

# Eshmuno® Q resin

# For efficient AEX chromatography

Eshmuno® Q resin is a strong anion exchange (AEX) resin, coupling our renowned tentacle structure with a hydrophilic polyvinyl ether base matrix. It offers outstanding results in typical anion exchange applications such as removing biomolecules' impurities in flow-through mode, or separating blood factors in plasma processing.

#### **Benefits**

- Superior productivity for downstream processing of biomolecules
- High flow rate versus pressure flow behavior
- Excellent removal of impurities
- Robust and safe packing procedures
- · Strong chemical stability

Table 1: Eshmuno® Q resin characteristics

	Eshmuno <sup>®</sup> Q Resin
Type of chromatography	Strong anion exchanger
Functional group	Trimethylammoniumethyl (TMAE)
Base material	Surface grafted rigid hydrophilic polyvinyl ether polymer
Mean particle size (d <sub>50</sub> )	85 µm
Dynamic protein binding capacity: 2 min. residence time, 10% breakthrough (BT)	≥ 80 mg BSA/mL packed resin
Ionic capacity	90-190 µmol/mL, settled resin
pK value	≥ 13
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 1000 cm/h (2.5 bar net pressure) 20 $\times$ 10 cm i.d. column, 8% compression, 150 mM NaCl as mobile phase
Storage conditions**	20% Ethanol/150 mM NaCl solution, at ambiant temperature
Shipping solution	20% Ethanol/150 mM NaCl solution

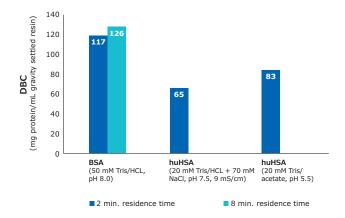
<sup>\*</sup> Recommended pH intervals where the resin can be operated at room temperature without significant change in function





<sup>\*\*</sup> Time interval between utilizations of the resin

Eshmuno® Q resin exhibits a superior binding capacity for various biomolecules. **Figure 1** shows the dynamic binding capacity (DBC) of Eshmuno® Q resin for selected macromolecules at different flow rates: High flow rates (2 min. residence time correspond to approximately 600 cm/h) do not significantly affect the high binding capacities obtained at lower flow rates.



**Figure 1.**Dynamic binding capacities (DBC) measured at 10% breakthrough

# **Applications**

# **Monoclonal Antibody (mAbs) Flow Through Polishing**

Two post protein A mAb feeds were tested for host cell protein (HCP) and leached protein A removal. Initial protein concentration and conductivity are listed in Table 2 below.

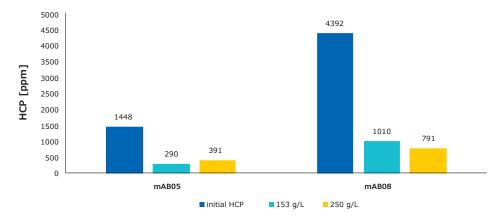
**Table 2: Feed Material Information** 

Feed Description (process stage)	Concentration (g/L)	Conductivity (mS/cm)
Post protein A pool mAb05	2.9	5
Post protein A pool mAb08	5.1	5

Device: 1 mL column (8 mm  $\times$  20 mm) prepacked with Eshmuno<sup>®</sup> Q resin Equilibration conditions: Buffer 25 mM Tris, pH 7.5 at 5 mS/cm

#### **HCP Removal**

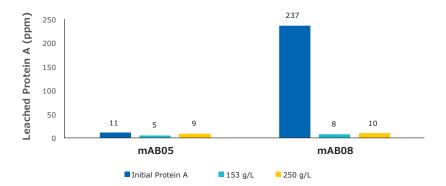
Figure 2 shows the amount of HCP remaining using Eshmuno $^{\$}$  Q resin at an intermediate loading point of 153 g/L and at the target loading of 250 g/L.



**Figure 2.** Eshmuno® Q resin HCP clearance at intermediate and target loading capacities

#### **Leached Protein A Removal**

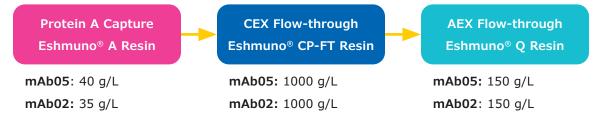
**Figure 3** shows the amount of leachable protein A remaining using Eshmuno<sup>®</sup> Q resin during the intermediate loading point of 150 g/L and at the target loading of 250 g/L.



