Rapid, Efficient Purification and Evaluation of His-Tagged Proteins

Eric Larson and Shane Petersen, Bio-Rad Laboratories, Inc.,
Hercules, CA 94547 USA

Introduction

Affinity purification is a routine method used to purify quantities of a target protein for use in experiments designed to characterize the protein and validate the results of gene expression experiments. It is desirable to purify protein quickly and efficiently in preparation for these downstream experiments.

A number of tags may be engineered into recombinant proteins to facilitate their purification. Among these, the polyhistidine (His) tag, comprised of six or more histidine residues, is commonly used for purification due to its small size and easy purification by immobilized metal ion affinity chromatography (IMAC). IMAC purification can be performed at various scales using nickel- or cobalt-containing gravity flow columns, spin columns, or cartridges. Following separation, collected fractions are usually analyzed by gel electrophoresis (for example, by SDS-PAGE).

In this tech note, we compare purification of a His-tagged protein on Bio-Scale™ Mini Profinity™ IMAC cartridges and on two other commercially available IMAC cartridges using the BioLogic DuoFlow™ chromatography system. To expedite this analysis, the fractions collected using all three cartridges were analyzed with the Experion™ automated electrophoresis system.

Methods

Recombinant His-tagged NIF3 from *Saccharomyces cerevisiae* (YGL221C, 32 kD) was expressed in *E. coli*. A 300 ml culture of *E. coli* expressing the recombinant protein was pelleted and lysed in a 10:1 volume of B-Per reagent (Pierce Biotechnology), yielding 12 ml of concentrated lysate.

Purification Protocol

Samples of cell lysate were purified at 2 ml/min through a 1 ml Bio-Scale Mini Profinity IMAC Ni^{2+} cartridge and two different IMAC cartridges from another supplier (supplier A cartridges 1 and 2) using a BioLogic DuoFlow chromatography system. Bead sizes were 60 µm for the Profinity IMAC cartridge and 90 µm and 34 µm for supplier A cartridges 1 and 2, respectively.

Each column was first rinsed with 5 column volumes (CV) of deionized water and then equilibrated in 5 CV of equilibration buffer (300 mM KCl, 50 mM KPO_4, 5 mM imidazole, pH 8.0). Cell lysate (4 ml) was then applied, and the columns were washed twice, the first time with 6 CV of equilibration buffer and the second time with 6 CV of wash buffer (300 mM KCl, 50 mM KPO_4, 10 mM imidazole, pH 8.0). The protein was eluted in 2.5 CV of elution buffer (300 mM KCl, 50 mM KPO_4, 250 mM imidazole, pH 8.0). (The running conditions were within the range recommended by supplier A.) The purification comparison was performed twice.

Fraction Collection and Analysis

Elution profiles were monitored at 280 nm, and fractions containing the flowthrough (4 ml) and wash fractions (5 ml for the first wash and 4 ml for the second wash) were collected based on the programmed method. The eluted peak (2.5 ml) was collected when A_{280} crossed a threshold of 0.100. The lysate (load) and collected fractions from each cartridge were then analyzed using the Experion automated electrophoresis system and Pro260 analysis kit to evaluate the resulting purity of the NIF3 protein from each cartridge. The Experion Pro260 analysis kit includes a protein ladder, sample buffer, gel solution, fluorescent stain, spin filters, and Pro260 microfluidic chips. Samples (4 µl) were mixed with 2 µl sample buffer containing β-mercaptoethanol, heated at 95°C for 5 min, diluted with ReadyPrep™ proteomics grade water, and loaded onto chips primed according to the protocols provided with the kit. The purity of the tagged NIF3 protein was evaluated using Experion software.

