Ni Sepharose High Performance

Prepacked HisTrap HP columns and HisTrap HP Kit

The preparative purification of His-tagged recombinant proteins by immobilized metal affinity chromatography (IMAC) is both popular and highly effective. IMAC exploits the ability of the amino acid histidine to bind chelated transition metal ions. His is globally the most used tag, often found as six histidine residues in series, but it is also present on the surface of many non-modified proteins. Of the metal ions used in this technique, nickel (Ni^{2+}) has generally been proven to be the most successful. Ni Sepharose™ High Performance, the new IMAC medium from Amersham Biosciences, should further increase the use and reliability of this valuable method of purification. Its performance characteristics include:

- Negligible leakage of the Ni^{2+} ion
- Compatibility with a very wide range of reducing agents, detergents and other additives
- Very high protein binding capacities
- Available in the convenient and time-saving prepacked HiTrap™ format as HisTrap™ HP 1 ml and 5 ml columns and HisTrap HP Kit

These features make Ni Sepharose High Performance the first-choice medium for the single-step purification of His-tagged recombinant proteins from cellular or cell-free systems.

Fig 1. Ni Sepharose High Performance, also prepacked as convenient HisTrap HP columns, is the first choice for purifying His-tagged recombinant proteins.

Fig 2. HisTrap HP Kit is a rapid and convenient choice for one-step purifications of His-tagged proteins.

Medium characteristics

Ni Sepharose High Performance consists of highly cross-linked 6% agarose beads to which a chelating group has been immobilized. This chelating group has then been charged with Ni^{2+} ions. The resulting medium selectively retains His-tagged recombinant proteins, allowing them to be purified from cellular contaminants or cell-free systems.

The 34 µm bead Sepharose High Performance matrix with the chelating ligand immobilized displays high chemical and physical stability, resulting in excellent flow rates and distinctly separated peaks containing concentrated material.

In addition, the chelating group charged with Ni^{2+} gives a binding capacity that is demonstrably superior to similar media from other manufacturers.

Ni Sepharose High Performance is compatible with all commonly used aqueous buffers, reducing agents and denaturants such as 6 M guanidine hydrochloride and 8 M urea, as well as a range of other additives. It is stable over a broad pH range. This high stability and broad compatibility maintains the biological activity and increases the yield of the purified product, at the same time as it greatly expands the range of suitable operating conditions, including procedures used to clean the medium. Table 1 lists the main characteristics of Ni Sepharose High Performance.

Amersham Biosciences

Table 1. Main characteristics of Ni Sepharose High Performance.

Matrix	Highly cross-linked spherical agarose, 6%
Mean particle size	34 µm
Metal ion capacity	~15 µmol Ni^{2+}/ml medium
Dynamic binding capacity*	At least 40 mg $(His)_6$-tagged protein/ml medium
Recommended flow rate**	<150 cm/h
Max back pressure	0.3 MPa, 3 bar
Compatibility during use	Stable in all commonly used buffers, reducing agents, denaturants and detergents. See Tables 4 and 5 for details.
Chemical stability (Ni^{2+}-stripped medium)	0.01 M HCl, 0.1 M NaOH, 6 M Gua-HCl, 8 M urea, sodium acetate, pH 4.0. Tested for 1 week at 40 °C. 1 M NaOH, 70% HAc. Tested for 12 hours. 2% SDS. Tested for 1 hour. 30% 2-propanol. Tested for 30 min.
Avoid (during purification)	Chelating agents, e.g. EDTA, EGTA
pH stability	short term (≤2 hours): 2–14 long term (≤1 week): 3–12
Storage	20% ethanol
Storage temperature	+4 to +8 °C

* Dynamic bindning capacity conditions:

Sample:	1 mg/ml $(His)_6$-tagged pure proteins (M_r 28 000 or 43 000) in binding buffer ($QB_{10\%}$ determination) and/or $(His)_6$-tagged protein bound from *E. coli* extract)
Column volume:	0.25 ml or 1 ml
Flow rate:	0.25 ml/min or 1 ml/min
Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4

** H_2O at room temperature.

Operation

Packing in laboratory columns

Ni Sepharose High Performance is supplied pre-swollen in 25 ml and 100 ml packs. The medium is easy to pack and use in laboratory columns from the Tricorn™ and XK series (see Ordering Information). Full user instructions are supplied with each pack.

