ХРОМАТОГРАФИЧЕСКИЕ СРЕДЫ ESHMUNO CPS/CMX

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Eshmuno® CPS resin

High capacity and salt tolerance for recombinant protein purification

Eshmuno® CPS cation exchange chromatography (CEX) resin combines high dynamic binding capacity and separation efficiency in downstream purification processes of recombinant protein feed streams at elevated salt concentrations.

The demonstrated salt tolerance of Eshmuno® CPS resin enables direct loading of high conductivity feed streams (conductivity ≥10 mS/cm), reducing the need of dilution. Direct savings can be made on buffer, time and manufacturing footprint. Streamlined process steps, associated with efficient purification result in an overall improved productivity.

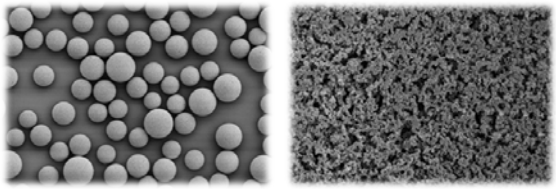
As a strong cation exchanger without hydrophobic groups, Eshmuno® CPS resin allows easy process development with straightforward binding and elution conditions and selection of process parameters.

Benefits

- Outstanding binding capacities at elevated conductivity levels (≥ 10 mS/cm)
- Strong cation exchanger chemistry without hydrophobic groups for easy process development
- Rigid base bead that enables higher flow rates and easier column packing
- Improved productivity through cost and time savings

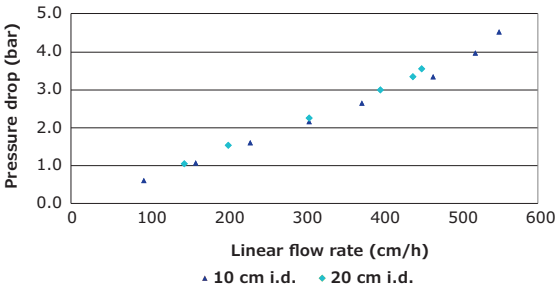
Proven technology

Eshmuno® CPS resin is a member of our high performance Eshmuno® platform, which is a family of chromatography resins designed to meet the demands of highly productive downstream processes. Eshmuno® base beads are composed of a hydrophilic polyvinyl ether polymer that enables high flow rates and therefore shorter processing times. Eshmuno® ion exchange resins also make use of our well-established tentacle ligand technology that provides a flexible multipoint interaction between the protein and resin resulting in higher binding capacities and superior selectivity.



SEM pictures of Eshmuno® CPS resin

Eshmuno® CPS resin can be easily packed into production-scale columns, either by simple flow packing or axial compression. The pressure-flow curves for 10cm and 20cm i.d. columns at 20cm bed height are shown below demonstrating linear scalability.



Flow packed in 0.15 M NaCl, 20 cm bed height, 14% compression, running buffer: 0.15 M NaCl

Process Development

Eshmuno® CPS resin is available in pre-packed, ready-to-use, disposable columns. MiniChrom columns can be used for lab-scale process development with any standard chromatography system, whereas RoboColumns® can be utilized for high-throughput process development in conjunction with a chromatography robot. These small scale columns are the ideal tool for performing initial resin screening, scaling and optimization studies.

	Eshmuno® CPS Chromatography Resin
Type of chromatography	Strong cation exchanger
Functional group	Sulfoisobutyl
Base material	Surface grafted rigid hydrophilic polyvinylether polymer
Mean particle size (d50)	50 µm
pK value	<1
pH stability	pH 2 to 14
Mechanical stability	8 bar
Linear flow rate	up to 300 cm/h (< 3.0 bar net pressure) 20 x 10 cm i.d. column, 12% - 14% compression equivalent to 1.14 to 1.16 compression factor, 150 mM NaCl as mobile phase
Storage conditions	20% EtOH + 150 mM NaCl solution, ambient temperature
Shipping solution	20% EtOH + 150 mM NaCl solution

