HiTrap Chelating HP

HiTrap™ Chelating HP is one of a range of prepacked, ready to use, 1 ml and 5 ml columns for preparative metal chelate affinity chromatography. Fast, simple, and easy separations are provided by the combination of a specially designed column and a high-performance affinity medium. HiTrap Chelating HP is particularly suitable for the isolation and purification of proteins and peptides containing exposed histidine residues.

- Fast and convenient to use
- Packed with Chelating Sepharose™ High Performance
- Simple operation with a syringe, pump or chromatographic system such as ÄKTA™design or FPLC™ System
- Economical

HiTrap Chelating HP columns are easily operated by a syringe. Alternatively, a laboratory pump, alone or within a chromatography system, can be advantageous, especially when linear gradients are required. 1 ml and 5 ml columns are available.

Column characteristics

The HiTrap column is made of polypropylene, a material which is biocompatible and does not interact with biomolecules. Top and bottom frits are manufactured from porous polyethylene. The column is delivered with a stopper on the inlet and a twist-off end on the outlet. Both ends have M6 connections (6 mm metric threads).

Medium characteristics

Sepharose High Performance is the base matrix for HiTrap Chelating HP. The carbohydrate nature of the agarose base provides a hydrophilic and chemically favourable environment for coupling, while the highly cross-linked structure of the 34 µm spherical beads ensures excellent chromatographic properties. Fast kinetics and high dynamic capacities are properties of all HiTrap affinity columns.

The metal chelate-forming ligand iminodiacetic acid is coupled to the Sepharose High Performance matrix by stable ether bonds via a seven-atom spacer arm. This gives a very stable adsorbent that can be used over the pH range 4–12.

Fig 1. Prepacked with Chelating Sepharose High Performance, HiTrap Chelating HP columns offer fast and simple affinity purifications of proteins and peptides containing exposed histidine residues.

When charged with a suitable metal ion, HiTrap Chelating HP will selectively retain proteins if complex-forming amino acid residues, e.g. histidine, are exposed on the surface of the protein.

HiTrap Chelating HP is supplied free of metal ions and has to be charged with a suitable ion before use. (Histidine forms complexes with many transition metal ions and it is not always possible to predict which ion is most appropriate.) The metal ions most often used are nickel (Ni^{2+}), copper (Cu^{2+}), zinc (Zn^{2+}), cobalt (Co^{2+}) and calcium (Ca^{2+}). Cu^{2+} affords strong binding and some proteins have specificity, Zn^{2+} normally gives weaker binding. See Application Note, 18-1145-18, ”Purification of $(His)_6$-tagged proteins using HiTrap Chelating HP columns charged with different metal ions”(1).
The metal ion capacity of HiTrap Chelating HP is about 23 µmoles Cu^{2+}/ml gel.

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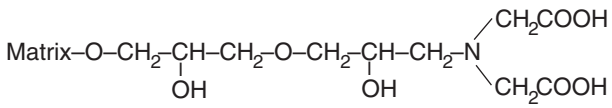
Figure 2 shows the partial structure of Chelating Sepharose High Performance. Table 1 summarizes the main media characteristics.



Chelating Sepharose High Performance

Fig 2. Partial structure of Chelating Sepharose High Performance.

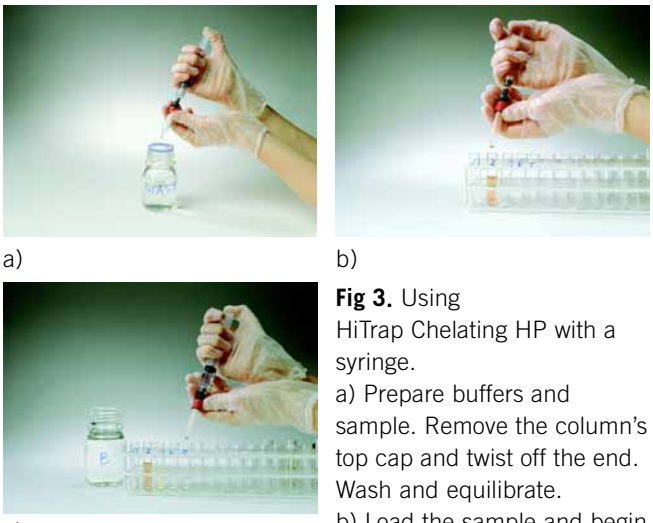
Table 1. Main characteristics of HiTrap Chelating HP.

