HisTrap FF crude columns
HisTrap FF crude Kit

TAGGED PROTEIN PURIFICATION

HisTrap™ FF crude is a ready to use column for convenient purification of histidine-tagged proteins from unclarified lysates by immobilized metal affinity chromatography (IMAC). HisTrap FF crude columns shorten purification time, which can minimize degradation and oxidation of sensitive target proteins, resulting in improved purification of histidine-tagged proteins. Filters incorporated in the column enable direct loading of unclarified samples without the need for centrifugation and filtration.

HisTrap FF crude columns provide the following benefits:

- Reliable purification of histidine-tagged proteins directly from unclarified lysates – simply sonicate and run
- Reduced sample preparation time, which minimizes risk of protein degradation by proteases
- Compatible with a wide range of reducing agents, detergents, denaturants, and other additives
- Available in convenient prepacked 1 ml and 5 ml HiTrap™ columns and HisTrap FF crude Kit
- Simple operation with a syringe, a pump, an ÄKTA™ system, or other chromatography systems

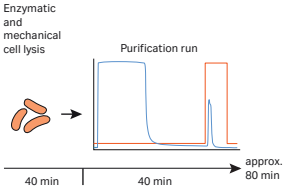
HisTrap FF crude columns are prepacked with Ni Sepharose™ 6 Fast Flow, which has high binding capacity for histidine-tagged proteins at high flow rates. Furthermore, the prepacked medium has low nickel (Ni^{2+}) ion leakage and is compatible with a wide range of additives used in the purification of histidine-tagged proteins.

Speed and convenience

HisTrap FF crude 1 ml and 5 ml columns permit rapid yet reliable separations with a minimum of sample preparation and equipment. Figure 2 illustrates the benefit of HisTrap FF crude in terms of the total sample preparation time saved.

Fig 1. HisTrap FF 1 ml and 5 ml crude columns, which are available individually or incorporated in HisTrap FF crude Kit, enable fast and convenient purification of histidine-tagged proteins by allowing loading of unclarified lysates directly on the column.

HisTrap FF crude



Conventional IMAC

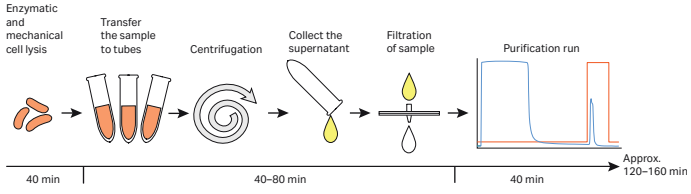


Fig 2. Schematic showing the time saved in purification of histidine-tagged proteins using HisTrap FF crude compared to conventional IMAC.

cytiva

Column characteristics

HisTrap FF crude columns are available in 1 ml and 5 ml formats. These columns are specifically designed to reduce the sample preparation needed for purification of histidine-tagged proteins. After cell disruption, it is possible to directly load the unclarified lysate on HisTrap FF crude columns without the need for laborious centrifugation and filtration. The novel filter system incorporated in the column makes direct loading of sonicated or homogenized lysates possible. The filter pore size is optimized to allow loading of sonicated lysates without causing backpressure problems or leakage of the Ni Sepharose 6 Fast Flow beads.

HisTrap FF crude columns are made of bio-inert polypropylene. Columns are delivered with a stopper on the inlet and a snap-off end on the outlet. Connectors for using the columns with a syringe, laboratory pump, or chromatography system are included in each package. Note that HisTrap FF crude columns cannot be opened and repacked.

Table 1. HisTrap FF crude characteristics

Matrix	Highly cross-linked spherical agarose, 6%
Average particle size	90 µm
Metal ion capacity	~15 µmol Ni^{2+}/ml medium
Dynamic binding capacity[1]	Approx. 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
Column hardware pressure limit	5 bar (70 psi, 0.5 MPa)
Compatibility during use	Stable in all commonly used buffers, reducing agents, denaturants, and detergents, see Table 2.
Chemical stability[3]	0.01 M HCl, 0.1 M NaOH. Tested for one 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.
Avoid in buffers	Chelating agents, e.g. EDTA, EGTA, citrate, see Table 2.
pH stability[3]	
Working[4]	3 to 12
Cleaning[5]	2 to 14
Storage	4°C to 30°C in 20% ethanol

[1] Dynamic binding capacity conditions:
Sample: 1 mg/ml $(histidine)_6$-tagged pure protein (M_r 43 000) in binding buffer
($QB_{10\%}$ determination) 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, 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

Note: Dynamic binding capacity is protein-dependent.

[2] H_2O at room temperature

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

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

[5] Refers to the pH interval for regeneration

Chromatography medium characteristics

HisTrap FF crude columns are delivered prepacked with Ni Sepharose 6 Fast Flow. This medium 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 such that charging it with Ni^{2+} ions ensures a high binding capacity for proteins. Furthermore, leakage of Ni^{2+} ions is negligible.

