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Sepapure FPLC columns
Short guide

Document no. V6016

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.

KNAUER Technical Support:
Phone: +49 30 809727-111 (9-17h, Central European Time)
Fax: +49 30 8015010
E-Mail: support@knauer.net
Languages: German, English

Publisher:
KNAUER Wissenschaftliche Geräte GmbH
Hegauer Weg 38
14163 Berlin
Germany
Phone: +49 30 809727-0
Fax: +49 30 8015010
Internet: www.knauer.net
E-Mail: info@knauer.net

Version information:
Article number: V6016
Version number: 1.0
Last update: October 26, 2021

The information in this document is subject to change without prior notice.
For latest version of the instructions, check our website: www.knauer.net/en/Support/User-manuals.

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1. Specifications

Sepapure Desalting columns are designed for separating larger biomolecules (i.e. proteins such as antibodies, enzymes or larger nucleic acids) from unwanted smaller molecules. Common separation processes include buffer exchange, desalting, removal of low molecular weight contaminants and reaction terminations. 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
Fittings	10/32 UNF
Bed volume	1 and 5 ml

1.2 Resin specifications

Resin name	Sepapure Desalting
Base matrix material	Dextran
Mean bead diameter	20 - 85 µm (hydrated)
Fractionation range	between 1 and 5 kDa
Recommended Flow rate	1 CV/min
pH stability	2-13

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

For desalting neutral compounds, a low ionic strength buffer is recommended. For separating charged compounds, a buffer with a higher ionic strength may be required.

2. Preparing the column

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 distilled water at a flow rate of no more than 1 CV/min to remove the storage ethanol.
3. Equilibrate the column with 3 to 5 CVs of buffer at 1 CV/min.

Result The column is now ready for use.

3. Sample preparation

The sample should be free of insoluble compounds and particulates. To extend the life of the column, pass the sample through a filter with a 0.45 µm pore size prior to column loading. Highly viscous samples will require a buffer having a viscosity of not more than 1.5-fold from that of the sample. As a general rule, keep the protein concentration below 65 mg/ml for proteins and 5 mg/ml for high (> 1000 kDa) molecular weight polymers.

4. Protein purification

1. Load the sample at a flow rate of approximately 1 CV/min.
2. Collect eluent fractions to recover the purified sample.

5. Column regeneration

When the sample processing is completed, flush the column with a minimum of 5 CV buffer at no more than 1 CV/min before processing the next sample. Monitor the column effluent using a UV, conductivity, fluorescence or other detection system to assure the column is ready.

6. Column storage

It is recommended to exchange the buffer in the column with 20% ethanol (in distilled water) prior to storage.

Note: Columns should be stored at +4°C.
Do not freeze!