Column dimensions	0.7×2.5 cm (1 ml) 1.6×2.5 cm (5 ml)
Ligand	Iminodiacetic acid
Binding capacity	Approx. 23 µmoles Cu^{2+}/ml gel
Mean particle size	34 µm
Bead structure	Highly cross-linked spherical agarose
Max. back pressure	0.3 MPa, 3 bar
Max. flow rate	4 ml/min (1 ml), 20 ml/min (5 ml)
Recommended flow rate	1 ml/min (1 ml), 5 ml/min (5 ml)
pH stability*	
Long term	4–12
Short term	3–13
Temperature stability	
Regular use	+4 °C to room temp.
Storage	+4 to +8 °C
Storage buffer	20% ethanol

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

Operation

Like all HiTrap columns, HiTrap Chelating HP is quick and easy to use. Instructions and connectors are included with each pack of columns. In general, the separation can be easily achieved with a syringe (using the luer adaptor provided). Figure 3 illustrates this technique. Alternatively, the column can be operated using a laboratory pump via an M6 tubing fitting or be connected to an ÄKTA design system via supplied connectors. For easy scale-up, two or more columns can be connected in series by screwing the end of one into the top of the next.



a) b) c)

Fig 3. Using HiTrap Chelating HP with a syringe.
a) Prepare buffers and sample. Remove the column's top cap and twist off the end. Wash and equilibrate.
b) Load the sample and begin collecting fractions.
c) Elute and continue collecting fractions.

Applications

Metal chelate affinity chromatography separates proteins and peptides on the basis of their affinity for metal ions that have been immobilized by chelation. Certain amino acids (e.g. histidine and cysteine) form complexes with the chelated metals around neutral pH (pH 6–8). It is primarily the histidine content of a protein that is responsible for its binding to a chelated metal, which makes the technique an excellent method for purifying recombinant proteins such as poly-histidine fusions, as well as many natural proteins. Metalloproteins are not usually suitable candidates for purification since they tend to scavenge the metal ions from the column.

His-tagged protein purifications can be grouped into several categories, such as the purification of soluble proteins, the purification of insoluble proteins expressed as inclusion bodies, and the refolding and purification of insoluble proteins expressed as inclusion bodies. Figures 5–7 show examples of all three categories. They also illustrate the use of different metal ions.

Figure 4 shows an example of the purification of naturally occurring proteins using a Cu^{2+}-loaded HiTrap Chelating HP column.

Natural proteins

Figure 4 shows the purification of egg white proteins.

Sample:	200 µl egg white (10% in binding buffer filtered through a glass filter)
Column:	HiTrap Chelating HP, 1 ml, Cu^{2+}-loaded according to the instructions
Metal ion:	Cu^{2+}
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
Flow rate:	0.5 ml/min
Gradient:	8 ml linear gradient 0–100% in elution buffer
Equipment:	FPLC System

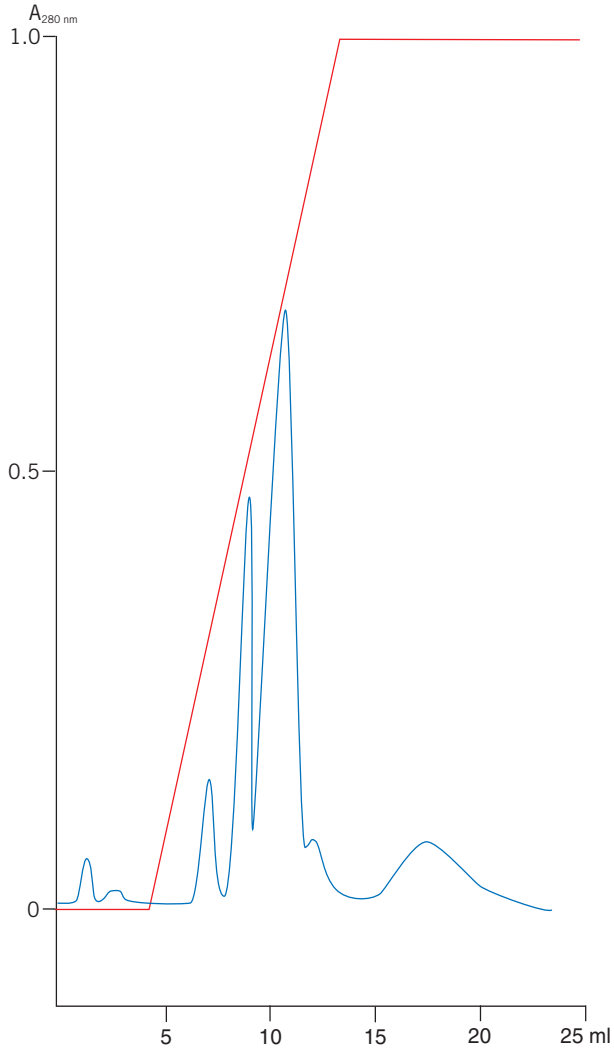
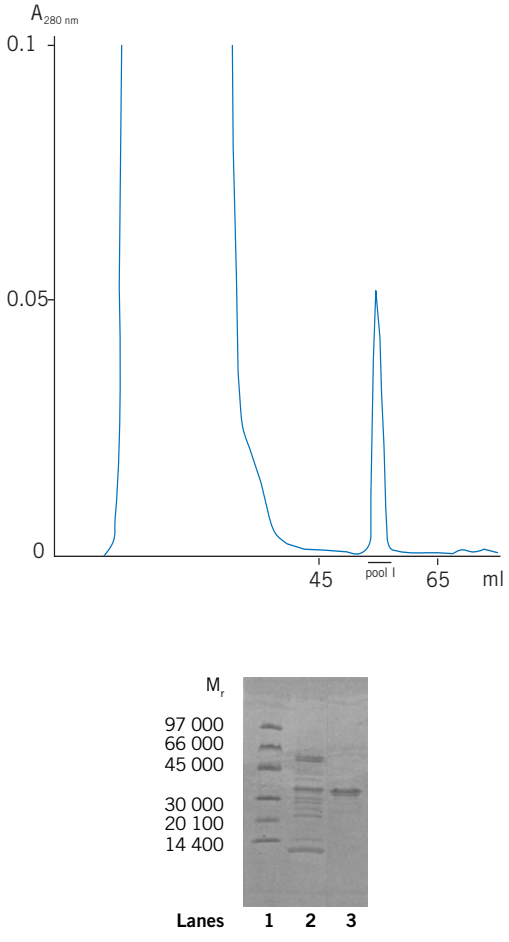