The medium is compatible with a wide range of additives commonly used in the purification of histidine-tagged proteins. Table 2 lists the compatibility of Ni Sepharose 6 Fast Flow with additives.

Table 2. Ni Sepharose 6 Fast Flow is stable in the following reducing agents, denaturing agents, detergents, additives, and buffer substances at the concentrations given

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	500 mM imidazole 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 11-0012-38 and 28-4036-83.

† Tested for one 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 $MgCl_2$ to the sample. Note that stripping effects may vary with applied sample volume.

No centrifugation or filtration required

HisTrap FF crude columns are designed to allow direct purification of unclarified cell lysates, without centrifugation or filtration in the sample preparation steps. Sample preparation methods such as sonication, homogenization, and freeze/thaw are, however, necessary prior to loading samples on HisTrap FF crude columns.

A general sample preparation protocol for use with HisTrap FF crude columns is:

1. Dilute cell paste in binding buffer.
2. Add lysozyme and DNAse for enzymatic lysis.
3. Perform mechanical lysis by sonication, homogenization, or freeze/thaw.
4. Adjust pH of the lysate and load on HisTrap FF crude column.

HisTrap FF crude Kit

HisTrap FF crude Kit (Fig 3) makes the purification of histidine-tagged recombinant proteins from unclarified cell lysates even more convenient. The kit includes three 1 ml HisTrap FF crude columns, ready-made binding and elution buffer concentrates, connectors, a syringe, and instructions.

The concentrates speed the rate at which results are obtained, and they help increase simplicity and reproducibility. Preparing sample, binding, and elution buffers and then running a basic purification protocol is done in a matter of minutes starting from an unclarified cell lysate. Purification can be performed simply using a syringe.

1. Lyse cells containing histidine-tagged protein.
2. Prepare buffers by mixing and diluting the concentrates.
3. Use the syringe to load unclarified sample to the column, wash, and elute the target protein.
4. Check purity by for example SDS-PAGE and/or Western blotting.

Fig 3. HisTrap FF crude Kit saves time and further increases convenience and simplicity when purifying histidine-tagged proteins from unclarified cell lysates.

The ready-made buffer concentrates in HisTrap FF crude Kit are made from high quality salts and water and filtered through a 0.45 µm filter. They comprise phosphate binding buffer, pH 7.4 (2 × 50 ml, 8 × concentrate), and 2 M imidazole elution buffer, pH 7.4. Easy-to-follow instructions, including sample preparation, purification and optimization protocols, as well as a troubleshooting guide are also included.

Figure 4 shows the purification of MBP-$(His)_6$ on a HisTrap FF crude 1 ml column using a syringe.

Evaluation of different cell lysis methods

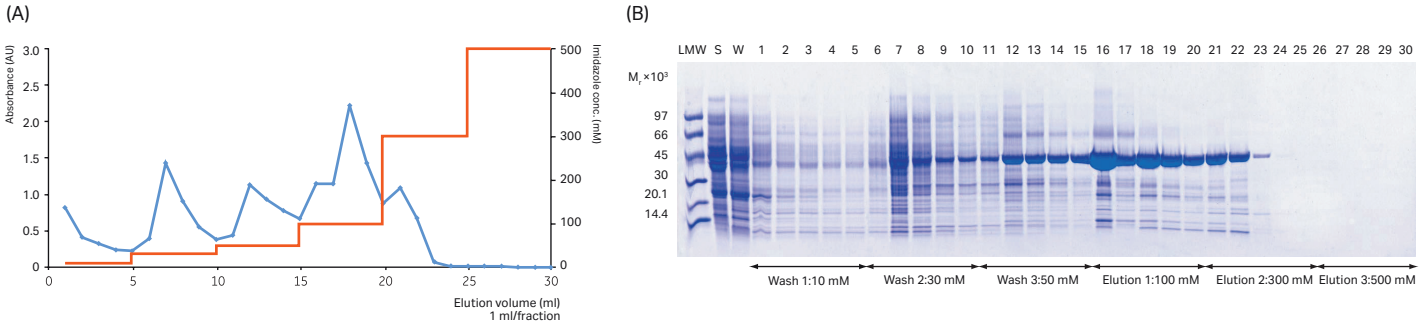
1. Sonication versus freeze/thaw

The effect of sonication and freeze/thaw on the purification performance of HisTrap FF crude was evaluated. $(Histidine)_6$ - tagged maltose binding protein (MBP-$[His]_6$) in *E. coli* DH5α lysate was sonicated or exposed to several cycles of freeze/thaw. For comparison, some of the sample was also clarified by centrifugation for 20 min followed by filtration. Purification was subsequently performed on HisTrap FF crude 1 ml. The sonication and freeze/thaw methods provided equally efficient purification of the MBP-$(His)_6$ as indicated in Figure 5. Moreover, the purification of unclarified MBP-$(His)_6$ after sample preparation by freeze/thaw was similar to that of the clarified sample (Fig 5).