Availability in HiTrap columns

Ni Sepharose High Performance is also available in the convenient HiTrap prepacked column format as HisTrap HP 1 ml and 5 ml columns and HisTrap HP Kit.

HisTrap HP columns

HisTrap HP 1 ml and 5 ml columns bring added time-saving, convenience and reliability to the purification of His-tagged recombinant proteins. The columns are simple to operate with a syringe and the supplied luer adaptor, a pump, or a chromatography system such as ÄKTA™design or FPLC™ System. ÄKTAdesign systems include preset method templates for HisTrap HP, which further simplifies operation, especially reproducibility.

The HiTrap column is made of biocompatible polypropylene. The columns have porous top and bottom frits that allow high flow rates. They are delivered with a stopper on the inlet and a twist-off end on the outlet. Table 2 lists the main characteristics of HisTrap HP 1 ml and 5 ml. Note that HisTrap HP columns cannot be opened or repacked.

Table 2. Main characteristics of HisTrap HP columns.

Matrix	Highly cross-linked spherical agarose, 6%
Mean particle size	34 µm
Metal ion capacity	~15 µmol Ni^{2+}/ml medium
Dynamic binding capacity*	At least 40 mg $(His)_6$-tagged protein/ml medium
Column volumes	1 ml or 5 ml
Column dimensions	i.d. x h: 0.7 x 2.5 cm (1 ml) 1.6 x 2.5 cm (5 ml)
Recommended flow rate	1 and 5 ml/min for 1 and 5 ml column respectively
Max. flow rates	4 and 20 ml/min for 1 and 5 ml column respectively
Max back pressure	0.3 MPa, 3 bar
Compatibility during use	Stable in all commonly used buffers, reducing agents, denaturants and detergents. See Tables 4 and 5 for details.
Chemical stability (Ni^{2+}-stripped medium)	0.01 M HCl, 0.1 M NaOH, 6 M Gua-HCl, 8 M urea, sodium acetate, pH 4.0. Tested for 1 week at 40 °C. 1 M NaOH, 70% HAc. Tested for 12 hours. 2% SDS. Tested for 1 hour. 30% 2-propanol. Tested for 30 min.
Avoid (during purification)	Chelating agents, e.g. EDTA, EGTA
pH stability	short term (≤2 hours): 2–14 long term (≤1 week): 3–12
Storage	20% ethanol
Storage temperature	+4 to +8 °C

* Dynamic bindning capacity conditions:

Sample:	1 mg/ml $(His)_6$-tagged pure proteins (M_r 28 000 or 43 000) in binding buffer ($QB_{10\%}$ determination) and/or $(His)_6$-tagged protein bound from *E. coli* extract)
Column volume:	0.25 ml or 1 ml
Flow rate:	0.25 ml/min or 1 ml/min
Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4

HisTrap HP Kit

HisTrap HP Kit makes the purification of His-tagged recombinant proteins even more convenient. The kit includes three HisTrap HP 1 ml columns plus ready-made binding and elution buffer concentrates (phosphate and imidazole) made from highest quality salts and water, and filtered through a 0.45 µm filter. These concentrates speed the rate at which results are obtained, and they help increase simplicity and reproducibility.

Performance benefits

Ni Sepharose High Performance and its prepacked companion products provide anyone purifying mg amounts His-tagged recombinant proteins with a broad spectrum of performance benefits. Researchers in industrial and academic labs will have no difficulty translating these advantages into higher protein purity, yield and activity, plus greater operational flexibility.

Negligible nickel leakage

The ability of Ni Sepharose High Performance to bind and hold nickel ions has been thoroughly tested by, for example, charging the matrix with Ni^{2+} and then exposing it to harsh acidic conditions (pH 4.0). The amount of nickel 'stripped-off' by this treatment was calculated as the ratio between the amount charged and that still bound.

Results show very low leakage over the wide interval of nickel capacities screened, a clear testimony that the synthesis and coupling procedures used when manufacturing Ni Sepharose High Performance give a highly homogeneous chelating ligand. Furthermore, when nickel leakage from similar IMAC media was compared with that of Ni Sepharose High Performance using the same test method, it was found to be considerably higher (Table 3).

