



# HisTrap FF crude for faster purification of histidine-tagged proteins

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# HisTrap FF crude for faster purification of histidine-tagged proteins

*A Bergh, L C Andersson, E Ancker, A Heijbel, A Karlsson, H Lindgren, J Lundqvist, K Torstenson and K Öberg*

GE Healthcare, SE-751 84 Uppsala, Sweden

## 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.

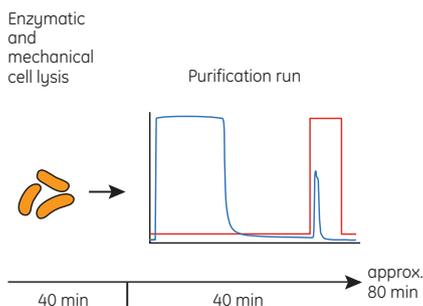
## Sample preparation

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.

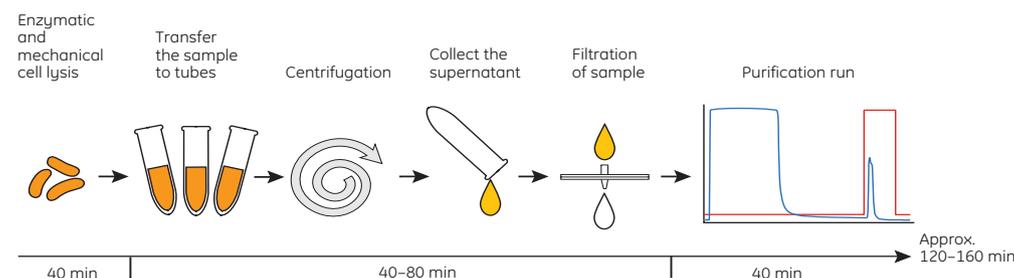
**No centrifugation or filtration required!**

## 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)

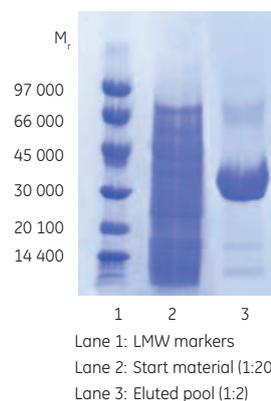
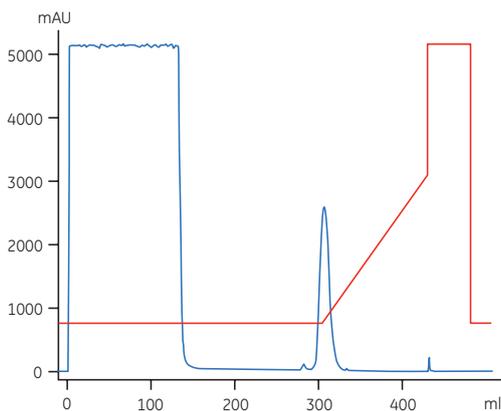
**Sample volume:** 130 ml

**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



## Results:

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

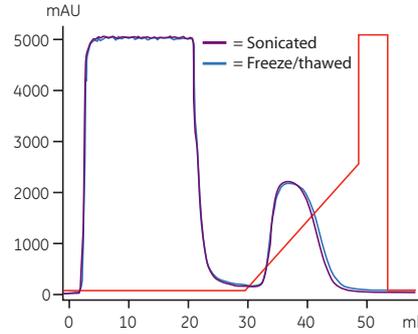
## Effect of different cell lysis methods

**Column:** HisTrap FF crude 1 ml  
**Sample:** Unclarified or clarified *E. coli* DH5 $\alpha$  lysate containing histidine-tagged maltose binding protein, MBP-(His)<sub>6</sub>, 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