2. Sonication versus homogenization

A sample of *E. coli* BL21 containing $(histidine)_6$-tagged green fluorescent protein (GFP-$[His]_6$) was prepared by sonication or homogenization. For comparison, an amount of the homogenized sample was also clarified by centrifugation/filtration. After purification on HisTrap FF crude 1 ml, eluted fractions and fraction pools were analyzed by SDS-PAGE (Fig 6). A comparison of the pooled fractions in lanes 18–20 shows similar purity and recovery of the protein for all the sample preparation methods used.

Purity and recovery of histidine-tagged protein purified using HisTrap FF crude is unaffected by either sonication or homogenization of the sample.



Column:	HisTrap FF crude 1 ml
Sample:	MBP-$(His)_6$, M_r 43 000
Flow rate:	Approx. 1 ml/min
Buffers:	Included in the HisTrap FF crude Kit, prepared according to the instructions
Fraction size:	1 ml
Equipment:	Manual purification using a syringe

Lanes	
LMW	LMW markers
S	Sample, unclarified, diluted 1:10
W	Wash 5 ml, 10 mM imidazole

Lanes	
Fraction 1-5	Wash 1, 10 mM imidazole in buffer
Fraction 6-10	Wash 2, 30 mM imidazole in buffer
Fraction 11-15	Wash 3, 50 mM imidazole in buffer
Fraction 16-20	Elution 1, 100 mM imidazole in buffer
Fraction 21-25	Elution 2, 300 mM imidazole in buffer
Fraction 26-30	Elution 3, 500 mM imidazole in buffer

Fig 4. (A) Purification of MBP-$(His)_6$ using HisTrap FF crude Kit. (B) Native SDS-PAGE (ExcelGel™ 8–18) of 1 ml fractions from the purification.

Column: HisTrap FF crude 1 ml
Sample: Unclarified or clarified *E. coli* DH5α lysate containing MBP-$(His)_6$ prepared by various methods (see text for details)
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
Elution: 5–250 mM imidazole (20 column volumes)
Flow rate: 0.5-1 mL/min
System: ÄKTAexplorer 100

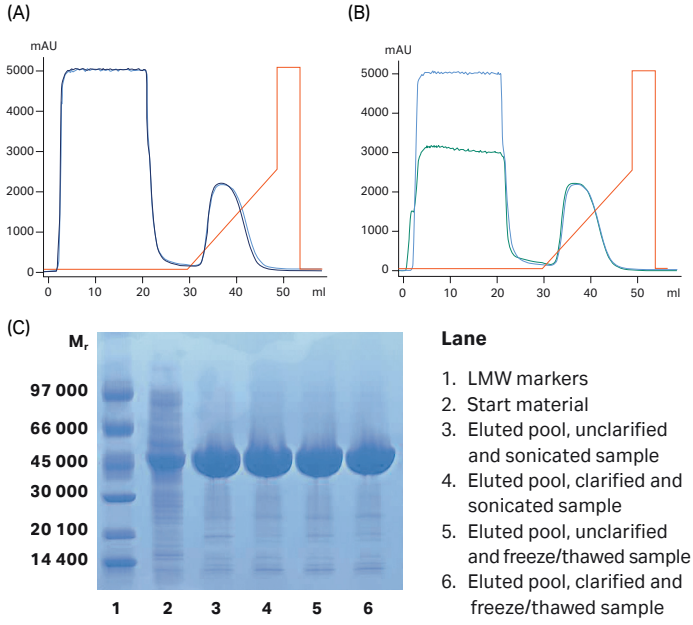


Fig 5. (A) Purification of unclarified MBP-$(His)_6$ sample prepared by sonication or freeze/thaw. Overlay of absorbance at 280 nm. *Purple*: sonicated sample. *Blue*: freeze/thaw sample. (B) Purification of unclarified and clarified MBP-$(His)_6$ sample prepared by freeze/thaw. Overlay of absorbance at 280 nm. *Blue*: freeze/thaw, unclarified sample. *Green*: freeze-thaw, clarified sample. (C) SDS-PAGE (ExcelGel SDS Gradient 8–18) under nonreducing conditions shows that the purity of protein from clarified and unclarified samples was unaffected by the sample preparation method used.

