Improved purification of histidine-tagged proteins with Ni Sepharose high performance

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Improved Purification of Histidine-tagged Proteins with Ni Sepharose High Performance

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Introduction

The $(histidine)_6$-tag has become the most used affinity tag due to its small size, strong metal ion binding, and ability to bind under denaturing conditions. Immobilized Metal Ion Affinity Chromatography (IMAC) with Ni^{2+} is the standard method for purifying histidine-tagged proteins. Ni Sepharose™ High Performance medium is optimized for purification of such proteins.

Ni Sepharose High Performance

- Is precharged with Ni^{2+} and available in different formats, including prepacked 1 ml and 5 ml HisTrap™ HP columns.
- Consists of 34 µm beads. The small bead-size gives narrow peaks with high target protein concentration.
- Is compatible with a wide range of buffers and additives, including denaturants, detergents, and reducing agents.
- Has very low nickel ion leakage.
- Has very high protein binding capacity.

For detailed information see Data File 18-1174-40.

Conclusions

The following results were obtained with Ni Sepharose High Performance:

- Low leakage of Ni^{2+} was confirmed. A low leakage minimizes potential problems such as Ni^{2+}-induced oligomerization and precipitation of target protein, and loss of binding capacity.
- The excellent binding properties of the medium allowed high flowrate purifications.
- A number of histidine-tagged proteins from different sources, with different M_r and with different expression levels were easily purified.
- 60 mg of purified protein could be obtained in a single run on a 1 ml column.

Material and Methods

Chromatography conditions were, unless otherwise stated:

Medium:	Ni Sepharose High Performance prepacked in HisTrap HP 1 ml columns or packed in 5×50 mm glass columns.
Samples: Clarified	Cell extracts from *E. coli* or *Pichia. pastoris.* by centrifugation and filtration; containing 0.5 M NaCl and imidazole at a concentration appropriate for each target protein.
Flow rate:	1 ml/min.
Binding buffer:	20 mM sodium phosphate, 0.5 M NaCl, x mM imidazole, pH 7.4.
Optimization:	The optimal imidazole concentration, to obtain the best purity and yield, differs from protein to protein. A small number of screening runs, including a run with an imidazole gradient from 5 mM, will facilitate finding a suitable imidazole concentration for optimal results.
Elution buffer:	20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4.

Chromatography was performed on ÄKTAexplorer™ 10 or 100 systems.

Elution was performed either with a step or a linear imidazole gradient.

Fractions of 1 ml were collected. SDS-electrophoresis was performed with ExcelGel™ SDS Gradient 8–18 gels.

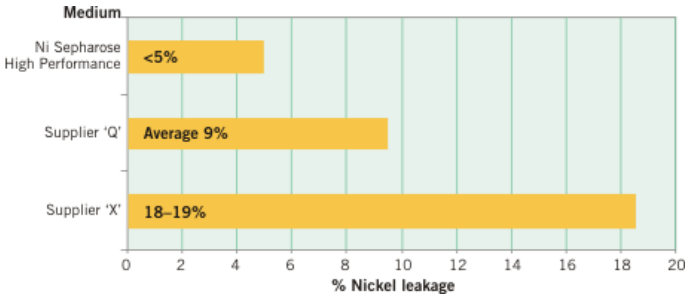
Low nickel ion leakage

A. Comparison of Ni^{2+} leakage from three IMAC media at pH 4

- 10 column volumes of pH 4.0 buffer (no protein).
- Ni^{2+} still bound was eluted with EDTA and the amount was determined spectrophotometrically (372 nm).

Results

- Ni^{2+} leakage was considerably lower for Ni Sepharose High Performance than for the other media.



B. Ni^{2+} concentration in purified target protein pools from two different IMAC media

- Samples: Histidine-tagged proteins in *E. coli* and *P. pastoris* cell extracts.
- IMAC: Equal volumes of identical extracts applied to two different IMAC media. 5 mM imidazole for equilibration, sample application, and wash. Elution with linear imidazole gradient (chromatograms not shown). Pool volumes differed due to different peak widths.
- Ni^{2+} concentration was determined by atomic absorption spectrophotometry in eluted pools of target protein (of very similar purity).

Results

- Lower Ni^{2+} content in pools from Ni Sepharose High Performance.

Note: Potential problems due to Ni^{2+} contamination may vary with the molar ratio Ni^{2+}/protein. The low ratio obtained for Ni Sepharose High Performance is due to the low Ni^{2+} concentration *and* the high target protein concentration obtained with small (34 µm) beads.

