Purification of histidine-tagged Maltose Binding Protein with Ni Sepharose 6 Fast Flow - method optimization and scale-up

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Purification of histidine-tagged Maltose Binding Protein with Ni Sepharose 6 Fast Flow—method optimization and scale-up

Abstract

This application note describes scale-up of the capture step with Ni Sepharose™ 6 Fast Flow in the purification of recombinant, histidine-tagged Maltose Binding Protein, MBP-$(His)_6$, expressed in *Escherichia coli*. Ni Sepharose 6 Fast Flow is an immobilized metal ion affinity chromatography (IMAC) medium with high protein binding capacity for histidine-tagged proteins. As member of the BioProcess™ media family, Ni Sepharose 6 Fast Flow meets the demands of industrial biotechnology with security of supply, comprehensive regulatory support, and all necessary support for process applications.

This study presents results from process optimization and scale-up work. The method was optimized at laboratory scale with HisTrap™ FF 1 ml column to find the best conditions for purification of the protein at high sample load. It was found that successful protein purification at 88 % of the binding capacity can be performed with an optimized imidazole concentration (5 mM). These conditions were then used for process optimization for purification of MBP-$(His)_6$ protein with HisPrep™ FF 16/10 column (20 ml). In two separate purification experiments more than 500 mg protein were purified per run with high recoveries. The protocol was then scaled-up 10-fold with ÄKTApilot™ system and AxiChrom™ 50 column packed with 210 ml Ni Sepharose 6 Fast Flow. At this scale, 5500 mg protein was loaded and purified with a 94% target protein recovery.

The results indicate that Ni Sepharose 6 Fast Flow is well suited for high productivity capture purification of histidine-tagged proteins.

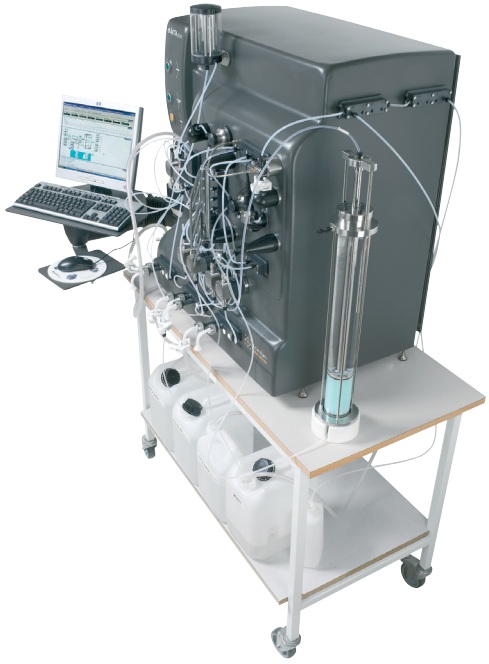


Fig 1. Scale-up of a histidine-tagged protein purification with ÄKTApilot.

Introduction

IMAC has become one of the most extensive affinity purification techniques for histidine-tagged proteins. Nickel (Ni^{2+}) is the most used metal ion since it has a high affinity for histidine tags. The protein binding can be disrupted by either competitive elution with imidazole that occupies the metal coordination sites or by protonation of the amino acid side chains involved in binding by reducing the pH of the elution buffer.

Ni Sepharose 6 Fast Flow is a chromatography medium specifically designed for purification of histidine-tagged proteins.

- Precharged with Ni^{2+} with low ion leakage
- High protein binding capacity
- Stable across the complete pH range and compatible with detergents, denaturing, and reducing agents commonly used for purification of histidine-tagged proteins.

The medium combines the advantages of Ni^{2+} with the established properties of Sepharose 6 Fast Flow matrix. The result is a reliable IMAC medium that is easy-to-use, that runs at high flow rates, and that ensures efficient and simple scale-up.