Column: HisTrap FF crude 1 ml
Sample: Unclarified or clarified *E. coli* BL21 lysate containing GFP-$(His)_6$ prepared by sonication/homogenization (see text for details)
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
Elution: 5–250 mM imidazole (20 column volumes)
Flow rate: 0.5–1 mL/min
System: ÄKTAexplorer 100

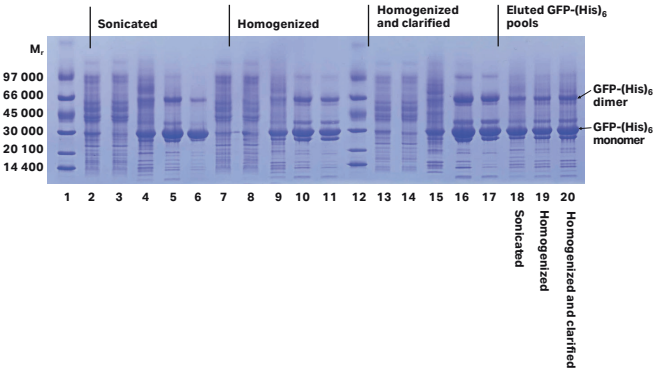


Fig 6. The purity and recovery of GFP-$(His)_6$ subjected to different sample preparation methods before purification on HisTrap FF crude 1 ml. SDS-PAGE of fractions under nonreducing conditions (ExcelGel SDS Gradient 8–18).

Purification of a histidine-tagged hydrolase expressed at low levels in *Pichia pastoris*

HisTrap FF crude columns are ideal for purification of histidine-tagged proteins expressed at low levels from hosts such as *Pichia pastoris*. Using HisTrap FF crude columns, highly pure protein can be obtained from unclarified lysates of *P. pastoris*.

Figure 7 shows the purification of a histidine-tagged *Saccharomyces cerevisiae* hydrolase expressed at low levels in *P. pastoris*. Unclarified sample was loaded onto a HisTrap FF crude 5 ml column. Purity of the protein from the unclarified sample was high as determined by SDS-PAGE.

Column: HisTrap FF crude 5 ml column
Sample: Unclarified lysate of YNR064c (*Saccharomyces cerevisiae* hydrolase) expressed in *P. pastoris*
Sample volume: 130 ml
Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 75 mM imidazole, pH 7.4
Elution buffer: 20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, 3 mM KCl, pH 7.4
Flow rate: 5 mL/min
System: ÄKTAexplorer 100

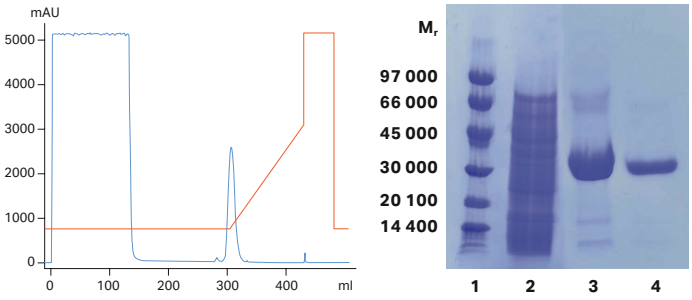


Fig 7. Purification of an unclarified sample of histidine-tagged *Saccharomyces cerevisiae* hydrolase expressed in *P. pastoris* on HisTrap FF crude 5 ml. SDS-PAGE under nonreducing conditions (ExcelGel SDS Gradient 8–18) shows the high purity obtained of the low-level expression protein.

Lane

1. LMW markers
2. Sonicated sample (diluted 1:10)
3. Flowthrough (diluted 1:10)
4. Eluted GFP-$(His)_6$ fractions
5. Eluted GFP-$(His)_6$ fractions
6. Eluted GFP-$(His)_6$ fractions
7. Homogenized sample (diluted 1:10)
8. Flowthrough (diluted 1:10)
9. Eluted GFP-$(His)_6$ fractions
10. Eluted GFP-$(His)_6$ fractions
11. Eluted GFP-$(His)_6$ fractions
12. LMW markers
13. Homogenized and clarified sample (diluted 1:10)
14. Flowthrough (diluted 1:10)
15. Eluted GFP-$(His)_6$ fractions
16. Eluted GFP-$(His)_6$ fractions
17. Eluted GFP-$(His)_6$ fractions
18. Eluted pool from sonicated sample
19. Eluted pool from homogenized sample
20. Eluted pool from homogenized and clarified sample

Negligible nickel leakage

The ability of Ni Sepharose 6 Fast Flow prepacked in HisTrap FF crude columns to bind and hold nickel ions was tested by charging the medium with Ni^{2+} ions and then exposing it to harsh acidic conditions (pH 4.0). The amount of nickel removed by this treatment was calculated as the difference between the amount charged and that still bound.

Low leakage was seen over a wide range 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 (Qiagen™) investigated using the same test was found to be 9%, while leakage from Ni Sepharose 6 Fast Flow was < 5%*.

