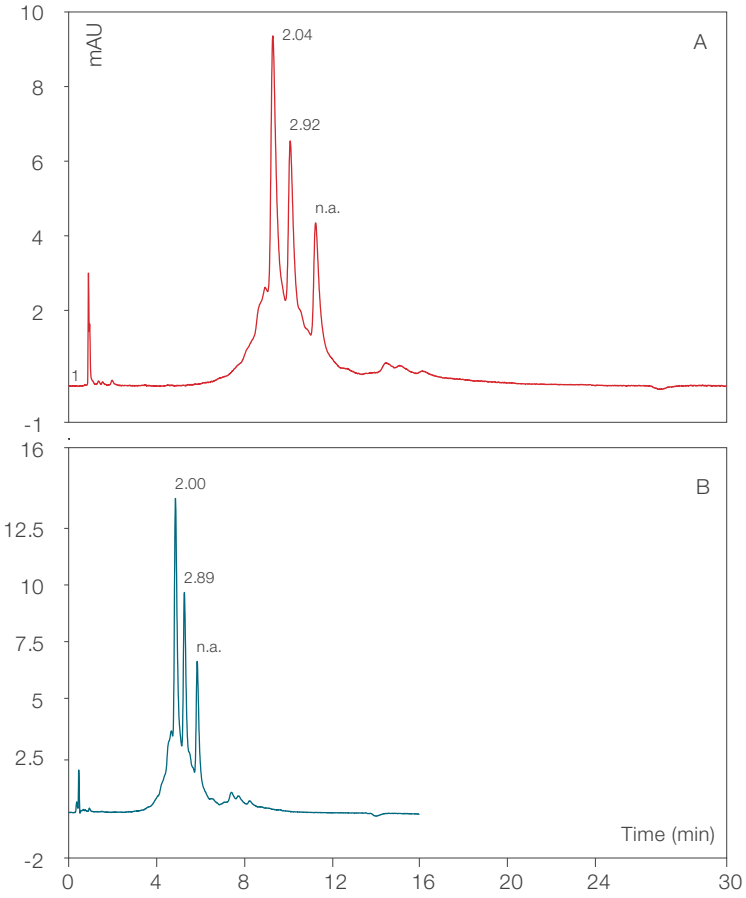
Ruggedness testing of MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm column at flow rate of 2 mL/min. Peak width at half height values in minutes are shown for lysine truncation peaks in the table below.

Sample no.	Peak 1	Peak 2	Peak 3
28	0.099	0.099	0.104
51	0.098	0.097	0.104
74	0.100	0.098	0.105
97	0.098	0.096	0.103
120	0.098	0.098	0.103
143	0.100	0.096	0.104
188	0.107	0.105	0.106
231	0.103	0.100	0.105
274	0.115	0.111	0.111
317	0.107	0.103	0.112
Average	**0.103**	**0.100**	**0.106**
RSD (%)	**5.47**	**4.75**	**3.03**

We have added 2.1 mm I.D. format columns in different lengths to our MAbPac SCX-10 RS, 5 µm portfolio. There are several advantages using these columns including sample and eluent conservation. However, it should be noted that the gradient method developed with the 4.6 mm column might not work with a 2.1 mm format column. Especially, this is evident if one is using lower flow rates due to influence of instrument delay volume. It is important to establish the separate gradient methods for 2.1 mm formats separately.

Figure 13 shows an example of mAb separation on MAbPac SCX-10 RS, 5 µm, 2.1 × 150 mm column at 0.21 mL/min and 0.42 mL/min flow rates (Panels A, B). Resolution values of lysine truncation peaks are shown. Resolution values of lysine variants are shown. These values are not much affected even at 0.42 mL/min. Therefore, run times can be shortened at this flow rate which can also improve productivity. An improvement in resolution of separation of both acidic and basic variants of a mAb can be achieved by using MAbPac SCX-10 RS, 5 µm, 2.1 × 250 mm columns (Figure 14). In this case mAb is separated using a sharp gradient and a shallow gradient. As expected, resolution values of lysine variants are higher when shallow gradients are used.