Fig 4. Purification of egg white proteins on Cu^{2+}-loaded HiTrap Chelating HP column, 1 ml.

Soluble recombinant proteins

Figure 5 shows the purification of a soluble $(HisGly)_4$His-tagged recombinant protein.

Sample:	9 ml *E. coli* periplasm containing Protein A-$(HisGly)_4$His. Diluted with 9 ml binding buffer.
Column:	HiTrap Chelating HP, 5 ml
Metal ion:	Zn^{2+}
Flow rate:	1.0 ml/min
Binding buffer:	50 mM sodium phoshate, 0.1 M NaCl, pH 8.0
Elution buffer:	50 mM sodium phosphate, 0.1 M NaCl, pH 4.0
Gradient:	20 ml elution buffer, step gradient
Electrophoresis:	SDS-PAGE, PhastSystem™, PhastGel™ Gradient 8–25, 1 µl sample, Coomassie™ stained



Lane 1: Low Molecular Weight Calibration Kit (LMW), reduced
Lane 2: Crude periplasmic fraction, reduced
Lane 3: Pool I, purified Protein A-$(HisGly)_4$His, reduced

Fig 5. Purification of recombinant proteins as poly-histidine fusions on Zn^{2+}-loaded HiTrap Chelating HP column, 5 ml.

Insoluble recombinant proteins

The heterologous expression of foreign genes in *Eschericia coli* can be engineered to lead either to the intracellular accumulation of recombinant protein, or to secretion and accumulation in the periplasmic space. The magnitude of protein production is generally much higher when intracellular expression is used.

However, recombinant protein accumulated intracellularly is frequently laid down in the form of inclusion bodies, which are insoluble aggregates of misfolded protein lacking biological activity.

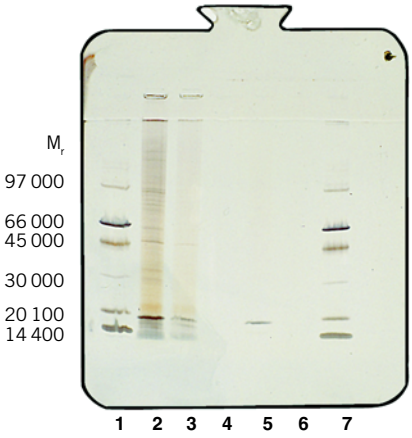
Figure 6 shows the analysis of a purification under denaturing conditions of a cell extract containing a $(His)_{10}$-tagged protein.