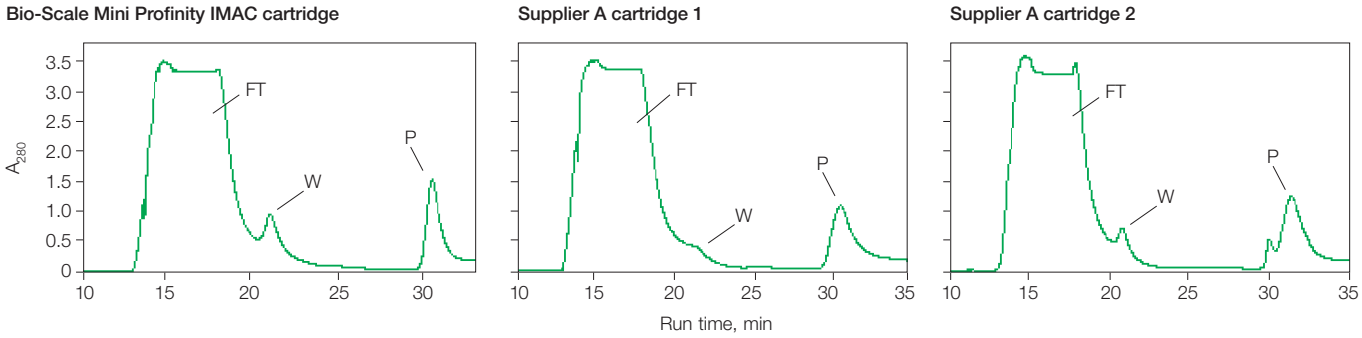


Fig. 1. Comparison of chromatographic separation of bacterial lysate on three different IMAC cartridges. FT, flowthrough; W, wash; P, eluted protein.

Results and Discussion

A previous report demonstrated the ability of Profinity IMAC media to deliver pure His-tagged protein (Wang et al. 2006). Profinity IMAC Ni^{2+} support has an optimized ligand density and open pore structure that interacts at high stringency with recombinant His-tagged proteins of a wide molecular weight range. Based on Bio-Rad's UNOsphere™ support, Profinity IMAC media exhibit excellent flow properties without compromising binding capacity, recovery, or purity. This report extends analysis to the Profinity IMAC media prepackaged in the Bio-Scale Mini cartridge format and compares it to another supplier's prepacked IMAC cartridges.

Bacterial cell lysate containing recombinant His-tagged NIF3 protein was applied to the three IMAC cartridges, and the chromatograms generated by the BioLogic DuoFlow software during purification are shown in Figure 1.

The load, flowthrough, and eluted fractions were collected and analyzed using the Experion automated electrophoresis system and Experion Pro260 analysis kit. The Experion system automates the separation, staining, and analysis steps of gel electrophoresis to generate results comparable or superior to those obtained by traditional SDS-PAGE (Chang et al. 2005, Zhu et al. 2005). This microfluidics-based system displays separation data in electropherograms and in a simulated gel image, with the peaks in the electropherograms corresponding to the bands in the simulated gel image. Figure 2 shows the simulated gel image obtained following separation of all the fractions collected from all three cartridges, and Figure 3 shows electropherograms from the separation of the fractions containing purified NIF3.