The objective of the work reported was to demonstrate the capture step purification of MBP-$(His)_6$ protein at high sample load followed by scale-up. Results from screening, process optimization and scale-up are presented.

Materials and Methods

Unless otherwise stated, all equipment and chromatographic media were obtained from GE Healthcare (Uppsala, Sweden) and the chemicals used were of analytical grade.

Preparation of starting material

Recombinant histidine-tagged Maltose Binding Protein, MBP-$(His)_6$, expressed in *E. coli* with a molecular weight of 44 000 and an isoelectric point (pI) of 4.4, was used as a target protein in this study.

E. coli cell paste (1830 g) was thawed in 3 volumes (5490 ml) of IMAC A buffer. Pefabloc™ (a protease inhibitor) was added to a concentration of 1 mM. The pH was measured and adjusted to 7.65 with Tris-base. The cell suspension was homogenized in a French press at 300-450 bar six times. After homogenization the pH was adjusted to 7.65 following centrifugation at 17 000 × g for 50 min.

Prior to use, the imidazole concentration was adjusted to 25, 15, or 5 mM for the imidazole binding optimization experiments. For HisTrap FF runs, 1 mM of Pefabloc was added and the sample was centrifuged for 20 min at 48 000 × g (18°C), followed by 0.22 µm filtration. No Pefabloc was added before the HisPrep FF16/10 runs and the sample was filtered through 0.45 µm filter. For the pilot-scale run on an AxiChrom 50 column the sample was centrifuged for 60 min at 17 000 × g (18°C) and suction filtered through Whatman™ GF/D and GF/B filters.

Columns and chromatography purification systems

Ni Sepharose 6 Fast Flow prepacked in HisTrap FF 1 ml and HisPrep FF 16/10, 20 ml columns were used in this study. ÄKTAexplorer™ 100 chromatography system with a 10 mm UV-cell was used at room temperature. For the pilot-scale experiment, Ni Sepharose 6 Fast Flow was packed in AxiChrom 50 column (inner diameter of 50 mm) and was run on ÄKTApilot™ chromatography system at room temperature. AxiChrom 50 column was loaded with Ni Sepharose 6 Fast Flow slurried in de-ionized water and packed at a linear flow velocity of 60 cm/h (19.6 ml/min). The packed bed was further compressed by 15% with axial compression to a bed height of 10.7 cm and a column volume of 210 ml.

The column effluent from ÄKTAexplorer system was fractionated using Frac-950 Fraction Collector whereas the effluent from ÄKTApilot system was collected manually.

Binding and elution buffers

IMAC A-buffer (binding): 20 mM sodium phopshate + 500 mM NaCl + x mM imidazole, pH 7.4

(where x = 5 mM, 15 mM or 25 mM depending on experiment)

IMAC B-buffer (elution): 20 mM sodium phopshate + 500 mM NaCl, + 500 mM imidazole, pH 7.4

Protein mass quantification

The MBP-$(His)_6$ was detected by monitoring UV absorbance at 280 nm. The fractions from the eluted peaks were diluted to obtain absorbance within the linear response range. The mass estimate of the protein was calculated from spectrophotometric absorbance measurements at 280 nm using the extinction coefficient for MBP-$(His)_6$ (ε=1.48 M^{-1} cm^{-1}).

Determination of binding capacity for MBP-$(His)_6$

The capacity of the medium was measured by loading HisTrap FF 1 ml column with a large amount of protein. The MBP-$(His)_6$ concentration in the cell extract was 2.6 mg/ml. Forty-five milliliters of diluted cell extract (1:3) in IMAC A-buffer, containing 5 mM imidazole, was loaded onto the column and the column was then washed with a further 30 column volumes (CV) of IMAC A-buffer. The bound MBP-$(His)_6$ was eluted from the column by a step elution using IMAC B-buffer (Fig 1).

The capacity was found to be 30 mg MBP-$(His)_6$ /ml medium.

