Millipore®

User Guide

MiniChrom Pre-packed Columns with Eshmuno® and Fractogel® EMD Cation Exchange Resins

Contents

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

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Introduction

MiniChrom Pre-packed Columns with Eshmuno® and Fractogel® EMD Cation Exchange Resins are designed for the rapid evaluation of resins for bioprocess development. The results obtained for biomolecule separations are generally comparable to the results achieved using laboratory-scale columns (e.g. 100 x 16 mm ID).

In bioprocess design, the MiniChrom columns can be used to rapidly determine whether a target biomolecule is bound to and can be eluted from the Eshmuno® and Fractogel® EMD resins, as well as determine whether an appropriate target yield may be achieved and whether major contaminants could potentially be removed.

MiniChrom columns are made from biocompatible polypropylene (PP) and polyethylene (PE) and are available in 1 and 5 mL bed volumes in the following geometries:

MiniChrom Column Dimensions

Column Dimensions (mm)		Column Bed Volume (mL)
Internal Diameter	Bed Length	
8	20	1
8	100	5

The 8mm diameter columns allow for scale-up from 1 to 5mL column volumes, with a constant internal diameter.

These columns are compatible with any chromatographic work station, LC system or peristaltic pump

Column Characteristics

MiniChrom Column Characteristics

Components	Column – Polypropylene
	Bed supports – Polypropylene/Polyethylene
Connections	Standard LC (M10-32 UNF for 1/16 in. tubing)
Maximum Working Pressure	30 bar
Flow rate	Up to 10 mL/min
Chemical Stability of Column Hardware	pH 1-14, organic solvents (except halogenated), minerals and salts, common eluents and additives (urea, antioxidants, thiols, detergents, tensides)
Temperature ranges	4 to 30°C

Eshmuno® Resin

The Eshmuno® chromatography resin platform is designed to meet the demands of highly productive downstream purification processes. Eshmuno® resins are surface grafted rigid hydrophilic polyvinylether polymer beads which provide high capacity, high resolution, high throughput, easy column packing, long life and high mechanical stability.

The most important advantage of the surface modification and its tentacle chemistry (polymer chains containing the functional groups), is the large number of sterically accessible ligands for binding. All tentacles are covalently attached to the polyvinylether backbone and are chemically stable under conditions commonly applied during processing, regeneration and sanitization.

The separation of proteins is based on reversible electrostatic interactions between the negatively charged regions of the protein's surface and the resin.

The strength of the binding depends on:

- the buffer system and strength
- pH value of the buffer which determines the surface charge of the protein
- the counter ions conductivity
- protein binding capacity

The Eshmuno® CPX resin combines high aggregate removal efficiency with an outstanding high dynamic binding capacity.

The Eshmuno® CP-FT cation exchanger is specifically developed for the flow-through removal of mAb aggregates using frontal chromatography.

The Eshmuno® CPS resin is a salt tolerant cation exchange resin which combines high dynamic binding capacity and separation efficiency in downstream purification processes of recombinant protein feed streams at elevated salt concentrations.

The Eshmuno® CMX chromatography resin is a mixed mode chromatography resin and combines weak cation exchange properties with hydrophobic interaction, providing high selectivity for Monoclonal Antibody (mAb), fusion protein and Antibody Drug Conjugate (ADCs) purification as well as separation of low molecular weight impurities and Host Cell Proteins (HCPs).

The Eshmuno® HCX mixed mode cation exchanger has a broad salt tolerance and is excellent for capture and purification steps in high conductivity applications.

The Eshmuno® S strong cation exchanger exhibits high flow rates and high binding capacities in capture and post protein A steps during the purification of basic and neutral proteins.

The cleanability of Eshmuno® ion exchangers assures long column lifetime.

