

## Eshmuno® Q resin

### For efficient AEX chromatography

Eshmuno® Q resin is a strong anion exchange resin, coupling our renowned tentacle structure with a hydrophilic polyvinyl ether base matrix. Consequently, Eshmuno® Q resin 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**

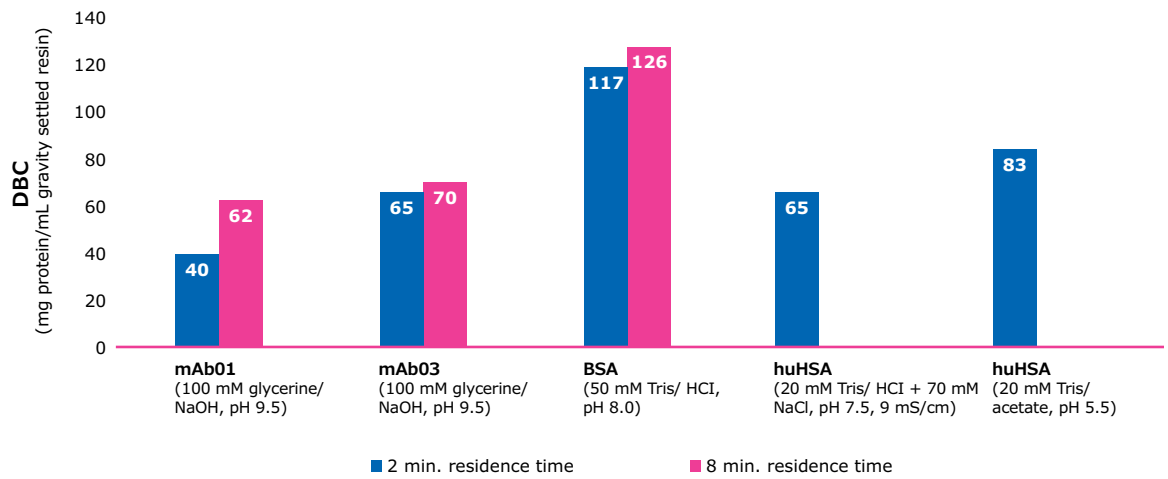
	<b>Eshmuno® Q Resin</b>
Type of chromatography	Strong anion exchanger
Functional group	Trimethylammoniummethyl (TMAE)
Base material	Surface grafted rigid hydrophilic polyvinyl ether polymer
Mean particle size (d <sub>50</sub> )	85 µm
Dynamic protein binding capacity: 2 min. residence time, 10% breakthrough (BT)	≥ 96 mg BSA/mL packed resin ≥ 48 mg IgG/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) 20x10 cm i.d. column, 8% compression, 150 mM NaCl as mobile phase
Storage conditions**	20% EtOH/150 mM NaCl solution, at ambient temperature
Shipping solution	20% EtOH/150 mM NaCl solution

\* Recommended pH intervals where the resin can be operated, at room temperature, without significant change in function

\*\* Time interval between utilizations of the resin

Eshmuno® Q resin exhibits a superior binding capacity for various biomolecules. Fig. 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) Impurities Removal

Table 2: Feed Material Information

Feed Description (process stage)	Concentration (g/L)	Conductivity (mS/cm)	Protein A (ppm)	HCP (ppm)
Post protein A pool mAb05	2.9	5	11	1448
Post protein A pool mAb08	5.1	5	237	4392

Device: 1 mL column (8 mm x 20 mm) prepacked with Eshmuno® Q resin  
Equilibration conditions: Buffer 25 mM tris, pH 7.5 at 5 mS/cm

### HCP removal

Table 3 shows the percentage of HCP removal using Eshmuno® Q resin at an intermediate loading point of 153 g/L and at the target loading of 250 g/L.

Table 3: HCP % removal

	mAb08 - 153 g/L	mAb08 - 250 g/L	mAb05 - 153 g/L	mAb05 - 250 g/L
Eshmuno® Q resin	77%	82%	80%	73%

### Leached Protein A removal

Table 4 shows the percentage of leached Protein A removal using Eshmuno® Q resin at an intermediate loading point of 153 g/L and at the target loading of 250 g/L.

Table 4: Leached Protein A % removal

	mAb08 - 153 g/L	mAb08 - 250 g/L	mAb05 - 153 g/L	mAb05 - 250 g/L
Eshmuno® Q resin	97%	96%	55%	20%

## Virus removal

Challenge solution was prepared by spiking the feed with Minute Virus of Mice (MVM) to target titer  $2.0E+06$  TCID<sub>50</sub>/mL (0.05% (v/v)) and then filtered over 0.22 µm GP Millipore Express® filter. Samples were collected at various points during the run and assayed for titer.

