Eshmuno™ Q - The Smart Resin

Scout Column Kit
Strong Anion Exchanger

1. INTRODUCTION

Eshmuno™ Q is a new and unique ion-exchange resin especially designed for highly productive downstream purification of monoclonal antibodies, any other recombinant proteins and plasma proteins. This anion exchanger is member of the Eshmuno™ family of smart resins and is highly productive in bind-and-elute and flow-through steps.
Eshmuno™ Q is a surface grafted rigid hydrophilic polyvinylether polymer bead for the purification of strong acid and neutral proteins. Eshmuno™ Q process media provides high capacity and resolution along with all the advantages of polymerbased chromatographic beads such as high throughput, easy column packing, long life-time and high mechanical stability.
The most important advantage of the tentacle chemistry, where long, linear polymer chains ("tentacles") carry the functional groups, is the large number of sterically accessible ligands for the binding of biomolecules. All tentacles are covalently attached to the polyvinylether backbone and are chemically stable under conditions applied during chromatography, regeneration and sanitization.
Due to the titration behaviour the ion exchange capacity can be used from pH 2 up to pH 12.
The separation of proteins is based on reversible electrostatic interactions between the negatively charged regions of the proteins surface and the resin. Proteins are retained efficiently on Eshmuno™ Q when the pH of the buffer is about 1 unit above their isoelectric points (pI).

The strength of the binding depends on:

- the buffer system
- pH value of the buffer which determines the surface charge of the protein
- the counter ions conductivity
- the charge density on the resin (protein binding capacity)

The cleanability of Eshmuno™ Q ion exchangers assures long column lifetime.

GENERAL CHARACTERISTICS OF ESHMUNO™ Q

Table 1: Resin properties Eshmuno™ Q

Particle size	50-120 µm >80%
Type of chromatography	Strong anion exchange chromatography
Functional group	Trimethylammoniumethyl (TMAE)
Protein binding capacity (static)	120-190 mg BSA/ml of settled resin
Ionic capacity	90-190 µeq/ml settled resin
IgG Dynamic Capacity	≥ 40 mg/ml (2 min residence time, 10% breakthrough))
pK value	≥ 13
pH stability range	pH 1 up to pH 13
Elution conditions	High salt concentrations
Pressure limit	8 bar
Pressure drop	≤ 1.0 bar (100x16mm i.D., 5ml/min, 150 cm/h, buffer)
Operating temperature	4 °C to room temperature
Storage, preservative	20 % ethanol, 150 mmol/l NaCl
Regeneration	1 – 2 M NaCl
Sanitization	0.1 – 1.0 M NaOH
Linear flow rate	Up to 1000 cm/h <2.5 bar net pressure (20x10 cm i.d. column, 8% compression, 150mM NaCl as mobile phase)

KIT CONTENT

Including:

Eshmuno™ Q - scout columns	4 x 1 ml
Connectors LuerLock female/ 1/16" male	4 pieces

Eshmuno™ Q scout columns are designed for the rapid evaluation of this resin for bioprocess development. The results obtained for biomolecule separation using Eshmuno™Q scout columns are generally comparable to the results achieved using laboratory-scale columns (e.g. 100 x 16 mm i.d.).
Thus, in bioprocess design, the scout columns can be used to rapidly determine whether a target biomolecule is bound to and can be eluted from Eshmuno™ Q resin. In addition, data from experiments using Eshmuno™ Q scout columns allow one to determine whether an appropriate target yield may be achieved and whether major contaminants could potentially be removed. After choosing Eshmuno™ Q resin, bulk resin is available for preparative work on larger scale.