Table 3. Ni Sepharose High Performance has a much lower nickel leakage compared to similar media tested. This will help minimize protein precipitation and other problems and thus increase yield.

Medium	Ni^{2+} leakage
Ni Sepharose High Performance	<5%
Medium from supplier 'Q'	Average 9%
Medium from supplier 'X'	18–19%

Ni^{2+} leakage was also compared in the presence of reducing agents such as 2 mM and 5 mM DTT, 5 mM TCEP and 20 mM ß-mercaptoethanol. In this case, leakage from Ni Sepharose High Performance was extremely low, up to ten-fold lower than from media supplied by manufacturer 'Q' as well as manufacturer 'X'. Figure 3 shows Ni^{2+} leakage from the compared media under reducing conditions.

Summary

The negligible nickel leakage from Ni Sepharose High Performance helps retain the activity of the purified protein and reduce its precipitation, which results in increased purity, activity and yield of the target His-tagged protein.

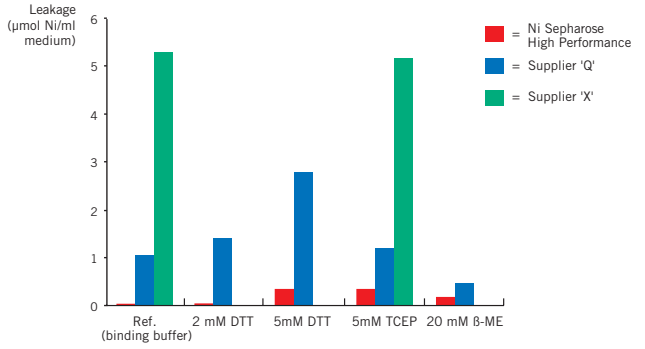


Fig 3. Leakage of Ni^{2+} from Ni Sepharose High Performance under reducing conditions (60 column volumes, CV) is negligible compared with two other media. (Manufacturer 'X' was only tested with 60 CV binding buffer and with 60 CV 5 mM TCEP).

High stability and compatibility increase usage

The wide interest in purifying His-tagged recombinant proteins makes medium stability and compatibility a key issue. Extensive studies have proven that Ni Sepharose High Performance is outstanding in both respects. For example, the medium is stable with reducing agents like DTT and DTE at concentrations up to 5 mM in sample and buffers.

Chromatographic and SDS-PAGE analyses also reveal that protein separations are not negatively affected by various reducing agents. The same purity and recovery were achieved as for reference purification without any reducing agents added (Fig. 4).

Unlike similar media from other suppliers, the color of Ni Sepharose High Performance is essentially unaltered by low concentrations of reducing agents like DTT (Fig. 5).

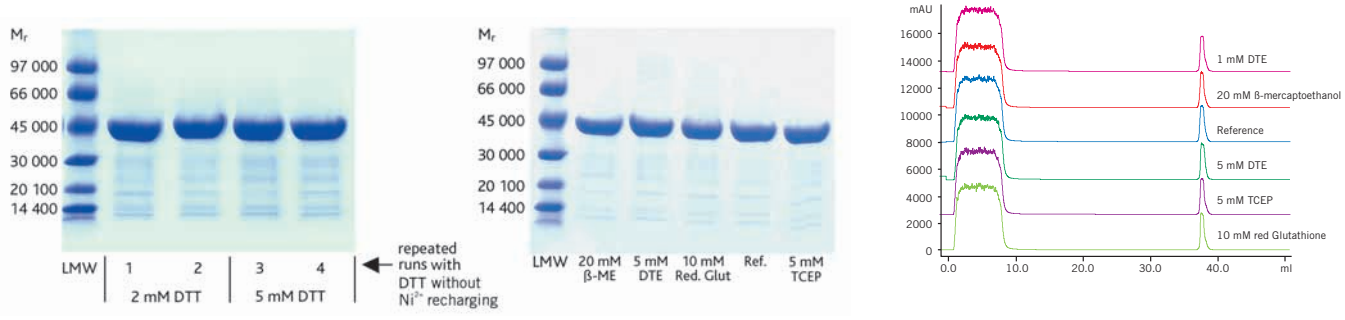
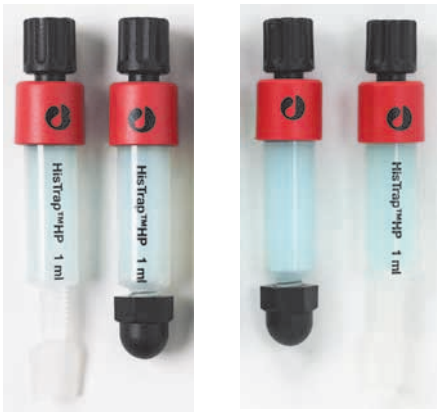


Fig 4. Reducing agents do not affect the purity or recovery of separation runs on Ni Sepharose High Performance.

