Ni Sepharose™ High Performance HisTrap™ HP

The preparative purification of histidine-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. Histidine is globally the most used tag, often found as six histidine residues in series, but it is also present on the surface of many unmodified 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 further increases the use and reliability of this valuable method of purification.

Ni Sepharose High Performance offers:

- 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
- Convenient and time-saving prepacked HiTrap™ format in the form of HisTrap HP 1 ml and 5 ml columns

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

Chromatography 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 histidine-tagged recombinant proteins, allowing them to be purified from cellular contaminants or cell-free systems.

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

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.

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[1]	At least 40 mg $(histidine)_6$-tagged protein/ml medium
Recommended flow rate[2]	<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. Tested for one week at 40°C. 1 M NaOH, 70% HAc. Tested for 12 h. 2% SDS. Tested for 1 h. 30% 2-propanol. Tested for 30 min.
Avoid in buffers	Chelating agents such as EDTA, EGTA, citrate (see Table 5)
pH stability	short term (at least 2 h): 2–14 long term (≤ one week): 3–12
Storage	20% ethanol
Storage temperature	4°C to 30°C

[1] Dynamic bindning capacity conditions:

Sample:	1 mg/ml $(histidine)^6$-tagged pure proteins (M_r 28 000 or 43 000) in binding buffer (capacity at 10% breakthrough) and/or $(histidine)_6$ 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

[2] 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.

HisTrap HP columns

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

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[1]	At least 40 mg $(histidine)_6$-tagged protein/ml medium
Column volumes	1 ml or 5 ml
Column dimensions	i.d. × h: 0.7 × 2.5 cm (1 ml) 1.6 × 2.5 cm (5 ml)
Recommended flow rate[2]	1 and 5 ml/min for 1 ml and 5 ml column respectively
Max. flow rates[2]	4 and 20 ml/min for 1 ml and 5 ml column respectively
Max. back pressure[2]	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. Tested for one week at 40°C. 1 M NaOH, 70% HAc. Tested for 12 h. 2% SDS. Tested for 1 h. 30% 2-propanol. Tested for 30 min.
Avoid in buffers	Chelating agents such as EDTA, EGTA, citrate (see Table 5)
pH stability	short term (at least 2 h): 2–14 long term (≤ one week): 3–12
Storage	20% ethanol
Storage temperature	4°C to 30°C

[1] Dynamic bindning capacity conditions:

Sample:	1 mg/ml $(histidine)^6$-tagged pure proteins (M_r 28 000 or 43 000) in binding buffer (capacity at 10% breakthrough) and/or $(histidine)_6$ 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

[2] H_2O at room temperature.

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 snap-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.

Performance benefits

Ni Sepharose High Performance and its prepacked companion products provide anyone purifying milligram amounts histidine-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 media was compared with that of Ni Sepharose High Performance using the same test method, it was found to be considerably higher (Table 3).

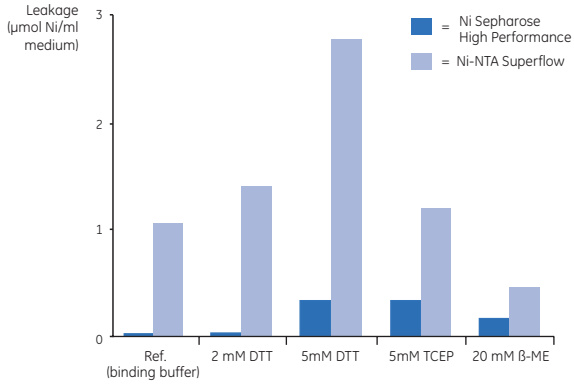


Fig 2. Leakage of Ni2+ from Ni Sepharose High Performance under reducing conditions (60 column volumes, CV) is negligible compared with Ni-NTA Superflow.

