HiTrap™ Chelating HP

HiTrap Chelating HP is one of a range of prepacked 1 ml and 5 ml columns for metal chelate affinity chromatography. Fast, simple, and easy separations are provided by the combination of the prepacked 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.

Key characteristics of HiTrap Chelating columns are:

- Fast and convenient use
- Packed with Chelating Sepharose™ High Performance
- Simple operation with a syringe, a pump, an ÄKTA™ system, or other chromatography systems

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.

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 favorable environment for coupling, while the highly cross-linked structure of the 34 µm spherical matrix ensures good 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 to 12. When charged with a suitable metal ion, HiTrap Chelating HP will selectively retain proteins if complex-forming amino acid residues, for example 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+}), and cobalt (Co^{2+}). Cu^{2+} affords strong binding and some protein specificity, while Zn^{2+} normally gives weaker binding (for further information on binding capacity, see Application Note, 18-1145-18, "Purification of $(His)_6$-tagged proteins using HiTrap Chelating HP columns charged with different metal ions"). The metal ion capacity of HiTrap Chelating HP is about 23 µmoles Cu^{2+}/ml medium.

Figure 2 shows the partial structure of Chelating Sepharose High Performance. The main characteristics of HiTrap Chelating HP are summarized in Table 1.

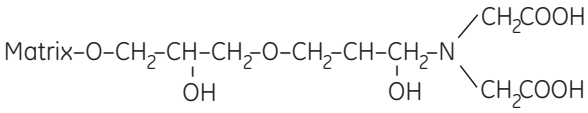


Fig 2. Partial structure of Chelating Sepharose High Performance.

Column characteristics

HiTrap columns are made of polypropylene, which is biocompatible with biomolecules. Top and bottom frits are manufactured from porous polyethylene. The columns are delivered with a stopper on the inlet and a snap-off end on the outlet.

Table 1. Characteristics of HiTrap Chelating HP columns

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 medium
Mean particle size	34 µm
Matrix	Highly cross-linked, spherical agarose
Column hardware pressure limit	5 bar (70 psi, 0.5 MPa)
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[1]	
Working	4 to 12
Cleaning	3 to 13
Temperature stability	
Regular use	4°C to room temp.
Storage	4°C to 8°C
Storage buffer	20% ethanol

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

Operation

Separations on HiTrap Chelating HP are easily performed using a syringe and the provided Luer adapter, a laboratory pump, or a chromatography system. Instructions and connectors are included with each pack of columns.

For quick scale-up, two or more columns can be connected in series by screwing the end of one column into the top of the next.

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 to 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.

Histidine-tagged protein purifications can be grouped into several categories, such as purification of soluble proteins, purification of insoluble proteins expressed as inclusion bodies, and refolding and purification of insoluble proteins expressed as inclusion bodies. Figures 4 to 6 show examples of all three categories. They also illustrate the use of different metal ions.

Figure 3 shows an example of purification of naturally occurring proteins using a Cu^{2+}-charged HiTrap Chelating HP column.

Column: HiTrap Chelating HP, 1 ml, Cu^{2+}-loaded according to the instructions
Sample: 200 µl egg white (10% in binding buffer filtered through a glass filter)
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% to 100% in elution buffer