2. GUIDELINES FOR USE OF ESHMUNO™ Q SCOUT COLUMNS

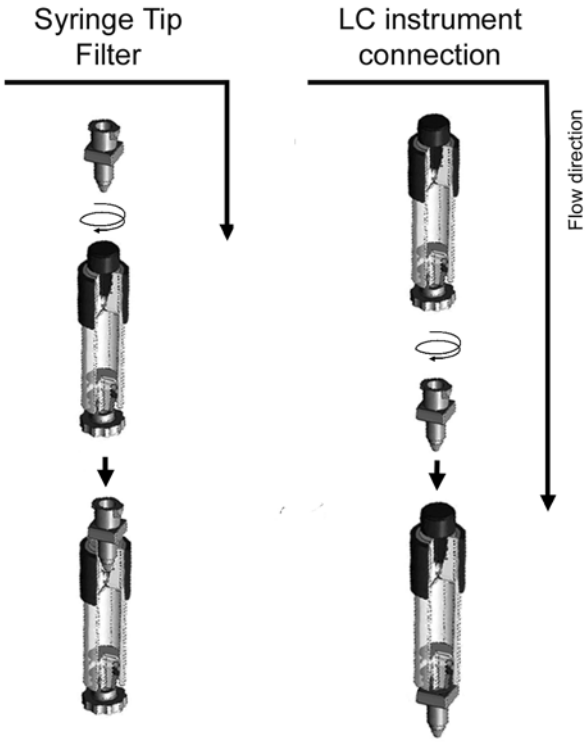
The Eshmuno™ Q scout columns are delivered in an aqueous solution comprised of 150 mM NaCl with 0,005% (w/v) Triton X 100 and stabilized with ProClin as a preservative. Prior to the first usage the preservation solution must be completely removed by washing the column with at least 5 column volumes (5 ml) of starting buffer.

All buffers should be filtered using a 0.22 µm filtration unit to remove particulate matter. All protein solutions should be filtered using a 0.45 µm filtration unit prior to injection
Do not operate the Eshmuno™ Q scout column at linear flow rates above 1500 cm/hour (13 ml/min).

The characteristics of the Eshmuno™ Q scout columns are summarized in Table 2.

Table 2: Characteristics of Eshmuno™ Q scout columns

Column volume	1 ml
Column dimension	0.82 x 1.92 cm
Maximum Flow Rate	1500 cm/hour (13 ml/min)
Pressure limit	5 bar
Column Pressure Drop	~ 0.2 bar at 200 cm/hour ~ 1,3 bar at 1000 cm/hour
Chemical stability (do not store under these conditions)	All commonly used aqueous buffers, 1 M NaOH 7 M urea 6 M guanidine hydrochloride 70 % ethanol



The Eshmuno™ Q scout columns are manufactured from polypropylene, which is biocompatible and non-interactive with biomolecules. The scout columns are delivered with a stopper at the inlet and a Luerlock stopper at the outlet. The column hardware is designed to allow for both manual and LC-instrument controlled usage.

When used manually, the scout columns are operated as syringe-tip filters, when used with LC-instrument control, the scout columns can be connected using the provided 1/16" adapters.

The Eshmuno™ Q scout columns cannot be opened or refilled.

To prevent leakage it is essential to ensure that the adaptors are tightly connected to the column.

3. STORAGE CONDITIONS

For long term storage the kit should be stored at 4° - 8°C.

Avoid freezing the scout columns!

For storage of Eshmuno™ Q scout columns after usage rinse the columns with three column volumes of 20 % ethanol in 150 mM NaCl (3 ml).

4. PRACTICAL HINTS

Selection of ion exchanger

Ion exchange chromatography is based on the amphoteric behaviour of proteins. At low acidic pH-values, all amino acids are cations due to protonation of the NH_2-groups and undissociated COOH-groups resulting in a positive net charge. At alkaline pH-values above pH 12, the amino-groups are uncharged, whereas the carboxyl-groups will be ionized, resulting in a negatively charged molecule.

Biomolecules are bound to ion exchangers when they carry a net charge opposite to the surface charge of the ion exchanger. The pH value at which a biomolecule has no net charge is called the isoelectric point (pI). At buffer pH values below the pI, a given biomolecule will carry a positive net charge and will bind to cation exchangers like Eshmuno™ S. At buffer pH values above the pI, the respective biomolecule will carry a negative net charge and will bind to the anion exchanger Eshmuno™ Q.

