Ni **Sepharose** 6 Fast Flow
HisPrep FF 16/10
HisTrap FF

AFFINITY CHROMATOGRAPHY

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Purifying histidine-tagged recombinant proteins by immobilized metal affinity chromatography (IMAC) continues to grow in popularity. Nickel (Ni^{2+}) is the most commonly used metal ion in IMAC purifications. Ni Sepharose™ 6 Fast Flow is a BioProcess™ medium (resin) that combines the advantages of using Ni^{2+} for purification of histidine-tagged proteins with the well-established properties of the Sepharose Fast Flow platform.

Key features include:

- Fast, reliable scale-up of histidine-tagged protein purifications
- High protein binding capacity, and minimal leakage of Ni^{2+} ions
- Compatible with a very wide range of reducing agents, detergents, and other additives
- As a BioProcess medium, Ni Sepharose 6 Fast Flow meets industrial demands with security of supply and comprehensive regulatory support
- Available as prepacked HisPrep™ and HisTrap™ columns for added speed, convenience, and reproducibility
- Suitable for gravity-flow purification using His GraviTrap™ columns, and multiwell plate screening using His MultiTrap™ plates

IMAC Sepharose 6 Fast Flow is a related affinity medium, supplied free of metal ions, allowing the user to optimize selectivity by charging the medium with the most appropriate metal ion (see data file 28-4041-06 for more details).

Chromatography medium characteristics

Ni Sepharose 6 Fast Flow consists of 90 µm beads of highly cross-linked agarose, to which a chelating ligand has been immobilized. The chelating ligand is immobilized to the Sepharose 6 Fast Flow matrix at a density that, when charged with Ni^{2+} ions, exhibits a high binding capacity for proteins. Furthermore, leakage of Ni^{2+} ions is minimized.



Fig 1. Ni Sepharose 6 Fast Flow is available for a broad range of applications, from convenient, prepacked HisTrap FF laboratory-scale columns to bulk quantities.

The medium is compatible with a wide range of additives commonly used in the purification of histidine-tagged proteins. Table 1 lists the main characteristics of Ni Sepharose 6 Fast Flow. The medium is easy to work with and convenient to pack in a wide variety of columns, from laboratory- to production-scale (Fig 1).

BioProcess media

BioProcess media are developed and supported for production-scale chromatography. Ni Sepharose 6 Fast Flow is a BioProcess medium and therefore fulfills essential criteria for validated production-scale manufacture, secure supply, scalability, and regulatory support. Regulatory Support Files (RSF) are available to assist process validation and submissions to regulatory authorities.

Ni Sepharose 6 Fast Flow is supplied preswollen in 5 mL, 25 mL, 100 mL, 500 mL, 1 L and 5 L packs. The medium is easy to pack in a wide range of columns (Table 2). Full user instructions for packing, optimization, operation, cleaning, and recharging are supplied with each pack.

Table 1. Main characteristics of Ni Sepharose 6 Fast Flow

Matrix	Highly cross-linked 6% spherical agarose
Average particle size	90 µm
Dynamic binding capacity[1]	Approx. 40 mg histidine-tagged protein/mL medium
Metal ion capacity	Approx. 15 µmol Ni^{2+}/mL medium
Max. linear flow velocity[2]	600 cm/h (20 mL/min) using XK 16/20 column with 5 cm bed height
Recommended flow velocity[2]	150 cm/h
Max. operating pressure†	0.1 MPa, 1 bar (when packed in XK columns. May vary if used in other columns)
Compatibility during use	Stable in all commonly used buffers, reducing agents, denaturants, and detergents, see Table 8
Chemical stability[3]	0.01 M HCl, 0.1 M NaOH Tested for 1 week at 40°C 1 M NaOH, 70% acetic acid Tested for 12 h. 2% SDS Tested for 1 h 30% 2-propanol, tested for 30 min
pH stability[3]	Cleaning[4]: 2 to 14 Working[5]: 3 to 12
	Storage 4°C to 30°C in 20% ethanol

[1] Dynamic binding capacity conditions:
Sample: 1 mg/mL $(histidine)_6$-tagged pure proteins (M_r 43 000) in binding buffer (capacity at 10% breakthrough) or $(histidine)_6$-tagged protein (M_r 28 000) bound from *E. coli* extract.
Column volume: 0.25 mL or 1 mL
Flow rate: 0.25 mL/min or 1 mL/min, respectively
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
Note: Dynamic binding capacity is protein-dependent.

[2] H_2O at room temperature.

[3] Ni^{2+}-stripped medium.

[4] Refers to the pH interval for regeneration

[5] Refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance

Prepacked columns

Ni Sepharose 6 Fast Flow is available in the prepacked column formats HisPrep FF 16/10 and HisTrap FF.

HisTrap FF and HisTrap FF crude columns

HisTrap FF and HisTrap FF crude 1 mL and 5 mL prepacked columns offer reliable and convenient purification of histidine-tagged recombinant proteins. The columns are simple to operate with a syringe and the supplied Luer adapter. The columns can also be used with a pump or chromatography system such as ÄKTA™ systems. Note that ÄKTA chromatography systems include preset method templates for HisTrap FF and HisTrap FF crude, which further enhances the simplicity of operation and reproducibility. In addition, multiple columns can be easily connected in series for increased purification capacity.

