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Sepapure ion exchange (IEX) columns

Short guide

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Note: For your own safety, read the instructions and observe the warnings and safety information on the device and in the instructions. Keep the instructions for future reference.

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For latest version of the instructions, check our website: www.knauer.net/en/Support/User-manuals.

Legal information

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1. Specifications

Sepapure ion exchange (IEX) columns are designed for purification and separation of biomolecules based on charge.

The columns are designed to be used with low-pressure FPLC-type automated purification systems and operated below 3 bar (0.3 MPa, 2.96 atm).

1.1 Hardware specifications

Column housing	Polypropylene
Frits	Polyethylene (nominal 20 µm porosity)
Fittings	10/32 UNF
Bed volume	1 and 5 ml

1.2 Resin specifications

Resin name	Sepapure Q FF	Sepapure DEAE FF	Sepapure SP FF	Sepapure CM FF
Functional Group	Quaternary amine	Diethylaminoethyl	Sulphonyl propyl	Carboxymethyl
Base matrix material	6% cross-linked, beaded agarose			
Mean bead diameter	100 µm			
Counter ion binding capacity (H+/Cl-)	8 – 16 mmol/ml			
Recommended flow rate for a 1 ml column	0.5-2 mL/min (60-240 cm/h)			
Recommended flow rate for a 5 ml column	2-10 mL/min (57-286 cm/h)			
pH stability	3 – 12 (long term) 2 – 14 (short term)			

Note: Before use, inspect the column for damage. If any damage is observed, do not use the column.

Note: Flow rates shown in this manual are for guidance only. Always ensure that system pressure is below the maximum for the column and resin.

1.3 Buffers

Sepapure columns are designed to be used with most aqueous phase chromatography buffers. A suggested buffer system is shown below, although other buffers may be used.

Equilibration buffer	20 mM Tris, pH 7.4; **or** 20 mM sodium phosphate, pH 6.8
Wash buffer	20 mM Tris, pH 7.4; **or** 20 mM sodium phosphate, pH 6.8
Elution buffer	20 mM Tris with 0.5 M NaCl, pH 7.4; **or** 20 mM sodium phosphate with 0.5M NaCl, pH 6.8

For proteins where the optimal purification conditions have not yet been established, then the following procedure can be used:

Strong anion exchange: Start at pH 8.0 and using a gradient elution using 1 M NaCl.

Strong cation exchange: Start at pH 6.0 and using a gradient elution using 1 M NaCl.

In both cases the gradient should run 0-100% .

If the results of the above are not satisfactory, then try weak ion exchange resins instead (DEAE and CM ligands).

2. Preparing the column

Sepapure ion exchange (IEX) columns are supplied with 20% ethanol as the storage buffer. This must be removed prior to purification.

Process

1. Remove the end-plugs and connect the column to the control system, taking care to avoid introduction of air into the system.

Note: Do not over-tighten fittings as this can strip the screw connections and lead to column leakage.

2. Flush the column with 3 to 5 Column Volumes (CVs) of binding buffer at high flow rate of to remove the storage buffer, ensuring that the system pressure remains below the given maximum.
3. Equilibrate the column with 3 to 10 CVs of binding buffer to ensure that pH, conductivity and UV_{280} signals are stable.

Result The column is now ready for use.

3. Sample preparation

Sepapure ion exchange (IEX) columns should only be used with clarified (particle-free) samples. If the protein is expressed in inclusion bodies, it can be released through the addition of denaturing reagents such as guanidine hydrochloride or urea.

To reduce the effects of sample viscosity when performing this step, it may be additionally necessary to treat the sample with DNAse before application of the sample to the column.

The pH of the sample depends on the target protein; your main impurities and selected IEX column. For general propose select the buffer conditions that your target protein will bind to the medium and most of impurities not bind.

Usually it is recommended to use buffer condition pH >7 for proteins that bind under this condition to anion exchange resins and buffer condition pH <7 for proteins that bind under this condition to cation exchange resins. In general, the binding condition relate to the isoelectric point (pI) of our target protein.

4. Protein purification

Process

1. Load the sample at a flow rate of approximately 0.5 CV/min.
   Solid phase - biomolecule binding kinetics and sample viscosities will require the flow rate to be adjusted.

Note: To avoid overloading the column, the amount of applied sample should not exceed the binding capacity of the solid phase to the target molecule.

2. Once the sample has been loaded, wash the column with binding buffer until the UV280 trace reaches baseline.
3. If a wash buffer is used, then also wash the column with 3 to 5 CVs of this reagent.
4. Elute the protein using the selected elution buffer. A stepwise or linear gradient may be used to determine the precise elution point of the target antibody. Initially, all elution fractions should be collected for further analysis. Buffer exchange and/or desalting might be required following elution and we recommend Sepapure Desalting Columns (010X460SPZ and 020X460SPZ) for this purpose.

5. Column regeneration

Process

To remove contaminants (lipids, non-specifically bound proteins, etc.) from the column following elution, wash with a further 3 to 5 CVs of elution buffer, followed by 3 to 5 CVs of equilibration buffer.

Ionically bound contaminants can also be removed using 2M NaCl.

Contaminants of a more hydrophobic nature may be removed by using detergents (0.1 – 2% non-ionic), 70% ethanol, 30% isopropanol under either acidic (eg.: acetic or phosphoric acids) or basic conditions. Combinations of the aforementioned reagents may also be used, with regeneration times of 30 minutes – overnight.

Next step

Following column regeneration, immediately re-equilibrate the column with 3 to 5 CVs equilibration buffer.

6. Sanitization

Sepapure ion exchange (IEX) columns can be sanitized with 0.5 – 1 M NaOH for 1 hour. Re-equilibrate the column before reuse or storage.

7. Column storage

Columns should be stored in 20% ethanol.

For Sepapure SP the storage buffer should be supplemented with 0.2 M sodium acetate.

Note: Columns should be stored at 2-8°C.
Do not freeze!

8. Sepapure IEX FF buffer compatibility

Sepapure IEX FF resins are compatible with commonly used aqueous buffers for protein separation including 1 M acetic acid, 1 M NaOH, 6 M guanidine HCl, 8 M urea, 30% isopropanol and 70% ethanol.

Oxidizing reagents should be avoided.

Avoid contact between anionic detergents and the anion IEX resins (Q, DEAE). Likewise, avoid contact between cationic detergents and the cation IEX resins (SP, CM).