Refolding and purification of an insoluble recombinant protein in a single step

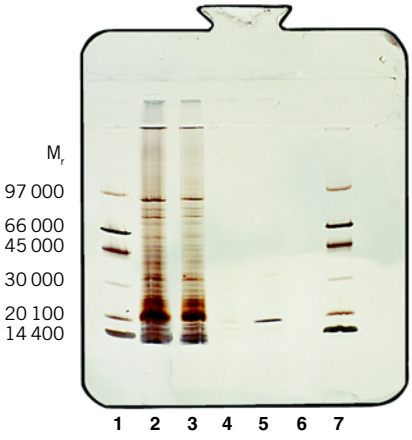
Conventional methods for refolding insoluble recombinant proteins are associated with several drawbacks. However, tagging the recombinant protein by adding several consecutive histidine residues opens the possibility of efficient purification and refolding in a single chromatographic step. Since binding of the histidine tract to immobilized divalent metal ions can occur in the presence of urea or guanidine hydrochloride, $(His)_6$-tagged inclusion body protein can be solubilized in urea or guanidine hydrochloride and bound directly to HiTrap Chelating HP. Removal of contaminating proteins and refolding by buffer exchange to non-denaturing conditions can then be performed before elution of the protein from the column (Ref. 2–6).

Figure 7 shows a general protocol for purifying and refolding a $(His)_6$-tagged recombinant protein produced in *E. coli* in a single step.

Sample:	8 ml cell extract containing $(His)_{10}$-tagged protein. The clone was a kind gift from Dr. C. Fuller and S. Brasher, Department of Biochemistry, University of Cambridge, UK
Column:	HiTrap Chelating HP, 1 ml
Metal ion:	Ni^{2+}
Binding buffers:	20 mM sodium phosphate, 0.5 M NaCl, 100 mM imidazole, 8 M urea or 6 M guanidine hydrochloride, pH 7.4
Elution buffers:	20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, 8 M urea or 6 M guanidine hydrochloride, pH 7.4
Flow rate:	Approx. 4 ml/min
Equipment:	Syringe
Electrophoresis:	SDS-PAGE, PhastSystem, PhastGel 10–15, 1 µl sample, silver staining



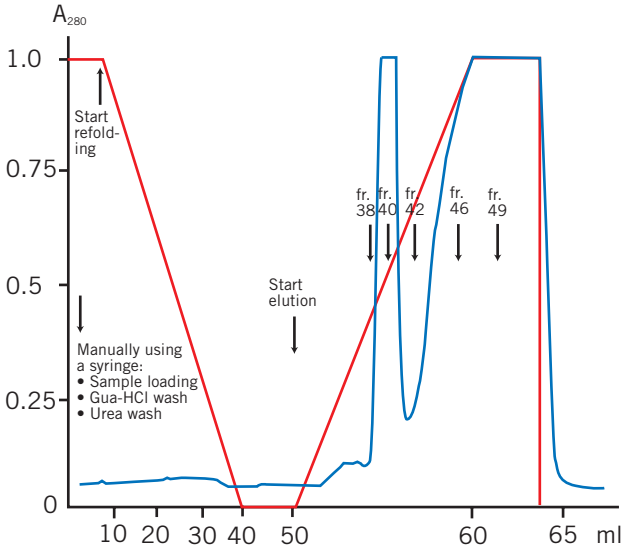
Purification in 8 M Urea
Lane 1: Low Molecular Weight Calibration Kit (LMW)
Lane 2: Starting material, cell extract, diluted 1:20
Lane 3: Flow-through, diluted 1:10
Lane 4: Wash
Lane 5: Elution (first two ml)
Lane 6: Elution (last two ml)
Lane 7: LMW

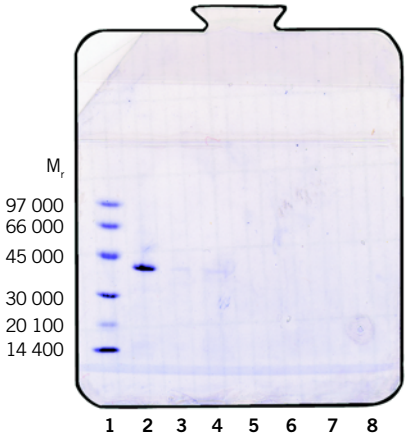


Purification in 6 M guanidine hydrochloride
Lane 1: Low Molecular Weight Calibration Kit (LMW)
Lane 2: Starting material, cell extract, diluted 1:10
Lane 3: Flow-through
Lane 4: Wash
Lane 5: Elution (first two ml)
Lane 6: Elution (last two ml)
Lane 7: LMW

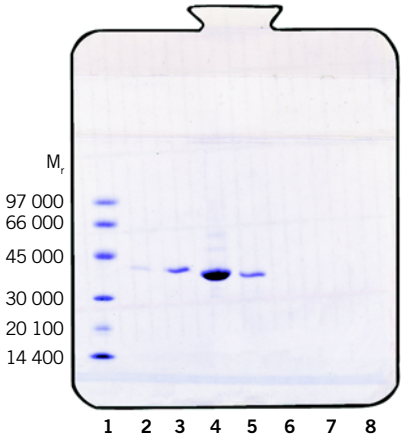
Fig 6. Purification of $(His)_{10}$-tagged protein from inclusion bodies on Ni^{2+}-loaded HiTrap Chelating HP column, 1 ml.