Table 3. Ni Sepharose High Performance has a much lower nickel leakage compared to similar media tested

Medium	Ni^{2+} leakage
Ni Sepharose High Performance	< 5%
Ni-NTA Superflow[1]	Average[2] 9%

[1] Details of all comparisons between Ni Sepharose High Performance and Ni-NTA Superflow described in this Data File are found at www.gehealthcare.com/protocol-his. All experiments were performed at GE Healthcare, Protein Separations' laboratories.

[2] Batch-to-batch variation was observed.

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 β-mercaptoethanol. In this case, leakage from Ni Sepharose High Performance was extremely low, up to ten-fold lower than from media supplied by other manufacturers. Figure 2 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 histidine-tagged protein.

High stability and compatibility

The wide interest in purifying histidine-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 such as 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 3). For best results run a blank run before applying samples and buffers including reducing agents.

Unlike similar media from other suppliers, the color of Ni Sepharose High Performance is essentially unaltered by low concentrations of reducing agents such as DTT.

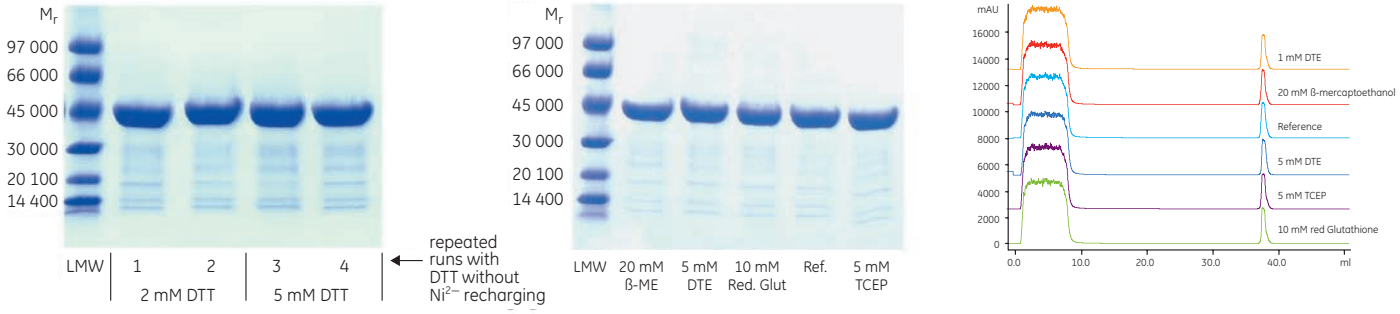
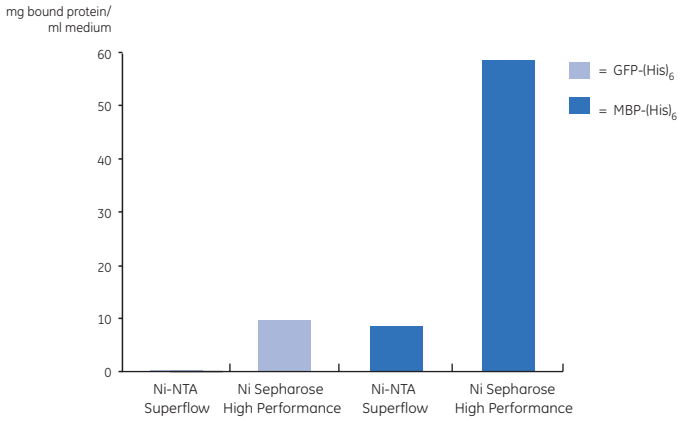


Fig 3. Reducing agents do not affect the purity or recovery of separation runs on Ni Sepharose High Performance.



Medium	MBP-$(His)_6$ mg/ml medium	GFP-$(His)_6$ mg/ml medium
Ni Sepharose High Performance	10	59
Ni-NTA Superflow[1]	0	9

[1] Further details of this comparison can be found at *www.gehealthcare.com/protocol-his*

Fig 4. Ni Sepharose High Performance has higher dynamic binding capacity ($QB_{10\%}$) for histidine-tagged maltose binding protein, MBP-$(His)_6$, and histidine-tagged green fluorescent protein, GFP-$(His)_6$, in the presence of 8 M urea compared with Ni-NTA Superflow. Note that binding capacity is dependent on the protein.

