

Column Care and Use Instructions

MacroSep IEX Q Column

Thank you for purchasing a YMC chromatography column for ion exchange chromatography. To ensure optimal performance and durability of the column, please read these instructions carefully before using this column.

1. Specification

Column specifications

Column size length x I.D. (mm)	50 x 5.0	50 x 11.3
Column volume (mL)	1	5
Column material	Polypropylene	
Max. pressure (MPa)	3	
Shipping solvent	20% ethanol/150 mM NaCl	

Media specifications

Item	MacroSep IEX Q
Particle size (μm)	30
Pore size (nm)	900
Matrix	Methacrylate-based hydrophilic porous polymer beads
Functional group	-R-N ⁺ (CH ₃) ₃
pH range	2–12

2. Recommendations for column connections and column use

- When installing the column, make sure to prevent air from entering the column.
- Use of a metal connector is not recommended. Inside parts of the column might be damaged if the metal connector is used.
- The correct direction of the solvent flow is indicated by an arrow on the column identification label.
- Avoid using a column repeatedly near the pressure limit, or with abrupt change in pressure to prevent shortening of the column life.

3. 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.
- When possible, the sample should be dissolved in a solvent that is the same composition as the initial mobile phase. Using a different buffer salts/additives concentration or a different pH solvent from the initial mobile phase for sample dissolution might result in decreased binding capacity and/or distorted peak shape.

- To prevent exposure of the column to excessive pressures, the mobile phase should be filtered through a 0.2–0.5 µm membrane filter.

4. Column cleaning

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 a new column.

4-1 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. 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.

4-2 Cleaning with surfactants and other additives

- Other additives such as urea (≤ 8 M) or guanidine hydrochloride (≤ 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.

5. Storage

When columns are not used for a long time, keep them in a cool place after replacing with the shipping solvent or mobile phase which is added water-soluble organic solvent (maximum of 30%), e.g. 20% ethanol solution.