**Figure 3.**Eshmuno® Q resin leached protein A clearance at intermediate and target loading capacities

Achieve HCP and aggregate removal at high loading in flow-through mode by combining Eshmuno<sup>®</sup> Q and Eshmuno<sup>®</sup> CP-FT resins

### Schematic of the flowthrough purification process for two mAb feed streams:



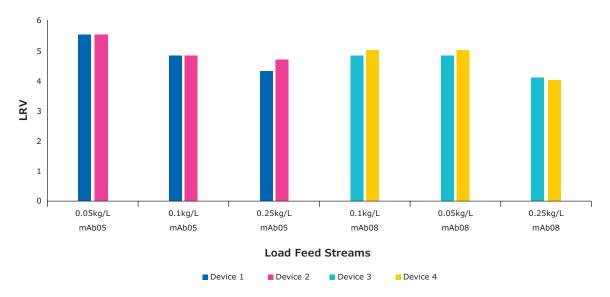
#### **Results:**

mAb Feed Stream	Chromatography Step	Resin	Loading (g/L)	Recovery	Dimer	Higher MW Aggregates	Total Aggregate	HCP (ppm)	mAb Concentration (g/L)
mAb 05	1. Capture	Eshmuno® A	40	88%	2.29%	0.77%	3.06%	47	15.1
HCP in supernatant =	2. CEX flow-through	Eshmuno® CP-FT	1000	92%	0.55%	0%	0.55%	17	13.6
47,819 ppm	3. AEX flow-through	Eshmuno® Q	150	>99%	0.61%	0%	0.61%	3	8.7
mAb 02	1. Capture	Eshmuno® A	35	97%	1.98%	0.45%	2.43%	302	15.4
HCP in supernatant = 128,657 ppm	2. CEX flow-through	Eshmuno® CP-FT	1000	91%	0.77%	0%	0.77%	181	13.7
	3. AEX flow-through	Eshmuno® Q	150	>99%	0.98%	0%	0.98%	9	8.5

Learn more about process intensification using integrated flow-through polishing for impurity and aggregate removal by downloading the poster "Flow-Through Removal of mAb Aggregates with Eshmuno® CP-FT Resin".

#### Virus removal

Challenge solution was prepared by spiking the feed with Minute Virus of Mice (MVM) to target titer 2.0E+06 TCID50/mL (0.05% (v/v)) and then filtered over 0.22  $\mu$ m Millex®-GP filter with Millipore Express® membrane. Samples were collected at various points during the run and assayed for titer.



**Figure 4.**MVM clearance for 2 mAb feeds measured in duplicate

Eshmuno $^{\circ}$  Q resin is able to provide consistent and stable reduction of impurities such as HCP, leached Protein A, and viruses in two different process feeds containing a broad range of impurity levels.

### **Immunoglobulin Purification**

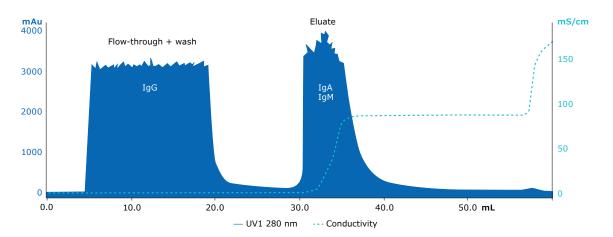
Eshmuno® Q resin achieves efficient purification of plasma-derived IgG.

### **Experimental conditions:**

- Column: Eshmuno® Q resin, 10 mm i.d. x 100 mm, 8% compression
- Buffer A1: 20 mM acetate, pH 6.0 (equilibration)
- Buffer B1: 20 mM acetate + 1 M NaCl, pH 6.0 (elution)
- Sample: Cohn Fr. II+III lyophilisate from human plasma, 30 mg/mL dissolved and dialyzed against buffer A1, pH 6.0, conductivity 1.8 mS/cm
- Load: 15 mL sample corresponding to 17 mg HuIgG /mL CV
- Wash: 1.3 CV buffer A1Elution: 3 CV buffer B1Flow rate: 150 cm/h
- Analytics were done using protein G-HPLC for IgG quantification-purity determination, and radial immunodiffusion (RID) for IgA/IgM quantitation.

Table 3: Immunoglobulin separation and recovery (IgG, IgA, IgM) with Eshmuno® Q resin

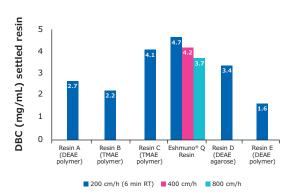
Fraction	IgG (mg)	IgA (mg)	IgM (mg)
Starting Material (Plasma containing IgG, IgA, IgM)	131 (100%)	20.3 (100%)	2.2 (100%)
Flow-Through + Wash	104 (79%)	0.4 (0.2%)	2.2 (5%)
Elute (1M NaCl)	23 (18%)	17.6 (87%)	2.2 (105%)
	IgG yield: 79% (Flow-through + wash) IgG Purity: increased from 77% to >98%	IgA yield (Eluate): 87%	IgM yield (Eluate): 105%



**Figure 5.**Eshmuno® Q resin is able to separate immunoglobulins from human plasma. A yield of 87% and 105% were achieved for IgA and IgM respectively in the eluate fraction while 79% of the IgG is recovered in the flow-through fraction.

### **Insulin purification**

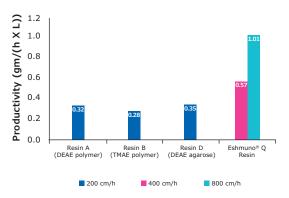
Eshmuno® Q resin delivers best capacities during capture of insulin compared to other commercially available anion exchange resins, even at much higher flow rates. This results in an improved overall productivity.



