HisTrap FF crude for faster purification of histidine-tagged proteins

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Introduction

HisTrap™ FF crude is designed for direct sample application of unclarified cell lysate during purification of histidine-tagged proteins. No centrifugation or filtration of the sample is required. The total purification time is decreased and the risk of degradation and oxidation of sensitive target proteins is minimized.

HisTrap FF crude

- Available as 1 ml and 5 ml HisTrap FF crude columns
- Prepacked with Ni Sepharose™ 6 Fast Flow medium
- Special column construction enables direct loading of unclarified lysates
- High protein binding capacity
- Compatible with a wide range of buffers and additives, denaturants, detergents and reducing agents
- Very low nickel ion leakage

Conclusions

Features of HisTrap FF crude include:

- No need for centrifugation or filtration of the samples. Direct sample application.
- No difference in final purity and recovery when using:
  - clarified or unclarified samples
  - different techniques for mechanical lysis
- Easy to scale up purifications from 1 ml to 5 ml columns.
- Histidine-tagged proteins from different sources with different M_r and expression levels can be easily purified.

Material and Methods

Chromatography conditions (unless otherwise stated):

Column: HisTrap FF crude, 1 ml or 5 ml

Samples: Unclarified cell lysates from *E. coli or P. pastoris*. See Sample preparation

Flow rate: 1 ml/min or 5 ml/min

Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, x mM imidazole, pH 7.4 (x = optimized for each target protein)

Optimization: The optimal imidazole concentration in sample and buffers, to obtain the best purity and yield, differs from protein to protein. A linear gradient from 5–500 mM imidazole will facilitate finding a suitable imidazole concentration for optimal results.

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

Purification was performed on ÄKTAexplorer™ 10 or 100 systems.

Elution was performed either stepwise or with a linear gradient.

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

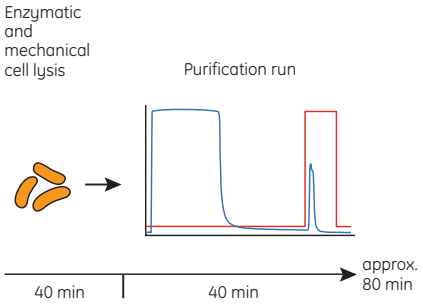
Sample preparation

1. Dilute cell paste in binding buffer.
2. Enzymatic lysis (DNase and lysozyme).
3. Mechanical lysis such as sonication, homogenisation or freeze/thawing (somewhat extended procedures).
4. Apply the sample directly.

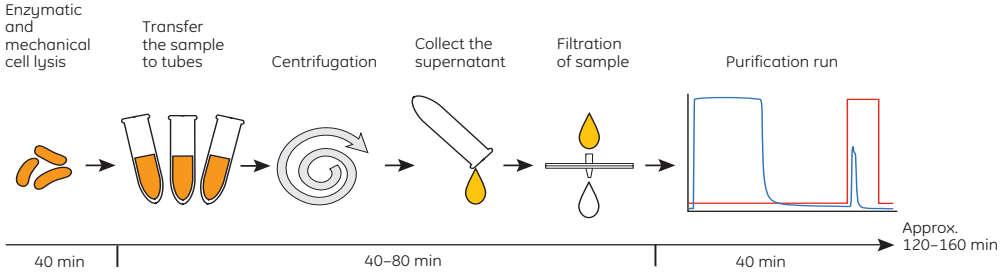
No centrifugation or filtration required!

Convenient and time-saving

HisTrap FF crude



Conventional IMAC



Purification of a low-expression histidine-tagged hydrolase from *Pichia pastoris* lysate

Column: HisTrap FF crude 5 ml

Sample: Unclarified sonicated *P. pastoris* lysate containing YNR064c *(Saccharomyces cerevisiae hydrolase)*

