Automated Purification of a His-Tagged Protein With the Profinia™ Protein Purification System: Comparison With Another Low-Pressure Chromatography System

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Introduction

Incorporation of polyhistidine (His) or glutathione S-transferase (GST) affinity tags into recombinant proteins is a routine step in the expression and purification of proteins. The use of fused His residues and immobilized metal affinity chromatography, or IMAC as the procedure is commonly referred to, has been used routinely since the mid-1970s (Porath et al.1975; Hochuli 1988). Multiple types of supports, buffers, and systems are commercially available to aid in the purification of His-tagged proteins. Under native conditions, soluble His-tagged proteins can be separated from crude bacterial lysates by following the three basic steps of binding, washing, and eluting target proteins. Crude lysates are applied to an IMAC support, and unbound host proteins are excluded from the column in the flow-through. Weakly bound contaminants are removed by washing with several column volumes of a low-imidazole buffer. The purified protein is then eluted with an increased concentration of imidazole in the elution buffer.

Traditional methods for purifying His-tagged proteins include the use of gravity-flow columns, spin columns, or low-pressure chromatography systems. Gravity-flow and spin-column purifications offer the benefit of low cost with the trade-off of increased hands-on time and poor reproducibility. Further, chromatographic traces are not generated with these methods, making analysis and method optimization more challenging. Alternatively, low-pressure chromatography systems can be programmed to purify affinity-tagged proteins; however, depending upon the system chosen, they require extensive user expertise and are typically more costly than gravity-flow and spin methods. With chromatography systems, the purified peak of interest is fractionated into multiple tubes, and identification and analysis of the purified protein requires higher levels of skill and understanding of the chromatographic separation process and methodology.

The Profinia protein purification system fills the niche between less sophisticated manual methods of purification and more technically challenging instrument-based separations. A large, informative touch screen guides the user through method selection and sample loading, and purified protein is automatically collected into a single tube. At the completion of a run, purified protein yield, volume, and concentration are calculated and displayed in the user interface. When the instrument is used with Profinia software, the real-time chromatographic profile is displayed on a computer screen, and the run data are transferred to the report and displayed in a tabular format. The software also generates publication-quality reports displaying the chromatogram, method steps, and data tables, which contain yield, concentration, and elution volume data.

The Profinia system performs both purification and buffer exchange of affinity-tagged proteins, simplifying and reducing the time required for a chromatographic cycle. Optimized preprogrammed methods for the most common affinity applications are available and can be used with the buffers and prepacked cartridges available in the Profinia purification kits. The preplumbed system is easily maintained through prepackaged reagents and automated cleaning and storage protocols. The automated methods separate crude lysates into flow-through, wash, and elution fractions, which allows determination of the effectiveness of purification. The option for automated detection, selection, and diversion of the main affinity peak onto a size exclusion column allows automatic buffer exchange without the need for user intervention. This consolidated, two-dimensional affinity/desalting step allows purification with reduced sample handling, reduced time to purified product, and overall reduced hands-on time.

A typical workflow for protein preparation, purification, and analysis with the Profinia system is illustrated in Figure 1. Lysis reagents provided in Profinia purification kits are used to prepare lysates. Following centrifugation and filtration, clarified lysates are loaded and purified using preprogrammed protocols. Eluted fractions are then analyzed to determine the concentration, yield, and percent purity of the target protein. The purified protein is then available for use in downstream assays.