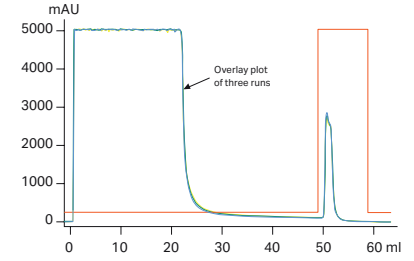
*Batch-to-batch variation was observed.

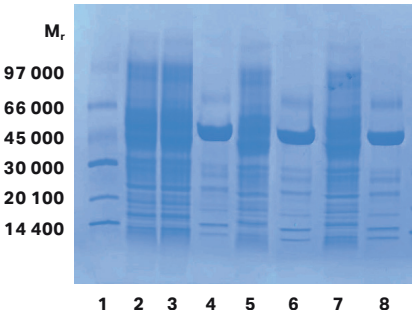
Repeated purification runs possible without Ni^{2+} recharging

Reproducibility of HisTrap FF crude columns in terms of purity, selectivity, and recovery over a number of repeated runs without stripping, cleaning, or Ni^{2+} recharging was investigated. $(Histidine)_6$-tagged maltose-binding protein (MBP-$[His]_6$) was purified from an unclarified lysate of *E. coli* in three consecutive runs without Ni^{2+} recharging of the chromatography medium (Fig 8). Enzymatic lysis and sonication were the only sample preparation steps applied to the sample prior to loading on the column.

Repeated purification runs of the unclarified lysate containing MBP-$(His)_6$ results in no significant change in purity and recovery as confirmed by SDS-PAGE (Fig 8). The overlay chromatogram of the three runs shows almost identical performance.

Column:	HisTrap FF crude 1 ml
Sample:	Unclarified *E. coli* BL21 lysate containing approx. 8 mg MBP-$(His)_6$
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 25 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Flow rate:	1 ml/min
System:	ÄKTAexplorer 10





Lane

1. LMW markers
2. Start material
3. Run 1 flowthrough
4. Run 1 eluted pool
5. Run 2 flowthrough
6. Run 2 eluted pool
7. Run 3 flowthrough
8. Run 3 eluted pool

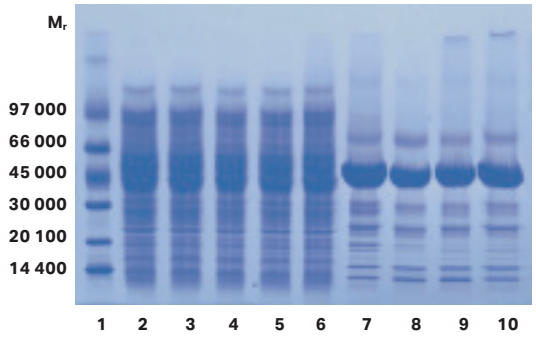
Fig 8. Three consecutive purifications of MBP-$(His)_6$ on HisTrap FF crude 1 ml (run 1 [orange], run 2 [green], run 3 [blue]) and SDS-PAGE of fractions under nonreducing conditions (ExcelGel SDS Gradient 8–18).

Column performance retained after repeated stripping and recharging of Ni^{2+} ions

The performance of HisTrap FF crude 1 ml during four repeated runs with Ni^{2+} ion stripping and recharging was assessed. The sample used was an unclarified lysate of *E. coli* BL21 containing MBP-$(His)_6$. Enzymatic lysis and sonication were the only sample preparation steps applied to the sample before loading on the column. After each purification run, Ni^{2+} ions were stripped from the column and cleaning-in-place (CIP) performed with 1 M NaOH (experimental conditions are described in Figure 9).

SDS-PAGE (Fig 9) reveals that a consistent level of protein purity is achieved over four purification runs with Ni^{2+} ion stripping, CIP, and recharging (lanes 7–10). The amount of protein recovered in the eluted pools of the four runs (lanes 7–10) was 7.8, 5.8, 6.4, and 6.8 mg respectively.

Column:	HisTrap FF crude 1 ml
Sample:	Unclarified *E. coli* BL21 lysate containing MBP-$(His)_6$
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 25 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Ni^{2+} ion stripping:	20 mM sodium phosphate, 500 mM NaCl, 50 mM EDTA, pH 7.4
Ni^{2+} ion recharging:	100 mM $NiSO_4$
Cleaning-in-place:	*1 M NaOH*
Flow rate:	1 ml/min
System:	ÄKTAexplorer 10



Lane

1. LMW markers
2. Start material (diluted 1:10)
3. Run 1 flowthrough (diluted 1:10)
4. Run 2 flowthrough (diluted 1:10)
5. Run 3 flowthrough (diluted 1:10)
6. Run 4 flowthrough (diluted 1:10)
7. Run 1 eluted pool (undiluted)
8. Run 2 eluted pool (undiluted)
9. Run 3 eluted pool (undiluted)
10. Run 4 eluted pool (undiluted)

Fig 9. Four repeated purifications of MBP-$(His)_6$ from *E. coli* extract on HisTrap FF crude 1 ml. The chromatography medium was subjected to Ni^{2+} ion stripping, CIP, and recharging between runs. SDS-PAGE (ExcelGel SDS Gradient 8–18) under nonreducing conditions shows that the purity of the protein obtained after each run was similar.