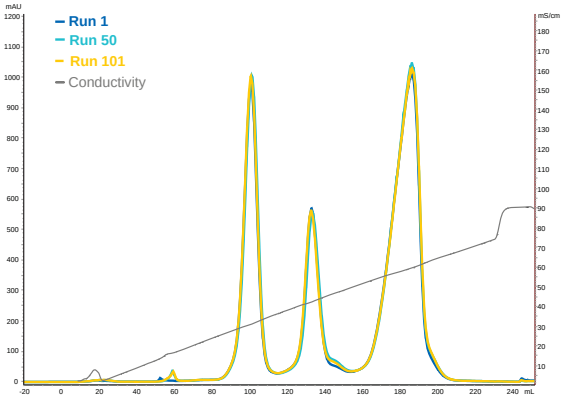
CIP Stability

Eshmuno® CPS resin can easily be cleaned or sanitized and is compatible over a wide range of pH conditions. It has excellent stability under both alkaline and acidic conditions. No significant differences in the separation of a three protein mixture were observed after 100 CIP cycles (60 minutes exposure to 1.0 M sodium hydroxide per cycle).

100 cycle study

Experimental conditions

- **Feed:** solution of Chymotrypsinogen A (14.3 mg/mL), Cytochrome C (7.2 mg/mL) and lysozyme (21.5 mg/mL) in buffer A (total protein concentration 43.0 mg/mL)
- **Load:** 5 mL sample volume corresponding to 215 mg total protein
- **Column:** 16 mm i.d. x 100 mm
- **Column volume:** 20.1 mL
- **Residence time:** 4 min
- **Buffer A:** 20 mM sodium phosphate, pH 6.0
- **Buffer B:** 20 mM sodium phosphate + 1 M NaCl, pH 6.0
- **Wash:** 1 CV Buffer A
- **Gradient elution:** 0 - 80% Buffer B in 10.4 CV
- **CIP:** 2 CV 1M NaOH at 20 cm/h (residence time 30 min)
- **Re-equilibration:** 1 CV buffer B + 5 CV buffer A



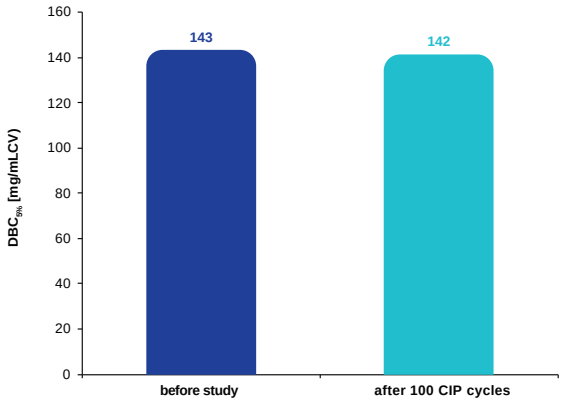
Separation of chymotrypsinogen A, cytochrome C and lysozyme on Eshmuno® CPS resin, overlaid chromatograms of run 1 (blue), run 50 (cyan), and run 101 (yellow)

Dynamic binding capacity measurements

Experimental conditions

- **Feed:** 5 mg/mL lysozyme in buffer A
- **Column:** 16 mm i.d. x 100 mm
- **Column volume:** 20.1 mL
- **Residence time:** 1 min
- **Buffer A:** 20 mM sodium phosphate, pH 6.0
- Determination of $DBC_{5\%}$

The resolution of protein separation remains unchanged over a multitude of runs. The dynamic protein binding capacity has not been altered after 100 chromatographic cycles.



