HiTrap Chelating HP, 1 ml and 5 ml

Instructions for Use

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Abstract

HiTrap™ Chelating 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™.

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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

Medium properties

HiTrap Chelating HP 1 ml and 5 ml are packed with 1 ml and 5 ml of Chelating Sepharose™ High Performance, respectively.

Chelating Sepharose High Performance consists of highly cross-linked agarose beads to which iminodiacetic acid has been coupled by stable ether groups via a spacer arm (seven-atoms). This coupling technique gives both high capacity and high performance. The medium is stable over the pH range 3–13, and tolerates all commonly used aqueous buffers and denaturants, such as 6 M guanidine hydrochloride, 8 M urea, and chaotropic salts. Several amino acids, for example histidine, form complexes with many metal ions. Chelating Sepharose High Performance, charged with suitable metal ions, will selectively retain proteins if complex forming amino acid residues are exposed on the surface of the protein.

The characteristics of the product are summarized in Table 2.

Table 2. HiTrap Chelating HP characteristics.

Chelating group	Iminodiacetic acid
Metal ion capacity	~ 23 µmol Cu^{2+}/ml medium
Binding capacity	~ 12 mg pure $(histidine)_6$-tagged protein (M_r ~ 27 600) per ml/medium
Mean particle size	34 µm
Bead structure	Highly cross-linked spherical agarose, 6%
Max back pressure	0.3 MPa, 3 bar
Max. flow rates	4 ml/min and 20 ml/min for 1 ml and 5 ml column respectively
Recommended flow rate	1 ml/min and 5 ml/min for 1 ml and 5 ml column respectively
Chemical stability	Stable in all commonly used buffers and denaturants such as 6 M guanidine hydrochloride, 8 M urea and chaotropic salts.
pH stability[1]	
short term	2 to 14
long term	3 to 13
Avoid (during purification)	Chelating agents, e.g. EDTA, EGTA Reducing agents, e.g. DTT, DTE
Storage	20% ethanol at 4°C to 30°C

[1] The ranges given are estimates based on our knowledge and experience. Please note the following:
pH stability, short term, refers to the pH interval for regeneration, cleaning-in-place and sanitization procedures.
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.

2 General considerations

HiTrap Chelating HP is supplied free of metal ions and has to be charged with a suitable metal ion before use. It is not always possible to predict which metal ion is most appropriate. The metal ions most often used are nickel (Ni^{2+}), copper (Cu^{2+}) and zinc (Zn^{2+}). A single exposed histidine residue may result in adsorption of the protein on Cu^{2+} while two vicinal histidine residues are needed for adsorption on Zn^{2+}. Ni^{2+} ions are often used for the purification of recombinant histidine-tagged proteins. In some cases iron (Fe^{2+}), cobalt (Co^{2+}), and calcium (Ca^{2+}) have been used with success.

It is a possibility to check different metal ions for optimizing the purification of histidine-tagged proteins, as different metal ions give different binding of the protein.

The choice of binding buffer depends on the properties of the chelated metal ion and the binding properties of the sample molecules. Adsorption at neutral to alkaline pH's in the presence of 0.5 M–1.0 M NaCl is recommended. Sodium acetate and sodium phosphate buffers are often used. Tris-HCl tends to reduce binding and should only be used when the metal-protein affinity is high. Chelating agents such as EDTA or citrate should not be included.

Note: *Phospate buffers can not be used in combination with Ca^{2+} because of the risk of formation of insoluble $Ca_3(PO_4)_2$-crystals.*

The addition of salt, e.g. 0.5–1.0 M NaCl, in the eluent is to eliminate any ion exchange effects. This can also have a marginal effect on the retention of proteins.

Elution of the proteins from the medium can be achieved by several different methods or combinations of methods. pH adjustment within the range of 2.5–7.5 is a frequently used technique. *At pH values below 4, metal ions will be stripped off the medium.*

A displacing agent such as ammonium chloride, imidazole or histidine are also frequently used. Chelating agents such as EGTA or EDTA will strip the metal ions from the medium and cause desorption.

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

Note: *Ni^{2+} may cause allergic reactions.*

3 Operation

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.

As a general method when Cu^{2+} is used as the metal ion, we recommend the following start and elution buffers.