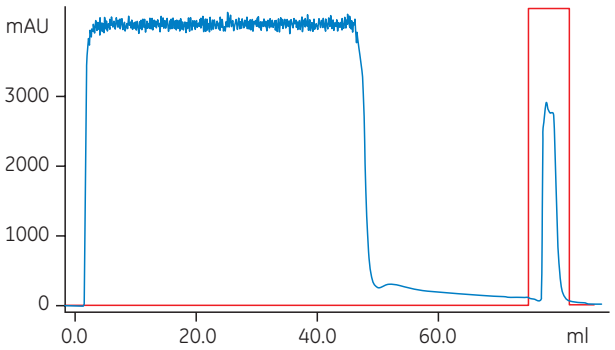


Fig 1. Determination of capacity for HisTrap FF 1 ml. Sample: 45 ml *E. coli* extract with MBP-$(His)_6$. Purification was monitored by absorbance at 280 nm. Wash was 30 CV.

Results and Discussion

Binding optimization

The recommended imidazole concentration in the binding buffer when using low protein load has been reported to be 25 mM with no protein leakage (See Data File 11-0008-86). However, one of the objectives in this study was to purify the protein at higher sample load. Therefore it was necessary to optimize the binding conditions. Experiments were performed on HisTrap FF 1 ml column using a sample load of about 88 % of the binding capacity of the medium at imidazole concentrations of 25, 15 and 5 mM. At this sample load the results showed protein leakage both in the flow through and washing fractions when 25 mM or 15 mM of imidazole were used in the sample and in the binding buffer as shown in Figures 2 and 3. However, at 5 mM imidazole no protein leakage was observed (chromatogram not shown here). The conclusion was to use 5 mM imidazole for the process optimization and scale-up experiments.

Column: HisTrap FF 1 ml
Sample: *E. coli* extract with MBP-$(His)_6$ (10 ml, 26 mg protein)
Flow: 156 cm/h (1 ml/min)
Binding buffer: A) IMAC A-buffer + 25 mM imidazole
B) IMAC A-buffer + 15 mM imidazole
Wash volume: 30 CVs of binding buffer
Elution buffer: IMAC B-Buffer
System: ÄKTAexplorer 100

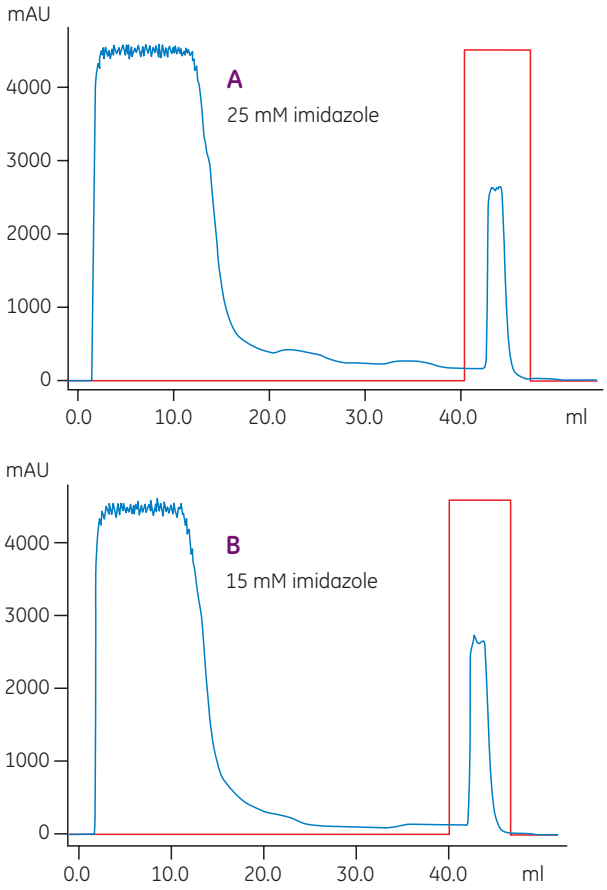
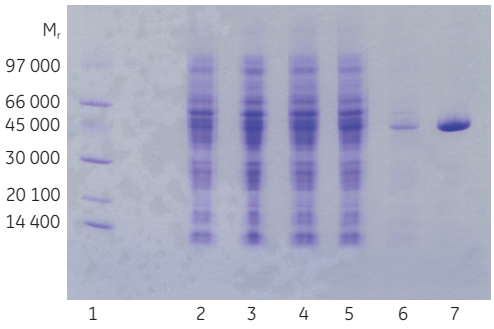