Table 2. Recommended columns for Ni Sepharose 6 Fast Flow at different scales of operation.

Column	Inner diam. (mm)	Bed volume	Bed height max (cm)
Laboratory-scale			
Tricorn™ 5/20	5	up to 0.55 mL	2.8
Tricorn 5/50	5	up to 1.1 mL	5.8
Tricorn 10/20	10	up to 2.2 mL	2.8
Tricorn 10/50	10	up to 4.5 mL	5.8
Tricorn 10/100	10	up to 8.5 mL	10.8
XK 16/20	16	up to 30 mL	15
XK 16/40	16	up to 70 mL	35
XK 26/20	26	up to 80 mL	15
XK 26/40	26	up to 190 mL	35
XK 50/20	50	up to 275 mL	15
XK 50/30	50	up to 510 mL	25
Production-scale			
BPG 100/500	100	up to 2.0 L	26
BPG 140/500	140	up to 4.0 L	26
BPG 200/500	200	up to 8.2 L	26
BPG 300/500	300	up to 18.0 L	26
BPG 450/500	450	17.2–34.4 L	23
Chromaflow™ 400/100–300	400	13–38 L	30
Chromaflow 600/100–300	600	28–85 L	30
AxiChrom™ 50/300	50	0.20–0.59 L	30
AxiChrom 70/300	70	0.38–1.15 L	30
AxiChrom 100/300	100	0.79–2.36 L	30
AxiChrom 140/300	140	1.54–4.62 L	30
AxiChrom 200/300	200	3.14–9.4 L	30
AxiChrom 400/300	400	13–38 L	30
AxiChrom 600/300	600	28–85 L	30
AxiChrom 800/300	800	50–151 L	30
AxiChrom 1000/300	1000	79–236 L	30

The main benefit of using HisTrap FF crude is that unclarified cell lysate can be directly loaded on the columns without the need for pretreatment of the sample, for example, by filtration or clarification. Detailed information is available in the instructions included with each pack.

HiTrap™ columns are made of biocompatible polypropylene. The porous top and bottom frits are fully compatible with the high flow rate property of Ni Sepharose 6 Fast Flow. Columns are delivered with a stopper on the inlet and a snap-off end on the outlet. Table 3 lists the main properties of HisTrap FF and HisTrap FF crude 1 mL and 5 mL. Note that the columns cannot be opened or repacked.

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Table 3. Main characteristics of HisTrap FF and HisTrap FF crude columns

Medium	Ni Sepharose 6 Fast Flow (see Table 1)
Column volumes	1 mL or 5 mL
Column dimensions (i.d. × H)	0.7 × 2.5 cm (1 mL column) 1.6 × 2.5 cm (5 mL column)
Recommended flow rate*	1 mL/min (1 mL column) 5 mL/min (5 mL column)
Maximum flow rates*	4 mL/min (1 mL column) 20 mL/min (5 mL column)
Column hardware pressure limit	5 bar (0.5 MPa, 73 psi)
Storage	20% ethanol
Storage temperature	4ºC to 30ºC

* H_2O at room temperature

HisPrep FF 16/10

HisPrep FF 16/10 prepacked columns with a 20 mL bed volume provide fast, reproducible purifications of histidine-tagged proteins. The columns are simple to operate and compatible with single-pump chromatography configurations, as well as ÄKTA systems.

HiPrep™ 16/10 columns are made of transparent, biocompatible polypropylene. Table 4 lists the main characteristics of HisPrep FF 16/10. Note that HisPrep FF 16/10 columns cannot be opened or repacked.

Table 4. Main characteristics of HisPrep FF 16/10 columns

Medium	Ni Sepharose 6 Fast Flow (see Table 1)
Bed volume	20 mL
Bed height	100 mm
Inner diameter	16 mm
Column hardware	Polypropylene
Recommended flow rate*	1 to 10 mL/min (30 to 300 cm/h)
Maximum flow rate*	10 mL/min (300 cm/h)
Column hardware pressure limit	5 bar (0.5 MPa, 73 psi)
Storage	20% ethanol
Storage temperature	4°C to 30°C

* H_2O at room temperature

Gravity-flow column purification

Ni Sepharose 6 Fast Flow is particularly useful for gravity-flow purification of histidine-tagged proteins using prepacked His GraviTrap columns. The single-use columns offer simple gravity-flow purifications without any need for a chromatography system. With a high binding capacity, large sample volumes can be applied and the histidine-tagged protein is purified rapidly in a small elution volume. For more information about His GraviTrap, see data file 11-0036-90.

Multiwell plate screening and purification

His MultiTrap FF are 96-well filter plates prepacked with Ni Sepharose 6 Fast Flow to simplify screening and small scale purification of up to 1 mg of histidine-tagged protein/well. The plates provide high well-to-well and plate-to-plate reproducibility, delivering consistent results.

His MultiTrap FF can be operated on a robotic system or manually by centrifugation or vacuum. The purification protocol can easily be scaled up for use with larger column formats such as HisTrap FF and HisPrep FF 16/10 or for use with larger laboratory- and production-scale columns (Table 2). For more information about His MultiTrap, see data file 11-0036-63.