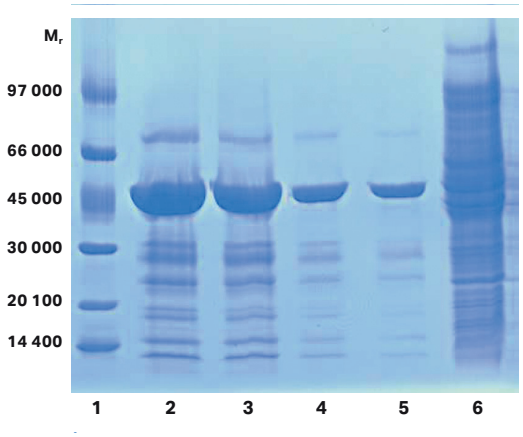
High stability and compatibility with reducing agents

Ni Sepharose 6 Fast Flow, the chromatography medium packed in HisTrap FF crude columns, is stable in many different reducing agents at relatively high concentrations (Table 2). For best results, we recommend performing a blank run without reducing agents before applying samples and buffers containing reducing agents.

To evaluate the effect of DTT on Ni Sepharose 6 Fast Flow packed in HiTrap columns, 5 mM DTT was included in an unclarified sample (*E. coli* BL21 extract containing MBP-$[His]_6$) and the buffers used for the purification of the protein. The sample underwent enzymatic lysis and sonication before the addition of DTT and purification of protein on HisTrap FF crude. Two repeated runs were performed without Ni^{2+} recharging or cleaning of the column between runs. No brown discoloration of the medium were observed. Note that a blank run was performed on the HisTrap FF crude column before loading of buffers/sample including DTT.

SDS-PAGE of fractions shows that purity and yield of the protein were unaffected by the DTT treatment (Fig 10).

Column:	HisTrap FF crude 1 ml
Sample:	Unclarified *E. coli* BL21 lysate containing MBP-$(His)_6$
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 25 mM imidazole, 5 mM DTT, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, 5 mM DTT, pH 7.4
Flow rate:	1 ml/min
System:	ÄKTAexplorer 100



Lane

1. LMW markers
2. Run 1 eluted pool (undiluted)
3. Run 2 eluted pool (undiluted)
4. Run 1 eluted pool (diluted 1:5)
5. Run 2 eluted pool (diluted 1:5)
6. Start material (diluted 1:10)

Fig 10. SDS-PAGE (ExcelGel SDS Gradient 8–18) of two repeated runs of MBP-$(His)_6$ on HisTrap FF crude in the presence of 5 mM DTT.

High protein binding capacity

Ni Sepharose 6 Fast Flow displays a histidine-tagged protein binding capacity that is higher than that found in comparative chromatography media from other manufacturers. The binding capacity of the medium is approximately 40 mg histidine-tagged protein/ml medium (Table 1). For more information on the binding capacity of Ni Sepharose 6 Fast Flow compared to products from other manufacturers, see Data File 11-0008-86, Ni Sepharose 6 Fast Flow.

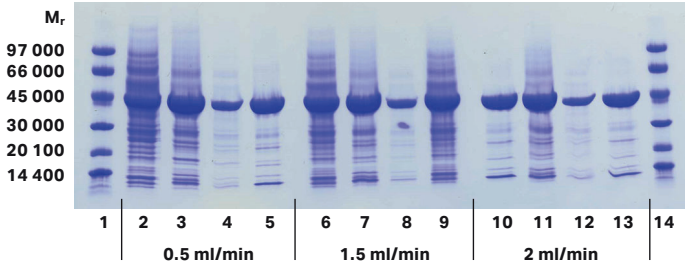
High flow rates

High flow rates can be used in purifications with HisTrap FF crude columns without affecting purity or increasing backpressure on the column.

MBP-$(His)_6$ expressed in *E. coli* BL21 was purified at three different flow rates (0.5, 1.5, and 2 ml/min) in a HisTrap FF crude 1 ml column. The sample was enzymatically lysed and sonicated before loading on the column. Figure 11 shows that HisTrap FF crude 1 ml could be used with flow rates up to 2 ml/min without adverse effects on the purity and recovery of MBP-$(His)_6$ purified from the unclarified cell lysate.

