Virus clearance using Eshmuno® HCX multi-mode cation exchange resin on minimally purified biopharmaceutical product feed

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Abstract

Eshmuno® HCX media is a new modified multi-mode cation exchanger (CEX) resin developed by Merck Millipore. The presence of ionic groups along a flexible tentacle type structure allows for a multipoint interaction between biopharmaceutical product and media resulting in higher binding capacities, while the hydrophilic polyvinyl ether base bead is designed to enable fast flow rates. This resin can be used in either bind/elute (capture) or flow-through (polishing) modes. The unique features of this resin allow it to capture monoclonal antibodies from high-salt, neutral pH solutions, such as bioreactor harvest material.

This study evaluated the virus clearance obtained for retrovirus and parvovirus using Eshmuno® HCX media on a clarified, pre-protein A capture, monoclonal antibody feed stream.

Figure 1. Chemical structure and features of Eshmuno® HCX media

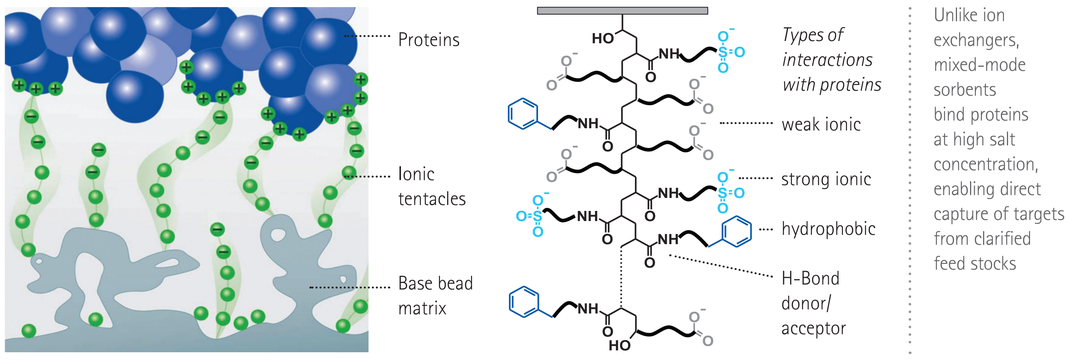
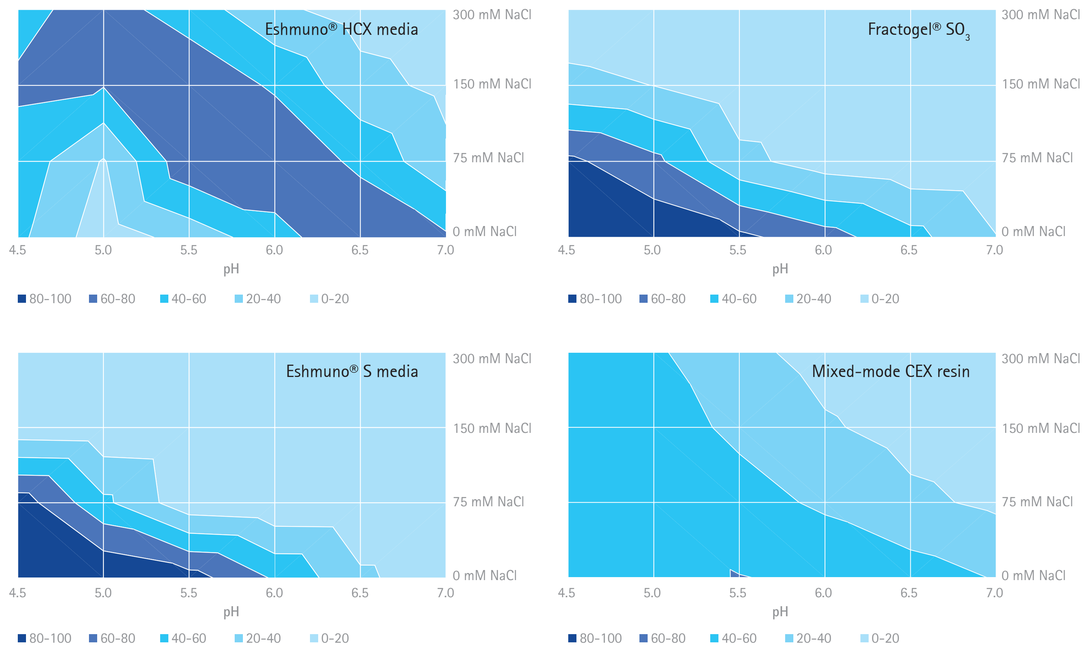


