Column Care and Use Instructions
MacroSep IEX Q Screening Kit

Thank you for purchasing MacroSep IEX Q Screening Kit. To ensure proper use of the kit, please follow these instructions.

1. Specifications

Column specifications

Item	1 mL type	5 mL type
Column volume (mL)	1	5
Column material	Polypropylene	
Column size length x I.D. (mm)	26 x 7.0	26 x 15.6
Recommended flow rate (mL/min)	1	5
Maximum flow rate (mL/min)	10	25
Max. pressure (MPa)	0.3	
Shipping solvent	20% ethanol aqueous solution	

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. Consideration for column connection

- The column is to be connected using 1/16" tubing. We recommend using Handy Connector 1（Product number：XRP0203）for connecting the column.
- The correct direction of the solvent flow is indicated by an arrow on the column identification label.
- When installing the column, make sure to prevent air from entering the column.

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.
- Take care to prevent the precipitation of salts when replacing shipping solvent with a buffer solution with high buffer/salt concentration.

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4. 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.

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

Flush the column with water, then with 20% ethanol solution. Make sure to close the end plug tightly to avoid drying out.

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