# HiTrap™ Heparin HP, 1 ml and 5 ml

HiTrap Heparin HP is a prepacked ready to use, column for preparative affinity chromatography. The special design of the column, together with the matrix, provide fast, simple and easy separations in a convenient format.

The columns can be operated with a syringe, peristaltic pump or liquid chromatography system such as ÄKTA™.

# Table of contents



Please read these instructions carefully before using HiTrap columns.

**Intended use**

HiTrap columns are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

**Safety**

For use and handling of the product in a safe way, please refer to the Safety Data Sheet.

# 1 Product description

## HiTrap column characteristics

The columns are made of biocompatible polypropylene that does not interact with biomolecules.

The columns are delivered with a stopper at the inlet and a snap-off end at the outlet. Table 1 lists the characteristics of HiTrap columns.

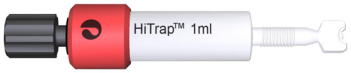


**Fig 1.** HiTrap, 1 ml column.


**Fig 2.** HiTrap, 5 ml column.

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

**Note:** *Make sure that the connector is tight to prevent leakage.*

**Table 1.** Characteristics of HiTrap columns.

| | | |
|---|---|---|
| Column volume (CV) | 1 ml | 5 ml |
| Column dimensions | 0.7 × 2.5 cm | 1.6 × 2.5 cm |
| Column hardware pressure limit | 5 bar (0.5 MPa) | 5 bar (0.5 MPa) |

**Note:** *The pressure over the packed bed varies depending on a range of parameters such as the characteristics of the chromatography medium, sample/liquid viscosity and the column tubing used.*

**Supplied Connector kit with HiTrap column**

| Connectors supplied | Usage | No. supplied |
|---|---|---|
| Union 1/16" male/ luer female | For connection of syringe to HiTrap column | 1 |
| Stop plug female, 1/16" | For sealing bottom of HiTrap column | 2, 5 or 7 |

## Medium properties

The nature of Heparin Sepharose™ High Performance makes it a very versatile tool for the separation of many proteins e.g., DNA binding proteins, growth factors, coagulation protein synthesis factors and steroid receptors.

The ligand in Heparin Sepharose High Performance is a naturally occurring sulfated glucosaminoglycan which is extracted from the native proteoglycan of porcine intestinal mucosa. Heparin consists alternating units of uronic acid and D-glucosamine, most of which are substituted with one or two sulfate groups. The molecular weight of the polymer is distributed over the range 5 000 to 30 000.

Heparin is covalently coupled to highly cross-linked agarose beads. The coupling method gives high capacity and high performance.

The medium is stable over the pH range 5 to 10, and tolerates all commonly used aqueous buffers.

The properties of the product are summarized in Table 2.

**Table 2.** HiTrap Heparin HP characteristics

| Ligand | Heparin |
|---|---|
| Degree of substitution | ~10 mg heparin/ml medium |
| Binding capacity | ~3 mg antithrombin III (bovine)/ml medium |
| Mean particle size | 34 µm |
| Bead structure | Highly cross-linked spherical agarose |
| Max. flow rates[1] | 4 ml/min and 20 ml/min for 1 ml and 5 ml column respectively |
| Rec. flow rates | 0.2 to 1 ml/min and 1.5 ml/min for 1 ml and 5 ml column respectively |
| Chemical stability | All commonly used buffers |
| pH stability[2] | |
| Long term | 5 to 10 |
| Short term | 5 to 10 |
| Storage | 4ºC to 30ºC in 20% ethanol |

[1] Water at room temperature

[2] The ranges given are estimates based on our knowledge and experience. Please note the following:
**pH stability, long term** refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
**pH stability, short term** refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.

# 2 Operation

The column can be operated with a syringe, peristaltic pump or a chromatography system.

Immobilized heparin has two main modes of interaction with proteins. It can operate as an affinity ligand; e.g., in its interaction with coagulation factors. Heparin has also a function as a high capacity cation exchanger due to its anionic sulphate groups.

Recommended elution conditions for both cases is to increase the ionic strength. Elution using a continuous or step gradient with NaCl, KCl or $(NH_4)_2SO_4$ up to 1.5 to 2 M is most frequently used.

