User Guide

# MiniChrom® Pre-Packed Columns

The life science business of
Merck KGaA, Darmstadt, Germany
operates as MilliporeSigma in
the US and Canada.

# Contents

# Introduction

MiniChrom® columns are prepacked chromatography columns intended for laboratory-scale process development evaluations. Results achieved for biomolecule separations using MiniChrom® columns are generally comparable to the results obtained using traditional laboratory scale columns from various vendors (i.e., MilliporeSigma Vantage® L columns).

# Column Specifications and Dimensions

## MiniChrom® Column Specifications

| | | |
|---|---|---|
| Materials of construction | Column | Polypropylene |
| | Bed supports | Polypropylene polyethylene |
| Connections | Standard LC: M10-32 UNF for $^{1}/_{16}$ inch tubing | |
| Mechanical pressure limit | 8 bar* | |
| Flow rate | ≤10 mL/min | |
| Chemical stability of column hardware | pH 1–14, organic solvents (except halogenated) | |
| | Minerals and salts | |
| | Common eluents | |
| | Additives (urea, antioxidants, thiols, detergents, tensides) | |
| Temperature range | 4–30 °C | |

* Operating pressure is dependent on process parameters and resin type. Typical pressure drop across MiniChrom® columns is 1–3 bar.

## MiniChrom® Column Dimensions

| Bed Volume (mL) | Bed Height (mm) | Internal Diameter (mm) |
|---|---|---|
| 0.2 * | 10 | 5 |
| 1 | 20 | 8 |
| 5 | 100 | 8 |

* Eshmuno® CP-FT resin only

# System Setup and Column Connection

MiniChrom® columns are compatible with most common liquid chromatography systems that use $^{1}/_{16}$ inch female connectors. Use the system with a back pressure regulator or flow restrictor to prevent degassing in the detector flow cell. Follow the system manufacturer's instructions.

## Buffers and Feedstock Solutions

All buffers and feedstock solutions applied to the column should be 0.22 µm filtered before application. Include a 2 µm in-line filter upstream of the column in the chromatography system.

## Connecting to a Chromatography System

MiniChrom® columns are shipped in a liquid storage buffer preservative.

1. Flush the system tubing with the desired equilibration buffer.
2. Remove the column's upper stopper and attach the column inlet to the system using a drop-to-drop connection to avoid introduction of air.
3. Remove the column's bottom stopper and attach column outlet to the system. Connections should be finger tight.
4. Before using the column for the first time, completely remove all storage buffer by washing the column with at least five column volumes of equilibration buffer.

# Protocol

This section provide general guidance and best practices for MiniChrom® columns evaluation in bioprocess development. Refer to Fractogel®, Eshmuno®, or ProSep® resin product datasheets for information on binding capacity, operating pressure, and other resin-specific information

Chromatography process development is dependent on characteristics of the product molecule, impurities, buffer conditions, and purification goals. Contact Technical Service for assistance in the development of purification processes.

## Evaluating Ion Exchange and Mixed Mode Resins

### Molecule Isoelectric Point (pI)

The isoelectric point (pI) is the pH at which the molecule of interest has zero net charge.

At a pH below the pI, the molecule has net positive charge and can bind to cation exchange media.

At a pH above the molecule's pI, the molecule has net negative charge and can bind to anion exchange media.

For optimal purification, consider the net charge of both product and impurities. Molecule isolelectric points can be determined experimentally through isoelectric focusing or though literature review.

## Operational Mode: Bind and Elute vs. Product Flowthrough

### Bind and elute Chromatography

Bind-and-elute chromatography involves binding the product molecule to the resin, washing unbound or weakly bound impurities, and selectively eluting the product.

Elution is achieved by modulating the mobile phase conductivity and/or pH. Linear increasing conductivity and/or pH gradients are an efficient way to identify optimal elution conditions during process development. Resin binding capacity is molecule specific and can vary with load pH, conductivity, flow rate, and feed impurity profile.

When bind and elute chromatography resins are evaluated for maximum selectivity (as opposed to maximum binding capacity), initial purification screening experiments can be conducted at 20–30% of the capacity values referenced in product datasheets. Bind and elute chromatography is typically used for capture and intermediate purification steps.

### Product Flowthrough Chromatography

In product flowthrough chromatography, impurities bind to the resin while the product is recovered in the load flowthrough pool. This mode of operation does not require a dedicated product elution step.