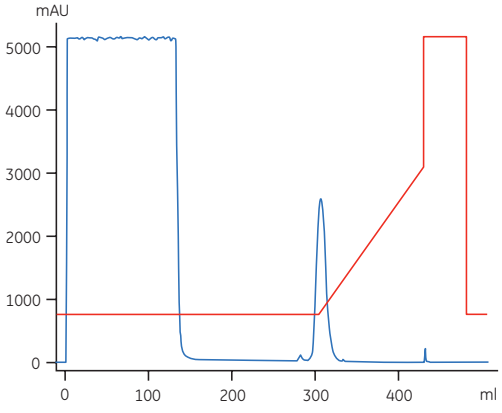
Sample volume: 130 ml

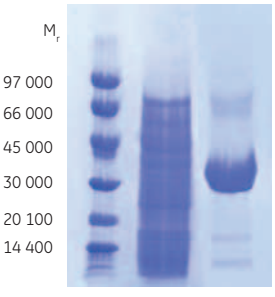
Binding buffer: 20 mM sodium phosphate, 500 mM NaCl, 75 mM imidazole, pH 7.4

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

Elution: 75–300 mM imidazole (25 CV)

Flow rate: 5 ml/min





Lane 1: LMW markers
Lane 2: Start material (1:20)
Lane 3: Eluted pool (1:2)

Results:

- Even with direct loading of an unclarified lysate, high purity target protein was obtained.

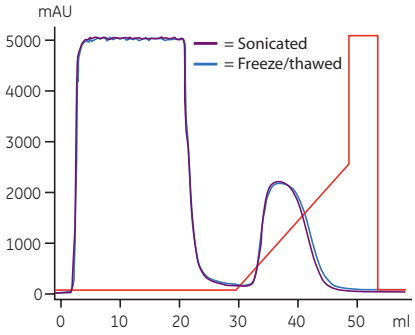
Effect of different cell lysis methods

Column:	HisTrap FF crude 1 ml
Sample:	Unclarified or clarified *E. coli* DH5α lysate containing histidine-tagged maltose binding protein, MBP-$(His)_6$, prepared by sonication or freeze/thaw.
Sample volume:	20 ml
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 CV)
Flow rate:	0.5–1 ml/min

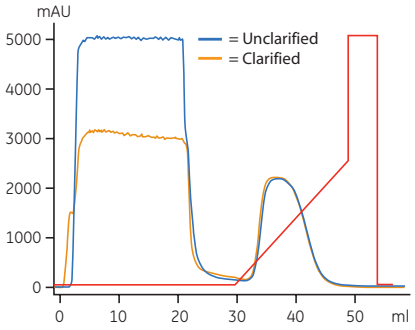
Results:

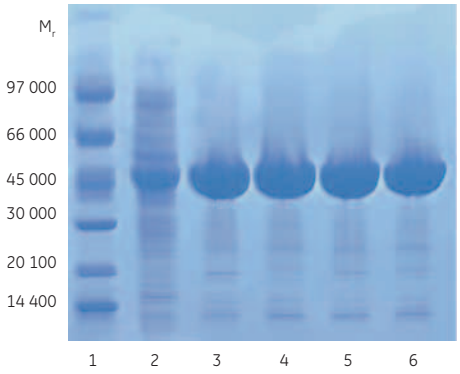
- Equal purity and recovery (50 mg target protein) were obtained.
- Similar results were obtained using different sample preparation techniques.
- Pressure during sample application was below the maximum pressure limit (0.3 MPa + system pressure).

Sonicated and freeze/thawed samples (both unclarified)



Clarified and unclarified samples (both freeze/thawed)





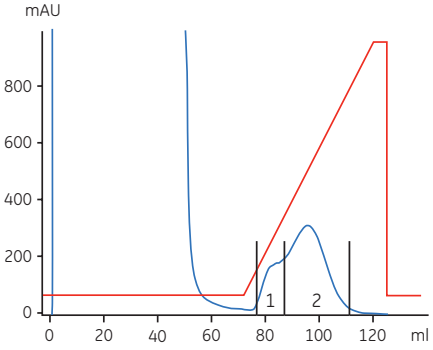
Lane 1: LMW markers
Lane 2: Start material, (1:10)
Lane 3: Eluted pool, unclarified and sonicated sample
Lane 4: Eluted pool, clarified and sonicated sample
Lane 5: Eluted pool, unclarified and freeze/thawed sample
Lane 6: Eluted pool, clarified and freeze/thawed sample