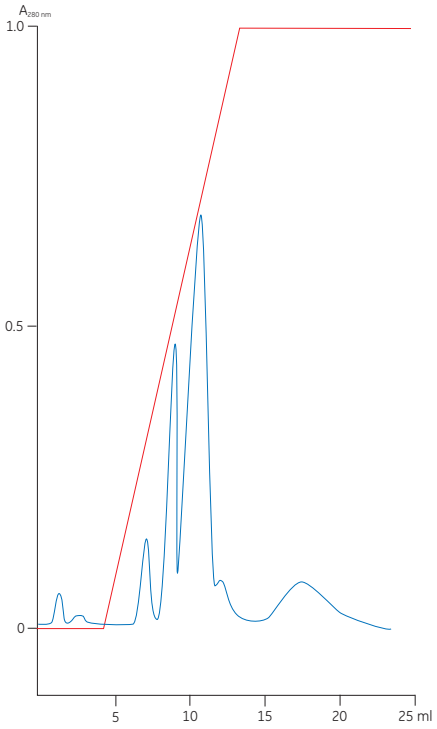
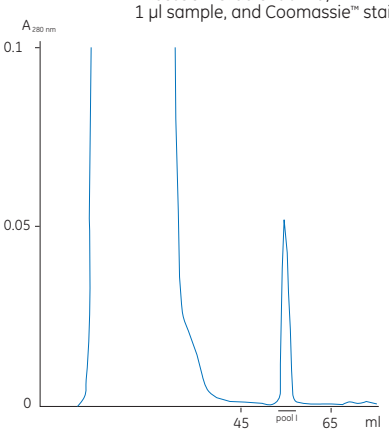


Fig 3. Purification of egg white proteins on Cu^{2+}-charged HiTrap Chelating HP column, 1 ml.

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

Lane 1: Low Molecular Markers, reduced
Lane 2: Crude periplasmic fraction, reduced
Lane 3: Pool I, purified protein A-$(HisGly)_4$His, reduced



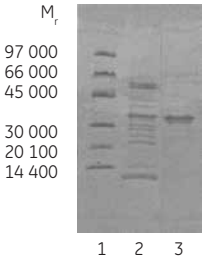


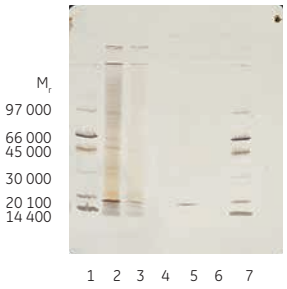
Fig 4. Purification of recombinant proteins such as poly-histidine fusions on Zn^{2+}-charged HiTrap Chelating HP column, 5 ml.

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.

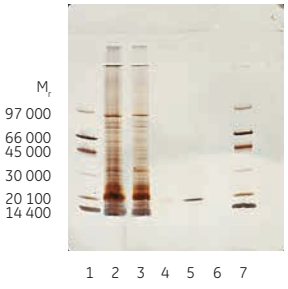
Figure 5 shows the analysis of a purification under denaturing conditions of a cell extract containing a $(histidine)_{10}$-tagged protein.

Column: HiTrap Chelating HP, 1 ml
Sample: 8 ml cell extract containing $(histidine)_{10}$-tagged protein. The clone was a kind gift from Dr. C. Fuller and S. Brasher, Department of Biochemistry, University of Cambridge, UK
Metal ion: Ni^{2+}
Binding buffers: 20 mM sodium phosphate, 0.5 M NaCl, 100 mM imidazole, and 8 M urea or 6 M guanidine hydrochloride, pH 7.4
Elution buffers: 20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, and 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, and silver staining



Purification in 8 M Urea

Lane 1: Low Molecular Marker
Lane 2: Starting material, cell extract, diluted 1:20
Lane 3: Flow-through, diluted 1:10
Lane 4: Wash
Lane 5: Elution (first 2 ml)
Lane 6: Elution (last 2 ml)
Lane 7: LMW



Purification in 6 M guanidine hydrochloride

Lane 1: Low Molecular Marker
Lane 2: Starting material, cell extract, diluted 1:10
Lane 3: Flow-through
Lane 4: Wash
Lane 5: Elution (first 2 ml)
Lane 6: Elution (last 2 ml)
Lane 7: LMW

Fig 5. Purification of a $(histidine)_{10}$-tagged protein from inclusion bodies on Ni^{2+}-charged HiTrap Chelating HP column, 1 ml.