Example applications: MAbPac SCX-10 RS, small particle columns (continued)

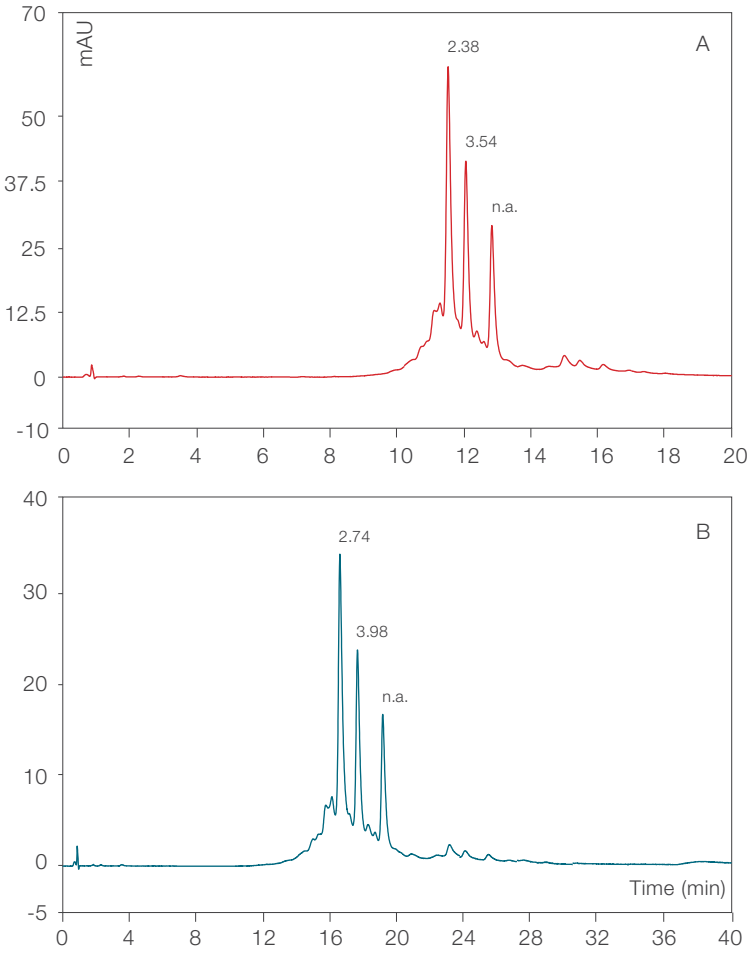


Column	MAbPac SCX-10 RS, 5 µm 2.1 × 150 mm
Cat. no.	088242
Mobile phase	A: 20 mM MES, pH 5.6 B: 20 mM MES, pH 5.6 + 300 mM NaCl
Flow rate	A: 0.21 mL/min B: 0.41 mL/min
Inj. volume	2 µL
Sample	mAb, 5 mg/mL
Gradient	A: 35-65% B in 24 min B: 35-65% B in 12 min

Resolution values are shown for lysine truncation variants

Figure 13. mAb separation on MAbPac SCX-10 RS, 5 µm 2.1 × 150 mm column using different flow rates and different gradient conditions

Example applications: MAbPac SCX-10 RS, small particle columns (continued)



Column	MAbPac SCX-10 RS, 5 µm 2.1 × 250 mm
Cat. no.	082515
Mobile phase	A: 20 mM MES, pH 5.6 B: 20 mM MES, pH 5.6 + 300 mM NaCl
Flow rate	0.32 mL/min
Inj. volume	3.3 µL
Sample	mAb, 5 mg/mL
Gradient	A: 30-60% B in 16.5 min B: 30-60% B in 33 min

Resolution values are shown for lysine truncation variants

Figure 14. mAb separation on MAbPac SCX-10 RS, 5 µm 2.1 × 250 mm column using different gradient conditions

Example applications: MAbPac SCX-10 RS, small particle columns (continued)

UHPLC based mAb separation: pH gradient based mAb separation

For pH gradient based separation, CX-1 pH gradient buffers were used (See Reference 3). The CX-1 pH gradient buffer kit is designed to generate a linear pH gradient on MAbPac columns. It consists of Buffer A and Buffer B which are titrated to pH 5.6 and pH 10.2 respectively. Therefore, running a gradient from 100% A to 100% B will generate a linear pH gradient from pH 5.6 to 10.2. If the pI of the mAb is not known, a broad range of pH gradient (0-100% B) may be employed to obtain a specific pH range of interest (Figure 15, 16; Panel A).