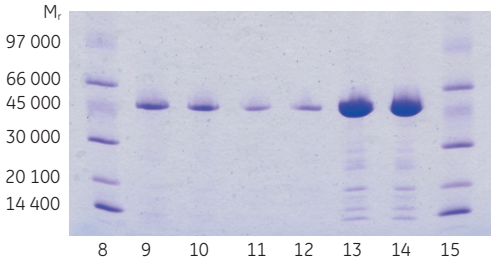
Fig 2 A and B. The effect of imidazole concentration in the binding buffer on the leakage of MBP-$(His)_6$ protein.

Process optimization

HisPrep FF 16/10 column was used for process optimization of lab-scale purification of MBP-$(His)_6$. The column was equilibrated with IMAC A-buffer containing 5 mM imidazole. The experiments were conducted twice to show the reproducibility of protein purification at high sample load (205 ml cell homogenate equivalent to 530 mg MBP-$(His)_6$). These samples, corresponding to 88% of the binding capacity, were applied at a linear flow velocity of 150 cm/h (5 ml/min) using IMAC A-buffer containing 5 mM imidazole as the binding buffer. The unbound material was washed from the column with the same IMAC A-buffer. The bound MBP-$(His)_6$ protein was eluted from the column by a step elution using IMAC B-buffer (Fig 4). The recovery of protein collected in the eluate peak was on average 98%, with an estimated purity of about 95%. The results from the SDS-PAGE in Figure 6 show similar purity of the MBP-$(His)_6$ protein in these two experiments which indicate the reproducibility of the method.



Lanes
1. LMW markers
2. Flow through fraction from fig. 2A
3. Flow through fraction from fig. 2A
4. Flow through fraction from fig. 2A
5. Flow through fraction from fig. 2A
6. Wash fraction from fig. 2A
7. Eluted fraction from fig. 2A



Lanes
8. LMW markers
9. Wash fraction from fig 2B
10. Wash fraction from fig 2B
11. Wash fraction from fig 2B
12. Wash fraction from fig 2B
13. Eluted fraction from fig. 2B
14. Eluted fraction from fig. 2B
15. LMW markers

Fig 3. Non-reduced SDS-PAGE analysis on PhastGel™ Gradient 8–25. Fractions from the eluted peaks after purification with HisTrap FF 1 ml column were collected (Fig 2A and 2B). Equal amount of protein was loaded in each lane. The gel was stained with Coomassie™.

Scale-up

A 10-fold scale-up was performed by increasing the column diameter while keeping other parameters such as linear flow velocity, sample load/ml medium and column bed height constant. AxiChrom 50 column was packed with 210 ml of Ni Sepharose 6 Fast Flow and used for the scale-up experiment.

Figure 5 shows the scale-up result of the purification of the MBP-$(His)_6$ from a sample load of 26 mg/ml medium which corresponded to 88% of the binding capacity. Thus, in this 10-fold scaled-up, 2125 ml starting material containing 5500 mg MBP-$(His)_6$ protein was applied at a linear flow velocity of 150 cm/h (50 ml/min) using ÄKTApilot chromatography system. IMAC A-buffer containing 5 mM imidazole was used as the binding buffer. The unbound material was washed from the column with the same IMAC A-buffer. The bound MBP-$(His)_6$ protein was then eluted from the column by step elution using IMAC B-buffer. The recovery of protein collected in the eluted peak was about 94%, with an estimated purity of 95%. The result from the SDS-PAGE (Fig 6) showed similar purity compared to the earlier results obtained from the experiments performed on the HisPrep FF 16/10 column. These results clearly demonstrate the scalability of the method (see Table 1).

