

# Care and Use Instructions

## Macro-Porous Ion Exchange Media

### MacroSep IEX Q

#### 1. Introduction

Thank you for purchasing YMC products for ion exchange media. MacroSep IEX Q is hydrophilic methacrylate polymer beads with high mechanical strength and macro-porous structure, modified with strong-anion exchanger (quaternary ammonium group).

MacroSep IEX Q, which is manufactured under highly controlled conditions, must pass a series of stringent tests before being accepted for shipment (Please refer to the certificate of analysis). To ensure optimal performance and durability of the media, please follow these instructions.

#### 2. Specification

Item	Strong-anion exchanger MacroSep IEX Q
Particle size (μm)	30
Pore size (nm)	900
Matrix	Methacrylate-based hydrophilic porous polymer beads
Functional group	-R-N <sup>+</sup> (CH <sub>3</sub> ) <sub>3</sub>
pH range	2-12
Temp. range (°C)	4-60
Pressure limit	Regular use: less than 2 MPa Upper limit: 3 MPa
Shipping solvent	20% ethanol aqueous solution
Application	Purification of large-sized biomolecules such as viral vectors

#### 3. Packing instructions

##### 3-1 Resin preparation (Removal of fines)

Recommended slurry solvent: Deionized water, buffer solution or 20% ethanol solution

Amount of media required: approximately 1.05X to 1.10X column volume

1. Suspend the settled resin in the shipping container(s) and transfer the slurry into a clean container with a capacity of 5 times or larger than the amount of resin.
2. Allow the slurry to settle for a certain time. Then measure the amount of the resin and decant the supernatant.
3. Add four times the resin volume of the slurry solvent stated above to the container.
4. Stir the solution with a rod or paddle to make homogeneous slurry. Do not use a sharp-edged paddle or a magnetic stirrer to avoid fine generation.
5. Allow the resin to settle for 120 minutes.
6. Once resin has settled, decant the supernatant.
7. Repeat Step 3-6 until the supernatant at the Step 6 becomes clear.

##### 3-2 Packing slurry preparation and column packing

Recommended packing solvent: A solution with high ionic strength (a component of mobile phase which has the highest ionic strength. For example, 1 M NaCl or 0.5 M Na<sub>2</sub>SO<sub>4</sub>)

1. Filter the slurry solution obtained after 3-1. If 20% ethanol is used as the slurry solvent at 3-1, rinse the resin by filtration with deionized water (This process can be skipped if deionized water or buffer is used at 3-1).
2. Rinse the resin on the filter with approximately three times the resin volume of the packing solvent stated above.
3. Transfer the resin to a clean container with a capacity of 5 times or larger than the amount of resin.
4. Add the packing solvent to adjust the slurry concentration to 30-50%. The slurry concentration can be calculated with a following equation. Slurry concentration (%) = Resin volume (L) / Total slurry volume (L) X 100
5. Stir the solution with a rod or paddle to make homogeneous slurry.
6. Once homogeneous slurry is formed, gently pour it into the column. Prevent air coming into the column during transfer.

7. Connect a pump to the column and start pumping the packing solvent at approximately two times of the actual flow rate in use. Maintain the flow rate until the packed bed becomes stable. Then disconnect the pump. The desired column volume can be obtained by flowing the packing solvent through the column and compressing the resin.
8. When using an axial compression column, the column performance may be improved by compressing the packed bed after pumping.

(NOTE) Also refer to the Instruction manual of the column hardware using, and follow the packing procedures described in it, when necessary.

### 3-3 Column performance evaluation

When the column packing is completed, evaluate the column performance by injecting a sample to the column, and determine the column theoretical plate number (N/m) and peak asymmetry factor (As). Typical evaluation condition is shown below. If there is a large difference between the value(s) obtained and typical performance listed below, adjust the packing parameter(s) (slurry concentration, flow rate of the slurry solvent etc.) and repeat the packing procedure.