Ordering information

Description	Catalog Number
Eshmuno® CPS, 10 ml	**1.20084.0010**
Eshmuno® CPS, 100 ml	**1.20084.0100**
Eshmuno® CPS, 500 ml	**1.20084.0500**
Eshmuno® CPS, 5L	**1.20084.5000**
MiniChrom prepacked column with Eshmuno® CPS resin, 1ml 8x20mm	**1.25164.0001**
MiniChrom prepacked column with Eshmuno® CPS resin, 5ml 8x100mm	**1.25165.0001**
RoboColumn® prepacked column with Eshmuno® CPS, 0.2ml 8PC 5x10mm	**1.25166.0001**
RoboColumn® prepacked column with Eshmuno® CPS, 0.6ml 8PC 5x30mm	**1.25167.0001**

Eshmuno® CMX Chromatography Resin

A highly selective mixed mode chromatography resin for difficult to purify mAbs, ADCs and fusion proteins

Eshmuno® CMX chromatography resin is a mixed mode chromatography resin built on the proven Eshmuno® resin technology. This innovative resin 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).

Eshmuno® CMX chromatography resin enables users to

- **Intensify processes and decrease costs** by reducing the number of chromatographic steps and buffer consumption
- **Improve performance** with higher recovery rates, high selectivity and superior dynamic binding capacity
- **Improve the user experience** with a broad operational window, simplified process development and easy column packing due to a rigid base bead

Proven Eshmuno® Technology

Eshmuno® CMX chromatography resin is a member of the Eshmuno® family of high-performance chromatography resins designed to meet the demands of highly productive downstream purification processes. Eshmuno® ion exchange resins carry an innovative surface tentacle structure which is able to bind target substances much more effectively. The resins combine this superior tentacle technology with the advantages of a rigid hydrophilic polyvinyl ether base matrix, enabling high flow rates and shorter processing times.

Application: Monoclonal Antibody and Fusion Protein Purification

Improvements in development processes for mAbs and fusion proteins have led to higher productivity and higher titers, increasing the number of high and low molecular weight impurities and the complexity of the purification process.

Traditional downstream processes for mAb and fusion proteins include capture, 2nd purification and final polishing steps. Studies using Eshmuno® CMX chromatography resin have shown that this process can be reduced from three to two chromatographic steps due to the high selectivity of the resin (Figure 1); this in turn reduces process time and costs while maintaining purity (Figure 2).

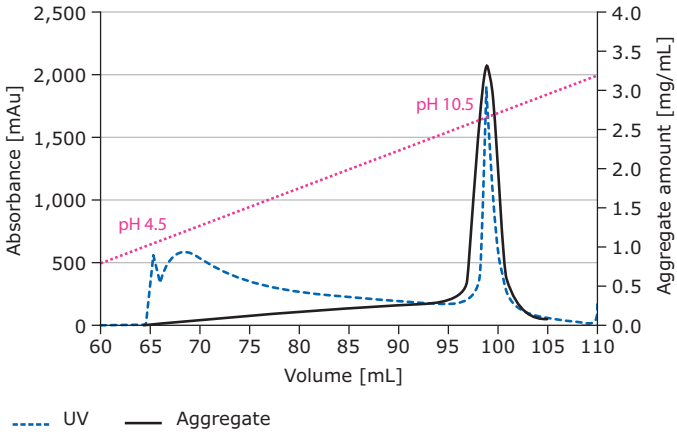


Figure 2 – Elution profile of an Fc fusion protein containing up to 30% aggregates on Eshmuno® CMX chromatography resin using linear pH gradient.

Compared to a traditional chromatography resin (Table 1), Eshmuno® CMX chromatography resin triples process yield while maintaining product purity.

Traditional downstream process

Capture		2nd Purification step		Polishing		Process	
Purity [%]	Yield [%]	Purity [%]	Yield [%]	Purity [%]	Yield [%]	**Purity [%]**	**Yield [%]**
81.06	96.39	92.54	36.18	97.37	36.56	**97.37**	**12.75**

Downstream process using Eshmuno® CMX chromatography resin

Capture		Polishing		Process	
Purity [%]	Yield [%]	Purity [%]	Yield [%]	**Purity [%]**	**Yield [%]**
82.94	100	97.15	41.26	**97.15**	**41.26**

Table 1 – Comparison of yield, fusion protein purification, traditional chromatography resin versus Eshmuno® CMX chromatography resin.