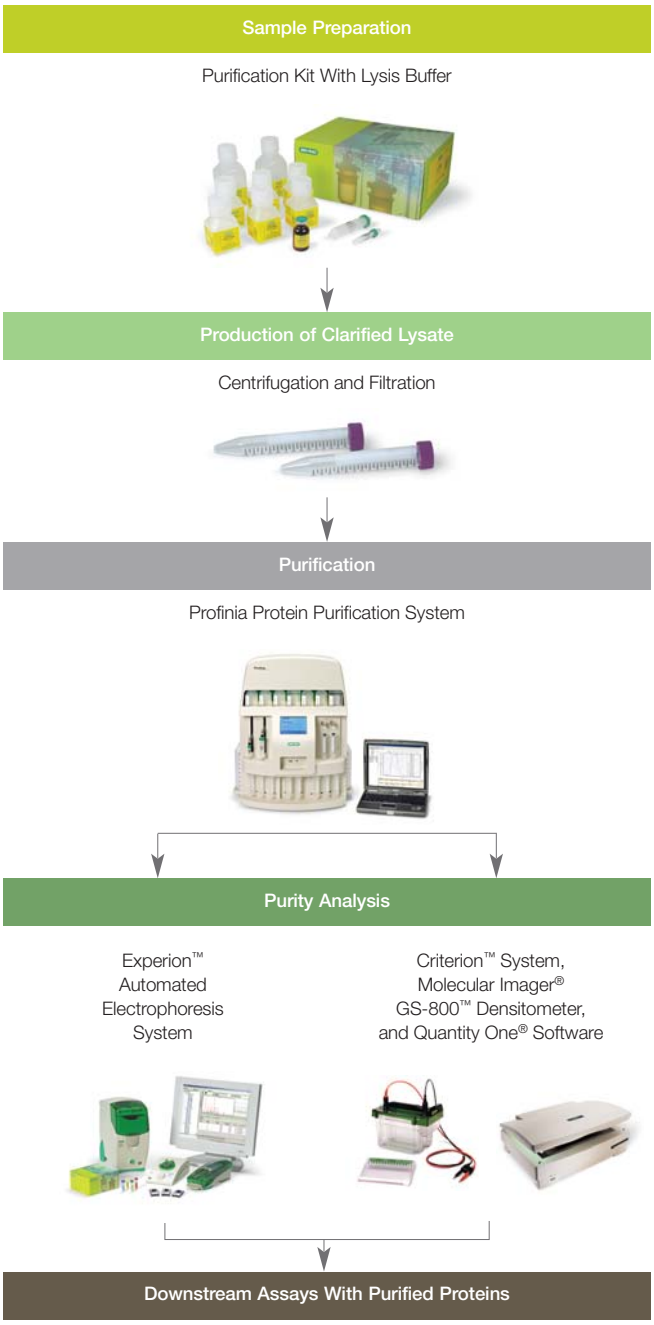

Fig. 1. Workflow from sample preparation to protein purification and analysis.

In this study, we compare the performance of the Profinia system to the ÄKTAprime system from GE Healthcare. The ÄKTAprime low-pressure chromatography system is routinely used for affinity purifications and is functionally comparable to the Profinia platform in that preprogrammed methods and cue cards guide the user through the separation. Included in this study are quantitative comparisons of protein yield, protein purity, and purification time. While the Profinia and ÄKTAprime systems generated purified protein with comparable yield and purity, user interaction and direct hands-on time were more streamlined and efficient on the Profinia system.

Methods

A 51 kD His- and GST-tagged protein was purified using both the Profinia and ÄKTAprime (GE Healthcare) low-pressure chromatography systems and their associated columns and reagents. A lyophilized *Escherichia coli* lysate containing the 51 kD protein was obtained from the Profinia IMAC starter kit (catalog #620-0229), and each vial of lysate was resuspended as directed in the kit instructions in 10 ml of Profinia lysis buffer. Five purification runs were performed using the same columns and buffers for each run. Flow-through, wash, and purified protein fractions from runs 1, 3, and 5 were then analyzed by SDS-PAGE. The concentration of the purified 51 kD protein was determined spectrophotometrically using the absorbance at 280 nm and the known absorbance of a 1 mg/ml solution of the protein. Purity of the 51 kD protein was evaluated using the Experion automated electrophoresis system.

Protein Purification and Desalting With the Profinia System

All purification reagents and columns were obtained from the Profinia IMAC starter kit. Purifications were performed with the Profinia preprogrammed method template for "Native IMAC + Desalting" and with the standard sample flow rate of 2.0 ml/min. The 1 ml IMAC cartridge and 10 ml desalting cartridge provided in the IMAC starter kit were used. For each purification run, 8 ml of sample was loaded and 4 ml of purified protein was collected. Real-time monitoring of each purification run and collection of the subsequent run data were done using Profinia software.