Alt. 1: **Competitive elution**

Binding buffer: 0.02 M sodium phosphate, 1 M NaCl, pH 7.2

Elution buffer: 0.02 M sodium phosphate, 1 M NH_4Cl, pH 7.2

Alt. 2: **Lowering of pH**

Binding buffer: 0.02 M sodium phosphate, 0.5 M NaCl, pH 7.2

Elution buffer: 0.02 M sodium phosphate, 0.5 M NaCl, pH 3.5

Alt. 3: **Stripping elution**

Binding buffer: 0.02 M sodium phosphate, 0.5 M NaCl, pH 7.2

Elution buffer: 0.02 M sodium phosphate, 0.5 M NaCl, 0.05 M EDTA, pH 7.2

Note: *Elution using "Alternative 2" leads to the eluted proteins being exposed to low pH. If the proteins are pH sensitive, it is recommended that the eluted fractions should be collected in tubes containing 1 M Tris-HCl, pH 9.0 (60-200 µl/ml fraction) to restore the pH to neutral.*

"Alternative 3" results in the presence of the metal ion bound to EDTA in the eluate. Thus, it may be necessary to perform a buffer exchange step, e.g. using a HiTrap Desalting column (Table 3), to achieve the correct buffer conditions. See *also "Purification of histidine-tagged recombinant proteins", page 12.*

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, *see* Table 3.

For optimal conditions for growth, induction and cell lysis conditions of your recombinant histidine-tagged clones, please refer to recommended protocols.

Table 3. Prepacked columns for desalting and buffer exchange

Column	Loading volume	Elution volume
HiPrep 26/10 Desalting[1]	2.5 to 15 mL	7.5 to 20 mL
HiTrap Desalting[2]	0.25 to 1.5 mL	1.0 to 2.0 mL
PD-10 Desalting[3]	1.0 to 2.5 mL[4]	3.5 mL
	1.75 to 2.5 mL[5]	Up to 2.5 mL
PD MiniTrap™ G-25	0.1 to 2.5 mL[4]	1.0 mL
	0.2 to 0.5 mL[5]	Up to 0.5 mL
PD MidiTrap™ G-25	0.5 to 1 mL[4]	1.5 mL
	0.75 to 1 mL[5]	Up to 1 mL

1 Prepacked with Sephadex™ G-25 Fine and requires a pump or a chromatography system to run.

2 Prepacked with Sephadex G-25 Superfine and requires a syringe or pump to run.

3 Prepacked with Sephadex G-25 and can be run by the gravity flow or centrifugation.

4 Volumes with gravity elution.

5 Volumes with centrifugation.

Column preparation

1. Fill the syringe or pump tubing with distilled water. 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 system.
2. Remove the snap-off end at the column outlet.
3. Wash the column with 5 ml or 15 ml distilled water for HiTrap 1 ml or 5 ml column, respectively. At this stage do not use buffer instead of water to wash away the 20% ethanol solution as metal ion precipitation can occur in step 4, depending on the buffer used.
4. Load 0.5 ml or 2.5 ml of 0.1 M metal salt solution (metal chloride and -sulphate salts e.g. 0.1 M $CuSO_4$ or 0.1 M $NiSO_4$ are commonly used) in distilled water on HiTrap 1 ml and 5 ml column respectively.
5. Wash with distilled water, 5 ml or 15 ml respectively.

Note: *When working with Fe^{3+} extra precautions have to be taken.*

In neutral solutions, Fe^{3+} is easily reduced and forms compounds that can be hard to dissolve. Media loaded with Fe^{3+} should not be left for long times in neutral solutions. Fe^{3+} should be immobilized in low pH, approximately pH 3, to avoid precipitation of insoluble compounds.

After charging the column and competitive elution is going to be used during the purification, perform a blank run to elute unspecifically bound metal ions that might otherwise be eluted during the desorption. Add 5 column volumes elution buffer, using the conditions planned for the separation step. Re-equilibrate the column with 5–10 column volumes of start buffer before sample application.

When performing lowering pH or stripping elution, no blank run should be done with elution buffer since this will remove metal ions from the medium.

4 Purification

1 After column preparation equilibrate the column with binding buffer by washing with 5–10 column volumes. Recommended flow rates are 1 ml/min or 5 ml/min for 1 ml and 5 ml column respectively.