Sample:	N-terminal $(His)_6$-tagged recombinant protein produced in *E. coli*
Column:	HiTrap Chelating HP, 1 ml
Metal ion:	Ni^{2+}
Flow rates:	0.1–1 ml/min sample loading and refolding, 1 ml/min washing and elution
Binding buffer:	20 mM Tris-HCl, 0.5 M NaCl, 5 mM imidazole, 6 M guanidine hydrochloride, 1 mM 2-mercaptoethanol, pH 8.0
Washing buffer:	20 mM Tris-HCl, 0.5 M NaCl, 20 mM imidazole, 6 M urea, 1 mM 2-mercaptoethanol, pH 8.0
Refolding buffer:	20 mM Tris-HCl, 0.5 M NaCl, 20 mM imidazole, 1 mM 2-mercaptoethanol, pH 8.0
Refolding gradient:	30 ml linear gradient
Elution buffer:	20 mM Tris-HCl, 0.5 M NaCl, 500 mM imidazole, 1 mM 2-mercaptoethanol, pH 8.0
Elution gradient:	10 ml linear gradient
Equipment:	FPLC System
Electrophoresis:	SDS-PAGE, PhastSystem, PhastGel Gradient 10–15, 1 µl sample, Coomassie staining





Lane 1: Low Molecular Weight Calibration Kit (LMW)
Lane 2: Starting material for HiTrap Chelating HP, 1 ml
Lane 3: Fraction 1 Gua-HCl wash (manually)
Lane 4: Fraction 2 Gua-HCl wash (manually)
Lane 5: Fraction 3 Gua-HCl wash (manually)
Lane 6: Fraction 4 Gua-HCl wash (manually)
Lane 7: Fraction 1 Urea wash (manually)
Lane 8: Fraction 2 Urea wash (manually)



Lane 1: LMW
Lane 2: Fraction 38
Lane 3: Fraction 39
Lane 4: Fraction 40
Lane 5: Fraction 41
Lane 6: Fraction 42
Lane 7: Fraction 46
Lane 8: Fraction 49

Fig 7. One-step refolding and purification of a $(His)_6$-tagged recombinant protein on Ni^{2+}-loaded HiTrap Chelating HP column, 1 ml.

References

1. Purification of $(His)_6$-tagged proteins using HiTrap Chelating HP charged with different metal ions. Application Note 18-1145-18, Amersham Biosciences.
2. Purification and renaturation of recombinant proteins produced in *Escherichia coli* as inclusion bodies. Application Note 18-1112-33, Amersham Biosciences.
3. Colangeli, R., Heijbel, A., Williams, A.M., Manca, C., Chan, J., Lyashchenko, K., Gennaro, M.L. Three-step purification of lipopolysaccharide-free polyhistidine-tagged recombinant antigens of *Myobacterium tuberculosis*. *J of Chromatography B,* **714**, 223–235 (1998).
4. Rapid and efficient purificaton and refolding of a $(His)_6$-tagged recombinant protein produced in *E. coli* as inclusion bodies. Application Note 18-1134-37, Amersham Biosciences.
5. The Recombinant Protein Handbook, Protein Amplification and Simple Purification, 18-1142-75, Amersham Biosciences.
6. Reference list, HiTrap Chelating HP, 18-1156-69. Available on www.chromatography.amershambiosciences.com

Ordering information

Item	Pack	Code No.
HiTrap Chelating HP	1 ml × 5	17-0408-01
HiTrap Chelating HP	5 ml × 1	17-0409-01
HisTrap™ Kit	1 kit	17-1880-01
Chelating Sepharose Fast Flow	50 ml	17-0575-01

Accessories

Item	Pack	Code No.
Domed nut*	4	18-2450-01
Union luerlock		
female/M6 female*	2	18-1027-12
female/M6 male*	2	18-1027-62
Tubing connector		
flangeless/M6 female*	2	18-1003-68
flangeless/M6 male*	2	18-1017-98
To connect columns with M6 connections to ÄKTA design:		
union female/1/16" male*	5	18-3858-01

* included in HiTrap package

Related literature

The Recombinant Protein Handbook	1	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29
Affinity Chromatography Columns and Media, Product Profile	1	18-1121-86
HiTrap Column Guide	1	18-1129-81

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