Table 3 shows that no increase in pressure on the column was observed during the purification.

Column:	HisTrap FF crude 1 ml
Sample:	Unclarified *E. coli* BL21 lysate containing MBP-$(His)_6$
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
Flow rate:	0.5–2.0 ml/min
System:	ÄKTAexplorer 100 with connected flow restrictor (0.2 MPa)



Lane

1. LMW markers
2. 0.5 ml/min fraction early in gradient
3. 0.5 ml/min eluted pool (undiluted)
4. 0.5 ml/min eluted pool (diluted 1:5)
5. 0.5 ml/min fraction late in gradient
6. 1.5 ml/min fraction early in gradient
7. 1.5 ml/min eluted pool (undiluted)
8. 1.5 ml/min eluted pool (diluted 1:5)
9. 1.5 ml/min fraction late in gradient
10. 2 ml/min fraction early in gradient
11. 2 ml/min eluted pool (undiluted)
12. 2 ml/min eluted pool (diluted 1:5)
13. 2 ml/min fraction late in gradient
14. LMW markers

Fig 11. Purity and recovery of MBP-$(His)_6$ purified from an unclarified lysate on HisTrap FF 1 ml. SDS-PAGE of pooled fractions was performed under nonreducing conditions on ExcelGel SDS Gradient 8–18. Fractions from early and late in the gradient, as well as eluted pools, were analyzed for each of the three flow rates tested.

Table 3. Effects of different flow rates on column pressure

Equilibration		Sample load		Wash		Elution	
Flow rate (ml/min)	Pressure (MPa)*	Flow rate (ml/min)	Pressure (MPa)*	Flow rate (ml/min)	Pressure (MPa)*	Flow rate (ml/min)	Pressure (MPa)*
1.0	0.22	0.5	0.34	0.5	0.27	1.0	0.32
1.5	0.28	1.5	0.39	1.5	0.28	1.5	0.28
2.0	0.27	2.0	0.39	2.0	0.30	2.0	0.30

* The total maximum pressure limit is 0.5 MPa when a flow restrictor is connected to an ÄKTA system; 0.2 MPa for the system and 0.3 MPa for the column.

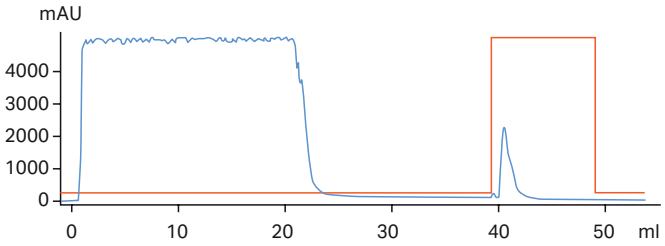
Scale-up from 1 ml to 5 ml columns

Figure 12 shows a scale-up study from 1 ml to 5 ml HisTrap FF crude columns. The sample was unclarified *E. coli* extract containing MBP-$(His)_6$, which had been subjected to enzymatic lysis in combination with homogenization prior to loading on the column. The samples contained approximately 8 and 40 mg MBP-$(His)_6$ for the 1 ml and 5 ml columns respectively.

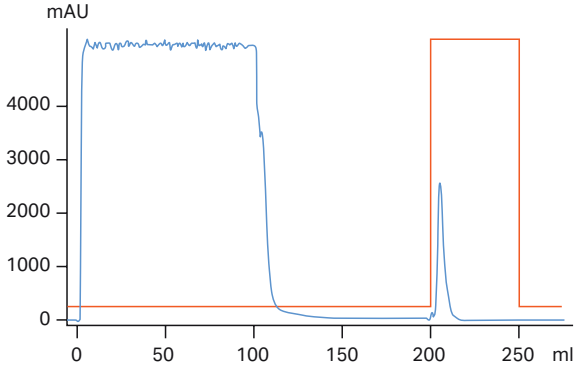
SDS-PAGE shows that the purity and recovery of the histidine-tagged protein purified on the two columns was almost identical.

