# Improving the Use of ProPac WCX-10 Column for Monoclonal Antibody Variant Analysis and Characterization

The ProPac® WCX-10 column Tips and Tricks Guide provides helpful information and suggestions for improving the use of this column for monoclonal antibody variant analysis and characterization. This guide highlights some key information and suggestions; please consult the ProPac WCX-10 column manual for more detailed information and suggestions.

The ProPac WCX-10 column is a weak cation-exchange column designed specifically for the high-resolution, high-efficiency analysis of monoclonal antibodies and associated variants. The unique nonporous pellicular resin provides exceptionally high resolving power, permitting the separation of monoclonal antibody 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. The reproducible resin chemistry and manufacturing processes eliminate column variability as a concern in stability and QA/QC testing

To ensure optimal performance of the ProPac WCX-10 columns, please observe the following suggestions.

## *Stainless Steel HPLC System & 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.

**Suggestions:**

Periodic passivation is required for stainless steel HPLC systems to reduce rust build up.

1. 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 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 h followed by a 20 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 h) before testing with your sample of interest. **Please note: treatment requires an inert device for pumping the oxalate or high pH eluents.**

# ProPac WCX-10 Column Tips and Tricks Guide

## *Improving Resolution and Efficiency for Monoclonal Antibody (MAb) Separations:*

If you notice a decline in your column performance you may try the wash methods described below. These methods help the ProPac WCX-10 column stationary phase grafts to extend and assume their most favorable conformation.

**ProPac WCX-10 Column Wash Treatments:**

**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® $H_2O$ or equivalent). Adjust the pH with NaOH to 5.6 and volume to 1 L. Filter before use.

1. **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 h.

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.

2. **Optional Extended Wash: MES/60° C Treatment Procedure:** Treat the ProPac WCX-10 column with 20 mM MES (pH 5.6) for 7 h 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 h.)

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

## *Method Development:*

Choice of buffer, pH, and ionic strength of the mobile phase and the temperature of the separation tremendously influences the MAb separations on the ProPac WCX-10 column.

- **Selection of buffers:** Choice of buffer plays a key role in establishing a robust method.
  - **Preferred buffers:** MES (pH 5.5–6.5); ACES or other GOOD'S buffers as they favor buffering of the stationary phase.
  - **Less preferred buffers:** Phosphate-based buffers.
- **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.
- **Ionic Strength:** Ion-exchange columns require certain minimum (20 mM) ionic strength to function reproducibly. NEVER USE $H_2O$ (WATER) 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.
- **Temperature:** The stability of your MAb/Protein at elevated temperatures should be established before routinely using high-temperature methods.
- **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.

**Please follow the procedures given in the ProPac WCX-10 column manual to recover a fouled column.**


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