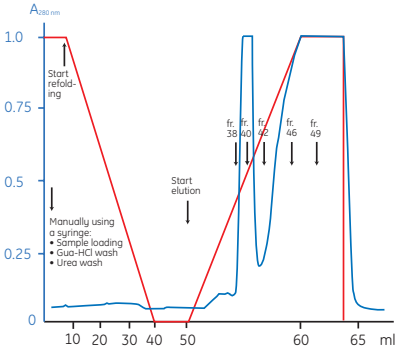
Refolding and purification of an insoluble recombinant protein in a single step

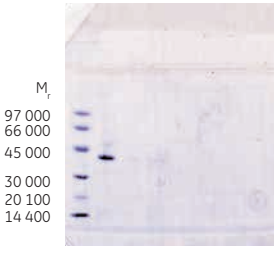
Conventional methods for refolding insoluble recombinant proteins are associated with several drawbacks. However, tagging the recombinant protein by adding several consecutive histidine residues opens the possibility of efficient purification and refolding in a single chromatographic step.

Since binding of the histidine tract to immobilized divalent metal ions can occur in the presence of urea or guanidine hydrochloride, a $(histidine)_6$-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.

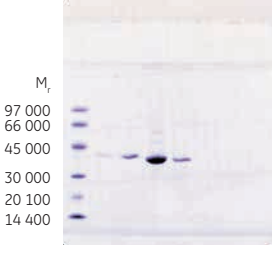
Figure 6 shows a general protocol for refolding and purifying a $(histidine)_6$-tagged recombinant protein produced in *E. coli* in a single step.

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





Lane 1: Low Molecular Marker
Lane 2: Starting material
Lane 3: Fraction 1, Gua-HCl wash (manual)
Lane 4: Fraction 2, Gua-HCl wash (manual)
Lane 5: Fraction 3, Gua-HCl wash (manual)
Lane 6: Fraction 4, Gua-HCl wash (manual)
Lane 7: Fraction 1, Urea wash (manual)
Lane 8: Fraction 2, Urea wash (manual)



Lane 1: Low Molecular Marker
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 6. One-step refolding and purification of a $(histidine)_6$-tagged recombinant protein on Ni^{2+}-charged HiTrap Chelating HP column, 1 ml.

Ordering information

Products	Quantity	Code number
HiTrap Chelating HP	5 × 1 ml	17-0408-01
HiTrap Chelating HP	1 × 5 ml	17-0409-01
HiTrap Chelating HP	5 × 5 ml	17-0409-03
HiTrap Chelating HP	100 × 5 ml[1]	17-0409-05

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

Related products	Quantity	Code number
Chelating Sepharose Fast Flow	50 ml	17-0575-01
HiTrap IMAC HP	5 × 1 ml	17-0920-03
HiTrap IMAC HP	5 × 5 ml	17-0920-05
IMAC Sepharose High Performance	25 ml	17-0921-07
IMAC Sepharose High Performance	100 ml	17-0921-08
HiTrap IMAC FF	5 × 1 ml	17-0921-02
HiTrap IMAC FF	5 × 5 ml	17-0921-04
HiPrep™ IMAC FF 16/10	1 × 20 ml	17-0921-06
IMAC Sepharose 6 Fast Flow	25 ml	17-0921-07
IMAC Sepharose 6 Fast Flow	100 ml	17-0921-08

Accessories	Quantity	Code number
1/16" male/Luer female*	2	18-1112-51
Tubing connector flangeless/M6 female	2	18-1003-68
Tubing connector flangeless/M6 male	2	18-1017-98
Union 1/16" female/M6 male	6	18-1112-57
Union M6 female/1/16" male	5	18-3858-01
Union luerlock female/M6 female	2	18-1027-12
HiTrap/HiPrep, 1/16" male connector for ÄKTAdesign	8	28-4010-81
Stop plug female, 1/16"†	5	11-0004-64
Fingertight stop plug, 1/16"‡	5	11-0003-55

* One connector included in each HiTrap package

† Two, five, or seven stop plugs female included in HiTrap packages depending on products.

‡ One fingertight stop plug is connected to the top of each HiTrap column at delivery.

Related literature	Code number
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Columns and Media, Selection Guide	18-1121-86
HiTrap Column Guide	18-1129-81

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