Table 5: Mean clearance of MVM for the two mAb feeds measured in duplicate

Eshmuno® Q sample description	Titer (Log <sub>10</sub> TCID <sub>50</sub> /mL)	LRV
mAb05: 5 mS/cm: Feed	5.88	n/a
mAb05: 5 mS/cm: Hold	6.38	n/a
Device 1: 0.05 kg/L	≤0.87	≥5.5
Device 1: 0.1 kg/L	1.54	4.8
Device 1: 0.25 kg/L	2.04	4.3
Device 2: 0.05 kg/L	≤0.87	≥5.5
Device 2: 0.1 kg/L	1.60	4.8
Device 2: 0.25 kg/L	1.66	4.7
mAb08: 5 mS/cm: Feed	6.06	n/a
mAb08: 5 mS/cm: Hold	6.13	n/a
Device 5: 0.05 kg/L	1.29	4.8
Device 5: 0.1 kg/L	1.29	4.8
Device 5: 0.25 kg/L	1.98	4.1
Device 6: 0.05 kg/L	1.16	5.0
Device 6: 0.1 kg/L	1.16	5.0
Device 6: 0.25 kg/L	2.10	4.0

Eshmuno® Q resin is able to provide consistent and stable reduction of impurities like HCP, leached Protein A and viruses in two different process feeds with a broad range of impurity levels.

## Immunoglobulin purification

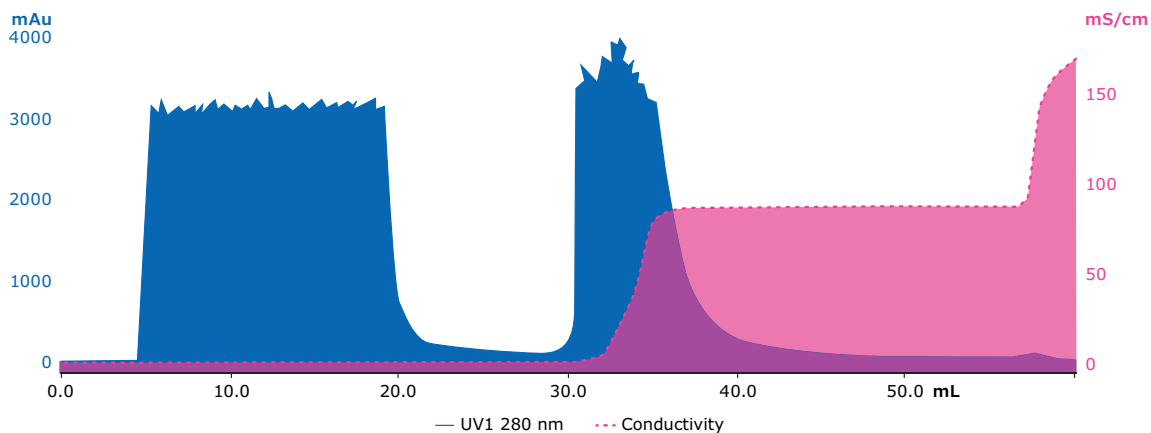
Eshmuno® Q resin's tentacle technology allows maximum binding of various immunoglobulins.

### 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 A1
- elution: 3 CV buffer B1
- flow 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 6: Immunoglobulin recovery (IgG, IgA, IgM) with Eshmuno® Q resin**

Fraction	Volume (mL)	IgG conc. (mg/mL)	IgG (mg)	IgA conc. (mg/mL)	IgA (mg)	IgM conc. (mg/mL)	IgM (mg)
Starting material	15.0	8.7	131 (100%)	1.356	20.3 (100%)	0.150	2.2 (100%)
Flow-through+wash	25.3	4.1	104 (79%)	0.015	0.4 (2%)	0.004	0.1 (5%)
Elute (1 M NaCl)	23.6	1.0	23 (18%)	0.749	17.6 (87%)	0.098	2.3 (105%)
	IgG recovery: 98% IgG yield: 79% (flow-through+wash) IgG purity: > 98% 77% (starting material)			IgA recovery: 89% IgA yield (eluate): 87%		IgM recovery: 107% IgM yield (eluate): 105%	



**Figure 2.**

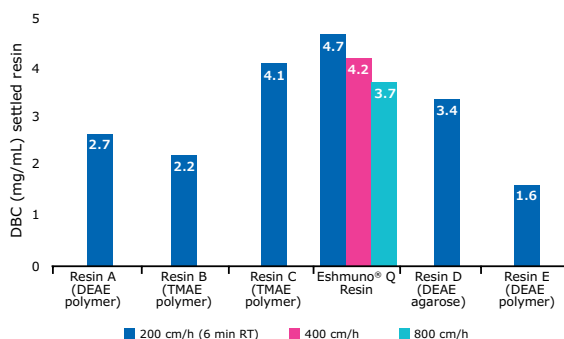
Yield and binding of IgA/IgM (retained impurities) with Eshmuno® Q resin. IgA/IgM binding capacity to the resin is above 87% and IgG yield is about 79%. To further increase IgG yield, operating conditions such as pH and conductivity can be optimized