Minimal nickel leakage

The ability of Ni Sepharose 6 Fast Flow to bind and hold nickel ions was tested by charging the medium 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 difference between the amount charged and the amount still bound.

Below, nickel leakage and other performance data for Ni Sepharose 6 Fast Flow have been compared with other products on the market also intended for histidine-tagged protein purification — Ni-NTA Superflow™ and HIS-Select™* (Qiagen GmbH and Sigma-Aldrich Co., respectively).

For Ni Sepharose 6 Fast Flow, very low leakage was seen over a wide interval of nickel capacities tested, demonstrating that the synthesis and coupling procedures used in manufacturing result in a highly homogeneous chelating ligand. In contrast, nickel leakage from Ni-NTA Superflow, investigated using the same test, was found to be 9% on average (batch-to-batch variation), compared to < 5% for Ni Sepharose 6 Fast Flow (data not shown). Minimal nickel leakage with Ni Sepharose 6 Fast Flow minimizes protein precipitation and other problems, leading to higher yields.

* All experiments followed the manufacturer's instructions and were performed at Cytiva's laboratories.

Ni^{2+} leakage during purification

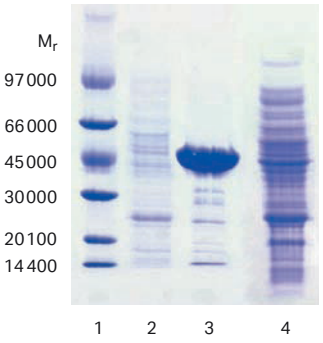
Purification of histidine-tagged maltose binding protein, MBP-$(histidine)_6$, was performed in combination with Ni^{2+} leakage determination. Figure 2 shows the SDS-PAGE results. Table 5 summarizes purity and yield as well as Ni^{2+} leakage data.

Table 5. MBP-$(histidine)_6$ purification results including Ni^{2+} leakage data

Amount eluted pool (mg)	Yield (%)	Ni^{2+} content (µg/L)	Total Ni^{2+} in pool (nmol)	Molar ratio Ni^{2+}/protein
6.5	82	341	17.4	0.12

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Column: HisTrap FF 1 mL
Sample: MBP-(histidine)$_6$ in *E. coli* extract
Binding Buffer: 20 mM sodium phosphate, 25 mM imidazole, 500 mM NaCl, pH 7.4
Elution Buffer: 20 mM sodium phosphate, 500 mM imidazole, 500 mM NaCl, pH 7.4
Flow rate: 1 mL/min



Lanes
1. LMW markers (LMW-SDS Marker Kit)
2. Flowthrough
3. Eluted pool
4. Start material

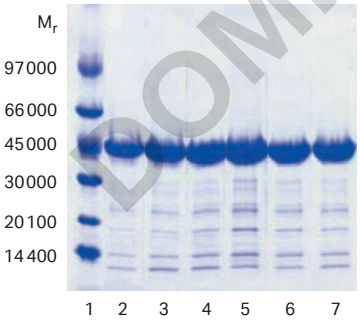
Fig 2. Purification of MBP-(histidine)$_6$ on HisTrap FF 1 mL, and SDS-PAGE analysis under nonreducing conditions (ExcelGel™ SDS Gradient 8–18).

Repeated purification without Ni^{2+} recharging

Reproducibility in terms of purity, selectivity, and recovery over a number of repeated runs without stripping, cleaning, or Ni^{2+} recharging was investigated. Six repeated purifications of MBP-(histidine)$_6$ and five repeated purifications of histidine-tagged green fluorescent protein, GFP-(histidine)$_6$, from *E. coli* extract, were run on HisTrap FF 1 mL and HisPrep FF 16/10 columns, respectively. The SDS-PAGE analysis and chromatograms (Figs 3 and 4) showed good reproducibility. The purity of the target protein did not change and no variation in recovery was observed. Furthermore, Ni^{2+} leakage was low in all cases (Table 6).

Note: The number of reproducible runs without Ni^{2+} recharging and cleaning will depend on the sample and protein used.

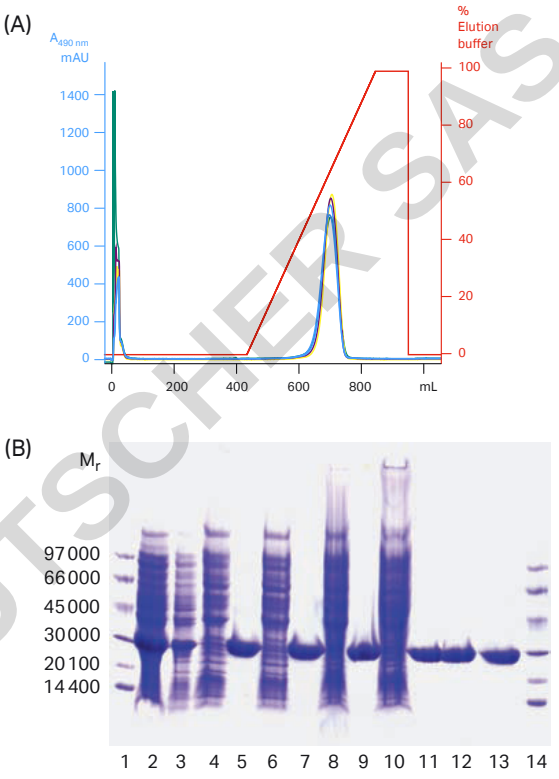
Column: HisTrap FF 1 mL
Sample: MBP-(histidine)$_6$ in *E. coli* extract
Binding Buffer: 20 mM sodium phosphate, 5 mM imidazole, 500 mM NaCl, pH 7.4
Elution Buffer: 20 mM sodium phosphate, 5-200 mM imidazole, 500 mM NaCl, pH 7.4 (linear gradient)
Flow rate: 1 mL/min



