

# Eshmuno® CPX resin

Efficient impurity removal and strong binding capacity at high flow rates

Eshmuno® CPX resin is a strong cation exchanger built on the proven Eshmuno® resin technology. Eshmuno® CPX resin combines high aggregate removal efficiency in downstream purification and an outstanding dynamic binding capacity, utilizing the 50 µm Eshmuno® base bead technology and our proprietary tentacle technology.



Figure 1.

Resin tentacles forming a three-dimensional ion exchange network, enable easy access of the proteins to the ligands



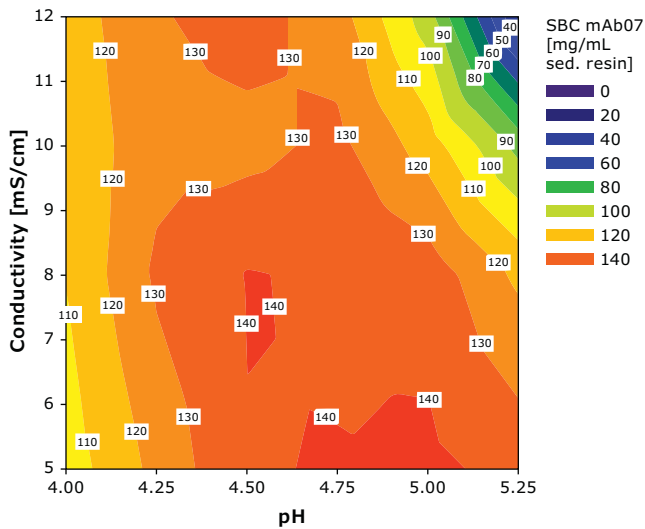
## Benefits

- High resolution resin for intermediate purification
- High binding capacity
- Effective mAb aggregate removal
- Efficient HCP removal
- Charge variant separation
- Viral clearance

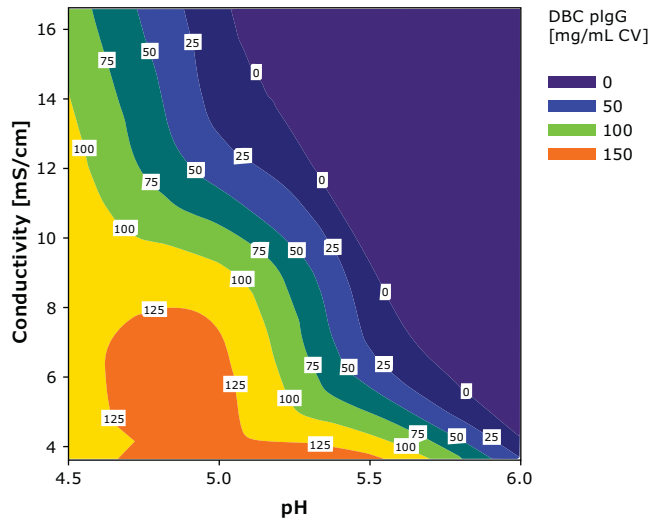
## Enhanced ease of use

- Ligand density optimized for bind-and-elute operation
- Wide operating range; compatible with pH elution for enhanced selectivity
- Caustic stable

Eshmuno® CPX resin allows binding of the target protein over a wide range of process conditions. (Figures 2 and 3)

