

HiPrep Heparin FF 16/10

Instructions for use

Introduction

HiPrep™ Heparin FF 16/10 is a prepacked ready to use column. The column provides fast, preparative separations of proteins and other biomolecules based on their affinity for heparin. Heparin is a naturally occurring glycosaminoglycan which is an effective affinity binding and ion exchange ligand for a wide range of biomolecules, including coagulation factors and other plasma proteins, lipoproteins, protein synthesis factors, enzymes that act on nucleic acids, and steroid receptors. Heparin is coupled to Sepharose™ 6 Fast Flow with a chemically optimized linkage.

Column data

Matrix	cross-linked agarose, 6%, spherical
Particle size, d_{50V}^1	~ 90 μm
Ligand concentration	~ 2 mg heparin/mL resin
Ligand	Heparin (procine origin)
Ligand coupling	Reductive amination
Bed volume	20 mL
Bed height	100 mm
i.d.	16 mm
Column composition	Polypropylene
Recommended operating flow rate ²	2–10 mL/min (60–300 cm/h)
Maximum operating flow rate ²	10 mL/min (300 cm/h)
Maximum pressure over the packed bed during operation, Δp^3	0.15 MPa (1.5 bar, 22 psi)
HiPrep column hardware pressure limit	0.5 MPa (5 bar, 73 psi)
pH stability, operational ⁴	4 to 12
pH stability, CIP ⁵	4 to 13
Storage	4°C to 30°C in 20% ethanol, 0.05 M sodium acetate

¹ Median particle size of the cumulative volume distribution.

² At room temperature using buffers with the same viscosity as water.

³ Many chromatography systems are equipped with pressure gauges to measure the pressure at a particular point in the system, usually just after the pumps. The pressure measured here is the sum of the precolumn pressure, the pressure drop over the resin bed, and the post-column pressure. It is always higher than the pressure drop over the bed alone. We recommend keeping the pressure drop over the bed below 1.5 bar. Setting the upper limit of your pressure gauge to 1.5 bar will ensure the pump shuts down before the resin is overpressured.

If necessary, post-column pressure of up to 3.5 bar can be added to the limit without exceeding the column hardware limit. To determine post-column pressure, proceed as follows:

⁴ pH range where resin can be operated without significant change in function.

⁵ pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function.

Note: To avoid breaking the column, the post-column pressure must never exceed 3.5 bar.

Step	Action
1	Connect a piece of tubing in place of the column.
2	Run the pump at the maximum flow you intend to use for chromatography. Use a buffer with the same viscosity as you intend to use for chromatography. Note the back pressure as total pressure.
3	Disconnect the tubing and run at the same flow rate used in step 2. Note this back pressure as pre-column pressure.
4	Calculate the post-column pressure as total pressure minus pre-column pressure.

If the post-column pressure is higher than 3.5 bar, take steps to reduce it (shorten tubing, clear clogged tubing, or change flow restrictors) and perform steps 1–4 again until the post-column pressure is below 3.5 bar. When the post-column pressure is satisfactory, add the post-column pressure to 1.5 bar and set this as the upper pressure limit on the chromatography system.

First time use

Ensure an appropriate pressure limit has been set. Equilibrate the column for first time use or after long storage by running:

- 100 mL binding buffer e.g. 20 mM Tris-HCl, pH 8 at 5 mL/min.
- 100 mL elution buffer, e.g. 20 mM Tris-HCl, 1 M NaCl, pH 8 at 5 mL/min.
- 100 mL binding buffer, e.g. 20 mM Tris-HCl, pH 8 at 5 mL/min.

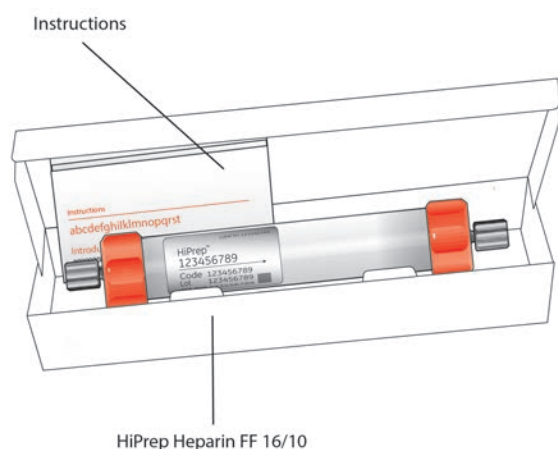
HiPrep Heparin FF 16/10 can be used directly on ÄKTA™ design systems without the need for any extra connectors.

Try these conditions first

Binding buffer	20 mM Tris-HCl, pH 8 (if the immobilized heparin interacts with the protein as a cation exchanger) or 20 mM Tris-HCl, 0.15 M NaCl, pH 8 (if the immobilized heparin interacts with the protein as an affinity ligand)
Elution buffer	20 mM Tris-HCl, 1 M NaCl, pH 8
Flow rate	5 mL/min
Linear gradient	0–100% elution buffer in 200 mL (10 × CV) (CV = column volumes)

Buffer and solvent resistance

De-gas and filter all solutions through a 0.45 µm filter to increase column life-time.