If the target biomolecule is most stable below the pI value, a cation exchanger should be used. If stability of the target biomolecule is higher at buffer pH values above its pI, an anion exchanger should be used. When the biomolecule is stable over a wide range of buffer pH values, either type of ion exchange resin can be used for purifications either alone or in consecutive combinations. It is important in ion exchange chromatography to choose optimal pH conditions so that the ion exchange groups are ionized. On the other hand, the buffer pH-value must be different enough from the isoelectric point of the target biomolecule in order to maintain sufficient net charge on the surface. Applying an increasing salt gradient or changing the pH of the buffers will reduce binding to the gel and elute proteins successively from the column when the charge differences have been neutralized.

Starting condition for ion exchange chromatography:

Highest salt concentration that permits binding of the target protein.

Equilibrate the Eshmuno™ Q scout columns with at least 5 column volumes of starting buffer prior to application of the sample.

NOTE:
It is strongly recommended that a typical gradient run without sample application is perfomed prior to the first use of an Eshmuno™ Q scout column to ensure removal of preservative compounds and to achieve a stable base line.

Washing step:

An ionic strength higher than the loading step and less than the elution step will remove contaminants that bind to the column.

Elution condition:

Lowest ionic strength which allows the elution of the protein of interest. In case of anion exchange chromatography, changing the buffer pH can also be used to elute the target biomolecule.

Stripping step
A higher concentration of salts in the buffer than the one used for elution is recommended for stripping the column after use.

Cleaning and regeneration:
Each column should be cleaned thoroughly after the separation step or at least after the experiments have been finished (especially if the back pressure increased during the separation).

Various methods are available for cleaning chromatographic media. Synthetic polymeric matrices are characterized by higher chemical stability than inorganic sorbents based on silica gels, which are unstable in the presence of NaOH. In contrast to media based on carbohydrates, synthetic polymeric matrices can also withstand treatment with acids.

Lipids or lipoproteins can be removed with organic solvents like ethanol, isopropanol or ethylene glycol. Denatured proteins can be effectively removed with sodium hydroxide (0.1 N up to 1 N NaOH). In addition to bases and acids, organic solvents can be used.

If contaminants are tightly bound, it may be necessary to clean the column with an acidic pepsin solution (0.1 % pepsin in 0.01 N HCl), 6 M guanidine hydrochloride or diluted sodium lauroyl sarcosinsate solution (2 % SLS in 0.25 M NaCl). The removal of SLS is achieved by treatment with 20 % 2-propanol in 0.01N HCl.

After cleaning and regeneration, Eshmuno™ Q scout columns must either be rinsed with three column volumes of 20 % ethanol in 150 mM NaCl (3 ml) for storage or reequilibrated for the next chromatographic run.

Avoid storage of columns in cleaning solutions!

Table 3. Recommended cleaning and regeneration steps

Reagent	Concentration	Bed volumes (BV)
NaCl	up to 2 M	2 BV
NaOH	0.1 - 1 N	1 - 5 BV
HCl	1 - 2 N	1 - 5 BV
Pepsin/HCl	0.1% / 0.01N HCl	1 - 2 BV
SLS/NaCl	2% / 0.25 M NaCl	1 - 2 BV
Isopropanol	20 %	1 - 2 BV
Acetonitrile	20 %	1 - 2 BV
Others	20 % ethanol guanidine HCl, 6 M urea	1 - 2 BV

5. ORDERING INFORMATION

Designation	Content	Ord.No.
Eshmuno™ Q	10 ml	1.20079.0010
	100 ml	1.20079.0100
	500 ml	1.20079.0500
	5000 ml	1.20079.5000

If you need any further information or support please do not hesitate to contact the specialists:

processing@merck.de