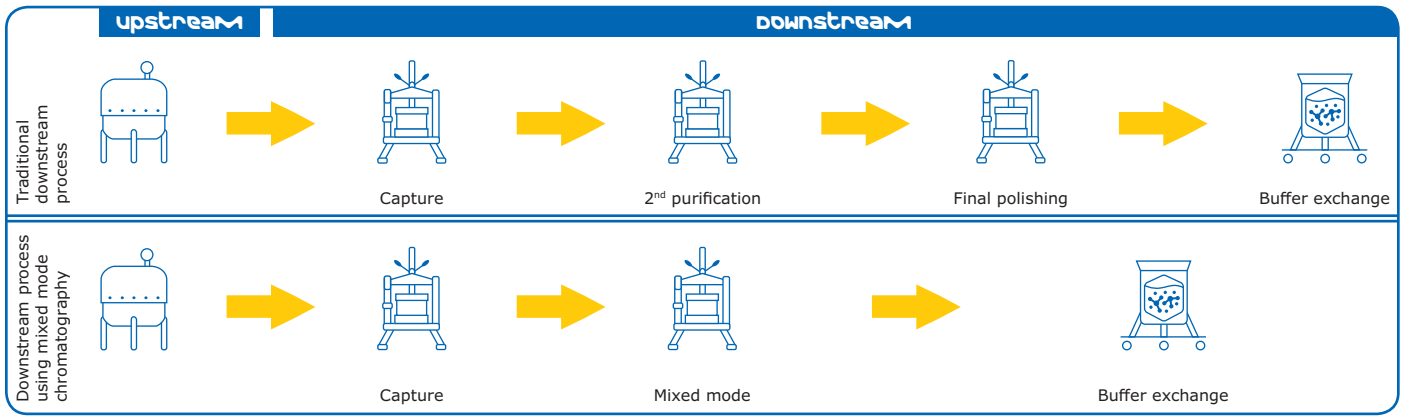


Figure 1 – Comparison of traditional downstream process and mixed mode chromatography process for fusion proteins.

Technical Information

Type of chromatography	Mixed Mode
Functional group	COO-(weak cation exchange) and alkyl-functional groups
Base material	Surface grafted rigid hydrophilic polyvinylether polymer
Mean particle size (d_{50})	50 µm
Dynamic protein binding capacity (4 min residence time, 5% BT)	60 mg pIgG/mL packed resin
Ionic capacity	120 µMol/mL, settled resin
pK value	<1
pH stability during operations	In working conditions (proteins/contaminants binding and elution): pH 2 to 12 In cleaning and sanitization: pH 0 to 14
Mechanical stability	8 bar
Linear flow rate	up to 300 cm/h 20 × 10 cm i.d. column, 12%–14% compression equivalent to 1.14 to 1.16 compression factor, 150 mM NaCl as mobile phase
Storage conditions	20% EtOH/150 mM NaCl solution, temperature between +2 °C to +30 °C
Shipping solution	20% EtOH/150 mM NaCl solution

Ordering information

Eshmuno® CMX resin, 10 mL	1.20650.0010
Eshmuno® CMX resin, 100 mL	1.20650.0100
Eshmuno® CMX resin, 500 mL	1.20650.0500
Eshmuno® CMX resin, 5 L	1.20650.5000
MiniChrom prepacked column with Eshmuno® CMX resin, 1 mL 8 × 20 mm	1.25185.0001
MiniChrom prepacked column with Eshmuno® CMX resin, 5 mL 8 × 100 mm	1.25186.0001
RoboColumn® prepacked column with Eshmuno® CMX resin, 0.2 mL 8PC 5 × 10 mm	1.25187.0001
RoboColumn® prepacked column with Eshmuno® CMX resin, 0.6 mL 8PC 5 × 30 mm	1.25188.0001

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