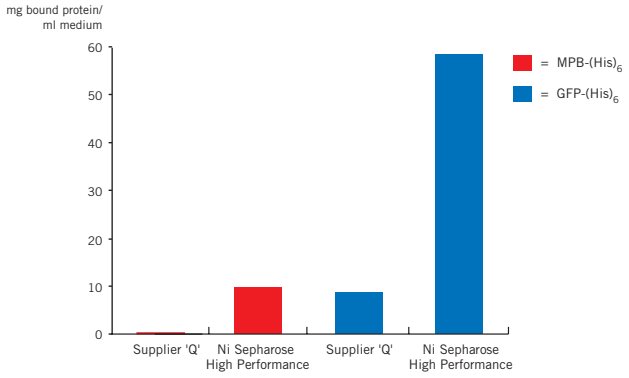


Ref. 1 mM DTT 5 mM TCEP Ref.

Fig 5. Low concentrations of reducing agents have minimal affect on the physical appearance of Ni Sepharose High Performance.

Fig. 6 shows that Ni Sepharose High Performance maintains its characteristically high dynamic binding capacity in the presence of 8 M urea, a strong denaturing agent (see later for native conditions). Note that a similar IMAC medium from other suppliers failed to match this performance.

Table 4 summarizes the stability of Ni Sepharose High Performance in common reducing and denaturing agents.



Medium	MBP-$(His)_6$ mg/ml medium	GFP-$(His)_6$ mg/ml medium
Ni Sepharose High Performance	10	59
Medium from supplier 'Q'	0	9

Fig 6. Ni Sepharose High Performance has the best dynamic binding capacity ($QB_{10\%}$) for His-tagged maltose binding protein (MBP-$(His)_6$) and His-tagged green fluorescent protein (GFP-$(His)_6$) in the presence of 8 M urea compared to Supplier 'Q'. Note that binding capacity will vary from protein to protein.

Table 4. The stability of Ni Sepharose High Performance is significantly better than similar media from other suppliers. The medium is stable in the following reducing and denaturing agents at least at the concentrations given.

5 mM DTE
5 mM DTT
20 mM ß-mercaptoethanol
5 mM TCEP
10 mM reduced glutathione
8 M urea
6 M guanidine hydrochloride

A similar story applies to detergents, additives and buffer substitutes. Little, if any change is seen in protein purity or recovery when additives or a range of buffer substances are used. Table 5 summarizes the compatibility of Ni Sepharose High Performance with such typical substances.

Table 5. Ni Sepharose High Performance is compatible with the following detergents, additives and buffer substances at least at the concentrations given.

2% Triton™ X-100 (non-ionic detergent)
2% Tween™ 20 (non-ionic detergent)
2% NP-40 (non-ionic detergent)
2% cholate (anionic detergent)
1% CHAPS (zwitterionic detergent)
500 mM imidazole
20% ethanol
50% glycerol
100 mM Na_2SO_4
0.5–1.5 M NaCl
1 mM EDTA*
1 mM EDTA + 10 mM $MgCl_2$*
60 mM citrate**
60 mM citrate + 80 mM $MgCl_2$**
50 mM sodium phosphate, pH 7.4
50 mM Tris-HCl, pH 7.4
100 mM Tris-HCl, pH 7.4
100 mM Tris-acetate, pH 7.4
100 mM HEPES, pH 7.4
100 mM MOPS, pH 7.4

* 1 mM EDTA has been used successfully in some cases (only in samples, not in buffers), but is not recommended. Metal stripping may be prevented by addition of $MgCl_2$.