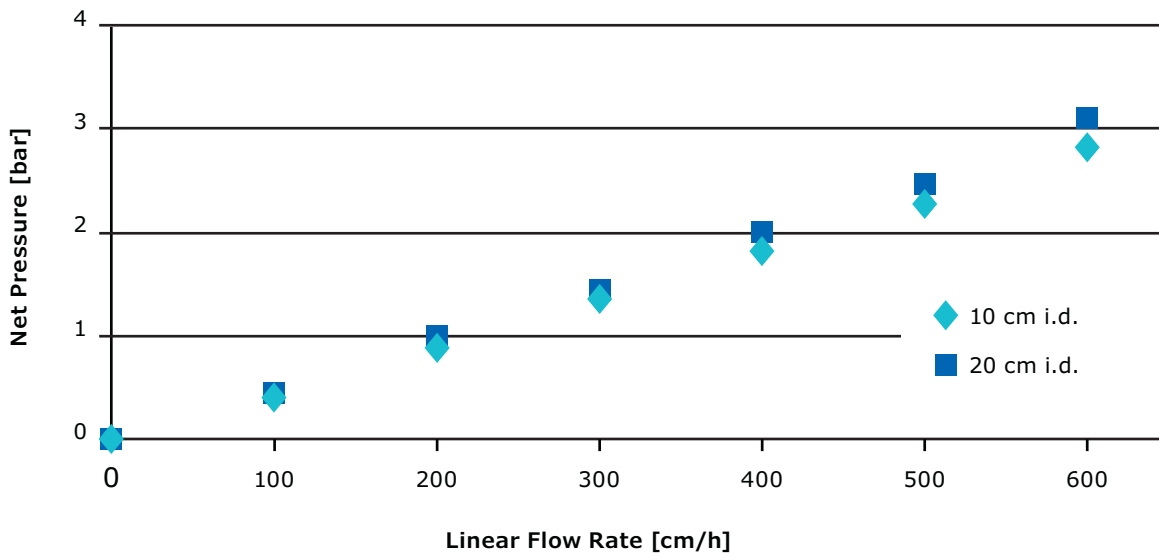


**Figure 2.** Flow packed in 0.15 M sodium chloride, 20 cm bed height, 15% compression, running buffer: 0.15 M sodium chloride. Static binding capacity of mAb07 across conductivity ranges of 5–12 mS/cm and a pH range of 4.00–5.25. The color scale represents the binding capacities, at various pH and conductivity ranges.



**Figure 3.** Dynamic binding capacity of plgG across a conductivity range of 5–12 mS/cm and a pH range of 4.00–5.25. The color scale represents the dynamic binding capacity at various pH and conductivity ranges.

The pressure–flow curves for 10 and 20 cm i.d. columns at 20 cm bed height are shown in **Figure 4**, demonstrating linear scalability.



**Figure 4.** Pressure versus flow curves for 10 and 20 cm i.d. columns at 20 cm bed height.

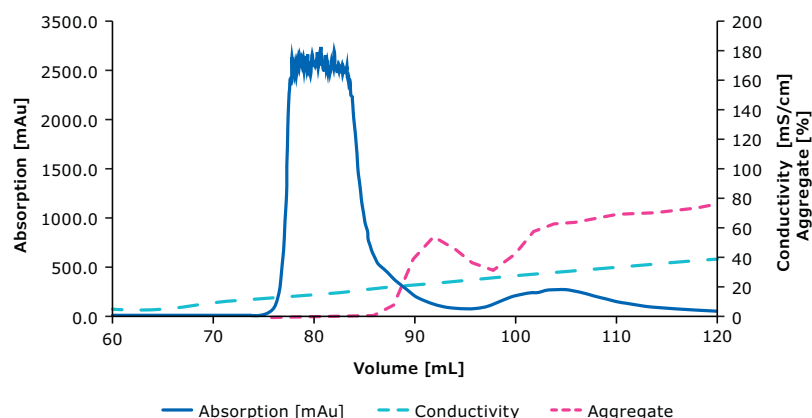
## Applications

Eshmuno® CPX resin is suitable for a variety of antibody feed streams as demonstrated in application studies that include:

- mAb monomer/aggregate separation
- charge variant separation
- virus removal
- HCP removal
- leached protein A clearance

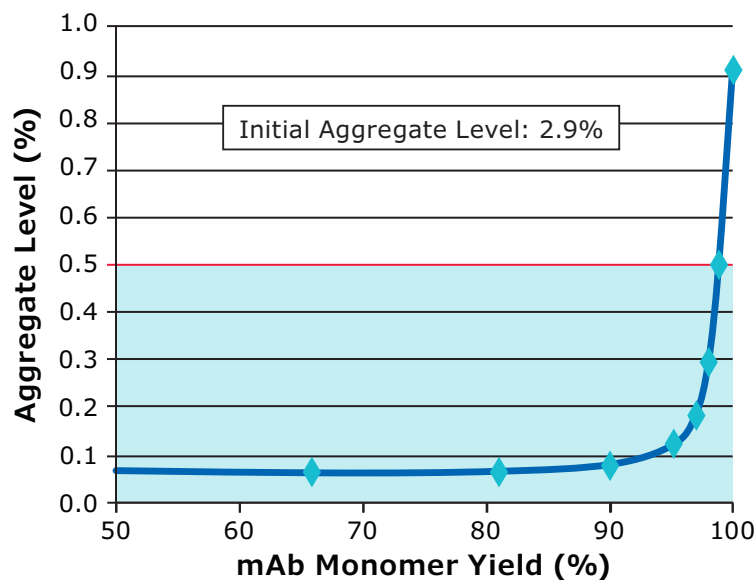
### mAb monomer/aggregate separation

Three different mAb post-protein A feedstreams have been tested in order to demonstrate excellent mAb monomer/aggregate separation using Eshmuno® CPX resin.