Figure 4 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 Ni-NTA Superflow™ failed to match this performance.

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

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 compounds commonly used in the purification of histidine-tagged proteins.

In most IMAC applications, imidazole is used for elution and, at lower concentrations, to increase the selectivity for histidine-tagged proteins. It is well known that the binding of untagged contaminants is suppressed with imidazole, and that too high a concentration of imidazole will also prevent binding of histidine-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 histidine-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.

Table 4. Ni Sepharose High Performance is stable in the following reducing and denaturing agents, at least at the concentrations shown

5 mM DTE[1]
5 mM DTT[1]
20 mM β-mercaptoethanol[1]
5 mM TCEP[1]
10 mM reduced glutathione[1]
8 M urea[2]
6 M guanidine hydrochloride[2]

[1] For best results, perform a blank run before including reducing agents in the sample/buffer. For details see the instructions 17-5027-68 and 71-5027-67.

[2] Tested for one week at 40°C.

Figure 5 presents a typical purification of a histidine-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 Ni-NTA Superflow. Also note that the amount of nickel ion in the eluted fractions is lower in the fractions pooled from Ni Sepharose High Performance, which confirms 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 over two-fold lower than Ni-NTA Superflow. In some cases, this can be very important for preventing precipitation of the target protein.

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

2% Triton™ X-100 (nonionic detergent)
2% Tween™ 20 (nonionic detergent)
2% NP-40 (nonionic detergent)
2% cholate (anionic detergent)
1% CHAPS (zwitterionic detergent)
500 mM imidazole
20% ethanol
50% glycerol
100 mM Na_2SO_4
1.5 M NaCl
1 mM EDTA[1]
60 mM citrate[1]
50 mM sodium phosphate, 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
100 mM sodium acetate, pH 4[2]

[1] The strong chelator EDTA has been used successfully at 1 mM. Generally, chelating agents should be used with caution (and only in the sample, not the buffer). Any metal-ion stripping may be counteracted by addition of a small excess of $MgCl_2$ before centrifugation/filtration of the sample. Note that stripping effects may vary with applied sample volume.

[2] Tested for one week at 40°C.

Column: 1) HisTrap HP 1 ml
2) 1 ml column, dimensions (i.d. × h) 5 mm × 5 cm

Media: 1) Ni Sepharose High Performance
2) Ni-NTA Superflow

Sample: 12 mg histidine-tagged green fluorescent protein, GFP-$(His)_6$, *E. coli* extract

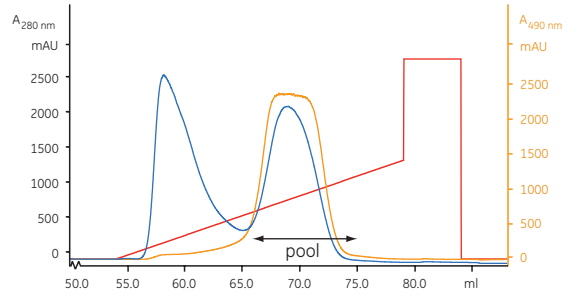
Sample volume: 19 ml (clarified extract)

Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 5 mM imidazole, pH 7.4

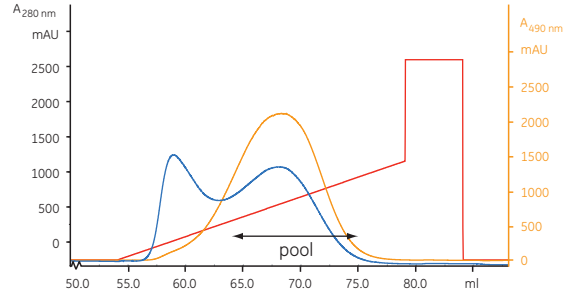
Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 M imidazole, pH 7.4