** Metal stripping may be prevented by addition of $MgCl_2$.

In most IMAC applications, imidazole is used for elution and, at lower concentrations, to increase the selectivity for His-tagged proteins. It is well known that the binding of non-tagged contaminants is suppressed with imidazole, and that a too high concentration of imidazole will also prevent binding of His-tagged proteins. The concentration of imidazole that will give optimal purification results (in terms of purity and yield) is protein-dependent, and is usually slightly higher for Ni Sepharose High Performance than for similar IMAC media on the market. Finding the optimal imidazole concentration for a His-tagged protein is a trial-and-error effort, but 20–40 mM in the sample and the binding and wash buffers is a good starting point for many proteins.

Fig. 7 presents a typical purification of a His-tagged protein expressed in *E. coli*. For clarity only the gradient parts of the chromatograms are shown. The pool eluted from Ni Sepharose High Performance has a much higher protein concentration than that eluted from a medium supplied by manufacturer 'Q'. Also note that the amount of nickel ion in the eluted fractions is lower in the fractions pooled from Ni Sepharose High Performance, thus confirming the low nickel leakage from this medium seen in other studies.

The low nickel content, in combination with the high concentration of the target protein in the eluted pool from Ni Sepharose High Performance, results in a molar ratio Ni^{2+}/protein that in this case is 2.2 times lower compared with supplier 'Q'. In some cases, this can be very important for preventing precipitation of the target protein.

Summary

The overall high chemical stability of Ni Sepharose High Performance also applies to the medium in its HisTrap HP column and kit formats. As well as helping maintain biological activity and increasing product yield, it greatly expands the range of conditions in which the medium can be used.

High protein binding capacity for more efficient purifications

The dynamic binding capacity of Ni Sepharose High Performance has been evaluated with pure His-tagged maltose binding protein (MBP-$(His)_6$, M_r 43 000) and with pure green fluorescent protein (GFP-$(His)_6$, M_r 28 000) using the parameter 10% breakthrough ($QB_{10\%}$).

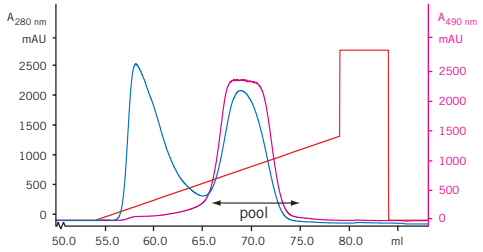
The absorbance at 280 nm is measured for a pure protein solution. The solution is then continuously loaded on the column and the absorbance of the eluate is followed until breakthrough occurs, i.e. column starts to get saturated. The volume of protein solution (or mg protein) that has been applied up to a certain breakthrough point (e.g. $QB_{10\%}$) is a measure of the dynamic protein binding capacity of the medium. (Protein is then eluted after a wash).

Comparative chromatograms (Fig. 8) demonstrate that Ni Sepharose High Performance binds 1 mg/ml MBP-$(His)_6$ for considerably longer time (approx. 70 column volumes) than a similar IMAC product from another supplier (approx. 20 column volumes) before the protein has started to enlarge from the column, i.e. before breakthrough has occurred. The determined dynamic binding capacity of Ni Sepharose High Performance is 65 mg/ml medium, which greatly exceeds that of the product from another supplier (14 mg/ml medium).

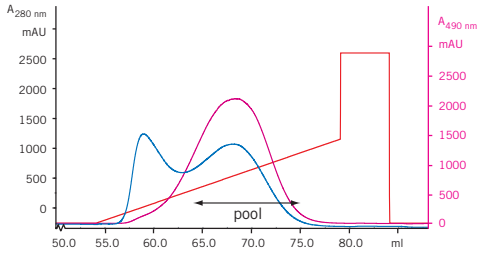
Figure 9 shows protein binding capacity data determined at room temperature and 4 °C. Once again, Ni Sepharose High Performance outperforms the product from supplier 'Q'.