2 Apply the sample, using a syringe or a pump. A partial displacement of chelated metal ions is often noted as the protein is adsorbed. This is visible, especially when using metal ions that are colored, such as Cu^{2+} and Ni^{2+}, as a downward extension of the zone of chelated ions.

The volume of the sample is not critical if substances are tightly bound under binding conditions. Weakly bound substances should be applied in a small volume (about 5% of bed volume) to avoid co-elution with non-adsorbed material.

3 Wash with 5–10 column volumes binding buffer. To increase the purity of eluted protein a wash with binding buffer containing 5–40 mM imidazole is often effective when working with recombinant $(histidine)_6$-tagged proteins (3–5 column volumes).

Note: *If the protein of interest is not bound tightly to the column the imidazole concentration should be kept low to avoid too early elution.*

4 Elute with elution buffer using a step or linear gradient. 2–5 column volumes is usually sufficient if the molecule of interest is rapidly eluted, e.g. a simple protein mixture eluted by a step gradient. Other volumes (or a different elution buffer) may be required if the interaction is difficult to break. A shallow gradient is used to separate proteins with similar binding strengths, e.g. a linear gradient of 10–20 column volumes.

Note: *500 mM imidazole has A_{280} ~ 0.5 (5 mm cell). Use the elution buffer as blank. If imidazole needs to be removed from the protein use HiTrap Desalting, HiPrep 26/10 Desalting or PD-10 columns.*

Note: *If a P1-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.*

Re-equilibration

Strip the column by washing with 5 column volumes start buffer containing 0.05 M EDTA. This should be followed by washing with 5–10 columns volumes of distilled water before re-charging the column following the instructions under column preparation. The loss of metal ions is more pronounced at lower pH. The column does not have to be stripped between each purification if the same protein is going to be purified. Then perform stripping and re-charging of the column after 5–10 purifications.

The reuse of HiTrap Chelating HP depends on the nature of the sample and should only be performed with identical recombinant proteins, to prevent cross-contamination.

5 Purification of histidine-tagged recombinant proteins

HiTrap Chelating HP, when charged with Ni^{2+} ions, will selectively retain proteins if complex-forming amino acid residues, in particular histidine, are exposed on the surface of the protein. Histidine-tagged proteins can be eluted from HiTrap Chelating HP with buffers containing imidazole.

Recommended buffers

Binding buffer:	0.02 M sodium phosphate, 0.5 M NaCl, 5–40 mM imidazole, pH 7.4
Elution buffer:	0.02 M sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4

If the recombinant histidine-tagged proteins are expressed as inclusion bodies add 6 M guanidine hydrochloride or 8 M urea to all buffers. For further information, *see* Ref. 1 and 2.

6 Scaling up

Two or three HiTrap 1 ml resp. 5 ml columns can be connected in series for quick scaling up of purifications (backpressure will increase).

7 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.*

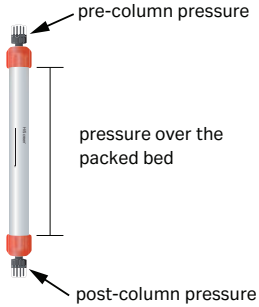

Fig 3. Pre-column and post-column measurements.

ÄKTA avant

The system will automatically monitor the pressures (pre-column pressure and pressure over the packed bed, 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.*

8 Storage

Store the column in 20% ethanol at 4°C to 30°C. For longer storage the column should be stripped of metal ions.

9 References

1. Colangeli R., et al. Three-step purification of lipopolys-accaridefree polyhistidine-tagged recombinant antigens of Myobacterium tuberculosis. J. of Chromatography B, 714 (1998), 223–235.
2. Rapid and efficient purification and refolding of a $(histidine)_6$-tagged recombinant protein produced in *E. coli* as inclusion bodies, 18-1134-37, GE.

10 Ordering information

Product	No. supplied	Product code
HiTrap Chelating HP	5 × 1 ml	17-0408-01
	1 × 5 ml	17-0409-01
	5 × 5 ml	17-0409-03
	100 × 5 ml[1]	17-0409-05

Related products	No. supplied	Product code
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 × 20 ml	17-5087-01
	4 × 20 ml	17-5087-02
PD-10 Desalting Column	30	17-0851-01

[1] Special pack size delivered on specific customer order.

Accessories	Quantity	Product code
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

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