Lanes
1. LMW markers (LMW-SDS Marker Kit)
2. Run 1
3. Run 2
4. Run 3
5. Run 4
6. Run 5
7. Run 6

Fig 3. Six repeated purifications of MBP-(histidine)$_6$ from *E. coli* extract on Ni Sepharose 6 Fast Flow without stripping, cleaning, or Ni^{2+} recharging. Analysis by SDS-PAGE (nonreducing conditions; ExcelGel SDS Gradient 8–18 of the eluted pools shows excellent reproducibility.

Column: HisPrep FF 16/10
Sample: GFP-(histidine)$_6$ in *E. coli* lysate
Sample volume: 20 mL, ~140mg GFP-(histidine)$_6$
Binding and wash buffer: 20 mM sodium phosphate, 500 mM NaCl, 5 mM imidazole, pH 7.4
Elution buffer: 20 mM sodium phosphate, 500 mM sodium chloride, 250 mM imidazole, pH 7.4
Flow rate: 5 mL/min (150 cm/h)
Gradient: 0% to 100% elution buffer in 400mL (20CV)
Detection: 490 nm
System: ÄKTAexplorer 100



Lanes
1. LMW markers (LMW-SDS Marker Kit)
2. Start material GFP-(histidine)$_6$ in *E.coli* lysate, diluted 5×
3. Start material GFP-(histidine)$_6$ in *E.coli* lysate, diluted 10×
4. Flowthrough, HisPrep 16/10 FF (first-generation column)
5. Eluted pool, HisPrep 16/10 FF (first-generation column)
6. Flowthrough, HisPrep 16/10 FF, run 1
7. Eluted pool, HisPrep 16/10 FF, run 1
8. Flowthrough, HisPrep 16/10 FF, run 2
9. Eluted pool, HisPrep 16/10 FF, run 2
10. Flowthrough, HisPrep 16/10 FF, run 3
11. Eluted pool, HisPrep 16/10 FF, run 3
12. Eluted pool, HisPrep 16/10 FF, run 4
13. Eluted pool, HisPrep 16/10 FF, run 5
14. LMW markers (LMW-SDS Marker Kit)

Fig 4. Purification of GFP-(histidine)$_6$ from *E. coli* extract using HisPrep FF 16/10 columns. (A) Five repetitive purifications on the same HisPrep FF 16/10 column. (B) Analysis by SDS-PAGE (under reducing conditions; ExcelGel SDS Gradient 8–18), stained with Coomassie™, shows high reproducibility.

Table 6. Summary of results from the six repeated purification runs on HisTrap FF 1 mL

Run	Amount eluted protein (mg)	Yield (%)	Molar ratio Ni^{2+}/protein
1	7.5	88	0.14
2	7.4	87	0.11
3	7.4	87	0.08
4	7.2	85	0.07
5	7.5	88	0.07
6	7.5	88	0.06

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High reproducibility, stability, and compatibility

In Figure 4, HisPrep FF 16/10 columns gave high reproducibility in five repetitive purifications of GFP-(histidine)$_6$ on the same column. The high stability and compatibility of Ni Sepharose 6 Fast Flow makes it well-suited for scaling up purifications of histidine-tagged recombinant proteins. For example, the medium is stable in reducing agents such as DTT at concentrations up to 5 mM.

For best results, we recommend running a blank run without reducing agents before applying samples and buffers containing reducing agents. The same purity and recovery were achieved during repeated runs with 2 and 5 mM DTT on the same column (Fig 5).

Table 7 summarizes the compatibility of Ni Sepharose 6 Fast Flow with commonly used reducing agents, denaturing agents, detergents, additives, and buffer substitutes. In addition, the color of Ni Sepharose Fast Flow is essentially unaltered by low concentrations of reducing agents.

Table 7. Ni Sepharose 6 Fast Flow is compatible with the following reducing agents, denaturing agents, detergents, additives, and buffer substances (at least at the given concentrations)

Reducing agents*	5 mM DTE 5 mM DTT 20 mM β-mercaptoethanol 5 mM TCEP 10 mM reduced glutathione
Denaturing agents	8 M urea† 6 M Gua-HCl†
Detergents	2% Triton™ X-100 (nonionic) 2% Tween™ 20 (nonionic) 2% NP-40 (nonionic) 2% cholate (anionic) 1% CHAPS (zwitterionic)
Other additives	20% ethanol 50% glycerol 100 mM Na_2SO_4 1.5 M NaCl 1 mM EDTA‡ 60 mM citrate‡
Buffer substances	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†

* For best results, it is recommended to perform a blank run before including reducing agents in the sample/buffers. For details, see Instructions 11-0008-87, 11-0008-88, or 11-0008-89.

