GE Healthcare

Instructions 28-9413-26 AD

HiPrep Heparin FF 16/10

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	6% highly cross-linked spherical agarose
Mean particle size	90 µm
Ligand	Heparin (porcine origin)
Ligand density	5 mg/ml medium
Ligand coupling	Reductive amination
Binding capacity	2 mg bovine antithrombin III/ml medium
Bed volume	20 ml
Bed height	100 mm
i.d.	16 mm
Column composition	Polypropylene
Recommended flow rate*	2–10 ml/min (60-300 cm/h)
Maximum flow rate*	10 ml/min (300 cm/h)
Maximum pressure over the packed bed during operation, Δp†	0.15 MPa, 1.5 bar, 22 psi
HiPrep column hardware pressure limit†	0.5 MPa, 5 bar, 73 psi
pH stability	
short term	pH 4–13
long term and working range	pH 4–12
Storage	4°C to 30°C in 20% ethanol, 0.05 M sodium acetate

* Water at room temperature. Flow rate is determined by $v \bullet \eta \leq 10$ ml/min where v = flow rate and η = viscosity.

† 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 pre-column pressure, the pressure drop over the gel 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 gel 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:

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

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

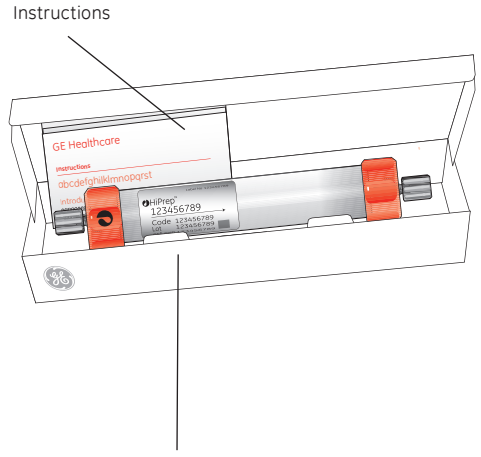
Ensure an appropriate pressure limit has been set. Equilibrate the column for first time use or after long storage by running:

1. 100 ml binding buffer e.g. 20 mM Tris-HCl, pH 8 at 5 ml/min.
2. 100 ml elution buffer, e.g. 20 mM Tris-HCl, 1 M NaCl, pH 8 at 5 ml/min.
3. 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)



HiPrep Heparin FF 16/10

Buffer and solvent resistance

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

Daily use

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

Cleaning

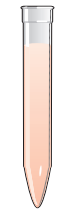
Sodium chloride, up to 2 M
Sodium hydroxide, up to 0.1 M
Guanidine hydrochloride, up to 6 M
Urea, up to 8 M
Ethanol, up to 70%
Non-ionic detergents, up to 0.1–0.5%

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)". 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.

HiPrep

Optimization

Perform your first run according to "Try these conditions first". If the results are unsatisfactory, consider the following:

Action	Effect
Change pH/buffer salt	Selectivity change, if the immobilized heparin works as a cation exchanger
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.
2. Removal of hydrophobically bound proteins:
   80 ml of 0.1-0.5% non-ionic detergent.
3. Inactivation of microbial contaminants:
   60 ml of 70% ethanol, stand for 12 hours.

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	28-9365-49

Related products

HiTrap™ Heparin HP	5 x 1 ml	17-0406-01
HiTrap Heparin HP	1 x 5 ml	17-0407-01
HiTrap Heparin HP	5 x 5 ml	17-0407-03
Heparin Sepharose 6 Fast Flow	50 ml	17-0998-01
Heparin Sepharose 6 Fast Flow	250 ml	17-0998-25
Heparin Sepharose 6 Fast Flow	1 liter	17-0998-03
HiPrep 26/10 Desalting	1 x 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 x 53 ml	17-5087-02

Accessories

HiTrap/HiPrep 1/16" male connector to ÄKTA design	8	28-4010-81
To connect columns with 1/16" connections to FPLC™ System:		
Union M6 female/1/16" male	5	18-3858-01

Related printed literature

Handbook, Affinity Chromatography, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media, Selection guide	18-1121-86
Prepacked chromatography columns for ÄKTA design, Selection guide	28-9317-78

Further information

For more information, please check:
www.gelifesciences.com/protein-purification
www.gelifesciences.com/purification_techsupport
Handbook is also available, see ordering information.

www.gelifesciences.com/protein-purification
www.gelifesciences.com/purification_techsupport

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

imagination at work

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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