Resin Properties

Property	Eshmuno® Resin					
	CPX	CP-FT	CPS	CMX	HCX	S
Mean particle size (d50)	50 µm				85 µm	
Chromatography type	Strong cation exchange			Mixed Mode	Multimodal cation exchange	Strong cation exchange
Functional group	Sulfoisobutyl			Carboxy and alkyl	Sulfo, carboxy and phenyl	Sulfoisobutyl
Protein binding capacity	~ 120 mg lysozyme/mL	~ 70 mg lysozyme/mL	~ 160 mg lysozyme/mL	50 mg pIgG/mL packed resin	≥ 50 mg pIgG / mL settled resin 5 min residence time, 10% breakthrough	~ 165 mg lysozyme/mL of gel
Ionic capacity	52.5 - 77.5 µeq/ mL	28 - 46 µeq/mL	115 - 155 µeq/ mL	115 - 165 µeq/mL	170 - 300 µeq/mL	50 - 100 µeq/mL
pK value	<1	<1	<1	<1	5	<1
pH stability range	2 to 12 pH	2 to 14 pH	2 to 14 pH	2 to 12 pH	2 to 12 pH	2 to 12 pH
Elution conditions	Moderate salt concentrations/pH >7				High salt concentrations/pH >7	
Mechanical Stability	8 bar					
Operating Temperature	2 to 30 °C					
Storage, preservative	20% ethanol and 150 mM NaCl solution					
Regeneration	1 to 2 M NaCl	1 to 2 M NaCl	1 to 2 M NaCl	1M NaOH	6 M Gu HCl or 1 M arginine	1 to 2 M NaCl
Sanitization*	0.1 to 1.0 M NaOH					
Linear flow rate (bar net pressure)	Up to 500cm/h (< 3.0)	Up to 400cm/h (< 3.0)	Up to 1000 cm/h (< 2.5)	Up to 400cm/h (< 3.0)	Up to 1000 cm/h (< 2.5)	

Detailed specifications can be found on the Certificate of Analysis of the resin.

* Use sanitization agents and conditions that are suitable for the process requirements. Sanitization agents must comply with applicable local regulations.

Fractogel® EMD Resin

Fractogel® resins are synthetic methacrylate based polymeric beads with pores of approximately 800 Å and excellent pressure stability resulting in high flow rate capabilities. The surface is hydrophilic due to ether linkages in the polymer matrix. As with Eshmuno® resins, tentacles carry the functional groups, providing high capacity, high resolution, high throughput, easy column packing, long life and high mechanical stability.

Due to the tighter binding of the target molecule, the capture or initial step in a purification is often more efficient than with other resins. This results in higher recovery of valuable biomolecules.

The cleanability of Fractogel® ion exchangers assures long column life.

Resin Properties

Property	Fractogel® Media			
	SO3 (S)	SO3 (M)	SE Hicap	COO
Particle size	20-90 µm	40-90 µm		
Type of chromatography	Strong cation exchange			Weak cation exchange
Functional group	Sulfoisobutyl		Sulfoethyl	Carboxy ethyl
Protein binding capacity (static)	~130 mg lysozyme/ mL of gel		~120-160 mg lysozyme/ mL of gel	~100 mg lysozyme/ mL of gel
pK value	<1			~4.5
pH stability range	pH 1 up to pH 13		pH 2 up to pH 12	pH 1 up to pH 12
Elution conditions	High salt concentrations			
Pressure limit	8 bar			
Pressure drop	< 1.0 bar (5mL/min)			
Operating temperature	+2 to 30 °C			
Regeneration	1 – 2 M NaCl			
Sanitization*	0.1 – 0.5 M NaOH			
Linear flow rate	Up to 100 cm/h	Up to 200 cm/h		Up to 300 cm/h

Detailed specifications can be found on the resin Certificate of Analysis.

* Use sanitization agents and conditions that are suitable for the process requirements. Sanitization agents must comply with applicable local regulations.

Chromatography System Set Up

The systems should be used with a back-pressure regulator to prevent degassing in the detector flow cell, following the system manufacturer's instructions.

All buffers or feedstock solutions being applied to the column should be 0.22 µm filtered.

Include a 2 µm in-line filter in the chromatography system upstream of the column.

Prior to the first usage the preservation solution must be completely removed by washing the column with at least 5 column volumes (5 ml) of starting buffer.