† Tested for 1 week at 40°C.

‡ The strong chelator EDTA has been used successfully in some cases at 1 mM. Generally, chelating agents should be used with caution (and only in the sample, not in buffers). 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.

Column: HisTrap FF 1 mL
Sample: MBP-(histidine)$_6$ in *E. coli* extract
Binding Buffer: 2 or 5 mM DTT, 20mM sodium phosphate, 25 mM imidazole, 500 mM NaCl, pH 7.4
Elution Buffer: 2 or 5 mM DTT, 20 mM sodium phosphate, 500 mM imidazole, 500 mM NaCl, pH 7.4
Flow rate: 1 mL/min

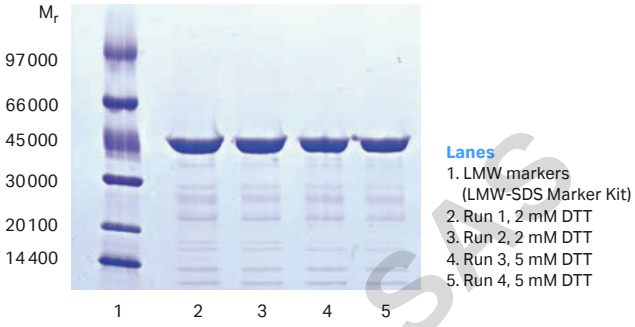


Fig 5. The purity and recovery of repeated separations using Ni Sepharose 6 Fast Flow are not affected by the reducing agent DTT.

High binding capacity

The binding capacity of Ni Sepharose 6 Fast Flow was compared to HIS-Select and Ni-NTA Superflow. Figure 6 presents a binding study using the maximum load of an *E. coli* extract containing GFP-(histidine)$_6$. Note the differences in loss of GFP-(histidine)$_6$ during sample application and wash. The purity of the eluted pools was similar according to SDS-PAGE analysis (Fig 6B), while the highest yield was demonstrated for Ni Sepharose 6 Fast Flow (Table 8).

Column volume: 1mL, (5 mm × 5 cm)
Media: 1) Ni Sepharoise 6 Fast Flow, 2) Ni-NTA Superflow, 3) HIS-Select
Sample: GFP-(histidine)$_6$ in *E. coli* extract
Binding Buffer: 20 mM sodium phosphate, 5 mM imidazole, 500 mM NaCl, pH 7.4
Elution Buffer: 20 mM sodium phosphate, 5-250 mM imidazole, 500 mM NaCl, pH 7.4
Flow rate: 1 mL/min

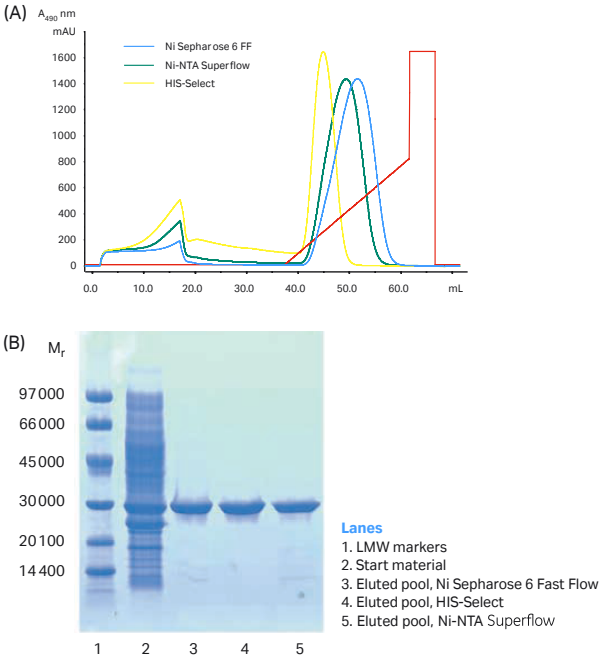


Fig 6. Maximum loading on Ni Sepharose 6 Fast Flow compared to HIS-Select and Ni-NTA Superflow. (A) Columns were loaded with 17 mL *E. coli* extract containing GFP-(histidine)$_6$. (B) SDS-PAGE (reducing conditions; ExcelGel SDS Gradient 8–18) of eluted pools following purification.

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Table 8. Amount of protein eluted after purification of GFP-(histidine)$_6$ on Ni Sepharose 6 Fast Flow, HIS-Select, or Ni-NTA Superflow

Medium	Amount eluted protein (mg)
Ni Sepharose 6 Fast Flow	40
HIS-Select	25
Ni-NTA Superflow	35

The dynamic binding capacity of Ni Sepharose 6 Fast Flow was also evaluated with pure MBP-(histidine)$_6$ (M_r 43 000) using the parameter 10% breakthrough ($Q_{B, 10\%}$). Comparative chromatograms demonstrate that the dynamic binding capacity of Ni Sepharose 6 Fast Flow was 49 mg/mL medium, which greatly exceeded that of Ni-NTA Superflow (14 mg/mL medium, Fig 7).