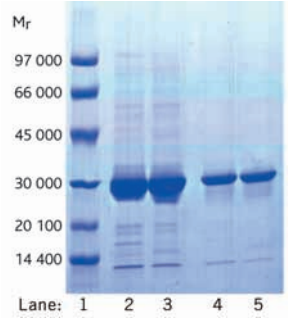
Column:	1) HisTrap HP 1 ml 2) 1 ml column, dimensions (i.d. x h) 5 mm x 5 cm
Medium:	1) Ni Sepharose High Performance, 2) Medium from supplier 'Q'
Sample:	12 mg His-tagged Green Fluorescent Protein (GFP-$(His)_6$), *E. coli* extract
Sample volume:	19 ml (clarified extract)
Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4
Gradient:	25 ml linear gradient of 5–250 mM imidazole, followed by a "push" with 500 mM imidazole

1. HisTrap HP



2. Supplier 'Q'





Lane 1: LMW, Low Molecular Weight marker
Lane 2: Eluted pool, HisTrap HP
Lane 3: Eluted pool, Supplier 'Q'
Lane 4: Eluted pool, HisTrap HP, dil. 1:4
Lane 5: Eluted pool, Supplier 'Q', dil. 1:4

Pooled fractions from:	µg Ni^{2+}/l (ppb)	Protein conc. (mg/ml)	Molar ratio Ni^{2+}/protein
1) HisTrap HP	185	1.60	0.055
2) Medium from supplier 'Q'	231	0.91	0.121

Fig. 7. Protein concentration is higher and nickel leakage lower in pooled fractions from Ni Sepharose High Performance compared to a medium from supplier 'Q' during linear gradient elution. Final protein purity was similar in both runs. (Black arrows indicate the final pools). For SDS-PAGE, pools were adjusted to the same volume to get equal intensity of target protein bands. This was confirmed by the 1:4 dilutions. Note that the peak areas are not comparable since the highest absorbance signals are above the linear range.

Column volume:	0.25 ml, dimensions (i.d. x h), 5 mm x 1.3 cm
Medium:	1) Ni Sepharose High Performance 2) Medium from supplier 'Q'
Sample:	Pure His-tagged maltose binding protein, MBP-$(His)_6$, M_r 43 000, 1 mg/ml
Flow rate:	0.25 ml/min
Binding buffer:	20 mM sodium phosphate, 5 mM imidazole, 0.5 M NaCl, pH 7.4
Elution buffer:	20 mM sodium phosphate, 0.5 M imidazole, 0.5 M NaCl, pH 7.4

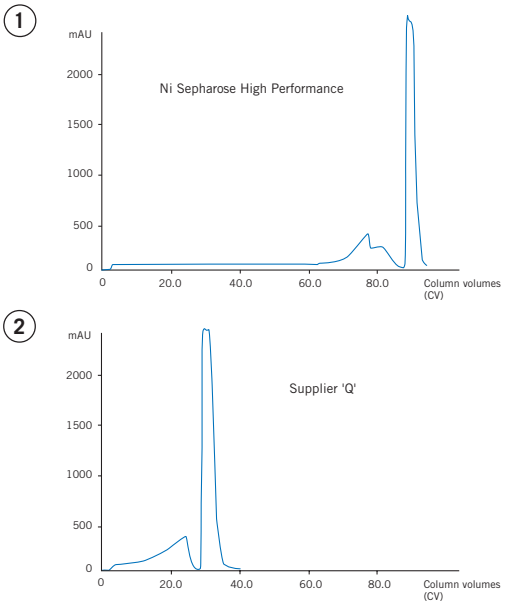
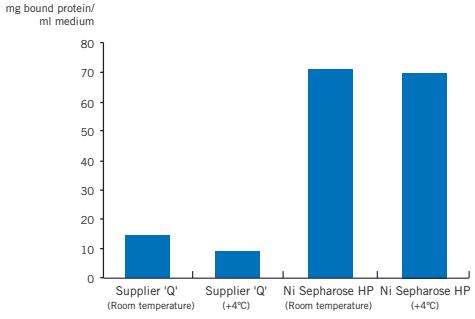


Fig 8. Ni Sepharose High Performance has a greater dynamic binding capacity (seen here as time of binding) for MBP-$(His)_6$ than a product from supplier 'Q'. Note that the areas of the elution peaks cannot be compared since the highest absorbance signals are above the linear range.



Medium	MBP-$(His)_6$ mg/ml medium
Ni Sepharose High Performance	
room temp.	68
4 °C	67
Medium supplied by manufacturer 'Q'	
room temp.	13
4 °C	8

Fig 9. Dynamic binding capacity data ($QB_{10\%}$) for Ni Sepharose High Performance determined for MBP-$(His)_6$ exceeds that of the supplier 'Q' product at room temperature and 4 °C. Note that binding capacity will vary from protein to protein and at different temperatures.