Histidine-tagged green fluorescent protein from *E. coli*. Data from final pool of purified protein:

	Ni Sepharose HP	Supplier 'Q'
volume of final pool	7 ml	11 ml
µg Ni^{2+}/L (=ppb)	185	231
total amount of Ni^{2+} in pool (nmol)	22	43
protein conc (mg/ml)	1.60	0.91
molar ratio Ni^{2+}/protein	0.055	0.121

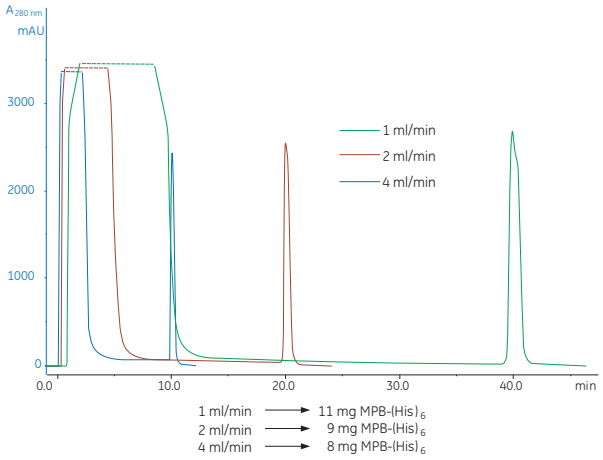
Histidine-tagged hydrolase from *P. pastoris*. Data from final pool of purified protein:

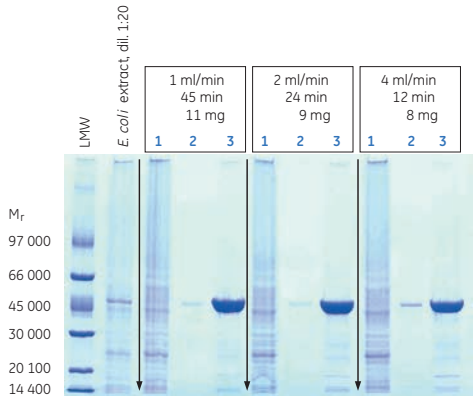
	Ni Sepharose HP	Supplier 'Q'
volume of final pool	5 ml	8 ml
µg Ni^{2+}/L (=ppb)	87	129
total amount of Ni^{2+} in pool (nmol)	7	18
protein conc (mg/ml)	n.d.	n.d.
molar ratio Ni^{2+}/protein	n.d	n.d

n.d. = not determined

Purifications at high flow rates

1, 2 or 4 ml/min on 1 ml HisTrap HP columns





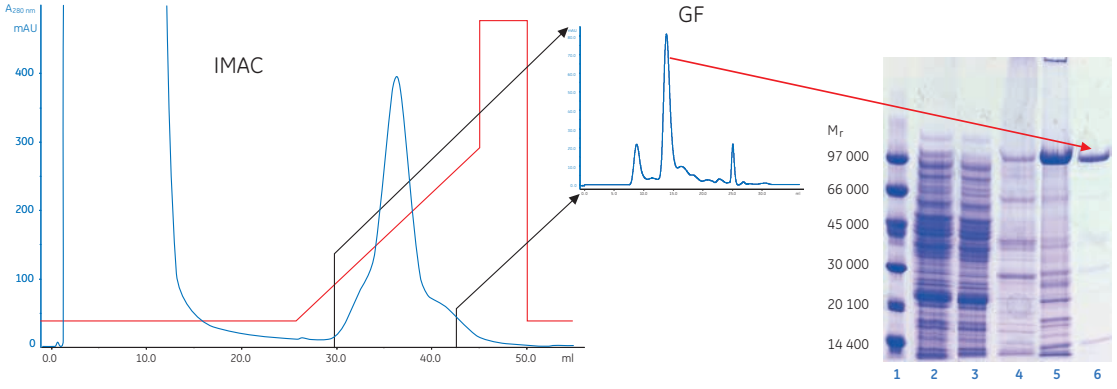
Lane 1: Flow-through diluted 1:10
Lane 2: wash; not diluted
Lane 3: Eluted histidine-tagged protein, diluted 1:5

- Samples: *E. coli* extract with histidine-tagged maltose binding protein (MBP-$(His)_6$, M_r ~ 44 000) at ~ 1.7 mg/ml.
- IMAC: At 1, 2 or 4 ml/min on 1 ml HisTrap HP. 8 ml extract applied (13.6 mg MBP-$(His)_6$). 35 mM imidazole during equilibration, sample application, and wash (see Optimization in Materials and Methods). Step elution with 500 mM imidazole.

Results

- Only a slight decrease in yield was seen in the high-flow runs.
- SDS-PAGE showed very similar purity at low and high flow rates.

Purification of a high-MW histidine-tagged mannanase expressed in *E. coli*



Lane 1: Low molecular weight marker
Lane 2: *E. coli* extract
Lane 3: IMAC flow-through
Lane 4: Early IMAC fraction
Lane 5: IMAC eluted pool
Lane 6: Gel filtration pool

Fig 4. Purification by IMAC and gel filtration. Black lines in IMAC chromatogram indicate the IMAC pool taken to gel filtration.

- Sample: *E. coli* extract with low-level expression of a histidine-tagged mannanase, Man 26A, from *Cellulomonas fimi* (M_r ~ 100 000).
- IMAC: 10 ml extract applied to a 1 ml column with Ni Sepharose High Performance. 30 mM imidazole during equilibration, sample application, and wash (see Optimization in Materials and Methods). Elution with a 25 ml linear imidazole gradient to 300 mM.
- Gel filtration: Superdex™ 200 10/300 GL.