Protein Purification and Desalting With the ÄKTAprime System

Identical volumes of rehydrated samples of the *E. coli* lysate containing the 51 kD dual-tagged protein used for the Profinia separations were used for the ÄKTAprime separations. All other reagents and software used in these analyses were obtained from GE Healthcare. The reagents for the IMAC affinity purification step were obtained from the HisTrap FF crude kit and used according to the instructions provided. The phosphate buffer and imidazole concentrates were used to prepare the binding, wash, and elution buffers, which were similar in formulation to Profinia buffers. HiTrap desalting columns were used according to manufacturer's instructions. The affinity purifications were performed with the ÄKTAprime plus cue card instructions (11-0027-48 AB) and programmed according to the step-elution instructions on the cue card. The main peak from the affinity purification was manually collected and prepared for the second ÄKTAprime desalting step. The affinity peak was desalted according to the desalting separations as described on the HiTrap desalting ÄKTAprime cue card (18-1138-03). Each run was monitored using PrimeView software and analyzed using UNICORN software.

SDS-PAGE and Experion Pro260 Analysis

SDS-PAGE was performed using the Criterion system and 8–16% Tris-HCl precast gels. Gels were stained with Bio-Safe™ Coomassie stain. The sample, flow-through, and wash fractions were diluted 7-fold into Laemmli sample buffer and loaded in a volume of 10 µl. Purified protein was diluted

in Laemmli sample buffer to 0.1 µg/µl and loaded in a volume of 10 µl (1 µg). Experion analysis of the purified protein fractions was performed using the Experion Pro260 analysis kit following the protocol described in the instruction manual.

Results and Discussion

The IMAC affinity and desalting chromatographic separations showed similar separation profiles on both systems, as illustrated in the chromatograms in Figure 2. Both systems produced single, sharp affinity and desalted peaks, but the key difference between the output chromatograms was in the combined affinity and desalting profile displayed in the Profinia chromatogram (Figure 2A). The Profinia run required no manual intervention, and the affinity peak was automatically diverted to the desalting column. With the ÄKTAprime methods, two independent affinity (Figure 2B) and desalting (Figure 2C) steps were displayed, and the manual intervention required between the two steps was represented by the separation of the two chromatograms.

Protein purity was assessed by SDS-PAGE and Experion Pro260 analysis. SDS-PAGE analysis of three representative runs revealed that the protein profiles of the Profinia and ÄKTAprime separations were nearly identical (Figures 3A and 3B). The Experion electropherograms in Figures 3C and 3D show the separation of the 51 kD protein, which was also analyzed by Experion software to generate the purity data shown in Table 1. Comparable amounts of purified protein were obtained from both chromatography systems, and the purity of the eluted and desalted 51 kD protein was nearly identical for both systems, differing by less than 0.1% (96.7% for the ÄKTAprime system and 96.6% for the Profinia system; see Table 1).

When time was the criterion being compared, however, the Profinia system outperformed the ÄKTAprime system (Table 2). In this study, two main matrices of time were examined: hands-on time required by the user and overall time to purified protein for downstream applications. With the Profinia system, the time from the push of the "start" button on the touch screen to collection of the purified and desalted protein was 34 min. With the ÄKTAprime system, the total time required was 75 min, which included time for purification, manual identification of the fractions containing the main affinity peak, manual pooling of those fractions, and desalting. When the time required for buffer preparation and peak collection were considered, the Profinia system saved an additional 30 min by offering comprehensive purification kits, which contain a complete portfolio of affinity, desalting, cleaning, and storage solutions that are preconcentrated and ready for direct placement into the buffer positions of the Profinia system. With the HisTrap kits used with the ÄKTAprime system, dilution of buffer concentrates and preparation of buffers added ~20 min of hands-on time. Moreover, the time saved with the Profinia system was a direct result of the integrated two-dimensional affinity/desalting run, where the peak collection algorithm automatically detects, selects, and diverts the affinity peak onto the installed desalting cartridge.