Fig. 10 presents further evidence of the excellent protein binding capacity of Ni Sepharose High Performance compared with other media. As can be seen in this binding study using a cell extract containing GFP-$(His)_6$ as the target protein, the yield for HisTrap HP 1 ml was about two times higher than the medium from supplier 'Q' and over three times higher than that from supplier 'S'. The SDS-PAGE analysis of equal volumes of the eluted peaks (Fig. 11) confirms this superiority.

Columns:	1. HisTrap HP 1 ml 2. Supplier 'Q' 1 ml 3. Supplier 'S' 1 ml
Sample:	GFP-$(His)_6$, 1.85 mg/ml (including 5 mM imidazole) in *E. coli* extract
Sample volume:	32 ml clarified extract
Flow rate:	1 ml/min
Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, 5 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4
Gradient:	25 ml linear gradient, 5-250 mM imidazole

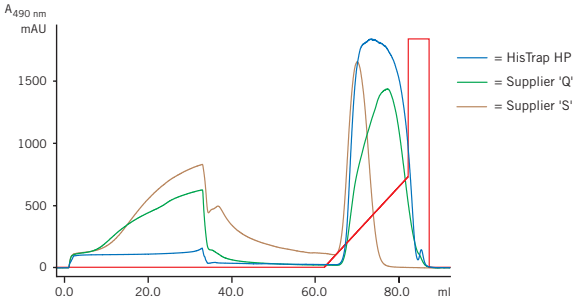
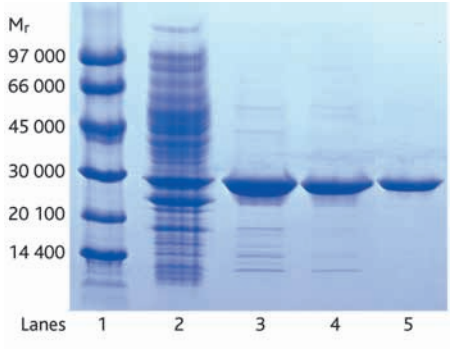


Fig 10. The superior binding capacity of Ni Sepharose High Performance for GFP-$(His)_6$ compared with two other media. Note the differences in loss of GFP-$(His)_6$ during sample application and differences in area of eluted peaks.



Lane 1: Low Molecular Weight marker (LMW)
Lane 2: Starting material, GFP-$(His)_6$
Lane 3: Eluted pool from HisTrap HP
Lane 4: Eluted pool from supplier 'Q'
Lane 5: Eluted pool from supplier 'S'

Fig 11. SDS-PAGE analysis of GFP-$(His)_6$ recovered in equal volumes of eluted peaks confirms the protein binding result seen in Fig. 10. Note that improved purity can be achieved with the use of increased imidazole concentration.

Summary

The high binding capacity of Ni Sepharose High Performance means greater efficiency. More sample can be applied and the target protein is obtained more concentrated at a lower cost/mg and in a shorter time.

Speed and convenience plus increased reproducibility

Ni Sepharose High Performance is easy to pack and use in laboratory columns such as Tricorn and XK columns. However, the greatest savings in time and ease-of-use come when the medium is prepacked as HisTrap HP columns.

HisTrap HP 1 ml and 5 ml columns

HisTrap HP 1 ml and 5 ml columns permit rapid yet reliable separations with the minimum of preparation and equipment. Fig. 12 illustrates a purification with a syringe, for which connectors are supplied with each column. HisTrap HP can also be operated with a simple laboratory pump by following a similar procedure.

Prepacked columns also mean consistently high quality and, above all, greatly increased reproducibility. This reproducibility is best achieved in practice by running the columns on ÄKTAdesign chromatography systems, which include pre-programmed method templates for HisTrap HP 1 ml and 5 ml columns. Method templates cover common techniques such as single-step purifications of His-tagged recombinant proteins, and they allow many user-defined protocols to be stored. Simply snap the HisTrap HP column into place and start purifying.