Once you know where it is eluting and the pI values of the mAb of your interest, you can run a shallow pH gradient that covers the pI of the mAb. An example of pH gradient based mAb separation with MAbPac SCX-10 RS, 5 µm 4.6 × 150 mm is shown in Figure 15. Similar, but another example of pH gradient based mAb separation with MAbPac SCX-10 RS, 5 µm 2.1 × 150 mm is shown in Figure 16. In the initial run, 0-100% B pH gradient was chosen (Panel A). Further optimization of the separation was achieved by simply running a shallower pH gradient (Figure 15, 16; Panel B).

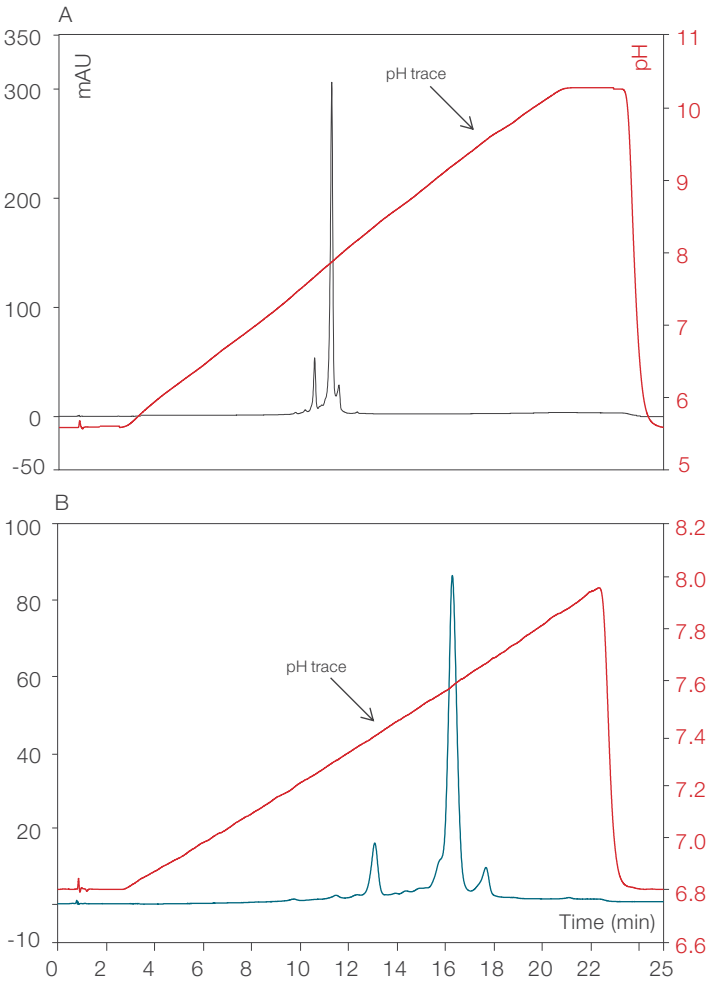
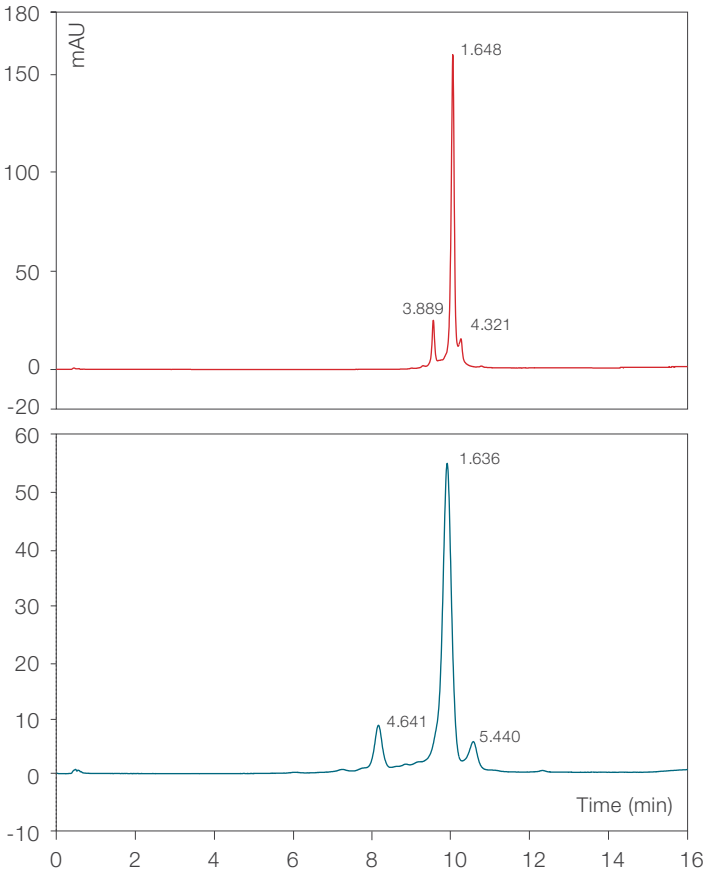


