Automated multistep purification of histidine-tagged proteins from unclarified cell lysates

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Automated multistep purification of histidine-tagged proteins from unclarified cell lysates

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Introduction

HisTrap™ FF crude is a ready to use column intended for purification of histidine-tagged proteins from unclarified cell lysates. No centrifugation or filtration of the samples is required before loading. The total purification time is thereby significantly reduced, which is an important advantage when working with many samples.

In this study, ÄKTAxpress™, a chromatography system designed for high-throughput protein purification, was used for the purification of unclarified samples. HisTrap FF crude was used in the first purification step, followed by up to three additional chromatography steps.

A comparison between unclarified sample purified on HisTrap FF crude and clarified sample purified on HisTrap FF is presented. Further, purification of a large volume of unclarified lysate as well as a large amount of target protein is shown.

Conclusions

- Significant time saving when using HisTrap FF crude together with ÄKTAxpress
- Comparable final purity and recovery when purifying clarified and unclarified samples
- More than 100 mg of purified target protein was obtained from a three-step purification
- Up to 100 ml of unclarified sample was loaded onto HisTrap FF crude, 1 ml, without any increase in backpressure

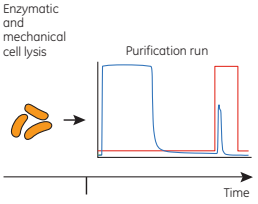
ÄKTA

HisTrap FF crude

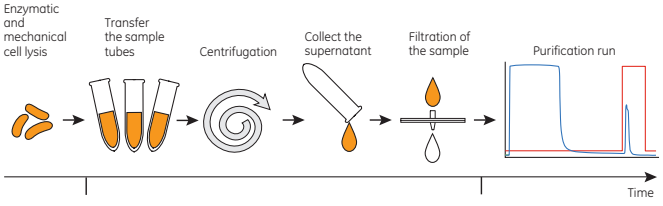
HisTrap FF crude is a prepacked column intended for immobilized metal affinity chromatography (IMAC). A special column construction enables direct loading of unclarified samples without the need for centrifugation and filtration. The use of HisTrap FF crude shortens the total purification time, which can minimize degradation and oxidation of sensitive target proteins. HisTrap FF crude is available as 1-ml and 5-ml columns.

Convenient and time saving

HisTrap FF crude

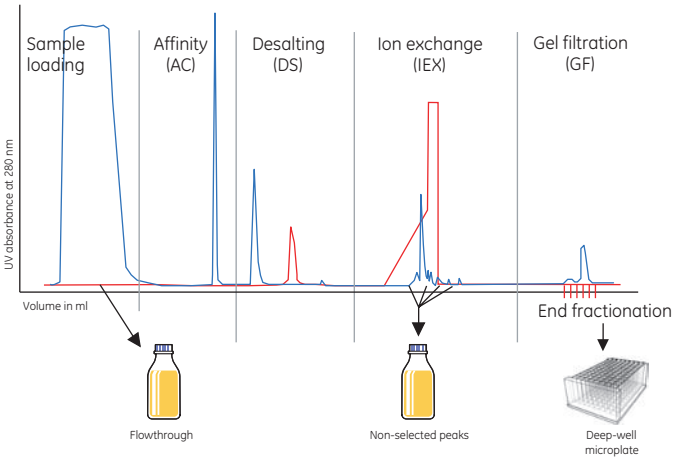


Conventional IMAC



ÄKTAxpress

- Automated multistep purification of affinity-tagged proteins
- Method wizard for easy creation of purification protocols
- Intelligent peak detection and collection of peaks in intermediate steps
- Optional on-column tag cleavage
- Up to four samples can be purified per module
- Up to 12 modules in parallel can be controlled from one computer



All protocols start with affinity or ion exchange chromatography followed by different combinations of desalting, affinity, ion exchange chromatography, and gel filtration. The selected peak from each step is transferred to the next column.

Table 1. Examples of typical run times, at room temperature and with maximum numbers of samples in four modules (sample loading not included)

Protocol	Number of samples	Typical run time (h)
AC-DS	16	5
AC-GF	16	11
AC-DS-IEX	12	6
AC-DS-IEX-DS	12	9
AC-DS-IEX-GF	8	8
DS	16	1
GF	16	6

Applications

Samples: The samples were expressed in *E. coli*. After harvest, cell lysis was performed by sonication. The unclarified samples were loaded directly onto the HisTrap FF crude columns. When using HisTrap FF columns, the lysates were centrifuged and filtered before sample loading.