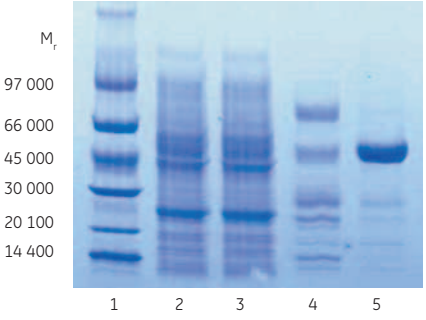
Purification of CaiB in β-mercaptoethanol at 4 °C

Column:	HisTrap FF crude 1 ml
Sample:	Unclarified sonicated *E. coli* BL-21 lysate containing histidine-tagged CaiB a Type III CoA Transferase
Sample volume:	48 ml applied using a Superloop™
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 10 mM β-mercaptoethanol, 10% glycerol, 25 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 10 mM β-mercaptoethanol, 10% glycerol, 500 mM imidazole, pH 7.4
Elution:	25–500 mM imidazole (50 CV)
Flow rate:	1 ml/min

Results:

- High purity target protein was obtained directly from unclarified cell lysate.
- Pressure during purification was below the maximum pressure even in the presence of glycerol and at +4 °C.





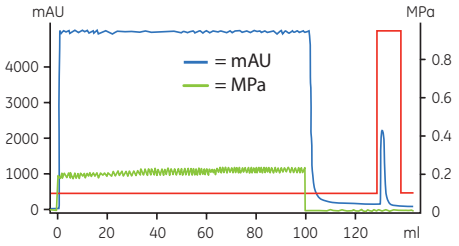
Lane 1: LMW markers
Lane 2: Start material (1:20)
Lane 3: Flowthrough (1:20)
Lane 4: Eluted Pool 1
Lane 5: Eluted Pool 2 (CaiB)

Scaling up purification

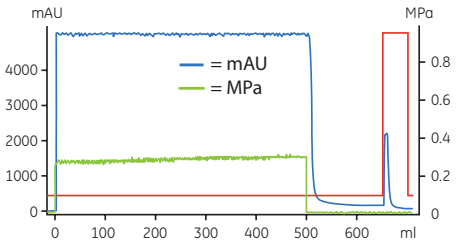
Large volumes of unclarified homogenised cell lysate

Column:	HisTrap FF crude 1 ml and 5 ml
Sample:	Unclarified homogenised *E. coli* BL-21 lysate containing histidine-tagged Green Fluorescent Protein, GFP-$(His)_6$
Sample volume:	100 ml (20 mg GFP-$(His)_6$) 500 ml (100 mg GFP-$(His)_6$)
Binding buffer:	20 mM sodium phosphate, 500 mM NaCl, 45 mM imidazole, pH 7.4
Elution buffer:	20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, pH 7.4
Flow rate:	1 and 5 ml/min

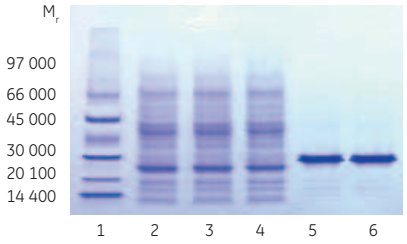
HisTrap FF crude 1 ml



HisTrap FF crude 5 ml



	HisTrap FF crude 1 ml	HisTrap FF crude 5 ml
Load (ml)	100	500
Load (mg)	20	100
Recovery (mg)	15	75
Recovery (%)	75	75



Lane 1: LMW markers
Lane 2: Start material (1:20)
Lane 3: Flowthrough 1 ml column (1:20)
Lane 4: Flowthrough 5 ml column (1:20)
Lane 5: Eluted pool, 1 ml column (1:5)
Lane 6: Eluted pool, 5 ml column (1:5)

Results:

- Scaling up at the same linear flow rate provided highly consistent results.
- Scaling up from a 1 ml to a 5 ml column did not significantly affect purity, recovery or total purification time.

Acknowledgement

We thank the owners of the clones used in this work: MBP-$(His)_6$ provided by Pharmacia Diagnostics, Uppsala, Sweden. GFP-$(His)_6$ provided by Dr. David Drew, Dept. of Biochemistry and Biophysics, Stockholm University, Sweden. *Saccharomyces* hydrolase provided by Dr. Mikael Widersten, Protein engineering and redesign, Dept. of Biochemistry, Uppsala University, Sweden. CaiB provided by Prof. Pär Nordlund, Dept. of Biochemistry and Biophysics, Stockholm University, Sweden.

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