The Eshmuno® Chromatography Family of Resins

The perfect accessory for highly productive downstream purification
Introduction

For over 300 years, Merck KGaA has been the leading specialist in high-quality products and services for the chemical and pharmaceutical industries. EMD Millipore, the life sciences division of Merck KGaA, continues this tradition with a long-term commitment to Research and Development investment has yielded game-changing innovations that have been proven time and time again. From discovery through manufacturing, EMD Millipore provides a complete suite of innovative solutions and support services designed to give you a performance edge at every step in the biopharmaceutical manufacturing process.

Chromatography Resins from EMD Millipore

When it comes to chromatography, we understand the challenges of efficiently capturing your final product, while minimizing costs and speeding time to clinic. Product titers are steadily increasing, placing even greater demands on downstream processing.

To help you succeed, we have focused on creating innovative, reliable chromatography solutions that meet regulatory and market demands. We were the first to manufacture silica products for chromatography, only one year after the discovery of the chromatographic principle in 1903. This legacy of commitment to substantial R&D investment is evident in the Eshmuno® family of chromatography resins, a series of chromatography resins designed for highly efficient downstream processing.

Eshmuno® Resin – The Resin for Efficient Downstream Processing

Eshmuno® chromatography resin is a unique family of ion-exchange resins specifically designed for highly productive downstream purification of monoclonal antibodies (mAb). In addition to its high binding capacity and excellent pressure-flow behavior, Eshmuno® resin features high selectivity and flexibility for your specific purification challenge, making it the most efficient resin available for host cell protein (HCP) removal. Using Eshmuno® resin, you can achieve an outstanding productivity in your downstream processing, resulting in substantial savings in development time and manufacturing costs.

Why Eshmuno® Resin?

- Superior productivity for downstream processing
- More selectivity and HCP removal
- Active tentacle adsorption
- Robust and safe packing procedures
- Tangible cost and time savings
How Does Eshmuno® Resin Work?

At the heart of Eshmuno® resin is an innovative, proprietary tentacle structure that is able to bind target substances much more effectively than previous technologies. Eshmuno® resin combines this superior tentacle technology with the advantages of a new hydrophilic polyvinyl ether base matrix. This combination allows the use of much higher flow rates while the biomolecule is still strongly bound by the tentacle.

Which Eshmuno® Resin is Right for Your Process?

Eshmuno® S Resin
- Cation exchange resin
- Designed for fast, efficient purification of antibodies in direct capture and post-protein A steps
- Superior binding capacity for antibodies compared to other modern cation-exchangers

Eshmuno® Q Resin
- Anion-exchange resin
- Outstanding results in typical anion-exchange applications, such as removing impurities in flow-through mode, or separating blood factors in plasma processing
- Increased stability during storage compared to traditional anion-exchange resins

Eshmuno® HCX Resin
- Multi-mode chromatography resin
- Specifically designed for direct capture of proteins at high salt concentration
- Excellent performance results at higher salt concentrations in typical ion exchange and flow-through applications
Eshmuno® S resin was the first member of the Eshmuno® family of ion-exchange resins. It is a strong cation exchanger and highly productive in direct capture and post-proteins A steps. Like all Eshmuno® resin products, Eshmuno® S resin combines a tentacle structure for more effective binding of the target substances with the advantages of a hydrophilic polyvinyl ether base matrix. It exhibits a superior binding capacity for antibodies compared to other modern cation-exchangers. Moreover, Eshmuno® S resin allows the use of much higher flow rates while the biomolecule is still strongly bound by the tentacle.

More Selectivity and HCP Removal

One advantage of using Eshmuno® S resin is its high selectivity for the biomolecule of interest. Eshmuno® S resin efficiently removes host cell proteins, resulting in greater selectivity compared to traditional chromatography resins. And thanks to the excellent pressure flow behavior of Eshmuno® S resin, you can achieve an outstanding productivity of more than 40 mg/mL x h in downstream processing, saving considerable manufacturing costs in your mAB production.

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**Eshmuno® S resin characteristics**

<table>
<thead>
<tr>
<th>Type</th>
<th>Strong cation exchanger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional group</td>
<td>-SO₃</td>
</tr>
<tr>
<td>Base matrix</td>
<td>Surface grafted rigid polyvinyl ether hydrophilic polymer</td>
</tr>
<tr>
<td>Lysozyme capacity</td>
<td>115–165 mg/mL settled resin</td>
</tr>
<tr>
<td>Ionic capacity</td>
<td>50–100 µg/mL settle resin</td>
</tr>
<tr>
<td>Mean particle xize</td>
<td>75–95 µm</td>
</tr>
<tr>
<td>IgG dynamic capacity</td>
<td>&gt; 60 mg/mL (2 min residence time)</td>
</tr>
<tr>
<td>Pressure drop</td>
<td>&lt; 1.0 bar</td>
</tr>
</tbody>
</table>