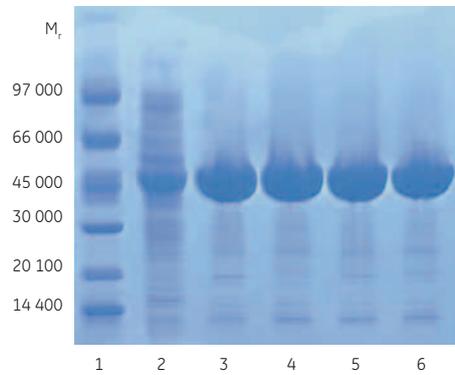
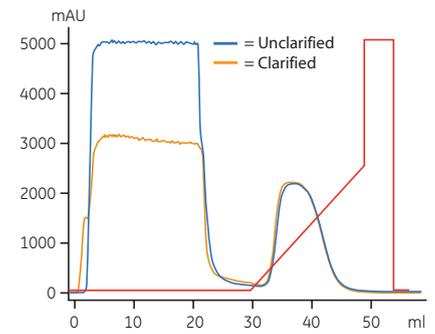
### Results:

- 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)



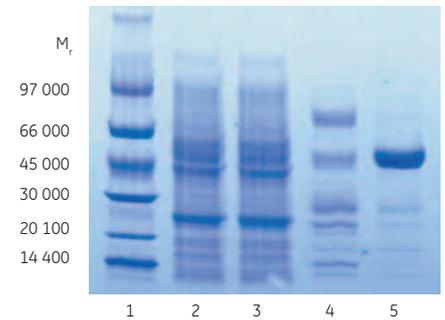
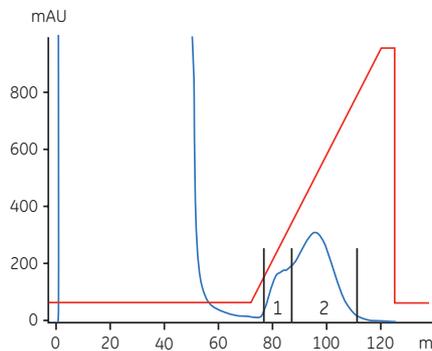
### Clarified and unclarified samples (both freeze/thawed)



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 $\beta$ -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  $\beta$ -mercaptoethanol, 10% glycerol, 25 mM imidazole, pH 7.4  
**Elution buffer:** 20 mM sodium phosphate, 500 mM NaCl, 10 mM  $\beta$ -mercaptoethanol, 10% glycerol, 500 mM imidazole, pH 7.4  
**Elution:** 25–500 mM imidazole (50 CV)  
**Flow rate:** 1 ml/min



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)

### Results:

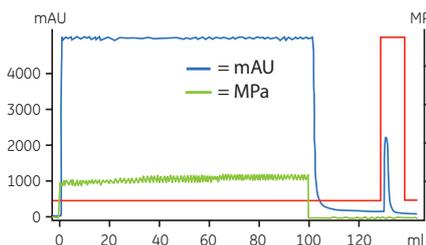
- High purity target protein was obtained directly from unclarified cell lysate.
- Pressure during purification was below the maximum pressure even in the presence of glycerol and at +4 °C.

# Scaling up purification

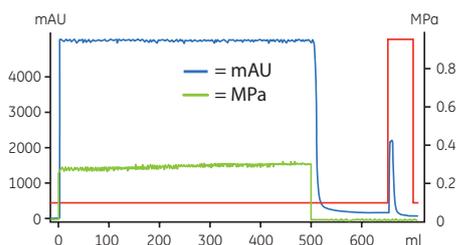
## Large volumes of unclarified homogenised cell lysate

**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)<sub>6</sub>  
**Sample volume:** 100 ml (20 mg GFP-(His)<sub>6</sub>)  
500 ml (100 mg GFP-(His)<sub>6</sub>)  
**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

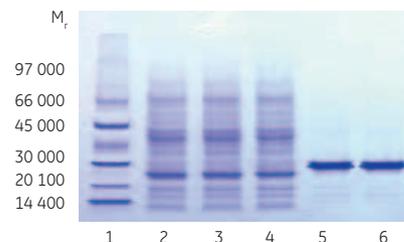
HisTrap FF crude 1 ml



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



### 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)<sub>6</sub> provided by Pharmacia Diagnostics, Uppsala, Sweden. GFP-(His)<sub>6</sub> 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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GE Healthcare

Amersham Biosciences AB

Björkgatan 30

751 84 Uppsala

Sweden

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