Buffers:

AC binding:	50 mM Tris-HCl, 0.5 M NaCl, 20 mM imidazole, pH 8.0
AC elution:	50 mM Tris-HCl, 0.5 M NaCl, 500 mM imidazole, pH 8.0
DS and IEX binding:	50 mM Tris-HCl, pH 8.0
IEX elution:	50 mM Tris-HCl, 1.0 M NaCl, pH 8.0
GF:	50 mM Tris-HCl, 0.1 M NaCl, pH 8.0

Analysis: Purity was analyzed by Coomassie™ stained SDS-polyacrylamide gels. The reduced samples were applied to 8–18 or ExcelGel SDS Homogeneous 7.5 gels.

Same final purity when using unclarified samples

In two experiments, *E. coli* BL21 lysates were divided into two parts. The first part was loaded directly onto HisTrap FF crude while the second part was centrifuged and filtered before it was loaded onto HisTrap FF. The comparison was performed with 1-ml and 5-ml columns.

Four-step protocol (AC-DS-IEX-GF)

Sample: *E. coli* BL21 lysate containing histidine-tagged cytochrome P-450 (M, 121 000)

Columns:
AC See Figures 1 and 2
DS HiPrep™ 26/10 Desalting
IEX Mono Q™ 5/50 GL
GF HiLoad™ 16/60 Superdex™ 200 pg

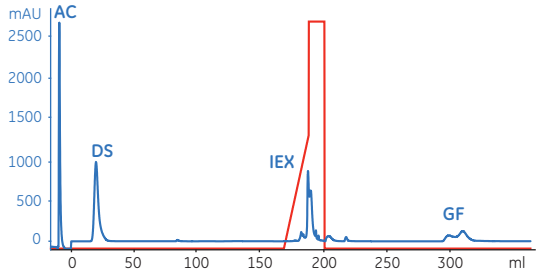


Fig 1. AC column: HisTrap FF crude, 1 ml. Yield: 7.2 mg purified target protein.

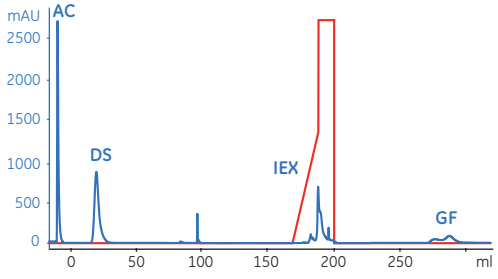
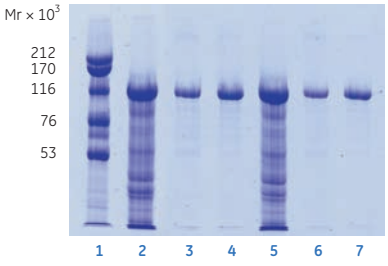


Fig 2. AC column: HisTrap FF, 1 ml. Yield: 4.8 mg purified target protein.



Lane 1: HMW markers
Lane 2: Start material, HisTrap FF crude[1]
Lane 3: Pool 1, HisTrap FF crude
Lane 4: Pool 2, HisTrap FF crude
Lane 5: Start material, HisTrap FF[1]
Lane 6: Pool 1, HisTrap FF
Lane 7: Pool 2, HisTrap FF

[1] Diluted ten-fold before analysis

Three-step protocol (AC-DS-IEX)

Sample: *E. coli* BL21 lysate containing histidine-tagged APB7 (M, 28 000). The plasmid was provided by SGX Pharmaceuticals.

Columns:
AC See Figures 3 and 4
DS HiPrep 26/10 Desalting
IEX RESOURCE™ Q, 6 ml

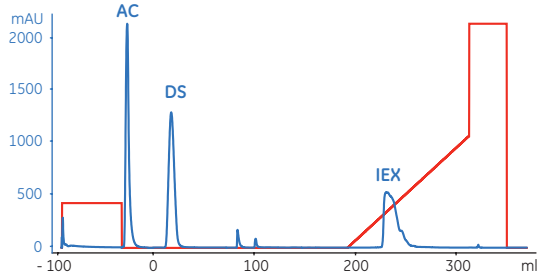


Fig 3. AC column: HisTrap FF crude, 5 ml. Yield: 102 mg purified target protein.

