GE Healthcare

A new IMAC medium for scaling up purification of Histidine-tagged proteins

Jon Lundqvist, Lars C Andersson, Ann Bergh, Ellinor Ancker, Marianne Carlsson, Anna Heijbel, Helena Lindgren, Karin Torstenson and Katarina Öberg

GE Healthcare, SE-751 84 Uppsala, Sweden

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. Here, a new medium optimized for scaling up purification, Ni Sepharose™ 6 Fast Flow, is presented.

Ni Sepharose 6 Fast Flow

- Pre-charged with Ni^{2+} and available in different formats, including pre-packed 1-ml and 5-ml HisTrap™ FF and 20-ml HisPrep™ FF 16/10 columns.
- The bead-size (~90-µm) makes it possible to scale-up purification maintaining high target protein concentration in the eluted peak.
- Compatible with a wide range of buffers and additives, including denaturants, detergents and reducing agents.

Conclusions

The following results were obtained with Ni Sepharose 6 Fast Flow:

- Easy to scale-up purification from 1-ml via 5-ml to 20-ml columns.
- Low leakage of Ni^{2+} and compatibility with the reducing agent DTT.
- A number of Histidine-tagged proteins from different:
  - expression systems
  - expression levels
  - with different M_r
  - with different lengths of the Histidine-tag

  were easily purified.

Material and Methods

Chromatography conditions were, unless otherwise stated:

Medium: Ni Sepharose 6 Fast Flow pre-packed in 1-ml and 5-ml HisTrap FF, and 20-ml HisPrep FF 16/10 columns

Samples: Cell extracts from *E. coli* or *P. pastoris* were clarified 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 (x = optimized for each target protein)

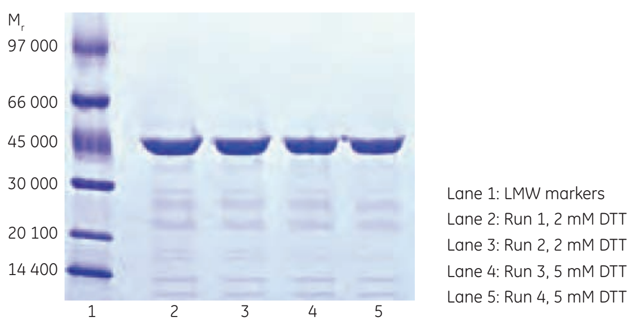
Optimization: The optimal imidazole concentration to obtain the best purity and yield differs from protein to protein. A small number of screening runs, including a run with a linear imidazole gradient from 5 mM, will facilitate finding a suitable imidazole concentration for optimal results.

Elution buffer: 20 mM sodium phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4

Purifications were performed on ÄKTAexplorer™ 10 or 100 systems. Elution was done either with a step or a linear imidazole gradient. SDS-PAGE was performed with ExcelGel™ SDS Gradient 8–18 Gels.

Purifications of Histidine-tagged proteins in the presence of DTT

Column: HisTrap FF 1-ml
Sample: $(Histidine)_6$-tagged Maltose Binding Protein in *E. coli* extract (conc. ~1 mg/ml, M_r ~43 000)
Sample volume: 7 ml
Binding buffer: 2 or 5 mM DTT, 20 mM 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
Note: A blank run without DTT was performed before the sample application.



Lane 1: LMW markers
Lane 2: Run 1, 2 mM DTT
Lane 3: Run 2, 2 mM DTT
Lane 4: Run 3, 5 mM DTT
Lane 5: Run 4, 5 mM DTT

Fig 1. Purity and recovery of repeated purification runs on Ni Sepharose 6 Fast Flow without Ni^{2+} recharging.

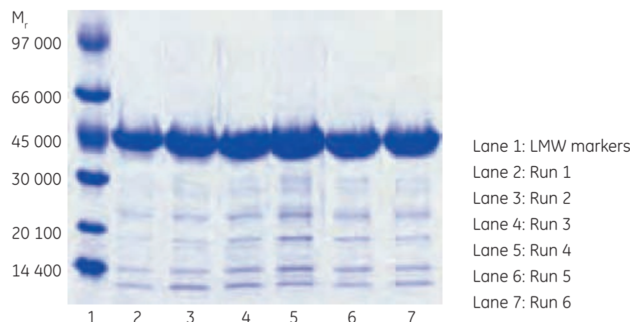
Results:

- Purity and recovery were not affected by DTT.