Figure 15. Separation of monoclonal antibody variants using pH gradienton a MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm column

Column	MAbPac SCX-10 RS, 5 µm 4.6 × 150 mm
Cat. no.	085209
Mobile phase	A: 1X CX-1, pH gradient buffer A (pH 5.6) B: 1X CX-1, pH gradient buffer B (pH 10.2)
Flow rate	1 mL/min
Inj. volume	12 µL
Sample	mAb, 5 mg/mL
Gradient	A: 0-100% B in 20 minutes B: 26-50% B in 20 minutes

Example applications: MAbPac SCX-10 RS, small particle columns (continued)



Column	MAbPac SCX-10 RS, 5 µm 2.1 × 150 mm
Cat. no.	088242
Mobile phase	A: 1X CX-1, pH gradient buffer A (pH 5.6) B: 1X CX-1, pH gradient buffer B (pH 10.2)
Flow rate	0.32 mL/min
Inj. volume	2 µL
Sample	mAb, 5 mg/mL
Gradient	A: 0-100% B in 12 minutes B: 30-60% B in 12 minutes

Figure 16. Separation of monoclonal antibody variants using pH gradient on a MAbPac SCX-10 RS, 5 µm, 2.1 × 150 mm column

Troubleshooting guide

Finding the source of high system backpressure

1. If you observe high back pressure, wash the column with an eluent containing high salt (Buffer containing 1M NaCl) at a lower flow rate (0.1 to 0.5 mL/min) until the pressure becomes normal.
2. A significant increase in the system backpressure may be caused by a plugged inlet frit (bed support).
3. Before replacing the inlet bed support assembly of the column, make sure that the column is the cause of the excessive backpressure.
4. Check for pinched tubing or obstructed fittings from the pump outlet, throughout the eluent flow path to the detector cell outlet. To do this, disconnect the eluent line at the pump outlet and observe the backpressure at the usual flow rate. It should not exceed 50 psi (0.3 MPa). Continue adding components (injection valve, column, detector) one by one while monitoring the system backpressure. The 4 × 250 mm MAbPac SCX-10, 10µm column should add no more than 2000 psi backpressure at 1 mL/min. The 4 × 50 mm MAbPac SCX-10, 10µm columns as expected should add no more than 1/5th of the analytical column (300-350 psi) back pressure at 1 mL/min. No other component should add more than 100 psi (0.7 mpa) to the system backpressure.
5. If the high backpressure is due to the column, first try cleaning the column. If the high backpressure persists, replace the column bed support at the inlet of the column.
6. Never use H_2O alone for washing the column. This will lead to significant increase in back pressure. This abnormality can be reversed by washing the column for long periods of time with buffered high ionic strength eluents. Please make sure to start at a low flow rate to keep the pressure under control. Gradually increase the flow rate as the column pressure drops further.

Warning

Never wash the MAbPac SCX-10 or MAbPac SCX-10 RS column with water. Always maintain minimum ionic strength (20 mM MES or equalent ionic strength) in the eluents.

