

ProPac WCX-10 Columns

Guidance for column use

Tips to maximize column lifetime

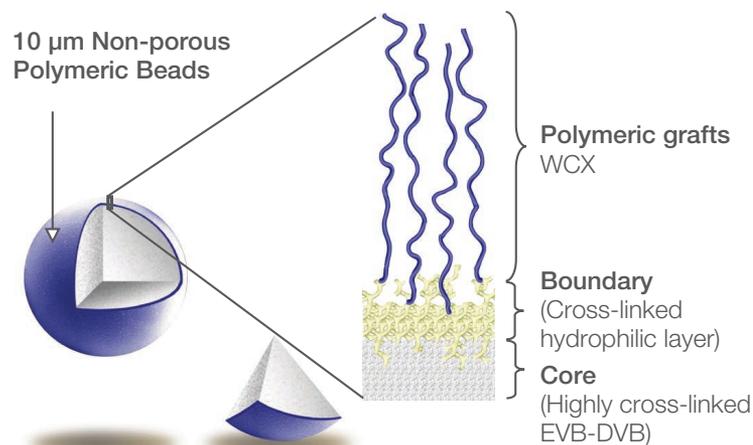
ProPac WCX-10 Column Tips and Tricks

This guide provides essential information and invaluable guidelines for mobile phases, wash procedures, system precautions, and column care information. Use these tips to maximize the lifetime of your Thermo Scientific™ ProPac™ WCX-10 column and achieve improved chromatography.

Column Description

The ProPac WCX-10 column is a weak cation-exchange column designed specifically for high-resolution, high-efficiency analysis of monoclonal antibodies (mAb), and associated variants.

The unique non-porous pellicular resin provides exceptionally high resolving power, permitting the separation of mAb variants that differ by as little as one charged residue. Hydrophobic interactions with the resin are essentially eliminated, resulting in highly efficient peaks. A proprietary grafted cation-exchange surface provides pH selectivity control, resulting in high-resolution separations. ProPac WCX-10 columns are stable between pH 2 and 12.





Mobile Phase Ionic Strength

Proteins are eluted using a gradient of increasing ionic strength. Optimum performance is obtained if a minimum salt concentration is maintained in buffer A at all times. The ProPac WCX-10 columns can be operated at any flow rate, as long as the backpressure remains below 4,000 psi. PEEK™ (polyetheretherketone) is used for the column hardware and has excellent resistance to aqueous solutions and most organic solvents.

NOTE:

Do not use the ProPac WCX-10 column in the absence of a minimal concentration of salt.

A minimum concentration of 20 mM NaCl or equivalent in buffer is recommended. Failure to maintain a minimum ionic strength in buffer A will result in alteration of the stationary phase conformation, causing 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. Replace the column and pump buffer B through the column at low flow rate (0.1–0.2 mL/min), until the backpressure falls back to normal.

Mobile Phase Optimization

The mobile phase 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 are widely used for various applications, usage of MES containing buffers is becoming increasingly popular for mAb separations between the pH range of 5–6.5. The advantage of using MES buffer is the buffering of the stationary phase of the column effectively with improved resolution.

Mobile Phase Constituent	Recommendations
Buffer	MES or other Goods Buffers, Tris
Salt	Potassium or sodium salts of chloride, acetate
pH Modifier	Phosphoric acid, HCl
Column Cleaning	Sodium hydroxide
Solvent*	Up to 80% acetonitrile
Detergent	Non-ionic, anionic, or zwitterionic detergents Do NOT use cationic detergents.
Anti-Microbial	0.1% sodium azide

* Alcohols should be avoided when using the ProPac WCX-10 column.

Adjusting the pH

Hint: The mobile phase should contain all the electrolytes before adjusting the pH. To make sure that the pH reading is correct, the pH meter should be calibrated at least once a day. Stirring and 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.

Filter the mobile phase

Hint: To extend the lifetime of your column as well as your HPLC pump, the buffers must be filtered using a 0.2 µm membrane filter to remove insoluble contaminants from the eluents. Degas the mobile phase before using it.

NOTE:
Cationic detergents (i.e. SDS) will irreversibly bind to the ProPac WCX-10 column and their use should be avoided.

Column Saturation

When first using the ProPac WCX-10 column, injection of your protein is recommended to stabilize the baseline for consistent initial use of the column.



Stainless Steel HPLC System and Column Poisoning

Metal poisoning of ProPac WCX-10 columns can cause problems when Stainless Steel (SST) HPLC systems are used. Metal components in SST systems corrode and form a metal complex (rust), when in contact with high salt concentration/low pH eluents. Metal complexes from the corrosion will leach onto the column and inhibit its performance. To avoid these problems, we highly recommend using an inert HPLC system for continued robust chromatography of monoclonal antibodies.