Six repeated purifications

without stripping, cleaning or Ni^{2+} recharging the column

Column: HisTrap FF 1-ml
Sample: $(Histidine)_6$-tagged Maltose Binding Protein in *E. coli* extract
Sample volume: 7 ml
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



Lane 1: LMW markers
Lane 2: Run 1
Lane 3: Run 2
Lane 4: Run 3
Lane 5: Run 4
Lane 6: Run 5
Lane 7: Run 6

Fig 2. SDS-PAGE analysis under nonreducing conditions of pooled fractions from different runs.

Table 1. Summary of purification results from the six repeated purification runs on Ni Sepharose 6 Fast Flow shown in Figure 2.

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

Results:

- Excellent reproducibility in purity and recovery without the need for stripping, cleaning, or Ni^{2+} recharging the medium.
- Low Ni^{2+}-leakage during purifications (low leakage minimizes potential problems such as Ni^{2+} induced oligomerization and precipitation of target protein, and loss of binding capacity).

Purification of a low expressed (Histidine)$_6$-tagged hydrolase from *Pichia pastoris*

Column:	HisTrap FF 1-ml
Sample:	*Pichia pastoris* extract with low expression of a (putative) *Saccharomyces cerevisiae* hydrolase (M_r ~34 000)
Sample volume:	50 ml (corresponding to 20 g cells)
Binding buffer:	20 mM sodium phosphate, 50 mM imidazole, 500 mM NaCl, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, pH 7.4, 25 ml linear gradient, 50–300 mM imidazole
Flow rate:	1 ml/min

Results:

- High-purity target protein was obtained despite low expression.
- ~ 90% of the target protein applied on the column was recovered.

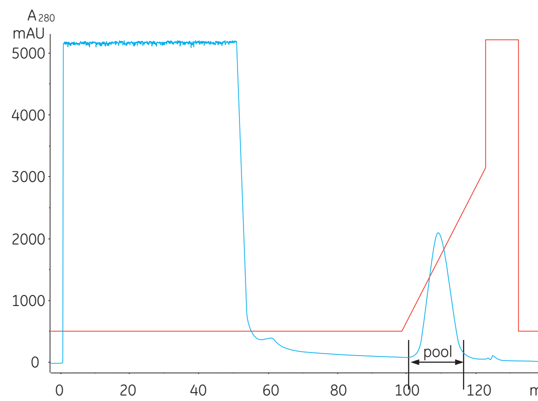
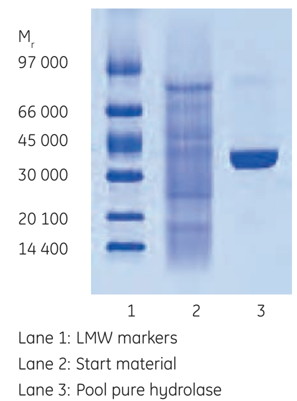


Fig 3. Black lines in the chromatogram indicate the final pool.



Lane 1: LMW markers
Lane 2: Start material
Lane 3: Pool pure hydrolase

Fig 4. Analysis by SDS-PAGE.

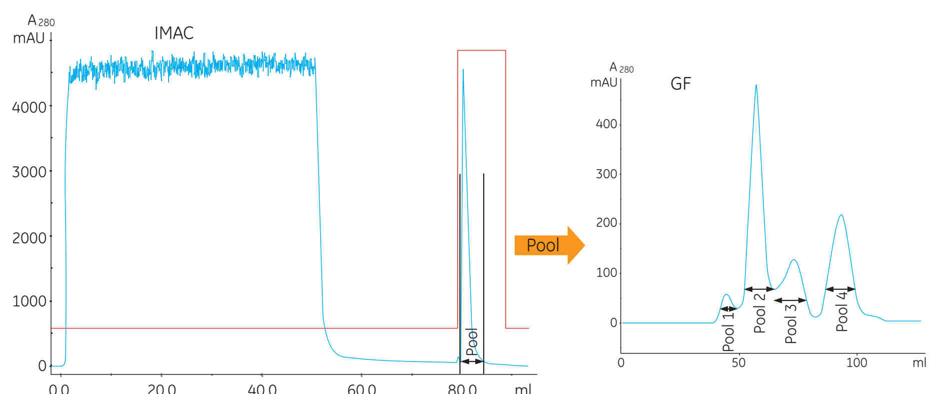
Two-step purification of a high molecular weight (Histidine)$_{10}$-tagged protein expressed in *E. coli*