## Buffer preparation

Water and chemicals used for buffer preparation should be of high purity. It is recommended to filter the buffers by passing them through a 0.45 µm filter before use.

**Recommended buffers:.**

**Binding buffer:** 10 mM sodium phosphate, pH ~7

**Elution buffer:** 10 mM sodium phosphate, 1-2 M NaCl, pH ~7

## Sample preparation

The sample should be adjusted to the composition of the binding buffer. This can be done by either diluting the sample with binding buffer or by buffer exchange using HiTrap Desalting, HiPrep™ 26/10 Desalting or PD-10 column. The sample should be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column.

# 3 Purification

The recommended flow rate for HiTrap Heparin HP is 0.1 to 1 ml/min or 1 to 5 ml/min for 1 ml or 5 ml column respectively.

**1** Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, "drop to drop" to avoid introducing air into the column.

**2** Remove the snap-off end at the column outlet.

**3** Wash out the preservative and equilibrate the column with 10 column volumes of binding buffer.

**4** Apply the sample, using a syringe fitted to the luer connector or by pumping it onto the column.

**5** Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.

**6** Elute with 5 to 10 column volumes of elution buffer using a continuous or step gradient.

**7** The purified fractions can be desalted using HiTrap Desalting, HiPrep 26/10 Desalting or PD-10 column if necessary (Table 3).

**Note:** *If a P-1 pump is used a max flow rate of 1 to 3 ml/min can be run on a HiTrap 1 ml column packed with Sepharose High Performance media.*

**Table 3.** Prepacked columns for desalting and buffer exchange

| Column | Code No. | Loading volume | Elution volume | Comments | Application |
|---|---|---|---|---|---|
| HiPrep 26/10 Desalting | 17-5087-01 | 2.5–15 ml | 7.5–20 ml | Prepacked with Sephadex™ G-25 Fine. Requires a laboratory pump or a chromatography system to run. | For desalting and buffer exchange of protein extracts ($M_r$ > 5000). |
| HiTrap Desalting | 17-1408-01 | 0.25–1.5 ml | 1.0–2.0 ml | Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run. | |
| PD-10 Desalting | 17-0851-01 | 1.0–2.5 ml[1]<br>1.75–2.5 ml[2] | 3.5 ml[1]<br>Up to 2.5 ml[2] | Prepacked with Sephadex G-25 Medium. Runs by gravity flow or centrifugation | For desalting, buffer exchange, and cleanup of proteins and other large biomolecules ($M_r$ > 5000). |
| PD MiniTrap™ G-25 | 28-9180-07 | 0.1– 0.5 ml[1]<br>0.2–0.5 ml[2] | 1.0 ml[1]<br>Up to 0.5 ml[2] | | |
| PD MidiTrap™ G-25 | 28-9180-08 | 0.5–1.0 ml[1]<br>0.75–1.0 ml[2] | 1.5 ml[1]<br>Up to 1.0 ml[2] | | |

[1] Volumes with gravity elution
[2] Volumes with centrifugation

# 4 Scaling up

For quick scale-up of purification, two or three HiTrap Heparin HP 1 ml or 5 ml columns can be connected in series (backpressure will increase).

It is also possible to scale up using the prepacked HiPrep Heparin 16/10 FF column (20 ml). This column is prepacked with Heparin Sepharose Fast Flow.

**Note:** *Heparin Sepharose Fast Flow is based on 90 µm beads and is another matrix compared to Heparin Sepharose High Performance (34 µm beads). It may therefore be necessary with some optimization of the purification method to get exactly the same purity of the target protein.*

# 5 Adjusting pressure limits in chromatography system software

Pressure generated by the flow through a column affects the packed bed and the column hardware, see Fig 3. Increased pressure is generated when running/using one or a combination of the following conditions:

- High flow rates
- Buffers or sample with high viscosity
- Low temperature
- A flow restrictor

**Note:** *Exceeding the flow limit (see Table 2) may damage the column.*

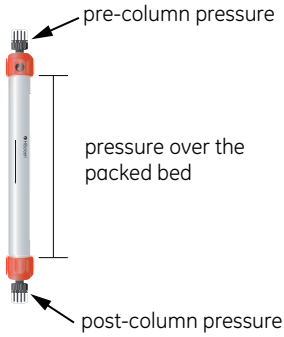


**Fig 3.** Pre-column and post-column measurements.

## ÄKTA avant

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, $\Delta$p). The pre-column pressure limit is the column hardware pressure limit (see Table 1).