In most product flowthrough applications, conditions are selected so that the product molecule has the same charge as the resin, while the charge of the impurities is opposite that of the resin. This enables impurities to bind to the resin while the product flows through unbound. In other flowthrough applications (such as those using Eshmuno® CP-FT resin), the product initially binds to the resin before being displaced by more strongly charged impurities, enabling product recovery in the load flowthrough pool.

Product flowthrough chromatography is most typically used for polishing applications.

## pH, Conductivity, and Buffer Selection

In general, negatively charged buffers such as sodium acetate, sodium phosphate, and sodium citrate are preferred for cation exchange chromatography. Positively charged buffers such as Tris and Bis-Tris are preferred for anion exchange chromatography. Common buffer concentrations are in the range of 20–50 mM. Consider molecule pH stability when selecting operating pH.

Select load conductivity to enable binding of the target molecule. Optimal binding occurs at low conductivity (in the range of 1–9 mS/cm) for most ion exchangers. Mixed-mode resins with hydrophobic binding sites (i.e., Eshmuno® CMX and Eshmuno® HCX) and salt-tolerant ion exchange resins (i.e., Eshmuno® CPS) may be operated at elevated conductivity.

Refer to resin datasheets for resin specific guidance.

## General Procedure for Ion Exchange and Mixed-Mode Chromatography

This table outlines common steps for operating ion exchange and mixed-mode chromatography.

Operating flow rates are selected to achieve a residence time between 4.5–6 minutes for Fractogel® resins and 3–5 minutes for Eshmuno® resins.

| Step | | Notes | Volume |
|---|---|---|---|
| 1 | Equilibrate pH and conductivity | Equilibrate the column with buffer formulated to match the load pH and conductivity. | 5 CV, or until pH and conductivity are stable. |
| 2 | Load | Bind target molecule(s) to the column. | Until target binding capacity is reached.<br>If binding capacity is unknown, monitor column effluent for breakthrough. |
| 3 | Wash | Wash the column with buffer to recover unbound product (product flowthrough mode) or remove weakly bound impurities (bind and elute mode). | 5 CV, or until UV signal is stable. |
| 4 | Elute (Bind and elute mode only) | Elute product with a stepwise or gradient change in pH and/or conductivity. Linear gradient elution is useful for optimization experiments. Consider collecting elution fractions in intervals of 0.5–1 CV for purity and yield analysis.* | Stepwise elution: 3-5 CV, or until product of interest is sufficiently eluted.<br>Linear gradient elution: At least 20 CV for initial screening. |
| 5 | Regenerate* | Remove residual bound material from the resin using high salt buffer (1–2 M NaCl) | 3–5 CV. |
| 6 | Clean-in-Place (CIP)* | Sanitize the resin with sodium hydroxide (0.1–1 N NaOH). | 3–5 CV. |
| 7 | Re-Equilibrate pH | Caustic CIP solution is most effectively flushed from the column with high salt buffer (1-2 M NaCl) formulated to match load pH conditions.<br>Following pH equilibration at high salt, either return to step 1 for conductivity equilibration, or store the resin in storage buffer. | 5 CV or until pH is stable. |

* See Tech Note 5773: Development Strategy for CEX Chromatography.

** Eshmuno® HCX resin requires a different cleaning strategy because the presence of strong hydrophobic interactions. See application note: “Regeneration and Cleaning of Eshmuno® HCX Chromatography Resin—A Screening Study.”

# Evaluating Protein A Affinity Resins

## Dynamic Binding Capacity Assessment

Dynamic binding capacity (DBC) is the amount of target protein the resin will bind under actual flow conditions before breakthrough of unbound protein occurs. The DBC of Protein A resin can be measured using either clarified monoclonal antibody (mAb) feedstock or mAb that has already been purified by Protein A affinity chromatography. In the case of pre-purified mAb, UV 280 nm absorbance is used to monitor mAb breakthrough at the column outlet.

Dynamic binding capacity is typically reported at a breakthrough point where the column outlet product concentration equals 5–10% of the column inlet product concentration. After identifying this breakthrough point, DBC is calculated using this formula:

$$\text{DBC}\left(\frac{\text{mass of mAb captured}}{\text{volume of resin}}\right) = \frac{C_O \times (V_L - V_O)}{V_C}$$

where:
$C_O$ = concentration of mAb in the feedstock
$V_L$ = feed volume loaded up to the breakthrough point
$V_O$ = void volume of the system
$V_C$ = column volume

Longer residence times (i.e., slower flow rate) can increase Protein A resin DBC. Residence times on the order of three to six minutes are generally used for Protein A resins.

See tech note: "Determination of Dynamic Binding Capacity for ProSep®-vA Media."