Daily use

Commonly used aqueous buffers, pH 4–12
Guanidine hydrochloride, up to 6 M
Urea, up to 8 M



Cleaning

Sodium chloride, up to 2 M
Sodium hydroxide, up to 0.1 M
Guanidine hydrochloride, up to 6 M
Urea, up to 8 M



Avoid

Solutions < pH 4

Sample preparation

Dissolve the sample in binding buffer, filter through 0.45 µm filter or centrifuge at 10 000 × g for 10 minutes.



Delivery/storage

The column is supplied in 0.05 M sodium acetate containing 20% ethanol. If the column is to be stored for more than two days after use, clean the column according to the procedure described under [Cleaning-in-place \(CIP\)](#), on page 2. Then equilibrate with at least 100 mL of 0.05 M sodium acetate containing 20% ethanol at a flow rate of 5 mL/min.



Note: HiPrep columns cannot be opened or refilled.

Optimization

Perform your first run according to [Try these conditions first](#), on page 2. If the results are unsatisfactory, consider the following:

Action	Effect
Change pH/buffer salt	Selectivity change, if the immobilized heparin works as a cation exchange
Change the ionic strength	Selectivity change, if the immobilized heparin works as a cation exchanger
Smaller sample volume	Improved resolution
Lower flow rate	Improved resolution
Shallower gradient	Improved resolution

Cleaning-in-place (CIP)

Regular cleaning

Wash the column with 40 mL of 2 M NaCl at a flow rate of 5 mL/min at room temperature to remove ionically bound proteins.

Re-equilibrate the column with at least 100 mL binding buffer at a flow rate of 5 mL/min at room temperature or until the UV base-line and pH/conductivity values are stable.

More rigorous cleaning

Reverse the flow direction and run at a flow rate of 1 mL/min at room temperature.

1. Removal of precipitated or denaturated proteins:

80 mL of 0.1 M NaOH or 40 mL of 6 M guanidine hydrochloride or 40 mL of 8 M urea.

After each cleaning procedure re-equilibrate the column with at least 100 mL binding buffer (see above).

Note: *HiPrep columns cannot be opened or refilled.*

Troubleshooting

Symptom	Remedy
Increased back pressure	Reverse the flow direction and pump 100 mL elution buffer through the column at a flow rate of 5 mL/min at room temperature. Return to normal flow direction and run 100 mL binding buffer at a flow rate of 5 mL/min. (Try different cleaning procedures described in section "Cleaning-in-place (CIP)".
Loss of resolution and/or decreased sample recovery	Try different cleaning procedures described in section "Cleaning-in-place (CIP)"
Air in the column	Reverse the flow direction and pump 100 mL of well de-gassed binding buffer through the column at a flow rate of 5 mL/min at room temperature.

Intended use

The HiPrep Heparin FF 16/10 is intended for research use only, and shall not be used in any clinical or in vitro procedures for diagnostic purposes.

Ordering information

Product	No. per pack	Code No.
HiPrep Heparin FF 16/10	1 x 20 mL	28936549

Related products		
HiTrap™ Heparin HP	5 x 1 mL	17040601
HiTrap Heparin HP	1 x 5 mL	17040701
HiTrap Heparin HP	5 x 5 mL	17040703
Heparin Sepharose 6 Fast Flow	50 mL	17099801
Heparin Sepharose 6 Fast Flow	250 mL	17099825
Heparin Sepharose 6 Fast Flow	1 liter	17099803
HiPrep 26/10 Desalting	1 x 53 mL	17508701
HiPrep 26/10 Desalting	4 x 53 mL	17508702

Accessories		
HiTrap/HiPrep 1/16" male connector to ÄKTA design	8	28401081
To connect columns with 1/16" connections to FPLC System:	5	18385801
Union M6 female/1/16" male		

Related printed literature		
Handbook, Affinity Chromatography, Principles and Methods		18102229
Affinity Chromatography Columns and Media, Selection guide		18112186
Prepacked chromatography columns for ÄKTA design, Selection guide		28931778

Further information

For more information, check:

cytiva.com/protein-purification.

cytiva.com/purification_techsupport.

Handbook is also available, see [Ordering information, on page 3](#).

cytiva.com

Cytiva and the Drop logo are trademarks of Global Life Sciences IP Holdco LLC or an affiliate.

ÄKTA, HiPrep, HiTrap, and Sepharose are trademarks of Global Life Sciences Solutions USA LLC or an affiliate doing business as Cytiva.

All other third-party trademarks are the property of their respective owners.

© 2020 Cytiva

All goods and services are sold subject to the terms and conditions of sale of the supplying company operating within the Cytiva business. A copy of those terms and conditions is available on request. Contact your local Cytiva representative for the most current information.

For local office contact information, visit cytiva.com/contact

28941326 AF V:5 10/2020