Column volume: 0.25 mL, (5 mm × 1.3 cm)
Media: 1) Ni Sepharoise 6 Fast Flow, 2) Ni-NTA Superflow
Sample: Pure MBP-(histidine)$_6$, M_r 43,000, 1 mg/ml
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
Flow rate: 0.25 mL/min

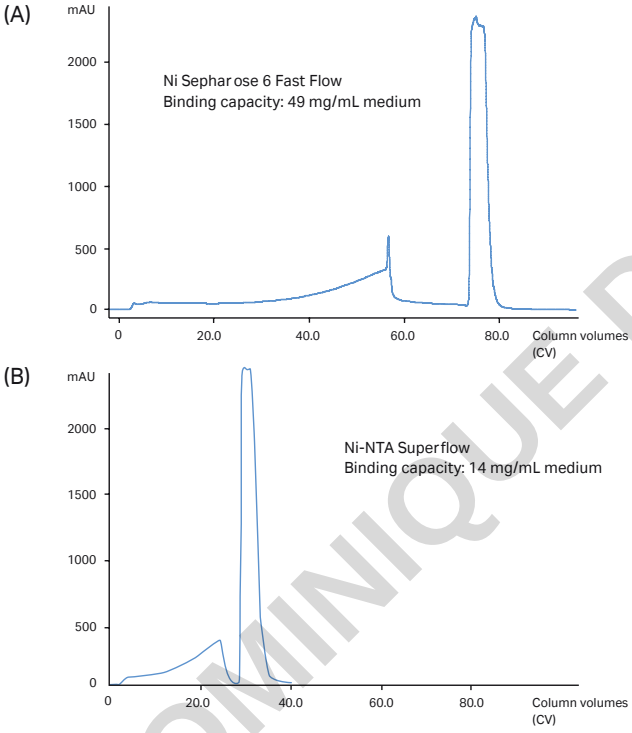


Fig 7. Ni Sepharose 6 Fast Flow has a greater dynamic binding capacity than Ni-NTA Superflow, measured as sample volume applied until 10% breakthrough for MBP-(histidine)$_6$. Note that the areas of the elution peaks cannot be compared since the highest absorbance signals are above the linear range.

High flow rates

Ni Sepharose 6 Fast Flow provides high performance even at high flow rates. Figure 8 shows the purification of MBP-(histidine)$_6$ in *E. coli* extract at three different flow rates (1, 2, and 4 mL/min; approx. 150, 300, and 600 cm/h, respectively) in a HisTrap FF 1 mL column. The results show minimal decrease in recovery and purity with increasing flow rate. The overall advantage of being able to increase flow rate is that the total time for protein purification is reduced considerably with a minimal loss in recovery (Table 9).

Column: HisTrap FF 1 mL
Sample: MBP-(histidine)$_6$ in *E. coli* extract
Binding Buffer: 20 mM sodium phosphate, 25 mM imidazole, 500 mM NaCl, pH 7.4
Elution Buffer: 20 mM sodium phosphate, 500 mM imidazole, 500 mM NaCl, pH 7.4
Flow rates: 1, 2 and 4 mL/min (approx. 150, 300, and 600cm/h, respectively)

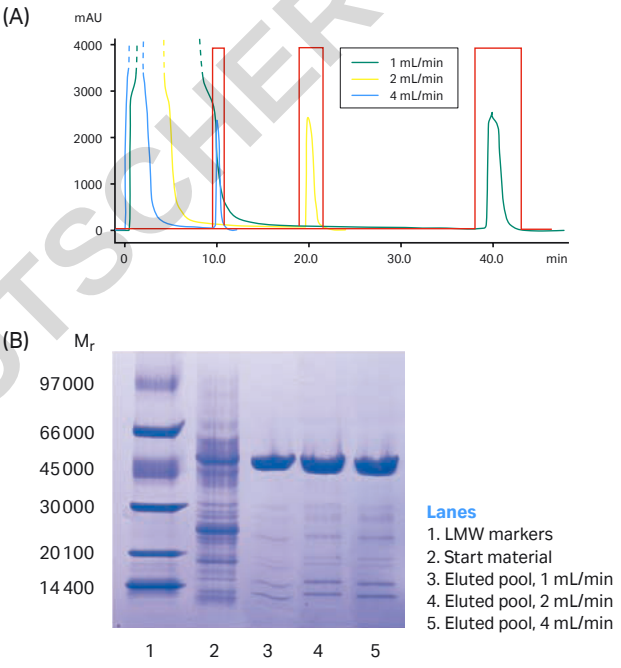


Fig 8. Ni Sepharose 6 Fast Flow provides high performance even at high flow rates. (A) Purification of MBP-(histidine)$_6$ from *E. coli* extract at three different flow rates. (B) Eluted material analyzed by SDS-PAGE (ExcelGel SDS Gradient 8–18), under nonreducing conditions, confirms that increasing flow rate does not significantly affect the recovery or purity of the purified material.