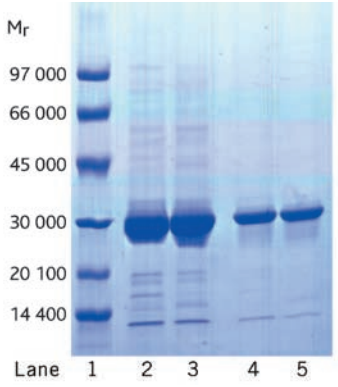
Gradient: 25 ml linear gradient of 5 to 250 mM imidazole, followed by a "push" with 500 mM imidazole

1. HisTrap HP



2. Ni-NTA Superflow





Lane
1. LMW markers
2. Eluted pool, HisTrap HP
3. Eluted pool, Ni-NTA Superflow
4. Eluted pool, HisTrap HP, dil. 1:4
5. Eluted pool, Ni-NTA Superflow, 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) Ni-NTA Superflow	231	0.91	0.121

Fig 5. Protein concentration is higher and nickel leakage lower in pooled fractions from Ni Sepharose High Performance 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 obtain equal intensity of target protein bands. This was confirmed by the 1:4 dilutions. The peak areas are not comparable since the highest absorbance signals are above the linear range.

Column volume: 0.25 ml, dimensions (i.d. × h), 5 mm × 1.3 cm

Medium: 1) Ni Sepharose High Performance. 2) Ni-NTA Superflow

Sample: Pure histidine-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, 500 mM NaCl, pH 7.4

Elution buffer: 20 mM sodium phosphate, 500 mM imidazole, 500 mM NaCl, pH 7.4

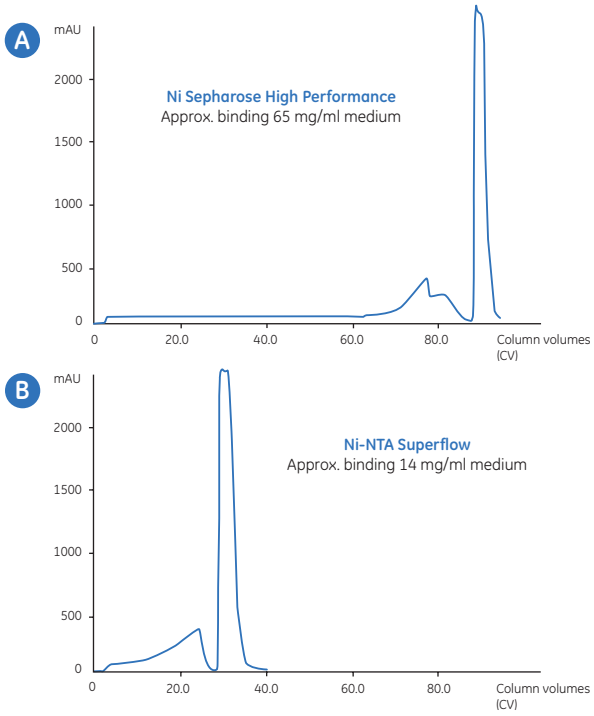


Fig 6. Ni Sepharose High Performance has a greater dynamic binding capacity (seen here as time of binding) for MBP-$(His)_6$ than Ni-NTA Superflow. The areas of the elution peaks cannot be compared since the highest absorbance signals are above the linear range.

Summary

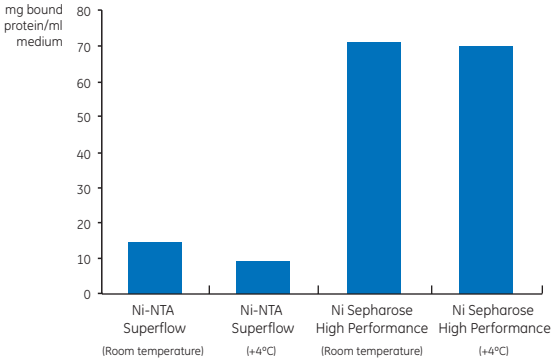
The overall high chemical stability of Ni Sepharose High Performance also applies to the medium in its HisTrap HP column formats. As well as helping maintain biological activity and increasing product yield, it greatly expands the range of conditions in which the medium can be used.