Table 1. Characteristic properties of Eshmuno® HCX media

Type of chromatography	Eshmuno® HCX media
Base material	cross-linked hydrophilic vinylether
Functional group	sulfo, carboxy, phenyl
Mean particle size (d_{50})	85 µm
Protein binding capacity (5 min residence time, 10 % breakthrough)	≥ 50 mg pIgG /ml settled resin
pK value	about 5
pH stability	pH 2 up to 12
Pressure limit	8 bar
Operating temperature	4 °C to room temperature
Storage	suspension in 20% ethanol / 150 mM NaCl
Elution conditions	high salt and/or pH > 7
Sanitization	0.1 - 0.5 M NaOH
Linear flow rate in 20 x 10 cm i.d. column	up to 1000 cm/h (< 2.5 bar net pressure)

Figure 2. Eshmuno® HCX media binding capacity: tolerance for salt and pH in comparison with other resins

These data demonstrate that Eshmuno® HCX media can bind mAb at higher salt levels and pH than other CEX resins examined. Static binding capacity of IgG in mg/ml settled resin was measured in 25 mM acetate/25 mM phosphate pH 4.5- 7.0 with 0 - 300 mM NaCl in micro-titer plates



Eshmuno® HCX media bind/elute purification of minimally purified monoclonal antibody (MAb)

Simulated MAb cell culture harvest feed material was produced by spiking serum free CHO cell harvest with purified MAb05 to a final concentration of 1.5 mg/mL.

Process feed characterization

Conductivity: 11.32 mS/cm pH 7.2 Total protein content: 2.2 mg/mL

MAb was eluted using step gradient elution method

(Step # 1: 60% 100mM NaCl; 40% 1M NaCl; Step # 2: 1M NaCl)

Figure 3. Step gradient elution profile for MAb05 purification on Eshmuno® HCX columns using bioreactor harvest feed material

Lane Blue: Absorbance 280nm

Lane Brown: Conductivity

Lane Green: pH

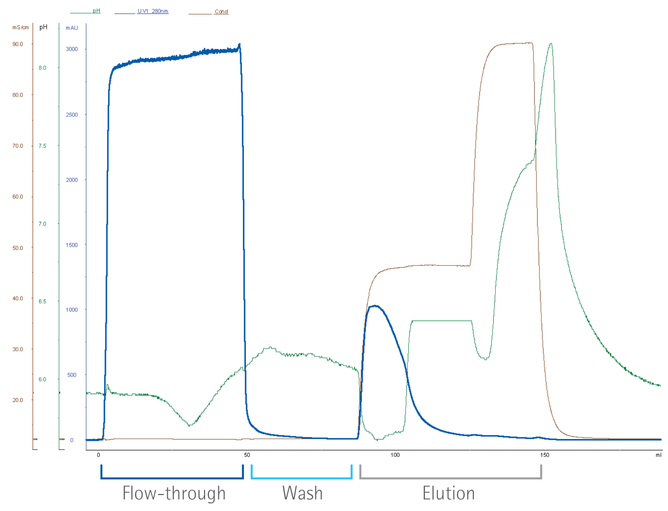
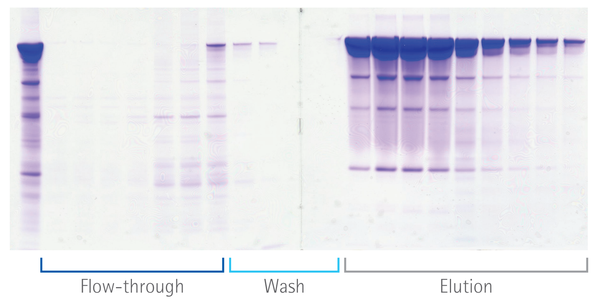


Figure 4. Analysis of selected fractions of flow-through, wash, and elution with SDS-PAGE (non-reducing conditions)

The typical MAb yield from Eshmuno® HCX media bind/elute purification is 70%.