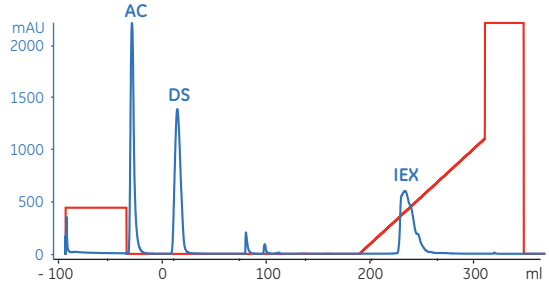
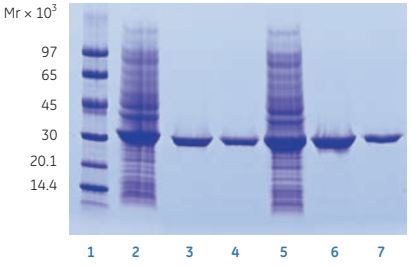


Fig 4. AC column: HisTrap FF, 5 ml. Yield: 104 mg purified target protein.



Lane 1: LMW markers
Lane 2: Start material, HisTrap FF crude[1]
Lane 3: Pool 1, HisTrap FF crude
Lane 4: Pool 2, HisTrap FF crude
Lane 5: Start material, HisTrap FF[1]
Lane 6: Pool 1, HisTrap FF
Lane 7: Pool 2, HisTrap FF

[1] Diluted ten-fold before analysis

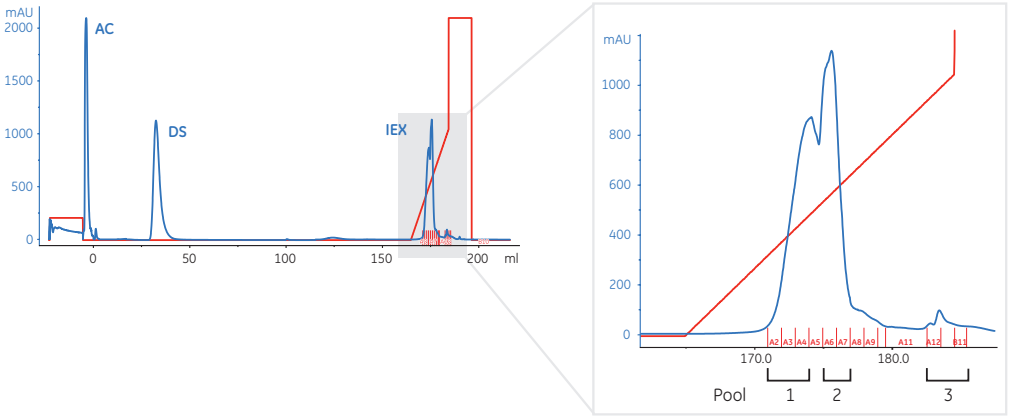
Results

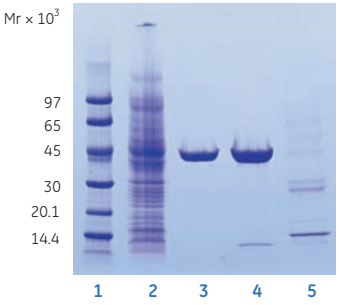
- Recovery as well as sample purity were similar for both column types.
- More than 100 mg was obtained from a three-step purification.

Purifying large volumes of unclarified cell lysates

A volume of 100 ml of unclarified cell lysate was purified using a three-step protocol, AC-DS-IEX. The sample was an *E. coli* DH5α lysate containing histidine-tagged maltose binding protein.

Columns:
AC HisTrap FF crude, 1 ml
DS HiPrep 26/10 Desalting
IEX Mono Q 5/50 GL





Lane 1: LMW markers
Lane 2: Start material[1]
Lane 3: Pool 1
Lane 4: Pool 2
Lane 5: Pool 3

[1] Diluted ten-fold before analysis

Fig 5. AC-DS-IEX with an enlargement of the IEX peaks and the collected pools to the right. Yield: 9.4 mg in pool 1 + 2.

Results

- A volume of 100 ml of unclarified cell lysate was loaded onto HisTrap FF crude, 1 ml, without any increase in backpressure.
- High purity target protein was obtained.

www.gelifesciences.com/protein-purification

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The plasmid expressing the APB7 protein was kindly donated by SGX Pharmaceuticals, Inc., San Diego, USA.

Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US patent numbers 5,284,933 and 5,310,663 and equivalent patents and patent applications in other countries (assignee: Hoffman La Roche, Inc).

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