

Purification of an N-terminal histidine-tagged protein using ÄKTA™ start

ÄKTA start eliminates the hassles of manual protein purification handling, such as loading large sample volumes and continually monitoring UV readings, and can give you consistency of yield and purity. Madhu here demonstrates how ÄKTA start aided him in purifying a recombinant N-terminal histidine-tagged protein.

Introduction

Expression of recombinant protein with affinity tags to aid purification is a common strategy. Histidine tags are the most common and versatile of these, as histidine is small and can bind to nickel-chelating chromatography media under denaturing conditions. This property is important because recombinant proteins expressed in *E. coli* sometimes form inclusion bodies, which are insoluble protein aggregates that lack biological activity. Denaturing (chaotropic) agents, such as urea or guanidine hydrochloride, are used to solubilize inclusion bodies, making the extraction process easier. However, slow removal of the denaturing agents is often required for proper refolding of these proteins to their functional form.

The ÄKTA start Pump can be used to load large sample volumes without manual intervention

Madhu was faced with a challenge of obtaining good yields with high purity of one such N-terminal histidine-tagged protein. The expressed protein formed inclusion bodies and to extract it, 6 M urea was used in the extraction buffer. When attempting to purify the protein Madhu could only get 60% to 80% pure protein with many closely associated impurities. After some initial tests,

Madhu realized that if he diluted his original sample ten times to start the refolding process, protein purity improved. However, manually handling large sample volumes was cumbersome and time-consuming. Madhu switched to ÄKTA start and a prepacked HisTrap™ FF crude column and saw dramatic improvements both in his purification results and in his workload.

ÄKTA start is compatible with a wide variety of prepacked columns

HisTrap FF crude is a ready-to-use column prepacked with precharged Ni Sepharose™ 6 Fast Flow, an immobilized metal affinity chromatography (IMAC) medium (resin). It has low nickel ion (Ni²⁺) leakage and is compatible with a wide range of reagents used in protein purification. The column also enables the loading of unclarified cell lysate, thus providing fast, simple, and convenient purification.

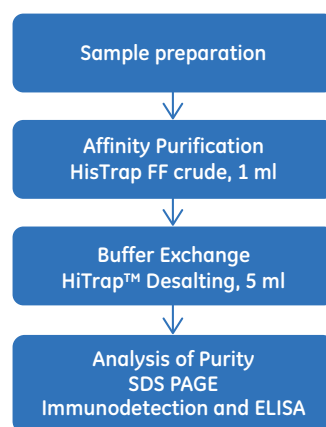


Fig 1. Work flow illustrating the steps involved in purifying and refolding histidine-tagged proteins from inclusion bodies.



Methods

Using the Method editor option in the UNICORN™ start 1.0 control software, Madhu selected the HisTrap FF crude 1 ml column from the column list provided in the software. Using the predefined affinity template he optimized the run parameters to suit his needs (Table 1). He followed the work flow illustrated in Figure 1 to complete the purification.

Table 1. UNICORN start method overview

Method Flow	Run parameters
Method settings	CV 1 ml* Pressure limit 0.3 MPa† Flow rate 1 ml/min
Prime and equilibration	10 CV*
Sample application	Apply sample using pump Sample volume 25 ml
Wash out unbound	10 CV*
Elution and fractionation	Gradient elution Step 1: 80% B, 5 CV* Step 2: 100% B, 15 CV* Peak Frac, 1 ml
Prime and equilibration	5 CV*

* Column volume

† 0.3 MPa = 3 bar (43.5 psi)

Madhu solubilized the inclusion bodies from *E. coli* containing the histidine-tagged protein in 50 mM Tris-HCl, 0.5 M NaCl, 6 M urea, pH 7.9. He then slowly diluted (15 min) the samples 10-fold with binding buffer (25 mM sodium phosphate, 150 mM NaCl, 20 mM imidazole, pH 7.4) and kept them at room temperature for 2 h before loading onto the column.

Use the Manual run option from UNICORN start to standardize purification protocols.

Madhu loaded 25 ml of 1:10 diluted sample onto a HisTrap FF crude 1 ml column. To accommodate large sample volumes and to reduce manual intervention, he loaded the sample through the system pump. He washed the column with 10 column volumes (CV) of binding buffer and eluted in a single step with 5 CV of 80% elution buffer. He collected 1 ml fractions using Frac30 fraction collector. Finally, Madhu washed the column with 15 CV of 100% elution buffer to ensure the removal of all tightly bound proteins. He re-equilibrated the column with 5 CV of binding buffer so that the column was ready for the next chromatography run.

Madhu pooled the peak fractions and removed the imidazole using a 5 ml HiTrap™ Desalting column. He used the predefined Desalting template available in UNICORN start. He analyzed the purity of the protein using 12.5% SDS-PAGE, and performed ELISA and immunodetection to confirm activity and specificity of the protein.

Results

Madhu purified the N-terminal histidine-tagged protein in a single purification step using affinity chromatography on ÄKTA start with UNICORN start control software and Frac30 fraction collector. Using UNICORN start Evaluation module, Madhu was able to identify and pool the peak fractions (Fig 2A). He used a desalting method to remove imidazole from the pooled fractions (data not shown).

The desalting step greatly reduced his time when compared to the conventional dialysis method.