High protein binding capacity for more efficient purifications

The dynamic binding capacity of Ni Sepharose High Performance has been evaluated with pure histidine-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, that is, the column starts to saturate. The volume of protein solution (or milligram protein) that has been applied up to a certain breakthrough point (for example, $QB_{10\%}$) is a measure of the dynamic protein binding capacity of the medium (protein is then eluted after a wash).

Comparative chromatograms (Fig 6) demonstrate that Ni Sepharose High Performance binds 1 mg/ml MBP-$(His)_6$ for a considerably longer period (Approx. 70 column volumes)



Medium	MBP-$(His)_6$ mg/ml medium
Ni Sepharose High Performance:	
Room temp.	68
4°C	67
Ni-NTA Superflow:	
Room temp.	13
4°C	8

Fig 7. Dynamic binding capacity data ($QB_{10\%}$) for Ni Sepharose High Performance determined for MBP-$(His)_6$ exceeds that of Ni-NTA Superflow at room temperature and 4°C. Note that binding capacity will vary from protein to protein and at different temperatures.

than Ni-NTA Superflow (approx. 20 column volumes) before the protein elutes from the column, that is before breakthrough has occurred. The determined dynamic binding capacity of Ni Sepharose High Performance is 65 mg/ml medium, which greatly exceeds that of Ni-NTA Superflow (14 mg/ml medium).

Figure 7 shows protein binding capacity data determined at room temperature and 4°C. Once again, Ni Sepharose High Performance outperforms Ni-NTA Superflow.

Figure 8 presents further evidence of the excellent protein binding capacity of Ni Sepharose High Performance compared with other media. 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 Ni-NTA Superflow and over three times higher than His-Select™. SDS-PAGE of equal volumes of the eluted peaks confirms the result (Fig 9)

Summary

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

Columns: 1. HisTrap HP 1 ml
2. Ni-NTA Superflow 1 ml
3. His-Select 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, 500 mM NaCl, 5 mM imidazole, pH 7.4

Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4

Gradient: 25 ml linear gradient, 5 to 250 mM imidazole

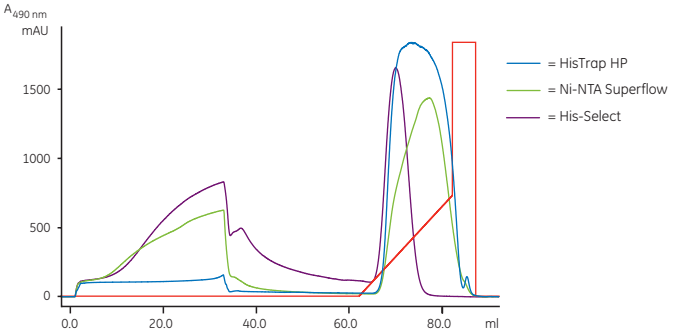
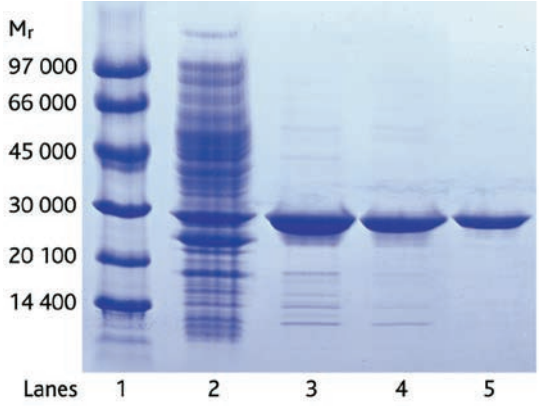


Fig 8. The binding capacity of Ni Sepharose High Performance for GFP-$(His)_6$ compared with two other media. Note the differences in loss of GFP-$(His)_6$ during sample application and differences in area of eluted peaks. Details of the comparison between Ni Sepharose High Performance, Ni-NTA Superflow, and His-Select are found at www.gehealthcare.com/protocol-his. This experiment was performed at GE Healthcare, Protein Separations' laboratories.