Table 9. Effects of different flow rates on total purification time and recovery

Flowrate (mL/min)	Time (min)	Eluted protein (mg)
1	48	5.4
2	24	5.3
4	12	5.2

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Columns:	HisTrap FF 1 mL, HisTrap FF 5 mL, HisPrep FF 16/10 (20 mL)
Sample:	MBP-(histidine)$_6$ in *E. coli* extract
Binding Buffer:	20 mM sodium phosphate, 25 mM imidazole, 500 mM NaCl, pH 7.4
Elution Buffer:	20 mM sodium phosphate, 500 mM imidazole, 500 mM NaCl, pH 7.4
Flow rates:	HisTrap FF 1 mL: 1 mL/min HisTrap FF 5 mL: 5 mL/min HisPrep FF 16/10: 5 mL/min

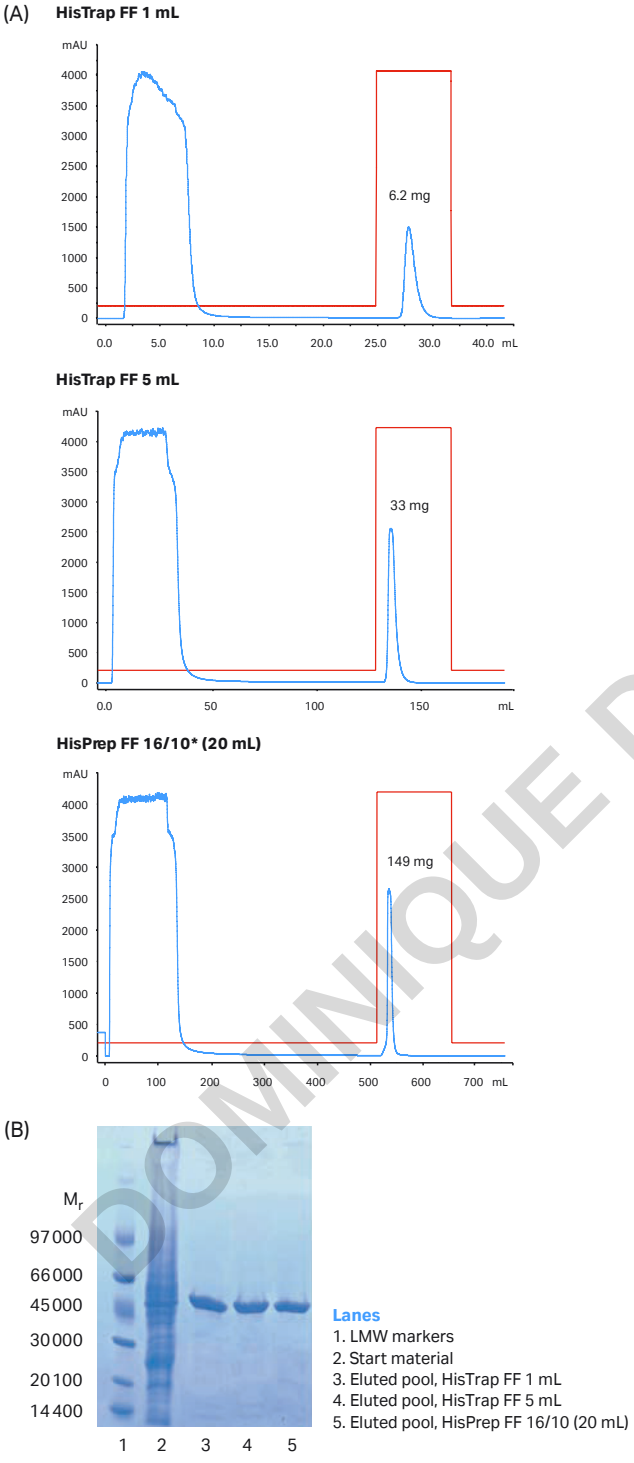


Fig 9. Scale-up from HisTrap FF 1 mL via HisTrap FF 5 mL to a HisPrep FF 16/10 (20 mL) prepacked column. The samples loaded contained approx. 8, 40, and 160 mg MBP-(histidine)$_6$, respectively. (A) Recovery in milligram is shown in each chromatogram. (B) SDS-PAGE (ExcelGel SDS Gradient 8–18) under nonreducing conditions confirms that scaling up from the 1 mL to the 20 mL column does not significantly affect the purification results.

* Note: Data was obtained using first-generation HisPrep 16/10 columns.

Easy scale-up

Laboratory scale

Scaling up column dimension using HisTrap and HisPrep columns, while running at the same linear flow rate provides highly consistent results. Figure 9 shows scale-up from HisTrap FF 1 mL via HisTrap FF 5 mL to HisPrep FF 16/10 (20 mL) prepacked columns. The sample used for purification was *E. coli* extract containing MBP-(histidine)$_6$. Pooled fractions analyzed by SDS-PAGE showed almost identical results in terms of purity and recovery (Fig 9B).