Column performance is deteriorated

Peak efficiency and resolution is decreasing; loss of efficiency

1. If changes to the system plumbing have been made, check for excess lengths of tubing, tubing diameters larger than 0.010 I.D. in., larger than normal tubing diameter, and for leaks.
2. Check the flow rate and the gradient profile to make sure your gradient pump is working correctly.
3. The column may be fouled. Clean the column using the recommended cleaning conditions.
4. If there seems to be a permanent loss of efficiency, check to see if headspace has developed in the column. This is usually due to improper use of the column such as submitting it to high backpressure. If the resin doesn't fill the column body all the way to the top, the resin bed has collapsed, creating a headspace. The column must be replaced.
5. If the peak shape looks good, but the efficiency number is low, check and optimize the integration parameters. If necessary, correct the integration manually, so the start-, maximum-, and end of the peak are correctly identified.

Warning

One possible source of decreased performance could be metal leaching from the system. To avoid denaturation of the protein samples and corrosion of components with halide-salt mobile phases we strongly recommend a metal-free inert system, including pump, tubing, ferrules, and bolt assemblies.

Troubleshooting guide (continued)

Unidentified peaks appear as well as the expected analyte peaks

1. The sample may be degrading. Proteins tend to degrade faster in solutions; therefore, store your protein samples in the freezer in dry form, and prepare only a small amount of solution/mixture for analysis.
2. The eluent may be contaminated. Prepare fresh, filtered eluent.
3. Run a blank gradient to determine if the column is contaminated. If ghost peaks appear, clean the column.

Peak efficiency and resolution is poor

1. Try to use different eluents (buffer, pH, concentration etc.), to make sure you are using the optimum conditions for your separation problem.
2. The column may be overloaded. Dilute the sample and/or inject smaller volumes.

Peak retention time varies from run-to-run

The column may not be adequately equilibrated or washed.

1. Make sure that the equilibration time is adequate (5 to 10 column volumes) and remains constant after every gradient run. Re-equilibration should be part of the method.
2. Column washing is usually not necessary between every run, unless your sample is extremely “dirty.” If you need to use a wash, a consistent and adequate method for washing and equilibrating should be part of the method.

Metal poisoning of columns causing reproducibility and recovery problems

1. Periodic passivation is a must for stainless steel HPLC systems to reduce rust build up.
2. If you have experienced, performance, reproducibility and recovery problems, it could be due to metal poisoning or rust from one or, several SST components of your HPLC system. Restoring the column to original metal free status is a tedious and time-consuming process. Potentially restoration can be achieved by treating the column with oxalic acid dihydrate (200mM) at 0.2 mL/min for 16 hrs or longer followed by a 100 mM NaOH wash for 30 min at 0.5 mL/min. Please equilibrate your column thoroughly for an extended period of time (1 to 2 hrs) before testing with your sample of interest. Please note that you need an inert device for pumping the oxalate or high pH eluents. Do not use stainless steel pumps to perform this step.

No peaks, small peaks, noisy baseline

Detection problem

1. Make sure that you are using the correct wavelength for your sample/buffer system.
2. Adjust the selected detector range (AU) according to your injected sample amount.
3. Check your lamp: aged UV lamps tends to give noisier response. Replace the lamp if necessary.

Chromatographic problem

Make sure that your sample can be eluted with the buffers and conditions you are using. Before trying a gradient separation, try isocratic elution with 100% B (high salt) buffer: the sample should elute at, or near to, t0 (void). If not, try a higher salt concentration or different pH.

Temperature stability problem of mAb/protein

Determine if your mAb/protein is stable at elevated temperatures prior to creating and using methods with elevated temperatures for routine sample analysis.

Column care

New column equilibration

MAbPac SCX-10 columns are shipped in a buffer containing 0.1% NaN_3. Before use, equilibrate the column with approximately 20 mL of the starting eluent (20 min at 1 mL/min).

Column clean up

1. For minor contamination, use a mild cleaning protocol by running consecutive gradient runs, using a high (1-2 M) salt concentration at the end of the gradient.
2. For more severe contamination, inject 100-500 µL (or more as needed) of 0.1-1 M NaOH consecutively.
3. If necessary, the column can be washed with strong acid such as 1.0 M HCl followed by base 0.1-0.5 M NaOH.
4. Usually 5-30 min at 1 mL/min is sufficient. Do not exceed 20 × the column volume of 0.5 M NaOH (60 mL). The use of high concentrations of base and/or larger volumes of base are not recommended. For the SCX-10 columns, the above mentioned strong acid or base cleaning solutions should be used at room temperature (<30 °C). After the wash, rinse the column with at least 20 mL of the starting buffer solution. Note: Do not store the column in strong acid or base solution.