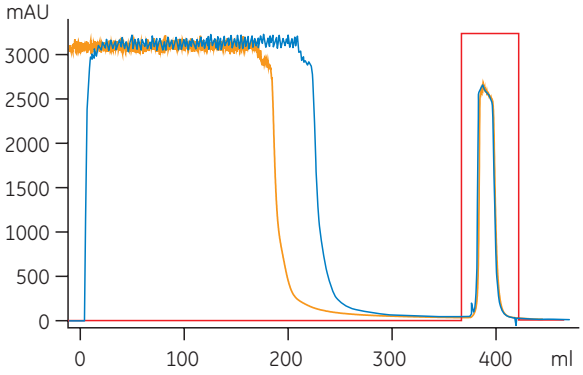


Fig 4. Overlay chromatograms from two purification experiments of MBP-$(His)_6$ protein on HisPrep FF 16/10 column. Blue chromatogram: run 1 and orange chromatogram: run 2. Sample: *E. coli* extract with MBP-$(His)_6$, 205 ml (530 mg protein). Purification was monitored by absorbance at 280 nm. The figure shows two repeated runs with different washing volumes 8 and 10 CV, respectively. The chromatograms are normalized to show similarity between the low absorbance in the wash and the size of the eluted peaks.

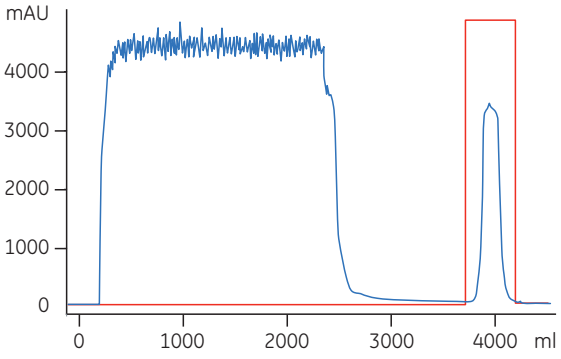
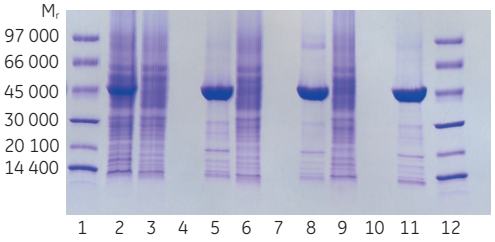


Fig 5. Ten-fold scale-up of MBP-$(His)_6$ purification on Ni Sepharose 6 Fast Flow using AxiChrom 50 column. Sample: 2125 ml *E. coli* extract containing 5500 mg MBP-$(His)_6$. Purification monitored by absorbance at 280 nm.



Lanes
1. LMW markers
2. Sample loaded *E. coli* extract
3. Flow through HisPrep FF 16/10 run 1
4. Wash HisPrep FF 16/10 run 1
5. Eluted pool HisPrep FF 16/10 run 1
6. Flow through HisPrep FF 16/10 run 2
7. Wash HisPrep FF 16/10 run 2
8. Eluted pool HisPrep FF 16/10 run 2
9. Flow through AxiChrom 50 run
10. Wash AxiChrom 50 run
11. Eluted pool AxiChrom 50 run
12. LMW markers

Fig 6. Non-reduced SDS-PAGE analysis on ExcelGel™ SDS Gradient: 8–18. Fractions from the eluted peaks after purifications with HisPrep FF 16/10 column and Ni Sepharose 6 Fast Flow in AxiChrom 50 column were collected and pooled (see figures 4 and 5). Samples used in purification: *E. coli* extracts expressing MBP-$(His)_6$. An equal amount of protein was loaded in each lane. The gel was stained with Coomassie.