The maximum pressure the packed bed can withstand depends on media characteristics and sample/liquid viscosity. The measured value also depends on the tubing used to connect the column to the instrument.

### ÄKTAexplorer, ÄKTApurifier, ÄKTAFPLC and other systems with pressure sensor in the pump

To obtain optimal functionality, the pressure limit in the software may be adjusted according to the following procedure:

1. Replace the column with a piece of tubing. Run the pump at the maximum intended flow rate. Note the pressure as *total system pressure*, P1.
2. Disconnect the tubing and run the pump at the same flow rate used in step 1. Note that there will be a drip from the column valve. Note this pressure as P2.
3. Calculate the new pressure limit as a sum of P2 and the column hardware pressure limit (see Table 1). Replace the pressure limit in the software with the calculated value.

   The actual pressure over the packed bed ($\Delta$p) will during run be equal to actual measured pressure - *total system pressure* (P1).

**Note:** *Repeat the procedure each time the parameters are changed.*

# 6 Storage

Wash the column with 5 column volumes of 20% ethanol at 1 ml/min (HiTrap 1 ml column) or at 5 ml/min (HiTrap 5 ml column). Store the column in 20% ethanol at 4ºC to 30ºC.

# 7 Ordering information

| Product | No. Supplied | Code No. |
|---|---|---|
| HiTrap Heparin HP | 5 × 1 ml | 17-0406-01 |
| | 1 × 5 ml | 17-0407-01 |
| | 5 × 5 ml | 17-0407-03 |

| Related products | No. Supplied | Code No. |
|---|---|---|
| HiPrep Heparin FF 16/10 | 1 × 20 ml | 28-9365-45 |
| HiTrap Desalting | 1 × 5 ml | 29-0486-84 |
| | 5 × 5 ml | 17-1408-01 |
| | 100 × 5 ml[1] | 11-0003-29 |
| HiPrep 26/10 Desalting | 1 × 53 ml | 17-5087-01 |
| | 4 × 53 ml | 17-5087-02 |
| PD-10 Desalting Column | 30 | 17-0851-01 |

[1] Special package. Delivered on specific customer order.

| Accessories | Quantity | Code No. |
|---|---|---|
| 1/16" male/luer female<br>*(For connection of syringe to top of HiTrap column)* | 2 | 18-1112-51 |
| Tubing connector flangeless/M6 female<br>(*For connection of tubing to bottom of HiTrap column)* | 2 | 18-1003-68 |
| Tubing connector flangeless/M6 male<br>*(For connection of tubing to top of HiTrap column)* | 2 | 18-1017-98 |
| Union 1/16" female/M6 male<br>*(For connection to original FPLC System through bottom of HiTrap column)* | 6 | 18-1112-57 |
| Union M6 female /1/16" male<br>*(For connection to original FPLC System through top of HiTrap column)* | 5 | 18-3858-01 |
| Union luerlock female/M6 female | 2 | 18-1027-12 |
| HiTrap/HiPrep, 1/16" male connector for ÄKTA design | 8 | 28-4010-81 |
| Stop plug female, 1/16"<br>*(For sealing bottom of HiTrap column)* | 5 | 11-0004-64 |
| Fingertight stop plug, 1/16" | 5 | 11-0003-55 |

| Related literature | Code No. |
|---|---|
| Affinity Chromatography Handbook, Principles and Methods | 18-1022-29 |
| Affinity Chromatography Columns and Media Selection Guide | 18-1121-86 |

For your local office contact information, visit:
www.gelifesciences.com/contact

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

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

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

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

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



imagination at work

71-7004-00 AU 00/2014