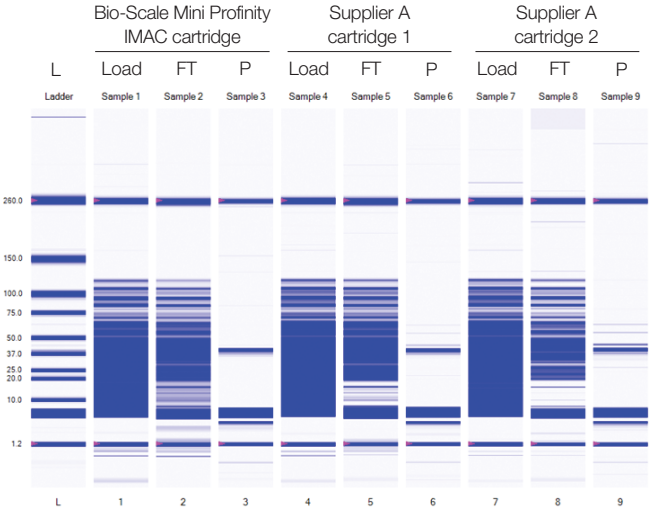
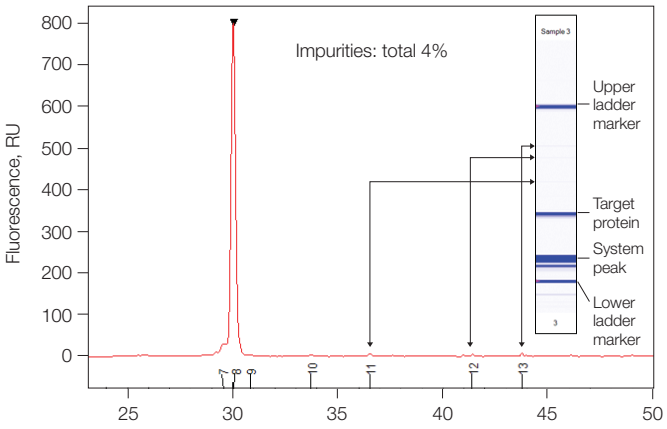
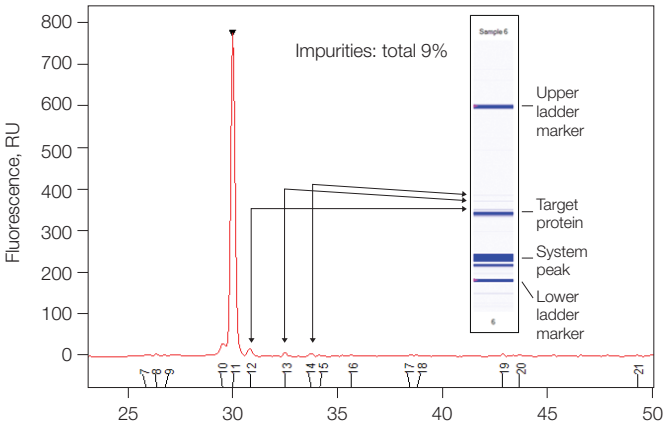


Fig. 2. Analysis of IMAC purification of His-tagged NIF3 protein. Samples collected during chromatographic purification of NIF3 from a crude cell lysate were analyzed using the Experion Pro260 analysis kit. For each cartridge used, the load (Load), flowthrough (FT), and eluted target protein (P) fractions were analyzed; the simulated gel view generated by Experion software is shown. L, Pro260 ladder. The upper (260 kD) and lower (1.2 kD) bands in each lane are internal standard markers.

Bio-Scale Mini Profinity IMAC cartridge



Supplier A cartridge 1



Supplier A cartridge 2

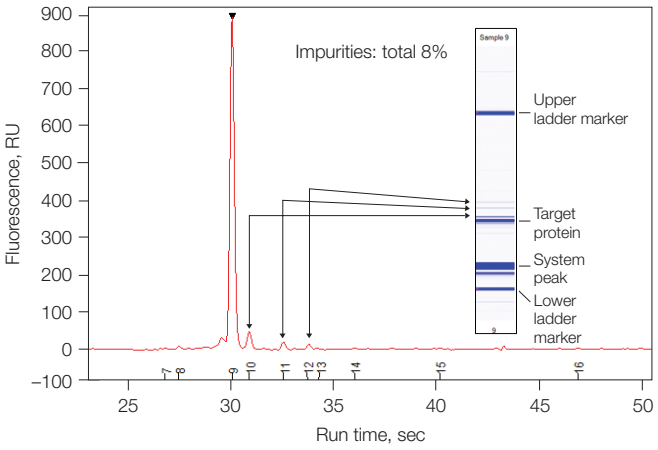


Fig. 3. Analysis of fractions containing purified NIF3. Electropherograms of the separation of samples containing His-tagged NIF3 purified from a crude cell lysate were analyzed using the Experion Pro260 analysis kit. Electropherograms are shown along with the simulated gel views (insets) for samples purified on each of the three IMAC cartridges.