Column:	HisTrap FF crude columns, 1 ml and 5 ml
Sample:	Unclarified *E. coli* BL21 lysate containing MBP-$(His)_6$
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 25 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Flow rate:	HisTrap FF crude 1 ml: 1 ml/min HisTrap FF crude 5 ml: 5 ml/min
System:	ÄKTAexplorer 100

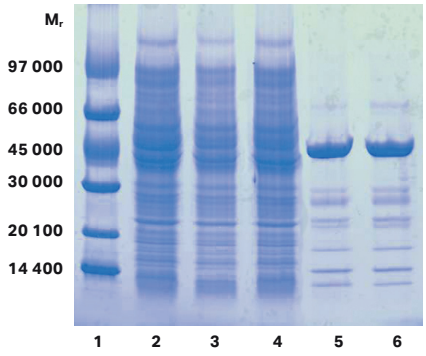
(A) **HisTrap FF crude 1 ml**



(B) **HisTrap FF crude 5 ml**



(C) **SDS-PAGE**



Lane

1. LMW markers
2. Start material (diluted 1:10)
3. Flowthrough, 1 ml column (diluted 1:10)
4. Flowthrough, 5 ml column (diluted 1:10)
5. Eluted pool 1 ml column (diluted 1:5)
6. Eluted pool 5 ml column (diluted 1:5)

Fig 12. Scale-up from (A) 1 ml to (B) 5 ml HisTrap FF crude columns. Recovery of protein was 6.3 and 35.2 mg for the 1 ml and 5 ml columns respectively. (C) SDS-PAGE under nonreducing conditions (ExcelGel SDS Gradient 8–18) confirms that scaling up from the 1 ml to the 5 ml column does not significantly affect the purification result.

Automated, multistep purification

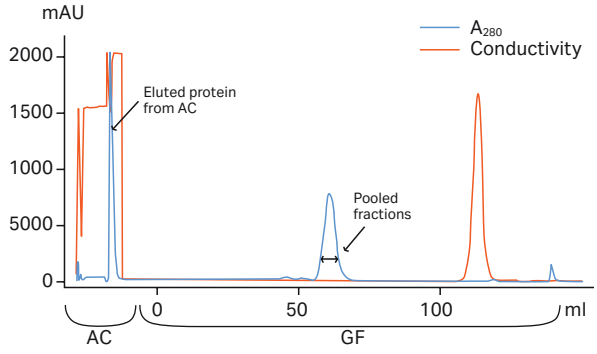
HisTrap FF crude columns can be run on systems for high-throughput purification of histidine-tagged proteins such as ÄKTAxpress. This system enables automated, parallel purification of histidine-tagged proteins with the capacity to run a number of different multistep protocols. A method wizard supplied with the UNICORN™ control software makes it easy to create methods for different purification protocols.

Figure 13 shows an automated two-step purification of an unclarified lysate of *E. coli* containing MBP-$(His)_6$. HisTrap FF crude 1 ml was used for the first step in the purification protocol, which was affinity chromatography (AC). The eluted peak from the affinity step was automatically collected in a loop and re-injected onto a HiLoad™ 16/60 Superdex™ 75 pg gel flitration column in the second step of the purification. Purity of the protein in fractions from the gel filtration step was confirmed by SDS-PAGE.

The results show that HisTrap FF crude together with ÄKTAxpress facilitates and enables significant time savings in the purification of histidine-tagged proteins without compromising sample purity.

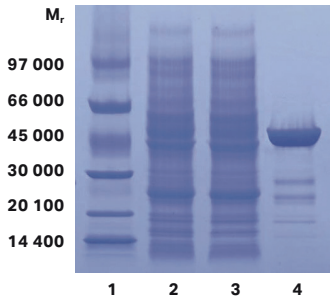
Affinity chromatography (IMAC) conditions

Column:	HisTrap FF crude, 1 ml
Sample:	Unclarified *E. coli* BL21 lysate containing MBP-$(His)_6$
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 25 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Flow rate:	1 ml/min
System:	ÄKTAxpress



Gel filtration (GF) conditions

Column:	HiLoad 16/60 Superdex 75 pg
Buffer:	10 mM sodium phosphate, 140 mM NaCl, 2.7 mM KCl, pH 7.4
Flow rate:	1.5 ml/min
System:	ÄKTAxpress



Lane

1. LMW markers
2. Start material (diluted 1:20)
3. Flowthrough, IMAC (diluted 1:10)
4. Pool from GF (diluted 1:10)

Fig 13. Automated two-step purification of MBP-$(His)_6$ from an unclarified lysate of *E. coli* using HisTrap FF crude on ÄKTAxpress. SDS-PAGE of pooled fractions of the protein from the gel filtration step was performed under nonreducing conditions on ExcelGel SDS Gradient 8–18.

Ordering information

Products	Quantity	Code number
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	3 × 1 ml + buffers	28-4014-77

*Pack size available by special order.

Related products	Quantity	Code number
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
HiScreen™ Ni FF	1 × 4.7 ml	28-9782-44
HisPrep™ FF 16/10	1 × 20 ml	28-9365-51
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
HiTrap Desalting	1 × 5 ml	29-0486-84
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml*	11-0003-29
PD-10 Desalting Column	30	17-0851-01
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 ÄKTA design	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
Ni Sepharose and IMAC Sepharose, Selection Guide	28-4070-92
Prepacked chromatography columns for ÄKTA systems	28-9317-78

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