Results

- Enzymatically active purified Man 26A.

Note: Several contaminants were co-purified with the full-length target protein. Previous results have indicated that these contaminants include various truncated, histidine-tagged forms of the target protein (data not shown). Accordingly, a second purification step was performed.

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

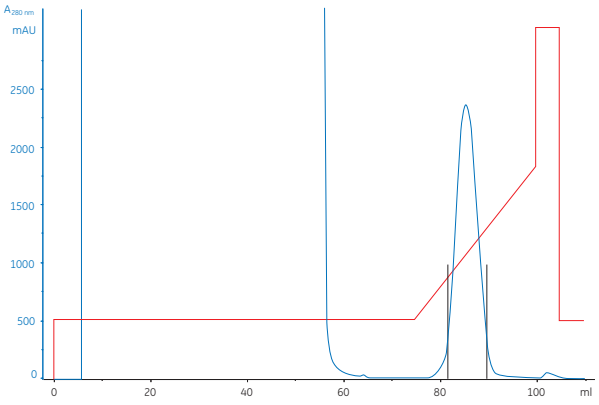
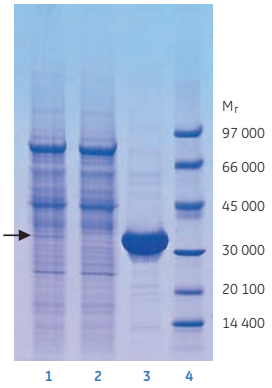


Fig 5. Black lines in chromatogram indicate the final pool. Arrow indicates the target protein band in the *Pichia* extract.



Lane 1: *Pichia* extract
Lane 2: Flow-through
Lane 3: Final pool
Lane 4: Low molecular weight marker

IMAC: 50 ml cell extract (corresponding to 20 g cells) applied to a 1 ml column with Ni Sepharose High Performance. 85 mM imidazole during equilibration, sample application and wash (see Optimization in Materials and Methods). Elution with a 25 ml linear imidazole gradient to 300 mM.

Fig 6. SDS-PAGE analysis of *Pichia pastoris* extract with low-level expression of a (putative) hydrolase from *Saccharomyces cerevisiae* (M_r ~ 34 000).

Results

- High-purity target protein was obtained from a low-expression sample.
- The Ni^{2+} concentration was low in the final pool of purified protein, 155 ppb (molar Ni^{2+}/protein ratio = 0.08).

Purification of 60 mg histidine-tagged protein on a 1 ml column

Comparison at high-load conditions between three different IMAC media

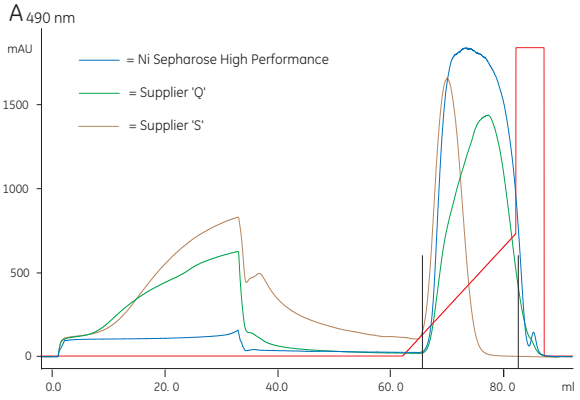
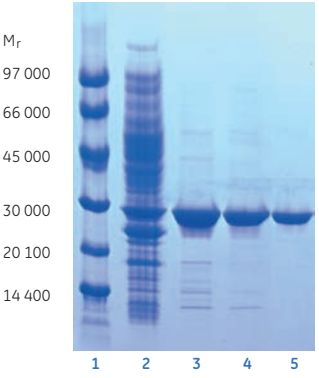


Fig 7. GFP-$(His)_6$ monitored at 490 nm. Black lines in chromatogram indicate the final pools.



Lane 1: Low molecular weight marker
Lane 2: *E. coli* extract with GFP-$(His)_6$
Lane 3: Eluted from Ni sepharose High Performance
Lane 4: Eluted from Supplier 'Q'
Lane 5: Eluted from Supplier 'S'

IMAC: 32 ml extract applied to each 1 ml column. 5 mM imidazole during equilibration, sample application and wash. Elution with a 25 ml linear imidazole gradient to 250 mM. Pools of 16 ml were analysed.

Fig 8. SDS-PAGE analysis of *E. coli* extract with hisitidine-tagged green fluorescent protein (GFP-$(His)_6$, M_r ~ 28 000) at ~ 2 mg/ml.

Results

- Due to the high dynamic binding capacity, the yield of eluted protein for Ni Sepharose High Performance was almost twice that from the Supplier 'Q' medium, and over three times higher than that from the Supplier 'S' medium.
- SDS-PAGE analysis of the final pools confirmed the superior binding capacity of Ni Sepharose High Performance.

Note: Improved purity can be achieved with a higher imidazole concentration than the 5 mM used in here binding buffer and sample.

Acknowledgements

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