**Figure 5.** mAb aggregate % removal during linear salt gradient elution.

A linear salt gradient elution of mAb07, from 0% (0 M sodium chloride) to 70% (0.7 M sodium chloride) elution buffer, with a gradient volume of 120 mL (28 CV) was applied at a 0.82 mL/min flow rate in a 5 mm i.d. x 200 mm length (3.93 mL CV) Eshmuno® CPX resin column. Aggregate % is depicted. Aggregates levels were reduced from >8% to <0.4% through gradient elution.



**Figure 6.** Eshmuno® CPX -HCP, leached protein A, and aggregate clearance.

**Feed:** mAb D (post-ProtA pool), aggregate level: 2.9%;  
**Load:** 50 mg/mL CV  
**Column size:** 8 mm i.d. x 100 mm  
**Column volume:** 5.0 mL  
**Linear flow rate:** 250 cm/h  
**Buffer A:** 25 mM sodium acetate, 50 mM sodium chloride, pH 4.75, 8 mS/cm  
**Buffer B:** 25 mM sodium acetate, 1 M sodium chloride, pH 4.75  
**Gradient:** 0 to 50% B, 20 CV

**Table 1.** Impurity levels prior and post Eshmuno® CPX chromatography step.

	mAb D post-ProtA (Load)	Elution pool*
HCP level [ng/mg mAb]	350	5
protA level [ng/mg mAb]	3	<0.06

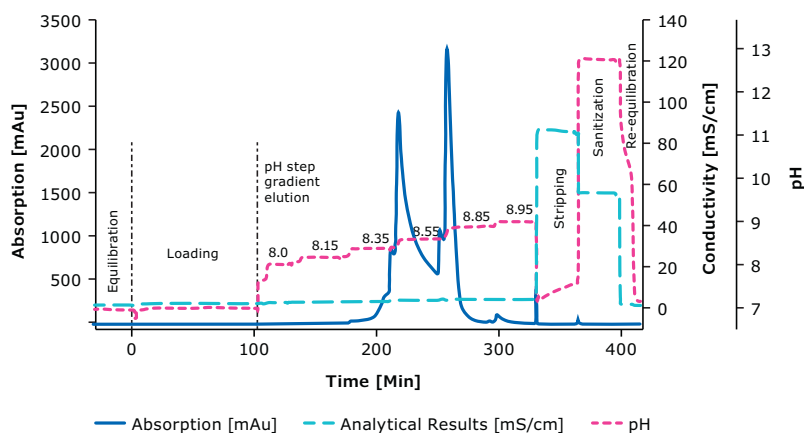
\*Pooling criterion: ≤ 0.5% aggregates

## Charge variant

Development of higher resolution chromatographic polishing operations enables targeted removal of product-related variants of monoclonal antibodies (mAbs) and debottlenecks other downstream purification steps. High resolution chromatography is useful for biosimilar mAbs where charge variant profile and glycoform profile would need to match the innovator molecule. The fractionation of an IgG-1 monoclonal antibody pool (mAb10) into sub-pools enriched with specific proteoforms is demonstrated below.

Charge variant separation with Eshmuno® CPX resin using a pH step gradient elution.

The number of mAb variants and peak resolution in our chromatographic method for Eshmuno® CPX is influenced by the number of elution steps, step length, and linear velocity through the column. Shorter elution step lengths lead to multiple peaks, while longer step lengths, as exemplified in **Figure 7**, result in fewer peaks. This demonstrates the tunability of our method, which has the potential to purify glycoproteins and selectively eliminate problematic product-related impurities and specific proteoforms. For additional information on charge variant separation with Eshmuno® CPX using a pH step gradient elution, please refer to Isu S, Vinskus L, Silva D, Cunningham K, Elich T, Greenhalgh P, Sokolnicki A, Raghunath B. Leveraging bioanalytical characterization of fractionated monoclonal antibody pools to identify aggregation-prone and less filterable proteoforms during virus filtration. *Biotechnol Prog.* 2024 Mar 7:e3451. doi: 10.1002/btpr.3451. Epub ahead of print. PMID: 38450976.