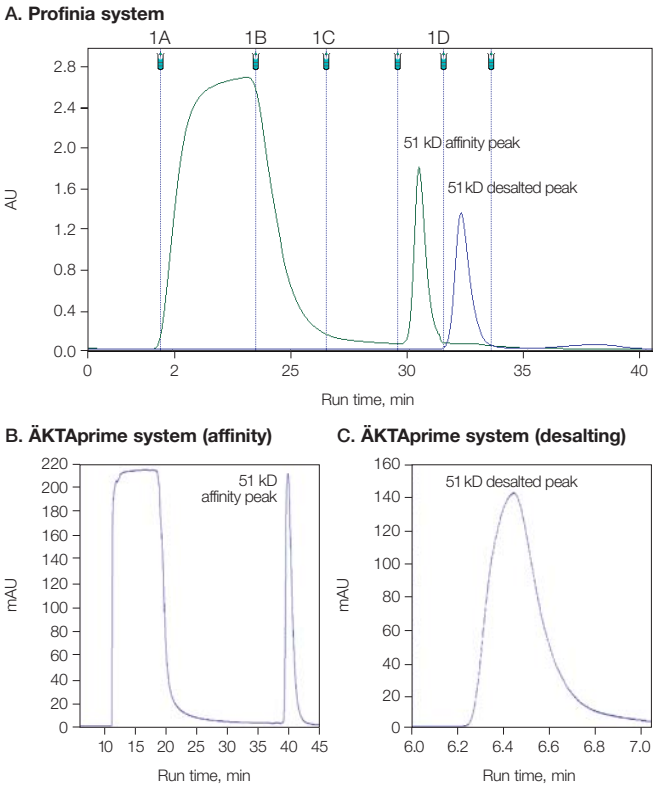

Fig. 2. Representative chromatographic traces from purifications of the 51 kD dual-tagged protein on the Profinia and ÄKTAprime systems. UV absorbance at 280 nm (AU or mAU) was collected over time for a representative purification. **A**, combined affinity and desalting traces generated by the Profinia system. (—), affinity trace; (—), desalting trace. Vertical lines indicate collection points of the fractions analyzed in Figure 3. 1A, flow-through; 1B, wash 1; 1C, wash 2; 1D, purified protein. **B** and **C**, affinity and desalting traces, respectively, generated by the ÄKTAprime system.

Table 1. Yield and purity of the 51 kD protein. Protein was purified and desalted with the Profinia protein purification system and with the ÄKTAprime system. Averages from all five purification runs are shown.

Purification Method	Average Yield (mg)	Average Purity (%)
Profinia	7.0	96.6
ÄKTAprime	7.3	96.7

Table 2. Time requirements for purification.

	Time Required (min)			
Purification Method	Affinity Step	Desalting Step	Buffer Preparation and Peak Collection/Application	Total
Profinia	34 (combined)		10	44
ÄKTAprime	55	20	40	115

Conclusions

The Profinia protein purification system has been tested against the ÄKTAprime system, a similar low-pressure chromatography instrument, for the purification and desalting of a His-tagged protein. The ÄKTAprime system was chosen due to the availability of preprogrammed methods by which to compare to the preprogrammed methods of the Profinia system. Preformulated buffers and chromatography cartridges are also available for both systems, which also allowed a comparison of ease of use and performance of the integrated systems.