Lane
1. LMW markers
2. Start material, GFP-$(His)_6$
3. Eluted pool, HisTrap HP
4. Eluted pool, Ni-NTA Superflow
5. Eluted pool, His-Select

Fig 9. SDS-PAGE analysis of GFP-$(His)_6$ recovered in equal volumes of eluted peaks confirms the protein binding result seen in Figure 8. Improved purity can be achieved by increasing the imidazole concentration.

Fig 10. Using HisTrap HP 1 ml with a syringe. A) Prepare buffers and sample. Remove the column's top cap and snap-off the end. Wash and equilibrate. B) Load the sample and begin collecting fractions. C) Wash and elute, continue collecting fractions.

Speed, convenience, and increased reproducibility

Ni Sepharose High Performance is easy to pack and use in laboratory columns such as Tricorn and XK columns. HisTrap HP prepacked columns, however, maximize time-saving and increase ease-of-use of Ni Sepharose High Performance.

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. Figure 10 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 ÄKTA design chromatography systems, which include pre-programmed method templates for HisTrap HP columns. Method templates cover common techniques such as single-step purifications of histidine-tagged recombinant proteins, and allow many user-defined protocols to be stored. Simply snap the HisTrap HP column into place and start purifying.

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. Note, however, that the backpressure will increase (Ni Sepharose High Performance packed in laboratory columns from the Tricorn or XK series offers an equally reliable alternative).

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 histidine-tagged recombinant protein purification into a simple and reliable everyday laboratory tool.

Acknowledgements

MBP-$(His)_6$ was provided by Phadia, 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[2]	17-5268-01
	100 ml[2]	17-5268-02
	5 × 1 ml	17-5247-01
HisTrap HP[1]	100 × 1 ml[3]	17-5247-05
	1 × 5 ml	17-5248-01
	5 × 5 ml	17-5248-02
	100 × 5 ml[3]	17-5248-05

[1] Includes connectors for easy connection to syringe, pump, or chromatography system.

[2] Larger quantities are available.

[3] Pack size available by special order.

Related products	Quantity	Code No.
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml[1]	11-0003-29
HiPrep™ 26/10 Desalting	1 × 53 ml	17-5087-01
	4 × 53 ml	17-5087-02

[1] Pack size available by special order.

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	No. Supplied	Code No.
1/16" male/luer female[1]	2	18-1112-51
Tubing connector flangeless/M6 female[1]	2	18-1003-68
Tubing connector flangeless/M6 male[1]	2	18-1017-98
Union 1/16" female/M6 male[1]	6	18-1112-57
Union M6 female /1/16" male[1]	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"[2]	5	11-0004-64
Fingertight stop plug, 1/16"[3]	5	11-0003-55

[1] One connector included in each HiTrap/HisTrap package.

[2] Two, five, or seven stop plugs female included in HiTrap/HisTrap packages depending on the product.

[3] One fingertight stop plug is connected to the top of each HiTrap/HisTrap column.

Related literature	Quantity	Code No.
Recombinant Protein Handbook, Principles and Methods	1	18-1142-75
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29
Affinity Chromatography Columns and Media, Selection Guide	1	18-1121-86
Ni Sepharose and IMAC Sepharose, Selection Guide	1	28-4070-92
HiTrap Column Guide	1	18-1129-81
Prepacked chromatography columns for ÄKTA design systems	1	29-9317-78

GE Healthcare Europe GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare UK Ltd
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Bio-Sciences Corp
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg. 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

For local office contact information, visit

www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com/protein-purification

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