**Figure 7.** Bind-and-Elute mAb10 charge variant fractionation.

**Bed Volume:** 20 mL

**Bed Height:** 20 cm

**Feed conditions:** 25 mM tris acetate buffer at pH 7, conductivity 1.4 mS/cm

**Elution buffer B:** 50 mM tris acetate pH 9, 20 mM sodium chloride

**Linear velocity:** 316 cm/hr

## Virus removal

**Table 2.** Eshmuno® CPX resin demonstrated >4.0 logs clearance for retrovirus. Removal of parvovirus MVM was less effective with less than 1 log removal.

Type of virus	Total virus hold ( $\log_{10}TCID_{50}$ )	Total virus eluted ( $\log_{10}TCID_{50}$ )	LRV
XMuLV (retrovirus)	6.47	$\leq 2.41$	$\geq 4.1$
MVM (parvovirus)	7.28	6.91	0.4

### Operating Conditions

**Feed:** mAb A post protein A pool + spiked virus

**Load:** 50 mg/mL CV

**Column size:** 8 mm i.d. x 100 mm

**Column volume:** 5.0 mL

**Linear flow rate:** 120 cm/h

**Buffer A:** 50 mM sodium acetate, 40 mM sodium chloride, pH 4.75, 8 mS/cm

**Buffer B:** 50 mM sodium acetate, 1 M sodium chloride, pH 4.75

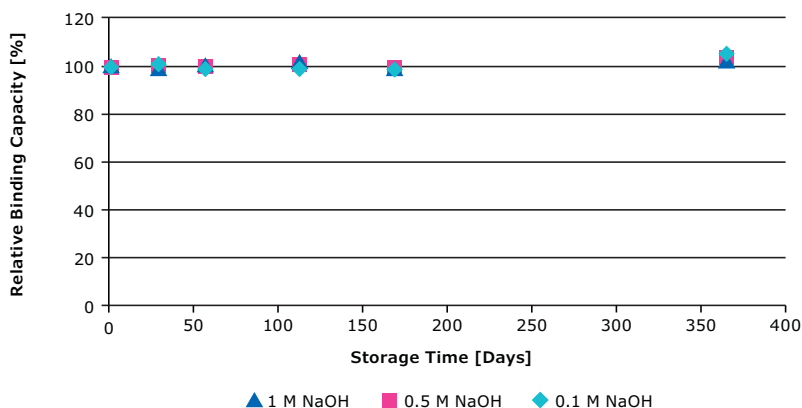
### Two-step salt elution:

**Step 1:** 73% A / 27% B

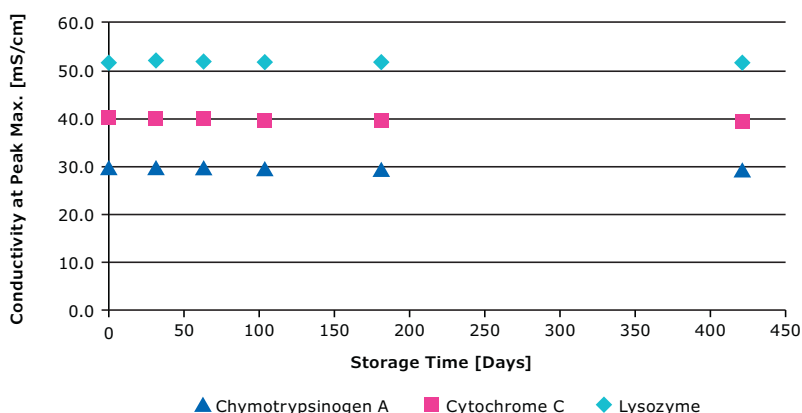
**Step 2:** 100% B

## Cleaning

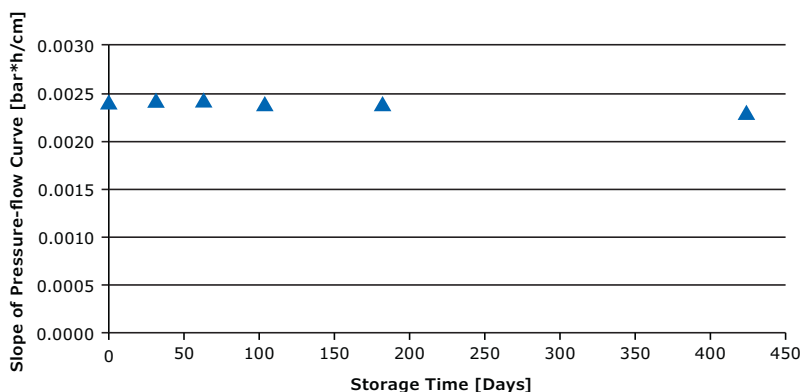
Eshmuno<sup>®</sup> CPX resin is compatible and has an excellent stability against caustic solutions.