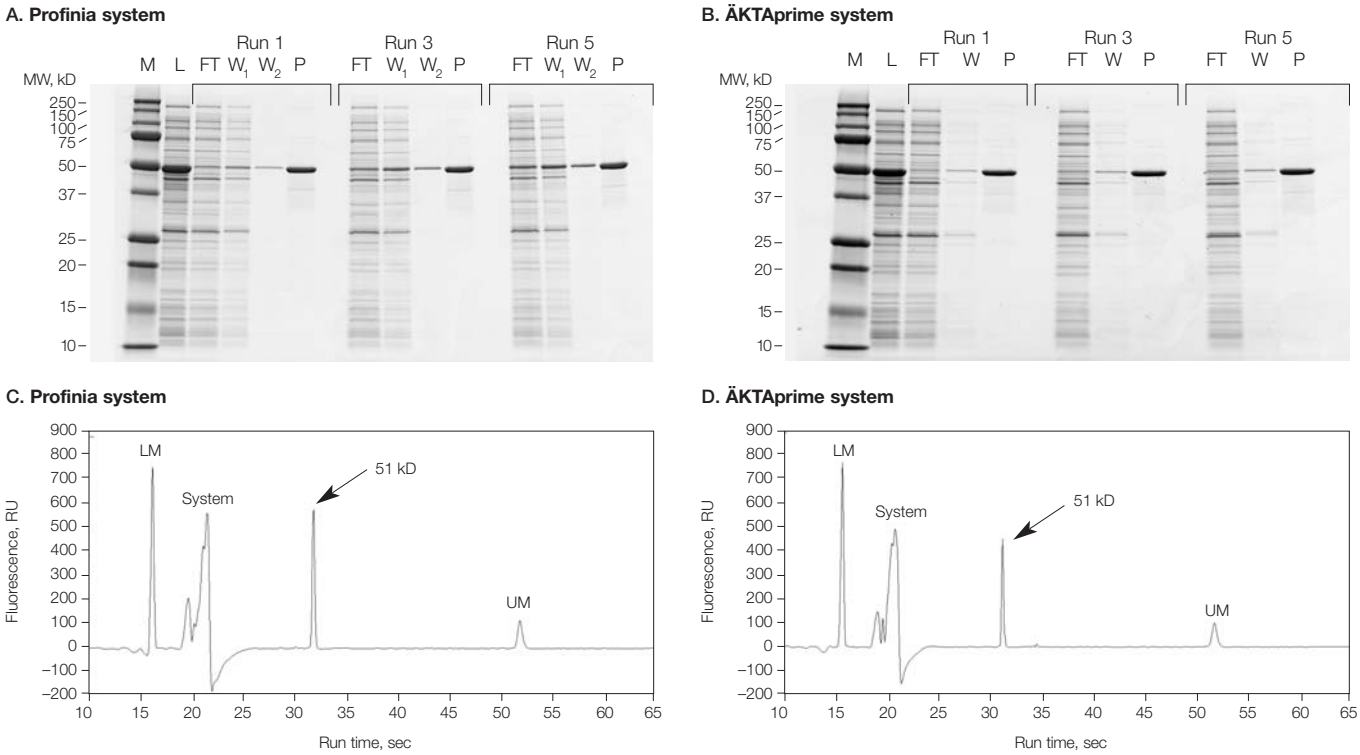

Fig. 3. SDS-PAGE and Experion analyses of purification fractions. Samples were separated by SDS-PAGE using 8–16% Tris-HCl Criterion precast gels or with the Experion Pro260 analysis kit. **A** and **B**, SDS-PAGE analysis of samples from the Profinia and ÄKTAprime separations, respectively. Lane designations are as follows: M, markers (Precision Plus Protein™ dual color standards); L, lysate (unfractionated); FT, flow-through fraction; W, wash fraction; P, purified protein. **C** and **D**, representative Experion electropherograms of separations of the purified 51 kD protein obtained with the Profinia and ÄKTAprime systems, respectively. LM, lower marker; UM, upper marker; System, system peaks.

The Profinia system is fully automated and requires no user handling or intervention between application of sample, affinity purification, and desalting of the purified protein. Moreover, the Profinia IMAC purification reagents, including solutions and cartridges, require no advanced preparation, while the ÄKTAprime HisTrap reagent kits provide buffer concentrates that must be formulated and diluted by the user.

While protein yields and purities generated by both systems were nearly equivalent, the Profinia system was superior in terms of the total time required for purification. The Profinia system saved over 1 hr from each affinity purification, which would result in 5 hr of savings over the course of five repetitive runs. Such a time savings would be advantageous in cases where multiple purifications must be performed in a single day.

References

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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. The ÄKTAprime plus system, a comparable low-pressure system with a more extensive selection of preprogrammed methods and cue cards, was not available at the time this study was conducted.

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