## Sodium Hydroxide (NaOH) Tolerance of Protein A Resins

ProSep® Ultra Plus resin is not compatible with NaOH. The compatible pH range for ProSep® Ultra Plus resin cleaning is pH 1–8.5.

Eshmuno® A resin is compatible with 0.1–0.3 M NaOH. The compatible pH range for Eshmuno® A resin cleaning is pH 1.5–13.5.

## Procedure for Protein A Chromatography

Operating flow rates are selected to achieve a residence times between three to six minutes for Protein A capture chromatography.

| Step | | Buffer | Volume |
|---|---|---|---|
| 1 | Equilibrate | Phosphate or Tris buffered saline (PBS or TBS), pH 7.4 | 5 CV, or until pH & conductivity are stable |
| 2 | Load | Clarified and 0.22 µm filtered monoclonal antibody feed | To target binding capacity |
| 3 | Wash | Phosphate or Tris buffered saline (PBS or TBS), pH 7.4 | 5 CV, or until UV signal is stable |
| 4 | Elute | 0.1M acetate or citrate, pH 3.0 | 3-5 CV, or until product of interest is sufficiently eluted. |
| 5 | Regenerate | 150mM phosphoric acid | 5 CV |
| 6 | Clean-in-Place (CIP) | Eshmuno® A resin: 0.1 – 0.3 M NaOH | 5 CV |
| | | ProSep® Ultra Plus: Either 6-8 M Urea or 6M Guanidine HCl | |
| 7 | Re-Equilibrate | Phosphate or Tris buffered saline (PBS or TBS), pH 7.4 | 5 CV, or until pH and conductivity are stable |

# Column Storage

Store columns at 4° to 30 °C. Do not freeze. For long-term storage, do not store in cleaning buffer.

Recommended storage solutions:

| Resin | Recommended Storage Buffer |
|---|---|
| Fractogel® resins | 20% ethanol + 150 mM NaCl |
| Eshmuno® ion exchange | |
| Eshmuno® mixed mode | |
| ProSep® Ultra Plus | 0.1M sodium acetate, pH 5.2 with 1–2% benzyl alcohol |
| Eshmuno® A | 20% Ethanol + 150 mM NaCl |
| | 0.1M sodium acetate, pH 5.2 with 1–2% benzyl alcohol |

# Troubleshooting

| Problem | Cause | Solution | |
|---|---|---|---|
| Low purity | Incorrect pH and/or load conductivity | Ensure that pH and/or load conductivity are at optimum levels. | |
| | Post load wash buffer is not adequate. | Identify wash conditions to remove weakly bound impurities from the resin prior to product elution. This may require multiple wash steps. | |
| | Resin mass loading | Note that conditions which maximize binding capacity may differ from conditions which maximize purity. | |
| Low product yield | Product is not collected in the expected elution or load flowthrough pool. | Construct a mass balance of collected feed, load, wash, elution, and regeneration pools to understand where the yield loss is occuring. | |
| | | Product bind and elute mode | Incomplete elution. Change elution conditions or increase elution volume. |
| | | | Mass loading exceeds resin binding capacity. Reduce loading or change load conditions. |
| | | Product flowthrough mode | Post load wash volume is insufficient to recover product. Increase wash volume. |
| | | | Load pH and conductivity conditions result in product binding to resin. |
| Reduced column performance and/or increased column backpressure with cycling | Resin fouling or insufficient cleaning | Investigate alternative strip and/or sanitization buffers | |
| | | Fractogel® and Eshmuno® ion-exchange or mixed-mode resins | For alternative cleaning methods, for Fractogel® and Eshmuno®, see tech note: “Cleaning and Regeneration of Fractogel® EMD and Eshmuno Ion Exchange Media.” |
| | | Protein A resins | Increasing sanitization frequency or contact time can improve resin cleaning. Cleaning in the upward flow direction can also improve cleaning effectiveness. |
| | | Optimal clarification and/or filtration of chromatography feed material can help to reduce resin fouling and extend column lifetime. | |

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

For technical assistance and worldwide contact information, visit:
**www.sigmaaldrich.com.**

For additional information and documentation, contact:

Merck KGaA, Darmstadt, Germany
Corporation with General Partners
Frankfurter Str. 250
64293 Darmstadt, Germany
Phone: + 49 6151-72 0

For requests from USA and Canada, contact:

MilliporeSigma
A subsidiary of Merck KGaA, Darmstadt, Germany
400 Wheeler Rd
Burlington, MA 01803
Phone: 1-800-645-5476



Publication No. MS_UG8178EN Rev. 1 09/2021