**Figure 8:** Relative static lysozyme binding capacity after prolonged treatment with 1.0 M, 0.5 M or 0.1 M sodium hydroxide.



**Figure 9:** Elution conductivities at peak maxima for a standard separation on a packed column (100 mm x 16 mm i.d., 12% compression) stored in 1.0 M sodium hydroxide for certain times after re-equilibration.



**Figure 10:** Pressure versus flow curves were recorded in 100 x 16 mm i.d. columns packed to 12% compression (150–1500 cm/h). The slope of the net pressure drop versus flow curve after treatment with 1.0 M sodium hydroxide for certain times was determined by linear regression. The coefficient of determination ( $R^2$ ) was always greater than 0.995 indicating a strong correlation between the variables.

## Conclusion

Eshmuno<sup>®</sup> CPX resin does not lose protein binding capacity even after exposure to 1.0 M sodium hydroxide solution for one year at room temperature. Concurrently, the resolution of standard proteins (see below) remains nearly unchanged within the period tested. Pressure versus flow properties (see below) are hardly altered during long term storage in 1.0 M sodium hydroxide.

## Technical Information

Eshmuno® CPX resin	
Type of chromatography	Strong cation exchanger
Functional group	Sulfoisobutyl
Base material	Surface grafted rigid hydrophilic polyvinylether polymer
Mean particle size (d <sub>50</sub> )	50 µm
Protein binding capacity (lysozyme)	85–135 mg/mL
Ionic capacity	52.5–77.5 µeq/mL
pK value	<1
pH stability, operational*	2 to 12
pH stability, CIP**	0 to 14
Mechanical stability	8 bar
Linear flow rate	Up to 500 cm/h (< 3.0 bar net pressure) 10 cm i.d. x 20 cm length column, 12%–15% compression, 150 mM sodium chloride as mobile phase
Storage conditions	20% EtOH/150 mM sodium chloride solution, +2 °C to +30 °C
Shipping solution	20% EtOH/150 mM sodium chloride solution

\*pH range in working conditions (proteins/contaminants binding and elution)

\*\* pH range when the resin is subjected to cleaning or sanitization

### Eshmuno® CPX Resin Formats

Eshmuno® CPX resin is available as bulk resin or in prepacked, ready-to-use, small-scale columns for research and lab development scale. The MiniChrom® and RoboColumns® are the ideal tools for performing initial resin screening, scaling and optimization studies. The easy-to-use, economical small-scale columns can be used with any chromatography system.

### Eshmuno® CPX Resin is Supported by the Emprove® Program – The Smart Way to Master Compliance and Control.

Complementing our product portfolio, the Emprove® Program provides convenient access to reliable technical, regulatory and supply information in Emprove® Dossiers to support your risk assessment continuum. A subscription to our Emprove® Suite can help you stay current: In addition to accessing the Emprove® Dossiers, you can also receive notification updates to document changes, as well as generate metrics and reports.

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## Ordering Information

Description	Cat. No.
Eshmuno® CPX resin, 10 mL	1.20083.0010
Eshmuno® CPX resin, 100 mL	1.20083.0100
Eshmuno® CPX resin, 500 mL	1.20083.0500
Eshmuno® CPX resin, 5 L	1.20083.5000
MiniChrom prepacked column with Eshmuno® CPX resin, 1 mL 8 × 20 mm	1.25156.0001
MiniChrom prepacked column with Eshmuno® CPX resin, 5 mL 8 × 100 mm	1.25157.0001
RoboColumn® prepacked column with Eshmuno® CPX resin, 0.2 mL 8PC 5 × 10 mm	1.25158.0001
RoboColumn® prepacked column with Eshmuno® CPX resin, 0.6 mL 8PC 5 × 30 mm	1.25159.0001

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