Supplier A cartridge 2

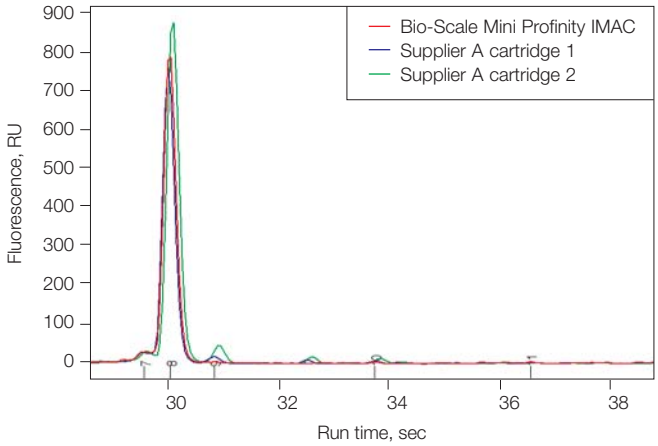


Fig. 4. Electropherogram overlay of separations of fractions containing purified NIF3. The zoomed-in overlay allows close comparison of impurities in the three samples.

Comparison of the electropherograms with the simulated gel image (Figure 3, insets) enables close analysis of small impurities in the samples, but a further comparison can be made using an overlay of the three electropherograms of the purified fractions (Figure 4), which shows that the impurities in the purified fractions were consistent from one cartridge to another.

Experion software automatically calculates the amount of total protein in a sample as well as the percentage of that total for each peak (Figure 3); it also enables a comparative analysis of specific peaks across different samples. In this way, the purity of the eluted peak is automatically calculated. Such analysis revealed that the impurities were more abundant in the fractions separated by the two cartridges from supplier A than from the Bio-Scale Mini Profinity IMAC cartridge (Table 1).

Table 1. Calculated purity of His-tagged NIF3 protein. Percent purity was calculated by Experion software.

Cartridge	Purity (%)
Bio-Scale Mini Profinity IMAC cartridge	96
Supplier A cartridge 1	91
Supplier A cartridge 2	92

Conclusions

The routine purification of His-tagged proteins is a useful and powerful technique in the process of preparing protein samples for functional and structural experiments. We demonstrate here that Bio-Scale Mini Profinity IMAC cartridges, when combined with the BioLogic DuoFlow chromatography system and Experion automated electrophoresis system, can be used to purify and analyze target protein in a rapid, efficient manner that still yields protein of high purity.

Acknowledgement

We thank Dr Ray Stevens, The Scripps Research Institute, for the vector containing the tagged NIF3 gene.

References

Chang B et al., Bio-Rad applies microfluidics to automate electrophoresis, Bio-Rad bulletin 5285 (2005)

Wang D et al., Profinity IMAC resin: an optimal support for His-tagged protein purification, Bio-Rad bulletin 5456 (2006)

Zhu K et al., Performance comparison of the Experion automated electrophoresis and SDS-PAGE for protein analysis, Bio-Rad bulletin 5299 (2005)

Information in this tech note was current as of the date of writing (2006) and not necessarily the date this version (rev A, 2006) was published.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD* ***Australia*** *02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 21 3237 9400* ***Canada*** *905 712 2771* ***China*** *86 21 6426 0808* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 455 8800* ***India*** *91 124 4029300/5013478* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 5811 6270* ***Korea*** *82 2 3473 4460* ***Mexico*** *55 5200 05 20* ***The Netherlands*** *0318 540666* ***New Zealand*** *64 9415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 095 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 0861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189/2578 7241* ***United Kingdom*** *020 8328 2000*