HiTrap Blue HP, 1 ml and 5 ml

Instructions for Use

HiTrap™ Blue HP is a prepacked ready to use, column for preparative affinity chromatography. The special design of the column, together with the medium, 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™.

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Important

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.

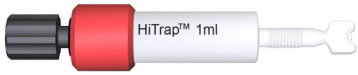

Fig 1. HiTrap, 1 ml column.

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 x 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

Chromatography medium properties

The nature of Blue Sepharose™ High Performance makes it a very versatile tool for the separation of many proteins e.g. enzymes requiring adenylcontaining cofactors (including NAD^+ and $NADP^+$), albumin, coagulation factors and interferon. The ligand is a dye, Cibacron Blue F3G-A, which is covalently attached to the highly cross-linked agarose medium via the triazine part of the dye molecule.

The medium is stable over the pH range 4–12, and tolerates all commonly used aqueous buffers.

The characteristics of the product are summarized in Table 2.

Table 2. HiTrap Blue HP characteristics

Chromatography medium	Blue Sepharose High Performance
Ligand	Cibacron Blue F3G-A
Degree of substitution	~ 4 mg Cibacron Blue F3G-A/ml medium
Binding capacity	~ 20 mg human albumin/ml medium
Mean particle size	34 µm
Bead structure	Highly cross-linked spherical agarose, 6%
Max. flow rates	4 ml/min and 20 ml/min for 1 ml and 5 ml column respectively
Rec. flow rates	1 ml/min and 5 ml/min for 1 ml and 5 ml column respectively
Chemical stability	All commonly used buffers, 70% ethanol, 8 M urea and 6 M guanidine hydrochloride
pH stability[1]	
Long term	4 to 12
Short term	3 to 13
Storage	4°C to 30°C in 0.1 M potassium phosphate and 20% ethanol

[1] 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

Blue Sepharose High Performance is a group specific adsorbent with affinity for a wide variety of enzymes. Some proteins interact biospecifically with the dye due to its structural similarity with nucleotide cofactors while others, such as albumin and interferon, bind in a less specific manner by electrostatic and/or hydrophobic interactions with the aromatic anionic ligand. Biospecifically adsorbed proteins can be eluted by low concentrations of the free cofactor, or increased ionic strength. Less specifically bound proteins require the use of much higher cofactor, or salt concentrations. Desorption with cofactors normally occurs in the range 1–20 mM. Desorption by increasing ionic strength is normally complete at salt concentrations 2 M or less (NaCl or KCl are suitable).

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.

These are some recommended buffers:

Binding buffer:	50 mM KH_2PO_4, pH 7.0 or 20 mM sodium phosphate, pH 7.0
Elution buffer:	50 mM KH_2PO_4, 1.5 M KCl, pH 7.0 or 20 mM sodium phosphate, 2 M NaCl, pH 7.0

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, PD-10 column or a HiPrep™ 26/10 Desalting column depending on the sample volume. The sample should be filtered through a 0.45 µm filter or centrifuged immediately before it is applied to the column.

Purification

1. Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided luer 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 5–10 column volumes of binding buffer.
4. Apply the sample at 0.5–1 ml/min or 2–5 ml/min for the HiTrap 1 ml or 5 ml column respectively, using a syringe fitted to the luer connector or by pumping it onto the column.
5. Wash with at least 5–10 column volumes of binding buffer or until no material appears in the effluent.
6. Elute with 5–10 column volumes of elution buffer.
7. The purified fractions can be desalted using HiTrap Desalting, PD-10 column or HiPrep 26/10 Desalting if necessary.

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

3 Scaling up

For quick scale-up of purifications, two or three HiTrap columns can be connected in series (backpressure will increase).

4 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.*

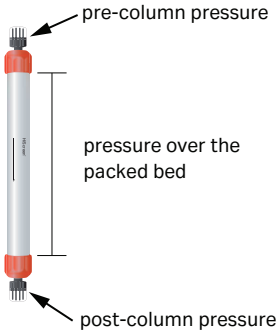

Fig 3. Pre-column and post-column measurements.

ÄKTA avant and ÄKTA pure

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, Δp). 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 (Δp) will during run be equal to actual measured pressure - *total system pressure* (P1).

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

5 Storage

Store the column at 4°C to 30°C in 0.1 M potassium phosphate and 20% ethanol.

6 Ordering information

Product	No. Supplied	Code No.
HiTrap Blue HP	5 × 1 ml	17-0412-01
HiTrap Blue HP	1 × 5 ml	17-0413-01

Related products	No. Supplied	Code No.
HiTrap Desalting	1 × 5 ml	29-0485-65
	5 × 5 ml	17-1408-01
PD-10 Desalting column	30	17-0851-01
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

Accessories	No. Supplied	Code No.
1/16" male/luer female *(For connection of syringe to top of HiTrap column*)	2	18-1112-51
Tubing connector flangeless/M6 female (*For connection of tubing to bottom of HiTrap column)*	2	18-1003-68
Tubing connector flangeless/M6 male *(For connection of tubing to top of HiTrap column)*	2	18-1017-98
Union 1/16" female/M6 male *(For connection to original FPLC System through bottom of HiTrap column)*	6	18-1112-57
Union M6 female /1/16" male *(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" *(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
Prepacked chromatography columns for ÄKTA systems, Selection guide	28-9317-78

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