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**Figure 1.**
Superior mAB binding capacity in direct capture step

a) Dynamic binding capacity (DBC) of Eshmuno® S resin for direct capture of monoclonal antibody mAB2 at 5% breakthrough and 5 minutes residence time from a real diluted feedstock

b) Excellent pressure flow behavior can be achieved with Eshmuno® S resin: 20 cm i.d. column; 19.5 cm bed height; 8% compression recorded in 150 mM NaCl
Eshmuno® Q resin couples the tentacle structure of Eshmuno® resin with a new hydrophilic polyvinyl ether base matrix. Eshmuno® Q resin offers outstanding results in typical anion-exchange applications, such as removing impurities in flow-through mode, or separating blood factors in plasma processing.

**Enhanced Stability**

Unlike conventional anion-exchange resins, Eshmuno® Q resin is intrinsically stable against alkaline solutions used in column sanitization. The result: Eshmuno® Q resin retains its high binding capacity, whereas other anion-exchange resins suffer considerable reductions.

**Tangible Savings in Cost and Development Time**

Eshmuno® Q resin shows remarkably sharp elution of the target molecule in a wide range of applications compared to other anion-exchangers. Higher concentrations of target molecules with significantly reduced elution volumes render further concentration steps unnecessary. This way, you save money on buffer solutions and consumables, as well as valuable time.

### Eshmuno® Q resin characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td>Strong anion-exchanger</td>
</tr>
<tr>
<td>Functional group</td>
<td>Trimethylammoniumethyl (TMAE)</td>
</tr>
<tr>
<td>Base matrix</td>
<td>Surface grafted rigid polyvinyl ether hydrophilic polymer</td>
</tr>
<tr>
<td>Mean particle size (d₅₀)</td>
<td>85 µm</td>
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<tr>
<td>Protein binding capacity (2 min residence time, 10% breakthrough)</td>
<td>≥ 80 mg BSA/mL settled resin ≤ 40 mg lgG/mL settled resin</td>
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<tr>
<td>Ionic capacity</td>
<td>90–190 µeq/mL settled resin</td>
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<tr>
<td>pK value</td>
<td>≥ 13</td>
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<tr>
<td>pH stability</td>
<td>pH 1 up to 13</td>
</tr>
<tr>
<td>Pressure limit</td>
<td>8 bar</td>
</tr>
</tbody>
</table>

**Figure 2.**

Dynamic binding capacities (DBC) measured at 10% breakthrough.
Eshmuno® HCX Resin

Direct Capture at High Salt Concentration

Eshmuno® HCX resin, the newest member of the Eshmuno® resin product offering, is a mixed-mode resin. Eshmuno® HCX resin was specifically designed for the direct capture of recombinant proteins at higher salt concentrations. The proven tentacles technology allows for a multipoint interaction between biopharmaceutical and media, resulting in higher binding capacities. The specially designed hydrophilic polyvinyl ether base bead allows for high flow rates, enabling faster processing in biopharmaceutical operations. As a result, Eshmuno® HCX resin offers outstanding performance results at higher salt concentration in typical ion exchange and flow-through applications.

Figure 3.
Unlike ion exchangers, mixed-mode resins bind proteins at high salt concentration, enabling direct capture of targets from clarified stocks.

Figure 4.
Dynamic binding capacity of polyclonal human IgG in mg/mL in 25 mM acetate, 25 mM phosphate, 0–300 mM NaCl.

Figure 5.
Highly cross-linked hydrophobic polyvinyl derivative created outstanding structure compared to other bead-based technologies.

Superior Capacity Over a Wide Range of Process Conditions

Both static and dynamic testing methods demonstrate the wide range of operating conditions of Eshmuno® HCX resin achieving high protein binding capacities at high salt levels. The open interconnected pore structure maintains rapid mass transfer, resulting in higher dynamic capacities over a wide range of process conditions.

Rigid Base Beads for Superior Packing

Take a close look at Eshmuno® HCX resin and you’ll see why it provides superior column packing. The spherical, regular shaped beads, and appropriate particle size distribution, allows for easy packing and scale-up. Its open and regular pore system enables a good accessibility of the proteins to the ligand.

