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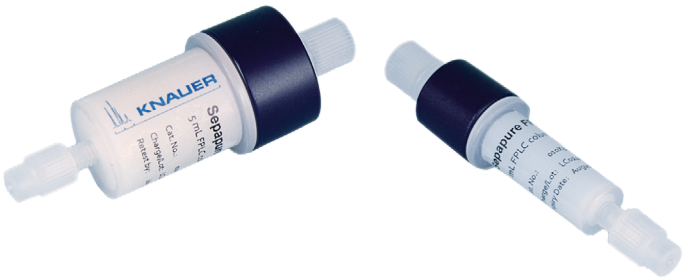
Sepapure Ni-NTA FPLC column

Short guide



Document no. V6013

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: V6013
Version number: 1.0
Last update: 2019/01/29

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Legal information

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1. Specifications

Definition Sepapure Ni-NTA columns are designed for the purification of poly-histidine tagged proteins. 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).

Hardware specifications

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

1.1 Resin specifications

Resin name	Sepapure Ni-NTA FF
Base matrix material	6% cross-linked, beaded agarose
Mean bead diameter	100 µm
Ligand	Nitrilotriacetic acid (NTA)
Metal ion capacity	> 15 µmol/mL
Static protein binding capacity	> 40 mg/mL (dependent on protein)
Flow rate	> 500 cm/hr
pH stability (with metal ion loaded)	2-14 (less than 1 hour) 3-12 (up to 1 week)

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.

1.2 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 please check the resin specifications and buffer compatibility table for further details.

Equilibration buffer	20 mM sodium phosphate with 0.5 M NaCl, pH 7.4
Wash buffer	20 mM sodium phosphate with 0.5 M NaCl, pH 7.4
Elution buffer	250-500 mM imidazole in 20 mM phosphate buffer with 0.5 M NaCl, pH 7.4

2. Preparing the column

Sepapure Ni-NTA columns are supplied pre-charged with nickel metal ions. The column can be stripped and re-charged again with nickel or another metal. Please refer to the Column Regeneration section below for this procedure.

Process Remove the end-plugs and connect the column to the control system, taking care to avoid introduction of air into the system. Do not over-tighten fittings as this can strip the screw connections and lead to column leakage. Flush the column with 3 to 5 Column Volumes (CVs) of distilled water at a flow rate of 1 to 2 CV/min to remove the storage buffer.

Equilibrate the column with 3 to 5 CVs of binding buffer at 1 CV/min.

Result The column is now ready for use.

3. Protein purification

Load the sample at a flow rate of approximately 1 CV/min. Some protein-metal binding kinetics and the sample viscosities will require the flow rate to be adjusted. To avoid overloading the column, the amount of sample that is applied to the column should not exceed the binding capacity of the solid phase to the target molecule.

Once the sample has been loaded, wash the column with binding buffer until the UV_{280} trace reaches baseline.

If a wash buffer is used, then also wash the column with 3 to 5 CVs of this reagent.

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 protein. 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.

4. Column regeneration

To remove contaminants from the column following elution, the best approach is to use 0.1 M EDTA with 0.5 M NaCl at pH 7.5. This will strip the solid phase of all metal ions and thus also remove any bound materials such as protein. The stripped resin should then be washed thoroughly with distilled water.

To re-charge the resin, prepare a solution of 0.1 M of a desired metal ion (Co^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, etc) in distilled water. For zinc, it is recommended to adjust the pH to approximately 5.5. Circulate this solution through the column for 10 to 30 minutes at 3 to 5 CV/min. Wash with 3 to 5 CVs of distilled water before using the column.

For sanitization of the column, a solution of 1 M NaOH may be used, as well as solutions containing low amounts of detergents (0.1 – 2%), 70% ethanol or 30% isopropanol. The time required to fully sanitize the column will vary (i.e. 30 minutes to overnight) after which the column should be washed thoroughly with distilled water prior to storage or further use.

5. Column storage

Columns may be stored either charged with metal or having been stripped with EDTA. In both cases, 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!

6. Sepapure Ni-NTA FlashFlow buffer compatibility

Buffer type	Name	Type
Chelating reagents	EDTA	Up to 1 mM, although care needs to be taken. Chelating reagents may strip the metal from the solid phase
	EGTA	
Denaturing reagents	Guanidine HCl	Up to 6M
	Urea	Up to 8M
Detergents	CHAPS	Up to 1%
	NP-40	Up to 2% (v/v)
	SDS	0.1 – 0.3%. Pre-test before use
	Tween-20	Up to 2% (v/v)
	Tween X-100	Up to 2% (v/v)
Reducing reagents	β-mercaptoethanol	Up to 20 mM
	DTE	Up to 2 mM
	DTT	Up to 2 mM
	Reduced glutathione	Up to 10 mM
Buffer reagents	HEPES	Up to 100 mM
	MOPS	Up to 100 mM
	Sodium acetate	pH 4, up to 100 mM
	Sodium phosphate	pH 7.5, up to 50 mM
	Tris-acetate	pH 7.5, up to 100 mM
	Tris-HCl	pH 7.5, up to 100 mM
Other additives	Citrate	Up to 60 mM
	Ethanol	Up to 20% (v/v)
	Glycerol	Up to 50% (v/v)
	Glycine	Not recommended
	Imidazole	Up to 500 mM
	Sodium bicarbonate	Not recommended
	Sodium sulphate	Up to 100 mM

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Wissenschaftliche Geräte GmbH
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Fax: +49 30 8015010
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Internet: www.knauer.net