HisTrap HP Kit

Supplied with ready-made highest quality phosphate (binding buffer) and imidazole (elution buffer) concentrates, HisTrap HP Kit adds a further dimension of convenience and reproducibility. Preparing sample, binding and elution buffers and then running a basic purification protocol is done in a matter of minutes.

1. Prepare the sample
2. Prepare buffers by mixing and diluting the concentrates:
   a) Binding buffer: 20 mM sodium phosphate, 0.5 M NaCl, 20–40 mM imidazole, pH 7.4
   b) Elution buffer: 20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4
3. Using a syringe:
   a) Wash the column with distilled water
   b) Equilibrate the column with binding buffer
   c) Apply the sample
   d) Wash with binding buffer
   e) Elute histidine-tagged protein with elution buffer and collect the eluate
   f) Check the purification on SDS-PAGE and/or Western blotting

Complete, easy-to-follow instructions, including sample preparation, purification and optimization protocols, and a trouble-shooting guide are included in the HisTrap HP Kit.

Scaling up

HisTrap HP columns are also simple to scale up for syringe, pump or system-based use. To increase capacity, just connect two or three 1 ml or 5 ml columns in series (Fig. 13). Note, however, that the back-pressure will increase. (Ni Sepharose High Performance packed in laboratory columns from the Tricorn or XK series offers an equally reliable alternative.)

Fig 13. Quick and predictable scale-up by coupling two HisTrap HP 1 ml or 5 ml columns in series.

Summary

HisTrap HP columns package all the performance benefits of Ni Sepharose High Performance in a highly attractive format. Their convenience and ease-of-use translate the inherent advantages of His-tagged recombinant protein purification into a simple and reliable everyday laboratory tool.

Fig 12. Using HisTrap HP 1 ml 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) Wash and elute, continue collecting fractions.

Acknowledgement

MBP-$(His)_6$ was provided by Pharmacia Diagnostics, Uppsala, Sweden. GFP-$(His)_6$ was provided by Dr. David Drew, Dept. of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden.

Ordering Information

Products	Quantity	Code no.
Ni Sepharose High Performance	25 ml*	17-5268-01
Ni Sepharose High Performance	100 ml*	17-5268-02
HisTrap HP 1 ml	5 x 1 ml	17-5247-01
HisTrap HP 1 ml	100 x 1 ml**	17-5247-05
HisTrap HP 5 ml	1 x 5 ml	17-5248-01
HisTrap HP 5 ml	5 x 5 ml	17-5248-02
HisTrap HP 5 ml	100 x 5 ml**	17-5248-05
HisTrap HP Kit	3 x 1 ml	17-5249-01

* Larger quantities are available. Please contact Amersham Biosciences for more information.

** Special pack delivered on specific customer order. Includes connector package, domed nuts and instructions. Please contact your local Amersham Biosciences representative.

Related products	Quantity	Code no.
HiTrap Desalting 5 ml	5 x 5 ml	17-1408-01
PD-10 Desalting Column	30	17-0851-01
HiPrep™ 26/10 Desalting	1 x 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 x 53 ml	17-5087-02

Empty lab-scale columns	Quantity	Code no.
Tricorn 5/20 column	1	18-1163-08
Tricorn 5/50 column	1	18-1163-09
Tricorn 10/20 column	1	18-1163-13
Tricorn 10/50 column	1	18-1163-14
Tricorn 10/100 column	1	18-1163-15
XK 16/20 column	1	18-8773-01
XK 16/40 column	1	18-8774-01
XK 26/20 column	1	18-1000-72
XK 26/40 column	1	18-8768-01

Accessories	Quantity	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 ÄKTAdesign:		
Union M6 female /1/16" male*	5	18-3858-01
Union 1/16" female/M6 male*	6	18-1112-57

* Included in HisTrap HP column packages.

Related literature	Quantity	Code no.
Recombinant Protein Handbook	1	18-1142-75
Affinity Chromatography Handbook, Principle and Methods	1	18-1022-29
Affinity Chromatography Columns and Media Product Profile	1	18-1121-86
HiTrap Column Guide	1	18-1129-81

For more information, visit:
www.chromatography.amershambiosciences.com
or www.hitrap.com

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Licensing information
Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US pat 5,284,933 and US pat 5,310,663, including corresponding foreign patents (assigne: Hoffman La Roche, Inc).

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