Hint: Periodic passivation is required for stainless steel HPLC systems to reduce rust build up. If you have experienced reproducibility and recovery problems, it could be due to the metal poisoning from one or several SST components in your HPLC system. Restoring the column to its original metal-free status is a tedious and time consuming process. However, it can be achieved by treating the column with oxalic acid dihydrate (200 mM) at 0.2 mL/min for 6 hours followed by a 20 mM sodium hydroxide (NaOH) wash for 30 min at 0.5 mL/min. Please equilibrate your column thoroughly for an extended period of time (1 to 2 hours) before testing with your sample of interest.

NOTE:
Treatment requires an inert device for pumping the oxalate or high pH eluents.





ProPac WCX-10 Column Wash Treatments

Short wash: NaOH/MES treatment procedure

Treat the ProPac WCX-10 column with 20 mM NaOH at 0.5 mL/min for 30 min at room temperature. After this, wash the column thoroughly with 20 mM MES (pH 5.6) for 2 hours. The column should now be ready for your analysis using routine conditions. If you do not see any improvement with your column performance, proceed to the optional extended wash treatment.

MES Buffer Preparation: Prepare 20 mM 2-(N-morpholino) ethanesulfonic acid (MES), pH 5.6, by adding 3.9 g of MES (Sigma) to 950 mL of purified DI water (Milli-Q® H₂O or equivalent). Adjust the pH with NaOH to 5.6 and volume to 1 L. Filter before use.

Extended wash: MES/60 °C treatment procedure

Treat the ProPac WCX-10 column with 20 mM MES (pH 5.6) for 7 hours at a flow rate of 0.2 mL/min at 60 °C. (Alternatively, you can fill up the column with an initial 30 min wash with 1 mL/min MES and leave it at 60 °C for 7 hours)

After treatment use your routine conditions for the analysis of your samples.

Optimizing Your Method

The separation is optimized by the buffer, pH, and ionic strength of the mobile phase and the temperature of the separation. These factors can have an enormous effect on the separations.

1. Selection of buffers: Choice of buffer plays a key role in establishing a robust method.

Preferred buffers: MES, ACES or other Good's buffers as they favor buffering of the stationary phase.

Less preferred buffers: Phosphate-based buffers.

2. pH: The ProPac WCX-10 column has weak cation-exchange functionality, therefore, slight changes in the pH can lead to significant differences in retention times of analytes of interest. It is important to prepare buffers gravimetrically (by weight) as much as possible and without need to adjust the pH each time. Slight pH meter adjustment variations can lead to substantial differences in the reproducibility of runs.

- 3. Ionic Strength:** Ion-exchange columns require certain minimum (20 mM) ionic strength to function reproducibly. Never use H₂O (water) alone for washing the column. This will lead to significant increase in backpressure. 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.
- 4. Temperature:** The stability of your mAb/protein at elevated temperatures should be established before routinely using high-temperature methods.
- 5. Sample Load:** The ProPac WCX-10 column is based on nonporous particles. Typically about 100 µg can be loaded on a 4 × 250 mm format column.

Column Care

1. Protect the column from contamination using a guard column.
2. Make sure that solvents are miscible when changing mobile phases.
3. When switching to a new mobile phase, the column should be equilibrated with at least 30 column volumes before injecting the sample.
4. 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.
5. The column can be stored in mobile phase for short term storage (e.g. overnight). For long term storage, it is highly recommended that the column be stored in 20 mM Na₂HPO₄/H₃PO₄, pH 6.5 with 0.1% sodium azide (more than 2 days).
6. The recommended operating maximum temperature is below 50 °C. In most cases, temperature control between ambient and 30 °C gives good results.
7. The recommended maximum backpressure is 4,000 psi (276 bar).

Ordering Information

Part Number	Description
063472	ProPac WCX-10 Analytical Column (2 × 250 mm)
063480	ProPac WCX-10G Guard Column (2 × 50 mm)
054993	ProPac WCX-10 Analytical Column (4 × 250 mm)
088779	ProPac WCX-10 Analytical Column (4 × 150 mm)
088778	ProPac WCX-10 Analytical Column (4 × 100 mm)
074600	ProPac WCX-10HT Analytical Column (4 × 50 mm)
054994	ProPac WCX-10G Guard Column (4 × 50 mm)
063474	ProPac WCX-10 Analytical Column (9 × 250 mm)
088766	ProPac WCX-10 Analytical Column (22 × 250 mm)
088767	Lot select column set – 3 columns from 1 resin lot (4 × 250 mm)
088768	Lot select column set – 1 column from each of 3 resin lots (4 × 250 mm)

Find out more at thermofisher.com/proteinLCcolumn

For technical assistance visit: thermofisher.com/chromexpert

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