### 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.

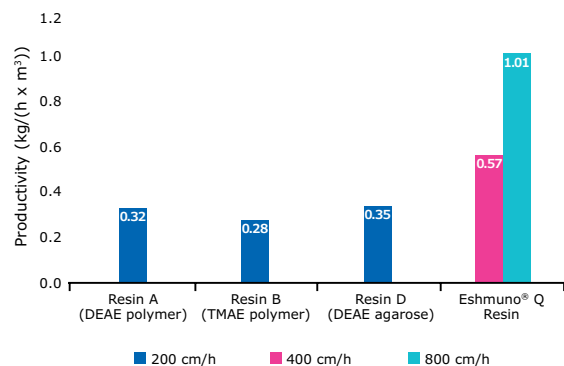
### Feed material information

Crude feed of refolded, recombinant human insulin analog expressed in *E. coli*, approximately 0.4 mg/mL, ≈10 % pure, pH 8.4, 5.2 mS/cm (from Bioton S.A., R&D Centre, Poland), 1 mL scout column.



**Figure 3.**

DBC (at 10% breakthrough) for different AEX resins

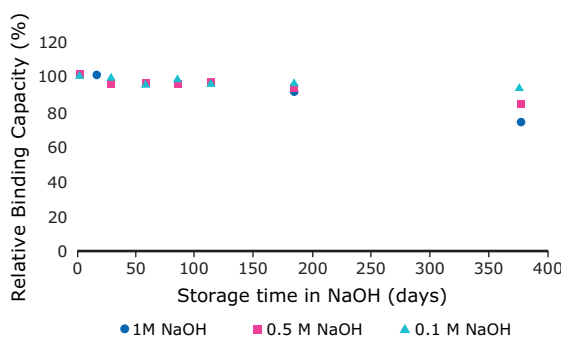


**Figure 4.**

Productivity of different AEX resins

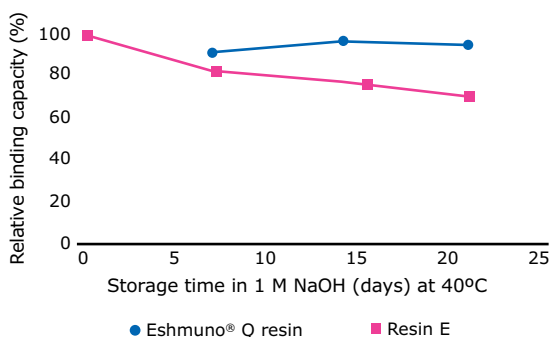
## Chemical stability

Unlike conventional anion exchange resins, Eshmuno® Q resin is intrinsically stable against alkaline solutions used in column sanitization. Fig. 5 shows the chemical stability and unaffected binding capacity of the resin after 6 months storage in 0.1, 0.5 and 1 M Sodium Hydroxide.



**Figure 5.** Relative static BSA binding capacity after prolonged treatment with 1.0 M, 0.5 M and 0.1 M sodium hydroxide

In addition, Fig. 6 shows superior stability of Eshmuno® Q resin compared to a competitive resin during storage in 1 M Sodium Hydroxide at 40°C.

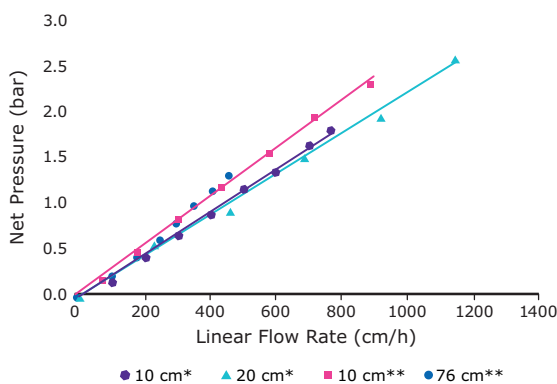


**Figure 6.** Static BSA binding capacity in 50 mM Tris/HCl pH 8.3 was measured after storage of resins in 1.0 M sodium hydroxide at 40 °C

## Robust and safe packing procedures

Eshmuno® Q resin can be easily packed into production scale columns either by simple flow packing or axial compression using 150 mM sodium chloride as packing buffer. To prevent corrosion of the tubing system, Eshmuno® Q resin can also be packed using 0.01 M sodium hydroxide solutions and even pure water resulting in plate numbers >2400/m with good peak symmetry.