Viral clearance by Eshmuno® HCX media in bioreactor harvest feed material

The MAb purification process described above was performed on identical feed spiked with either Xenotropic murine leukemia virus (X-MuLV) to $5x10^5$ $TCID_{50}$/mL or Minute virus of mice (MVM) to $2x10^6$ $TCID_{50}$/mL. The amount of virus present in each process fraction was quantified. The process was run on duplicate columns for each virus tested.

Table 2. Viral content of Eshmuno® HCX media MAb purification process fractions

XMuLV	Sample	Log ($TCID_{50}$/mL)	$TCID_{50}$/mL	Volume (mL)	Virus Load $TCID_{50}$	Total virus Log ($TCID_{50}$)	LRV
	Load	5.75	5.62E+05	38	2.14E+07	7.33	
	Hold	5.63	4.22E+05	38	1.60E+07	7.20	
Column #1	Wash	4.98	9.49E+04	37	3.51E+06	6.55	
	Fraction # 1	2.60	4.00E+02	35	1.40E+04	4.15	
	Fraction # 2	3.41	2.60E+03	15	3.90E+04	4.59	
	Total Virus Eluted			50	5.30E+04	4.72	**2.48**
	Regeneration	≤0.87	n/a				**ND**
	Re-equilibration	≤0.87	n/a				**ND**
Column #2	Wash	4.48	3.00E+04	37	1.11E+06	6.05	
	Fraction # 1	3.60	4.00E+03	35	1.40E+05	5.15	
	Fraction # 2	3.60	4.00E+03	5	2.00E+04	4.30	
	Total Virus Eluted			40	1.60E+05	5.20	**2.00**
	Regeneration	≤0.87	n/a				**ND**
	Re-equilibration	≤0.87	n/a				**ND**

MVM	Sample	Log ($TCID_{50}$/mL)	$TCID_{50}$/mL	Volume (mL)	Virus Load $TCID_{50}$	Total virus Log ($TCID_{50}$)	LRV
	Load	6.63	4.22E+06	38	1.60E+08	8.20	
	Hold	6.75	5.62E+06	38	2.14E+08	8.33	
Column #1	Wash	6.23	1.69E+06	37	6.24E+07	7.80	
	Fraction # 1	5.54	3.46E+05	35	1.21E+07	7.08	
	Fraction # 2	6.04	1.10E+06	15	1.64E+07	7.22	
	Total Virus eluted			45	2.86E+07	7.46	**0.87**
	Regeneration	≤0.87	n/a				**ND**
	Re-equilibration	≤0.87	n/a				**ND**
Column #2	Wash	6.04	1.10E+06	37	4.05E+07	7.61	
	Fraction # 1	5.41	2.60E+05	35	9.09E+06	6.96	
	Fraction # 2	6.23	1.69E+06	13.5	2.28E+07	7.36	
	Total Virus eluted			48.5	3.19E+07	7.50	**0.83**
	Regeneration	≤0.87	n/a				**ND**
	Re-equilibration	≤0.87	n/a				

Table 3. Viral clearance results for Eshmuno® HCX media in bioreactor harvest feed material

Virus	Virus in column load ($log_{10}TCID_{50}$)	Virus in eluate ($log_{10}TCID_{50}$)	LRV
X-MuLV	7.2 ± 0.0	5.0 ± 0.2	2.3 ± 0.1
MVM	8.3 ± 0.0	7.5 ± 0.0	0.9 ± 0.0

Conclusion

Eshmuno® HCX media is a cationic exchange resin that can bind monoclonal antibody under relatively high salt and pH conditions. The viral clearance of Eshmuno® HCX media was tested using a monoclonal antibody feed stream representing clarified bioreactor harvest material. Bind and elute purification of MAb using this resin provided virus reductions of 2.3 logs for retrovirus (X-MuLV) and 0.9 logs for parvovirus (MVM). These data demonstrate that CEX technologies with large operating windows for salt levels and pH can contribute to overall viral clearance even in a minimally purified, undiluted process stream.

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