Eshmuno® HCX resin characteristics

<table>
<thead>
<tr>
<th>Type</th>
<th>Multi-modal cation exchange chromatography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional group</td>
<td>Sulfon, carboxy and phenyl groups</td>
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<tr>
<td>Base matrix</td>
<td>Surface grafted rigid polyvinyl ether hydrophilic polymer</td>
</tr>
<tr>
<td>Mean particle size (d₅₀)</td>
<td>75–95 µm</td>
</tr>
<tr>
<td>IgG Dynamic Capacity (pH 5.5, 5 min residence time, 10% breakthrough)</td>
<td>≥ 50 mg/mL</td>
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<tr>
<td>Ionic capacity</td>
<td>170–300 µeq/mL</td>
</tr>
<tr>
<td>Linear flow rate</td>
<td>up to 1000 cm/h &lt; 2.5 bar net pressure</td>
</tr>
<tr>
<td>pH stability</td>
<td>pH 2 up to 12</td>
</tr>
<tr>
<td>Pressure limit</td>
<td>8 bar</td>
</tr>
</tbody>
</table>

Types of interactions with proteins

- Weak ionic
- Strong ionic
- Hydrophobic
- H-Bond donor/acceptor

H-O
H-O
H-N
H-N
H-N
H-N
H-N
H-O
S
S

Figure 3.

Types of interactions with proteins

- Weak ionic
- Strong ionic
- Hydrophobic
- H-Bond donor/acceptor

H-O
H-O
H-N
H-N
H-N
H-N
H-N
H-O
S
S

Figure 4.

Dynamic binding capacity of polyclonal human IgG in mg/mL in 25 mM acetate, 25 mM phosphate, 0–300 mM NaCl.

Figure 5.

Highly cross-linked hydrophobic polyvinyl derivative created outstanding structure compared to other bead-based technologies.

50 µm
10 µm
500 µm
Eshmuno® Resin and Virus Removal

Biopharmaceutical manufacturing carries the risk of viral contamination. Chromatography is a widely accepted approach for removing viruses, irrespective of their size and morphology. Depending on the parameter of your process, you have the option of implementing a viral adsorption strategy utilizing membrane- or bead-based technologies. Typically, both approaches are used at different process steps. Membrane-based chromatography is ideal for process steps with high flow rates and large volumes, while bead-based technologies provide specificity.

How does Eshmuno® resin work for virus removal? Multiple experiments indicate significant viral clearance for Eshmuno® S and and HCX resins. Cell culture supernatant taken from a production process was spiked with known titers of viruses. The data reveals significant viral clearance for all resins using ion exchange chromatography resins. Anion-exchange chromatography operated in the non-binding and binding mode is capable of effectively removing potential viral contaminants, and can be considered as a rather robust step. The use of fresh and close to the end of lifetime chromatographic material did not affect the viral clearance capabilities, demonstrating the robustness of the Eshmuno® resins in viral clearance process steps. These data demonstrate that IEX technologies with large operating windows can contribute to overall viral clearance.

Results of Virus Removal on Different Chromatographic Ion Exchange Media

<table>
<thead>
<tr>
<th>Chromatography Resin</th>
<th>Type of Chromatography and Mode</th>
<th>Virus</th>
<th>Virus (log$_{10}$) product eluate (run 1)</th>
<th>Virus (log$_{10}$) product eluate (run 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eshmuno® S</td>
<td>CEX, binding, pH 5.0</td>
<td>MuLV</td>
<td>≥ 5.47</td>
<td>≥ 5.36</td>
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<tr>
<td></td>
<td></td>
<td>PPV</td>
<td>6.56</td>
<td>5.73</td>
</tr>
<tr>
<td>Eshmuno® HCX</td>
<td>Multi-mode</td>
<td>X-MuLV</td>
<td>2.48</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MVM</td>
<td>0.87</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Making Eshmuno® Resin Work for You

Eshmuno® resin is backed by EMD Millipore’s unmatched global expertise and customer support services. Our dedicated service organizations are available in 64 countries, providing technical and application expertise worldwide. Count on us to provide on-site training and support, including validation services; or, utilize one of our eight Biomanufacturing Science and Training facilities worldwide to leverage our expertise in your process. Our goal is to help you:

- Move predictably and efficiently from bench scale to manufacturing scale processing
- Ensure production at planned rates, with desired yield and purity
- Create a robust, consistent and transferable process
- Ensure timely and successful startup and operation of plant
Manufacturing Excellence

Eshmuno® resins are developed and manufactured in Germany. Product quality is rigorously controlled from start to finish in accordance with cGMP. You can count on Eshmuno® resin for:

- Excellent batch-to-batch reproducibility
- Outstanding regulatory support
- Unrivalled expertise and application support

Ordering Information

<table>
<thead>
<tr>
<th>Eshmuno® S</th>
<th>1.20078</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eshmuno® Q</td>
<td>1.20079</td>
</tr>
<tr>
<td>Eshmuno® HCX</td>
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To Place an Order or Receive Technical Assistance

In the U.S. and Canada, call toll-free 1-800-645-5476

For other countries across Europe and the world, please visit: www.emdmillipore.com/offices

For Technical Service, please visit: www.emdmillipore.com/techservice