Table 1. Summary of results of MBP-$(His)_6$ protein purified at different scales.

Purification scale	Column dimensions (mm)	Column volume (ml)	Load (% of binding capacity)	Load (mg)	Recovery* (%)
Lab scale (HisPrep FF 16/10)	16 (i. d.) × 100	20	88	530	99
Lab scale (HisPrep FF 16/10)	16 (i. d.) × 100	20	88	530	97
Pilot scale (Ni Sepharose 6 Fast Flow packed in AxiChrom 50 column)	50 (i. d.) × 107	210	88	5500	94

* Recovery of the target protein with a purity of 95%

Conclusions

The work presented here describes the process optimization for the capture step for purification of MBP-$(His)_6$ protein at high sample load using Ni Sepharose 6 Fast Flow. By reducing the concentration of imidazole in the binding buffer to 5 mM no leakage of MBP-$(His)_6$ was observed in the flow through and the washing fractions even at high protein loading (88% of the binding capacity of the medium). Samples containing more than 500 mg MBP-$(His)_6$ were purified with high recovery using HisPrep FF 16/10. The protocol was then scaled-up 10-fold using AxiChrom 50 column packed with 210 ml of Ni Sepharose 6 Fast Flow. At this scale, 5500 mg protein was loaded and purified with a 94% target protein recovery using ÄKTApilot system. The results from the SDS-PAGE showed similar purity at the different scales. The results clearly demonstrate the scalability of the method and that Ni Sepharose 6 Fast Flow is suitable for high productivity capture purification of histidine-tagged proteins.

Acknowledgement

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www.gehealthcare.com/protein-purification
www.gehealthcare.com/his
www.gehealthcare.com

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

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

Asia Pacific Tel: +85 65 6 275 1830 Fax: +852 2811 5251 • Australasia Tel: + 61 2 9899 0999 Fax: +61 2 9899 7511 • Austria Tel: 01/57606-1619 Fax: 01/57606-1627 • Belgium Tel: 0800 73 888 Fax: 03 272 1637 • Canada Tel: 800 463 5800 Fax: 800 567 1008 • Central, East, & South East Europe Tel: +43 1 982 3826 Fax: +43 1 985 8327 • Denmark Tel: 45 16 2400 Fax: 45 16 2424 • Finland & Baltics Tel: +358 (0)9 512 39 40 Fax: +358 (0)9 512 39 439 • France Tel: 01 69 35 67 00 Fax: 01 69 41 96 77 • Germany Tel: 0761/4903-490 Fax: 0761/4903-405 • Italy Tel: 02 27322 1 Fax: 02 27302 212 • Japan Tel: +81 3 5331 9336 Fax: +81 3 5331 9370 • Latin America Tel: +55 11 3933 7300 Fax: +55 11 3933 7304 • Middle East & Africa Tel: +30 210 9600 687 Fax: +30 210 9600 693 • Netherlands Tel: 0165 580 410 Fax: 0165 580 401 • Norway Tel: 815 65 555 Fax: 815 65 666 • Portugal Tel: 21 417 7035 Fax: 21 417 3184 • Russia & other C.I.S. & N.I.S Tel: +7 (095) 232 0250 Fax: +7 (095) 230 6377 • South East Asia Tel: 60 3 8024 2080 Fax: 60 3 8024 2090 • Spain Tel: 93 594 49 50 Fax: 93 594 49 55 • Sweden Tel: 018 612 1900 Fax: 018 612 1910 • Switzerland Tel: 0848 8028 12 Fax: 0848 8028 13 • UK Tel: 0800 616928 Fax: 0800 616927 • USA Tel: 800 526 3593 Fax: 877 295 8102

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