When cleaning an analytical and guard column in series, move the guard column after the analytical column in the eluent flow path. Otherwise contaminants that have accumulated on the guard column will be eluted onto the analytical column.

Column storage

Short term storage

For short term storage, use the low salt concentration eluent (pH = 3-8) as the column storage solution. Do not store the column in high pH buffers (>pH 8.0) even for short term storage.

Long term storage

For long term storage, use storage eluent (or other low salt concentration eluent with pH = 6.0-7.5) with 0.1% sodium azide added to prevent bacteria growth on the column. Never store columns in high pH (>pH 8.0) buffers.

Flush the column with at least 10 mL of the storage eluent. Cap both ends, securely, using the plugs supplied with the column.

Replacing column bed support assemblies for PEEK columns (only)

1. Carefully unscrew the inlet (top) column fitting. Use two open end wrenches.
2. Remove the bed support. Tap the end fitting against a hard, flat surface to remove the bed support and seal assembly. Do not scratch the wall or threads of the end fitting. Discard the old bed support assembly.
3. Removal of the bed support may permit a small amount of resin to extrude from the column. Carefully remove this with a flat surface such as a razor blade. Make sure the end of the column is clean and free of any particulate matter. Any resin on the end of the column tube will prevent a proper seal. Insert a new bed support assembly (p/n 057804) into the end fitting and carefully thread the end fitting and bed support assembly onto the supported column.
4. Tighten the end fitting finger-tight, then an additional ¼ turn (25 in x lb.). Tighten further only if leaks are observed.

If the end of the column tube is not clean when inserted into the end fitting, particulate matter may prevent a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or break the end fitting.

Replace the inlet bed support only if the column is determined to be the cause of high system backpressure, and cleaning of the column does not solve the problem.

Do not replace column bed support assemblies for RS columns

These parts are not user-replaceable, and may be damaged by attempting to swage SST ferrules into the PEEK cone. For column-to-tubing connections we recommend the use of biocompatible Viper fingertight fittings or NanoViper fused-silica-lined PEEK tubing equipped with Viper connection fittings. If these are not available, other tubing materials may be connected using SST bolts equipped with captive PEEK ferrules according to the manufacturer's instructions.

Avoid use of SST ferrules on UHPLC MAbPac SCX-10 RS columns.

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Ordering information

MAbPac SCX-10 and SCX-10 RS columns

Description	Particle size	Dimensions	Cat. no
MAbPac SCX-10 columns			
MAbPac SCX-10, semi-preparative column	10 µm	22 x 250 mm	SP6947
MAbPac SCX-10, semi-preparative column	10 µm	9 x 250 mm	088784
MAbPac SCX-10, analytical column	10 µm	4 x 250 mm	074625
MabPac SCX-10, analytical column, 3 columns from 1 lot	10 µm	4 x 250 mm	088782
MabPac SCX-10, analytical column, 3 columns from 3 lots	10 µm	4 x 250 mm	088783
MAbPac SCX-10, analytical column	10 µm	4 x 150 mm	075602
MAbPac SCX-10, guard column	10 µm	4 x 50 mm	074631
MAbPac SCX-10HT, analytical column	10 µm	4 x 50 mm	075603TS
MAbPac SCX-10, analytical column	10 µm	2 x 250 mm	075604
MAbPac SCX-10, guard column	10 µm	2 x 50 mm	075749
MAbPac SCX-10, analytical column	5 µm	4 x 250 mm	078655
MAbPac SCX-10, analytical column	5 µm	4 x 150 mm	085198
MAbPac SCX-10, analytical column	5 µm	4 x 50 mm	078656
MAbPac SCX-10 RS columns			
MAbPac SCX-10 RS, analytical column	5 µm	4.6 x 250 mm	082673
MAbPac SCX-10 RS, analytical column	5 µm	4.6 x 150 mm	085209
MAbPac SCX-10 RS, analytical column	5 µm	4.6 x 50 mm	082674
MAbPac SCX-10 RS, analytical column	5 µm	2.1 x 250 mm	082515
MAbPac SCX-10 RS, analytical column	5 µm	2.1 x 150 mm	088242
MAbPac SCX-10 RS, analytical column	5 µm	2.1 x 50 mm	082675

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