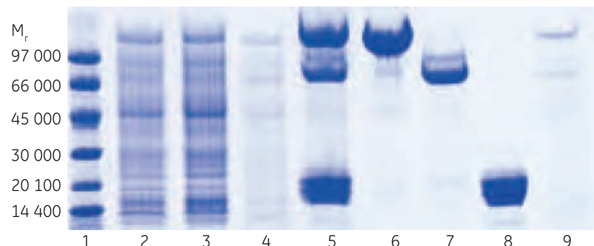
Columns:	IMAC:	HisTrap FF 1-ml
	Gel filtration (GF):	HiLoad™ 16/60 Superdex™ 200 pg
Sample:	*E.coli* extract with Histidine-trx-p450, a large (Histidine)$_{10}$-tagged protein (M_r ~130 000)	
Sample volume:	50 ml	
Binding buffer:	IMAC:	20 mM sodium phosphate, 60 mM imidazole, 500 mM NaCl, pH 7.4
Elution buffer:	IMAC:	20 mM sodium phosphate, 500 mM NaCl, pH 7.4 and 500 mM imidazole
	GF:	20 mM phosphate, 0.28 M NaCl, 6 mM KCl pH 7.4
Flow rate:	IMAC:	1 ml/min
	GF:	0.5 ml/min

Results:

- Three major bands were detected after IMAC purification.
- A very good separation between the contaminants and (His)$_{10}$-trx p450 was obtained by gel filtration.

Note: Contaminants after IMAC were truncated forms of (His)$_{10}$-trx-p450 (as determined by N-terminal sequencing, data not shown).





Lane 1: LMW markers
Lane 2: Start material, 1:30
Lane 3: Flowthrough IMAC, 1:20
Lane 4: Wash IMAC, 1:10
Lane 5: Pool IMAC,1:10
Lane 6: Pool 2, GF, 1:2
Lane 7: Pool 3, GF, 1:2
Lane 8: Pool 4, GF, 1:2
Lane 9: Pool 1, GF

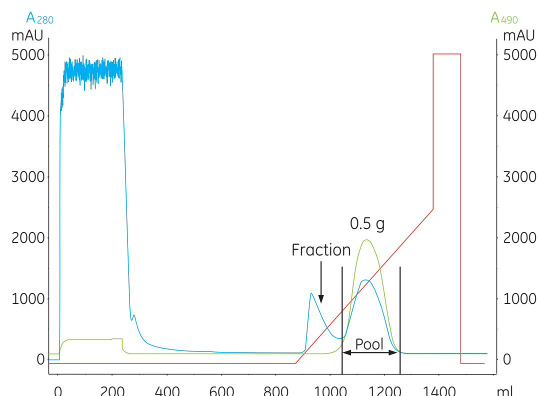
Fig 5. Two-step purification by IMAC and gel filtration. SDS-PAGE analysis under reduced conditions.

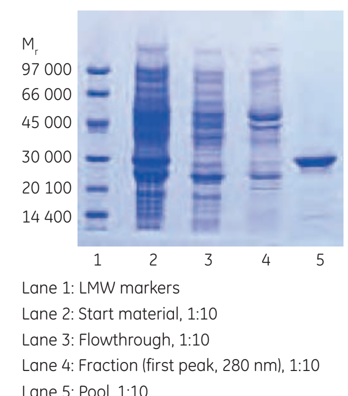
Purification of (Histidine)$_6$-tagged protein on a 20-ml HisPrep FF 16/10 column

Column:	HisPrep FF 16/10, 20-ml
Sample:	(Histidine)$_6$-tagged Green Fluorescent Protein (GFP-(His)$_6$, M_r~28 000) in *E. coli* extract
Sample volume:	230 ml
Binding buffer:	20 mM sodium phosphate, 5 mM imidazole, 500 mM NaCl, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, pH 7.4, 500 ml linear gradient, 5–250 mM imidazole
Flow rate:	5 ml/min

Results:

- 0.5 g GFP-(His)$_6$ was purified on HisPrep FF 16/10.
- GFP-(His)$_6$ was eluted in 180 ml (conc. 2.7 mg/ml).