#### Example of test conditions for the column packing evaluation

	Condition 1	Condition 2	Column performance	
Mobile phase	0.5 M NaCl	20 mM Tris-HCl buffer (pH 8)	Particle size	30 $\mu$ m
Sample	1 M NaCl	Formamide (2 $\mu$ L/mL)	Theoretical plate number (N/m)	$\geq$ 5,000
Detection	Conductivity	UV at 220 nm	Asymmetry factor (As)	0.7~1.7
Flow rate	Approximately 70 to 90 cm/hour			
Temperature	Room temperature (25°C)			
Injection volume	1% of bed volume			

\* These are just some guide values. Desired separation could be achieved even if the values obtained are out of the range shown above, depending on your usage and/or application.

\* Sample diffusion in the system flow path (extra-column volume) greatly affects column performance. If the packing conditions do not change the column performance, check if the inner diameter of connecting tubes and equipment performance are appropriate.

## 4. Equilibration and elution

- Equilibrate with about 5–10 column volumes of initial mobile phase before using a column for chromatographic separations.
- Adsorb the target samples onto the column using a 20-50 mM buffer as the initial eluent, then elute and separate the targets using a salt-concentration gradient. A linear gradient that increases the salt concentration from 0 to 1 M is typically used. However, if the targets do not elute effectively, it may be beneficial to elute at even higher salt concentrations, provided this does not inactivate the targets. Changing the type of salt used or using pH gradient may also improve separation. After each separation run, it is recommended to flush the column with an eluent having higher salt concentration to remove any impurities that remain uneluted during the purification run.
- Water-soluble organic solvent (maximum of 30%) can be added to the mobile phase. Before adding such solvent, make sure that salt in the buffer will not precipitate.

## 5. Cleaning and regeneration

A change of retention time or peak shape and/or pressure increase might result from the adsorption of fat-soluble substances or precipitated impurities in a sample. In such cases, follow these steps for column cleaning and regeneration. If these procedures will not solve the problem, we recommend using new media.

### 5-1 Common cleaning methods

- Cleaning in place (CIP) - The following CIP is effective when there is a change in column performance or before long-term storage (it is recommended to wash the column without connecting to a detector).  
First, flush the column with 3 to 5 CV (CV : Column Volume) of 1 to 2 M NaCl. Then, flush the column with 3 to 5 CV of 0.1 to 0.5 M NaOH. The cleaning efficiency of the column can be improved by increasing the concentration of NaOH (up to 1 M) and/or the exposure time by reducing the flow rate. In order to neutralize the column, flush the column with 3 to 5 CV of 1 to 2 M NaCl. After neutralization, equilibrate the column thoroughly with the same mobile phase used for the next step.  
If the column shows high pressure due to impurity accumulation, reduce the flow rate to wash.
- Batch method  
Soak and agitate the media in washing solution about 3–5 times of the media volume. After leaving it stationary, remove the supernatant fluid by decantation. Repeat the process 2–3 times. As a washing solution, the solvents used for CIP can be used.

### 5-2 Cleaning with surfactants and other additives

- Other additives such as urea ( $\leq 8$  M) or guanidine hydrochloride ( $\leq 6$  M), which are commonly used as protein denaturants are useful. Avoid solvents containing oxidant for the mobile phase.
- Nonionic surfactants or cationic surfactants are useful, but avoid anionic surfactants for MacroSep IEX Q.
- Some washing solution (high viscosity, etc.) may cause high pressure. In such case, reduce the flow rate.

## 6. Storage

Store the bulk media in 20% ethanol aqueous solution. Store the bulk media in the original container at temperatures from 4 to 35°C. Keep the container closed tightly.

NOTE) Product quality is maintained even if a product bottle is not stored or transported in the upright position, or the resin is adhering on wall or neck of the bottle.

However, it is recommended to store the product (bottle) in the upright position, and immerse all the resin in 20% ethanol solution (storage solvent in the bottle), from a standpoint of prevention of resin drying.