Laboratory- to pilot-scale

To go from laboratory- to pilot-scale, higher sample load is necessary. Scale-up was conducted with a high sample load (88% of the binding capacity) of MBP-(histidine)$_6$. The high sample load required optimization of the binding and wash buffer to avoid loss of MBP-(histidine)$_6$ during the wash step, and an imidazole concentration of 5 mM was found to give the best recovery and purity results. Two separate runs were conducted using HisPrep FF 16/10 columns to show the reproducibility of the purification. The protocol was then scaled up 10-fold using an AxiChrom 50 column (Fig 10A). Pooled fractions analyzed by SDS-PAGE gave almost identical results in terms of recovery and purity between the different runs and different scales, indicating a successful process scale-up (Fig 10B).

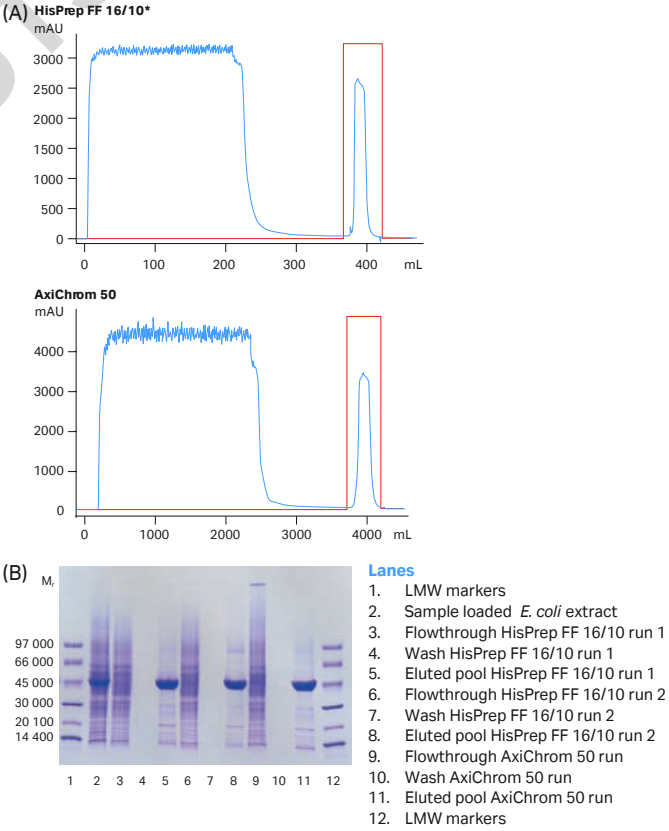


Fig 10. Scale-up from HisPrep FF 16/10 (20 mL) to AxiChrom 50 (210 mL) column. (A) ÄKTAexplorer 100 was used for the purification runs on HisPrep FF 16/10 columns and ÄKTApilot™ was used for AxiChrom 50 purification. All systems were controlled by UNICORN™ software. (B) SDS-PAGE (ExcelGel SDS Gradient 8-18) under nonreducing conditions shows the reproducibility of HisPrep runs and demonstrates that scaling up did not significantly affect the purity or recovery.

* Note: Data was obtained using first-generation HisPrep 16/10 columns.

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Ordering information

Product	Quantity	Code number
Ni Sepharose 6 Fast Flow	5 mL	17-5318-06
Ni Sepharose 6 Fast Flow	25 mL	17-5318-01
Ni Sepharose 6 Fast Flow	100 mL	17-5318-02
Ni Sepharose 6 Fast Flow	500 mL	17-5318-03
Ni Sepharose 6 Fast Flow	1 L	17-5318-04
Ni Sepharose 6 Fast Flow	5 L	17-5318-05
HisTrap FF	5 × 1 mL	17-5319-01
HisTrap FF	100 × 1 mL*	17-5319-02
HisTrap FF	5 × 5 mL	17-5255-01
HisTrap FF	100 × 5 mL*	17-5255-02
HisPrep FF 16/10	1 × 20 mL	28-9365-51
HisTrap FF crude	1 × 1 mL	29-0486-31
HisTrap FF crude	5 × 1 mL	11-0004-58
HisTrap FF crude	100 × 1 mL*	11-0004-59
HisTrap FF crude	5 × 5 mL	17-5286-01
HisTrap FF crude	100 × 5 mL*	17-5286-02
HisTrap FF crude Kit	1 kit	28-4014-77
HiTrap Desalting	1 × 5 mL	29-0486-84
HiTrap Desalting	5 × 5 mL	17-1408-01
HiPrep 26/10 Desalting	1 × 53 mL	17-5087-01
HiPrep 26/10 Desalting	4 × 53 mL	17-5087-02

* Pack size available by special order.

Use
20-40 mM Imidazole
in sample and binding buffer
FOR HIGHEST PURITY

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.

Empty laboratory-scale columns	Quantity	Code number
Tricorn 10/20 column	1	28-4064-13
Tricorn 10/50 column	1	28-4064-14
Tricorn 10/100 column	1	28-4064-15
XK 16/20 column	1	18-8773-01
XK 26/20 column	1	18-1000-72
XK 50/20 column	1	18-1000-71
XK 50/30 column	1	18-8751-01

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, Selection guide	18-1121-86
Ni Sepharose and IMAC Sepharose, Selection guide	28-4070-92
HiTrap Column Guide	18-1129-81
Prepacked chromatography columns for ÄKTA systems, Selection guide	28-9317-78

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