Lane 1: LMW markers
Lane 2: Start material, 1:10
Lane 3: Flowthrough, 1:10
Lane 4: Fraction (first peak, 280 nm), 1:10
Lane 5: Pool, 1:10

Fig 6. Total protein monitored at 280 nm (blue line) and GFP-(His)$_6$ monitored at 490 nm (green line). Black lines in the chromatogram indicate the fraction and pool collected for SDS-PAGE analysis.

Scaling up purification of $(Histidine)_6$-tagged protein

Column:	HisTrap FF 1-ml, HisTrap FF 5-ml, HisPrep FF 16/10 20-ml
Sample:	$(Histidine)_6$-tagged Maltose Binding Protein (M_r ~43 000) 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

Results:

- Scaling up provided highly consistent results.
- Scaling up from a 1-ml to a 20-ml column did not significantly affect the purity and recovery.

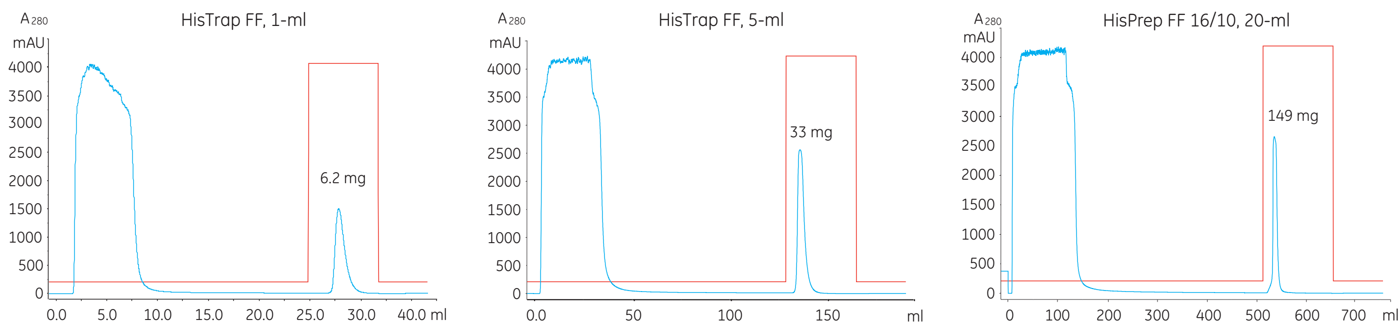


Fig 7. Scaling up from HisTrap FF 1-ml via HisTrap FF 5-ml to a HisPrep FF 16/10, 20-ml prepacked column. The samples loaded contained approximately 8, 40, and 160 mg MBP-$(His)_6$ respectively. Yield in milligrams is shown in each chromatogram.

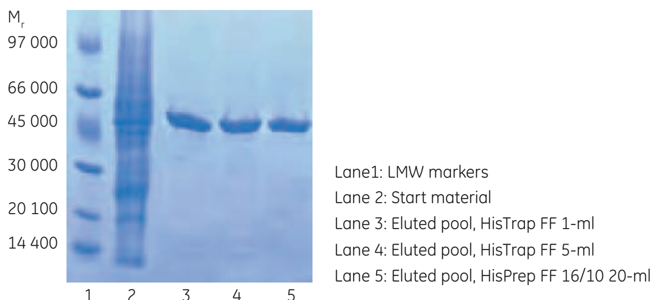


Fig 8. SDS-PAGE analysis under nonreducing conditions.

Acknowledgement

MBP-$(His)_6$ was provided by Pharmacia Diagnostics, Uppsala, Sweden. GFP-$(His)_6$ was provided by Dr. David Drew, Dept. of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden.

www.chromatography.amershambiosciences.com

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

ÄKTAexplorer, ExcelGel, HiLoad, HisTrap, HisPrep, Sepharose and Superdex are trademarks of GE Healthcare Ltd, a General Electric Company. GE and GE monogram are trademarks of General Electric Company.

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. GE Healthcare reserves the right, subject to any regulatory and contractual approval, if required, to make changes in specifications and features shown herein, or discontinue the product described at any time without notice or obligation. Contact your local GE Healthcare representative for the most current information.

Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US pat 5,284,933 and US pat 5,310,663, including corresponding foreign patents (assigne: Hoffman La Roche, Inc).

© 2005 General Electric Company – All rights reserved.

GE Healthcare Bio-Sciences AB, a General Electric company going to market as GE Healthcare.

11-0032-78 AA