Column:	HisTrap FF crude 1 ml
Sample:	Solubilized inclusion bodies from <i>E. coli</i>
Sample load:	25 ml
Binding buffer:	25 mM sodium phosphate, 150 mM NaCl, 20 mM imidazole, pH 7.4
Elution buffer:	25 mM sodium phosphate, 150 mM NaCl, 500 mM imidazole, pH 7.4
Flow rate:	1 ml/min
Gradient:	80% step
System:	ÄKTA start, UNICORN start, and Frac30 fraction collector
Detection:	UV (280 nm)

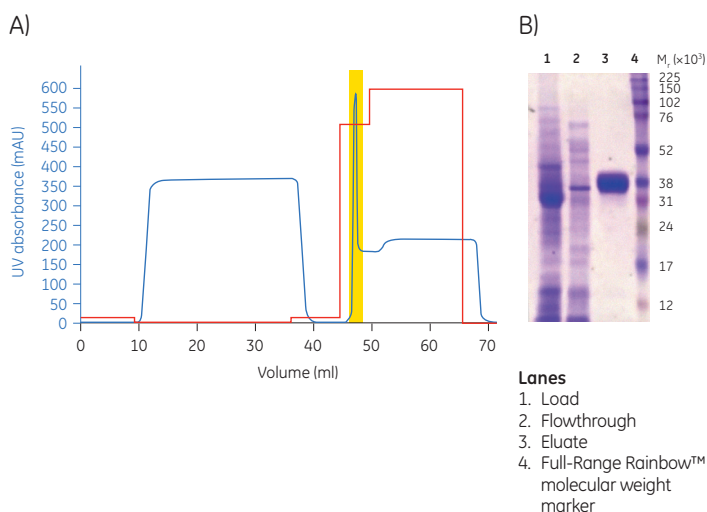


Fig 2. A) Chromatogram showing the purification profile of N-terminal histidine-tagged protein on ÄKTA start. Area highlighted (yellow) represents the pooled fractions. The high UV signal after the peak is due to the absorbance of imidazole at 280 nm. B) 12.5% SDS-PAGE profile of the protein fractions.

Analyze the results using single click peak integration and report generation features of UNICORN start.

Madhu evaluated the results using UNICORN start and generated a report containing information about the protein peak, such as retention time, area and height of the peak. He also used the report to identify and pool the peak fractions, which he later analyzed for purity using 12.5% SDS-PAGE (Fig 2B). The affinity purification method Madhu developed yielded excellent separation of the target protein from contaminating cellular proteins. Using ÄKTA start he could use the same method to consistently obtain a high level of purity (> 95%) of the target protein. Madhu achieved 1.6-fold higher purity of protein when compared to his conventional manual method. The quality of the purified protein was found to be high as revealed by SDS-PAGE (Fig 3A). He further analyzed the specificity of the protein by immunoblot (Fig 3B) and ELISA (data not shown).

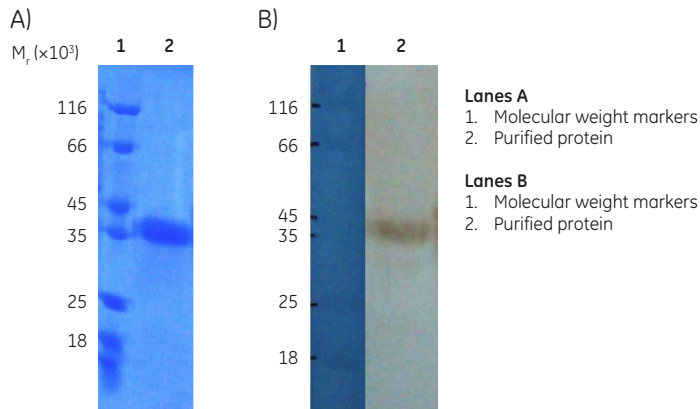


Fig 3. A) SDS-PAGE and B) Immunodetection profile of purified N-terminal histidine-tagged protein with protein-specific polyclonal antibodies..

Conclusions

Purification of histidine-tagged protein on ÄKTA start using a prepacked HiTrap FF crude column, Frac30 fraction collector, and UNICORN start software reduced Madhu's time and effort, giving him higher yield and purity with greater consistency.

Acknowledgments

We thank Dr. Shama Bhat, Chairman & Managing Director, Bhat Bio-Tech India Pvt. Ltd., Bangalore, India, for kindly providing us with antibody samples and supporting us with analytical data for the purified recombinant proteins. We wish to extend our appreciation for the insights Bhat Bio-Tech team has shared about the challenges faced while purifying recombinant proteins manually.

Ordering information

Product	Quantity	Code number
ÄKTA start	1	29-0220-94
Frac30 Fraction collector	1	29-0230-51
UNICORN start software	1	29-0187-51
HiTrap FF crude	5 × 1 ml	11-0004-58
HiTrap Desalting	5 × 5 ml	17-1408-01
Full-Range Rainbow Molecular Weight Markers	250 µl	RPN800E

Related literature

Product	Code number
Depletion of albumin from serum samples using ÄKTA start, Application note	29-0642-95
Purification of GST-tagged protein using ÄKTA start, Application note	29-0642-98
Purification of antibodies using ÄKTA start and HiTrap Protein G HP column, Application note	29-0643-02

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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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First published Sep. 2013.

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