The pressure-flow curves for different column diameters at 20 cm bed height are shown in figure 7, demonstrating linear scalability. Eshmuno® Q resin can be operated at very high flow rates (1000 cm/h) respecting conventional pressure drop levels, associated to standard chromatography columns and systems.



\* 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 7.** 85 µm base bead Eshmuno® resin pressure-flow curve

## Eshmuno® Q resin available in diverse prepacked column formats

Eshmuno® Q resin is available either as bulk media or in prepacked columns to conveniently meet your needs.



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. The easy-to-use, economical small scale columns can be used with any chromatography system.

For MiniChrom columns, please visit:  
[www.merckmillipore.com/MiniChromColumns](http://www.merckmillipore.com/MiniChromColumns)

For RoboColumns®, please visit:  
[www.merckmillipore.com/RoboColumns](http://www.merckmillipore.com/RoboColumns)

Chromabolt® is a family of prepacked chromatography columns containing our comprehensive chromatography resin portfolio, including Eshmuno® Q resin. Chromabolt® columns have been optimized for early clinical stage manufacturing and are available in 3 sizes – 10, 20 and 32 cm inner diameter all with 20 cm bed heights. These prepacked columns have been intelligently designed for ease of use, ergonomics and transportation and will free up your valuable time and resources by eliminating manual packing and cleaning.

Learn more about Chromabolt® columns at:  
[www.merckmillipore.com/Chromabolt](http://www.merckmillipore.com/Chromabolt)

## Chromatography columns and systems

Chromatography columns and systems are critical factors to the successful separation of your valuable molecule. Merck provides columns and systems from lab scale to pilot and process scale. From screening to large-scale production, our columns, systems and single-use solutions are designed to provide robust, consistent performance while providing you with the processing flexibility required in today's changing production environment.

## Ordering information

### Eshmuno® Q Resins

Product Description	Catalogue No.
Eshmuno® Q resin	1.20079
Eshmuno® Q bulk media	
10 mL	1.20079.0010
100 mL	1.20079.0100
500 mL	1.20079.0500
5000 mL	1.20079.5000
<b>Eshmuno® Q resin in prepacked columns</b>	
MiniChrom Column	
1 mL	1.25065.0001
5 mL	1.25074.0001
RoboColumn®	
0.2 mL	1.25133.0001
0.6 mL	1.25141.0001
Chromabolt® Columns	
10 cm	CHRPC10791
20 cm	CHRPC10792
32 cm	CHRPC10793

### Buffer preparation

Product Description	Catalogue No.
Potassium dihydrogen phosphate suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, NF	137039
di-Potassium hydrogen phosphate anhydrous suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, USP	137010
Sodium chloride suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, JP, USP	137017
Sodium dihydrogen phosphate dehydrate suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, USP, JPE	137018
Sodium hydroxide pellets suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, JP, NF, ACS	137020
Sodium hydroxide solution 1 mol/L suitable for biopharmaceutical production EMPROVE® bio	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 suitable for biopharmaceutical production EMPROVE® bio Ph Eur, BP, JPC, USP, ACS	108307
Tris(hydroxymethyl)aminomethane hydrochloride TRIS-HCl suitable for biopharmaceutical production EMPROVE® bio	108219
2-Morpholinoethanesulfonic acid monohydrate (MES) suitable for biopharmaceutical production	137074
2-Morpholinoethanesulfonic acid sodium salt (MES Na) suitable for the biopharmaceutical production	137073
2-[4-(2-Hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid Buffer substance HEPES	110110
2-[4-(2-Hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid sodium salt (HEPES Na)	137075
MOPS suitable for the biopharmaceutical production	137078
MOPS Na salt suitable for the biopharmaceutical production	137079

### Column cleaning & storage of Eshmuno® IEX Resins

Product Description	Catalogue No.
Ethanol 20% for cleaning of biochromatography resins	480910
Ethanol 20% v/v with 150 mMol/L sodium chloride solution for storage of chromatography resins	480940
Guanidinium hydrochloride suitable for biopharmaceutical production EMPROVE® bio Ph Eur	137037
Sodium hydroxide solution 0,1 mol/L suitable for biopharmaceutical production EMPROVE® bio	137058
Sodium hydroxide solution 0,5 mol/L suitable for biopharmaceutical production EMPROVE® bio	137060

## To place an order or to receive technical assistance

In Europe, please call Customer Service:

**France:** 0825 045 645

**Germany:** 069 86798021

**Italy:** 848 845 645

**Spain:** 901 516 645 Option 1

**Switzerland:** 0848 645 645

**United Kingdom:** 0870 900 4645

For other countries across Europe,  
please call: +44 (0) 115 943 0840

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