Storage Conditions

For long term storage the kit should be stored between 4 and 30° C.

Avoid freezing.

Avoid long-term storage of columns in sanitization solutions. For storage after usage, rinse the columns with three column volumes of 20% ethanol in 150 mM NaCl (3 mL).

Using the MiniChrom® Columns

Ion Exchanger Selection

Ion exchange chromatography is based on the amphoteric behavior of proteins. At low acidic pH values, all amino acids are cations due to protonation of the NH2 groups and undissociated COOH groups resulting in a positive net charge. At alkaline pH-values above pH 12, the amino groups are uncharged, whereas the carboxyl groups will be ionized, resulting in a negatively charged molecule.

Biomolecules are bound to ion exchangers when they carry a net charge opposite to the surface charge of the ion exchanger. The pH value at which a biomolecule has no net charge is called the isoelectric point (pI). At buffer pH values above the pI, a given biomolecule will carry a negative net charge, and will bind to Anion exchangers.

If the target biomolecule is most stable below the pI value, a cation exchanger should be used. If stability of the target biomolecule is higher at buffer pH values above its pI, an anion exchanger should be used. When the biomolecule is stable

over a wide range of buffer pH values, either type of ion exchange resin can be used for purifications either alone or in consecutive combinations. It is important in ion exchange chromatography to choose optimal pH conditions so that the ion exchange groups are ionized. On the other hand, the buffer pH-value must be different enough from the isoelectric point of the target biomolecule in order to maintain sufficient net charge on the surface. Applying an increasing salt gradient or changing the pH of the buffers will reduce binding to the gel and elute proteins successively from the column when the charge differences have been neutralized.

Equilibration

Equilibrate the MiniChrom columns with at least 5 column volumes of starting buffer prior to application of the sample.

The starting condition for ion exchange chromatography is the highest salt concentration that permits binding of the target protein.

NOTE

A typical gradient run without sample application should be perfomed prior to the first use of any MiniChrom column to ensure removal of preservative compounds and achieve a stable base line.

Washing Step

An ionic strength higher than the loading step and less than the elution step will remove impurities that bind to the column.

Elution Condition

Lowest ionic strength which allows the elution of the protein of interest. In case of cation exchange chromatography, changing the buffer pH can also be used to elute the target biomolecule.

Regeneration

A higher concentration of salts in the buffer than the one used for elution is recommended for stripping the column after use.

Cleaning

Each column should be cleaned thoroughly after the separation step or at least after the experiments have been finished (especially if the back pressure increased during the separation).

Various methods are available for cleaning chromatographic media. Synthetic polymeric matrices are characterized by high chemical stability. In contrast to media based on carbohydrates, synthetic polymeric matrices can also withstand treatment with acids.

Lipids or lipoproteins can be removed with organic solvents like 20% ethanol or 20% isopropanol. Denatured proteins can be effectively removed with sodium hydroxide (0.1 N up to 1 N NaOH). In addition to bases and acids, organic solvents can be used. It is recommended that lower flow rates be used with organic solvents and that the back pressure be monitored carefully.

Use sanitization agents and conditions that are suitable for the process requirements. Sanitization agents must comply with applicable local regulations.

After cleaning and regeneration, MiniChrom columns must either be rinsed with three column volumes of 20 % ethanol in 150 mM NaCl (3 ml) for storage or re-equilibrated for the next chromatographic run.

Ordering Information

Resin	Catalog No.
Eshmuno® CPX	1.20083
Eshmuno® CP-FT	1.20093
Eshmuno® CPS	1.20084
Eshmuno® CMX	1.20650
Eshmuno® HCX	1.20087
Eshmuno® S	1.20078
Fractogel® EMD COO (M)	1.16886
Fractogel® EMD SE Hicap (M)	1.14894
Fractogel® EMD SO3 (M)	1.16882
Fractogel® EMD SO3 (S)	1.16890

Standard Product Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms (within the “Terms and Conditions of Sale” applicable to your purchase transaction).

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For technical assistance and worlwide contact information please visit:

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