**Figure 6.**DBC measured at 10% breakthrough for different AEX resins

#### **Feed material information**

Crude feed of refolded, recombinant human insulin analog expressed in *E. coli*, approximately 0.4 mg/mL,  $\approx 10$  % pure, pH 8.4, 5.2 mS/cm, 1 mL scout column.

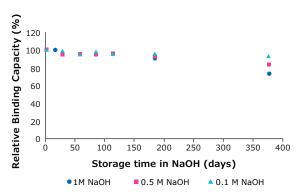


**Figure 7.**Productivity of different AEX resins

# **Chemical stability**

Unlike conventional anion exchange resins, Eshmuno® Q resin is intrinsically stable in alkaline solutions used in column sanitization. **Figure 8** shows the chemical stability and binding capacity of the resin after 6 months storage in 0.1, 0.5 and 1 M sodium hydroxide.

**Figure 9** shows superior stability of Eshmuno® Q resin compared to a competitive resin during storage in 1 M sodium hydroxide at 40 °C.



Storage time in 1 M NaOH (days) at 40°C

• Eshmuno® Q resin

• Resin E

Figure 8.

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Relative static BSA binding capacity after pronlonged treatment with 1.0 M, 0.5 M and 0.1 M sodium hydroxide

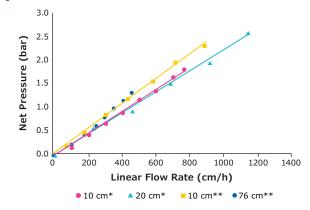
Figure 9.

Static BSA binding capacity in 50 mM Tris/HCL pH 8.3 was measured after storage of resins in 1.0 M sodium hydroxide at 40  $^{\circ}$ C

# Robust and safe packing procedures

Eshmuno® Q resin can easily be packed into production scale columns either by flow packing or axial compression using 150 mM sodium chloride as packing buffer. If corrosion of stainless steel hardware is a concern, 0.01M sodium hydroxide or purified water may be used as an alternative packing solution to achieve plate numbers >2400/m with good peak symmetry.

Eshmuno® Q resin can be operated at high flow rates (1000 cm/hr). The pressure-flow curves for different column diameters at 20 cm bed height are shown in **Figure 10**, demonstrating linear scalability.



\* flow packed in 0.15 M NaCl, 20 cm bed height, 8% compression \*\* flow packed in 0.01 M NaOH, 20.5 cm bed height, 10.5% compression

Figure 10. 85 μm base bead Eshmuno® Q resin pressure-flow curve

# Eshmuno® Q resin formats

Eshmuno® Q resin is available either as bulk resin or small-scale prepacked columns.

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



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# **Ordering information**

#### Eshmuno® Q Resins

Product Description	Catalogue No.
	Catalogue No.
Eshmuno® Q bulk resin	
10 mL	1.20079.0010
100 mL	1.20079.0100
500 mL	1.20079.0500
5000 mL	1.20079.5000
Eshmuno® Q resin in prepacked columns	
MiniChrom Column	
1 mL	1.25065.0001
5 mL	1.25074.0001
RoboColumn® Column	
0.2 mL	1.25133.0001
0.6 mL	1.25141.0001

#### **Buffer Preparation**

Product Description	Catalogue No.
Potassium dihydrogen phosphate cryst. Emprove® Expert, Ph Eur, BP, JPC, NF	137039
di-Potassium hydrogen phosphate anhydrous Emprove® Expert, Ph Eur, BP, USP	137010
Sodium Chloride Emprove® Expert	137017
Sodium Dihydeogen Phosphate Dehydrate Emprove® Expert	137018
Sodium hydroxide pellets suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, JP, NF, ACS	137020
Sodium Hydroxide Solution 1 mol/L Emprove® Expert	137031
Tris(hydroxymethyl)aminomethane (Trometamol) TRIS suitable for use as excipient EMPROVE® exp Ph Eur, BP, USP	108386
Tris(hydroxymethyl)aminomethane (Trometamol) (TRIS) high purity EMPROVE® Expert, Ph Eur, BP, ChP, JPC, USP, ACS	108307
TRIS((hydroxymethyl)aminomethane (Trometamol) (TRIS) hydrochrolide high purity EMPROVE® Expert	108219
Tris Hydrochloride Emprove® Evolve	108319
Tris Hydrochloride Emprove® Evolve	108315
MES	137074
MES	PHG0003
MES	RES0113M-A7
HEPES, Emprove® Expert	110110
HEPES	PHG0001
HEPES	RES6003H-B7

### Column Cleaning & Storage of Eshmuno® IEX Resins

Product Description	Catalogue No.
Ethanol 20% w/w Emprove® Expert	480910
Ethanol 20% v/v with 150 mMol/L NaCl Emprove® Expert	480940
Guanidine HCL (GuaHCL) Emprove® Expert	137037
Sodium Hydroxide Solution 0.1 mol/L Emprove® Expert	137058
Sodium Hydroxide Solution 0.5 mol/L Emprove® Expert	137060

### To place an order or receive technical assistance

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