Automated Purification of a GST-Tagged Protein With the Profinia™ Protein Purification System: Comparison to Manual Protein Purification Using Commercial Kits

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Introduction

Fusion tags are widely used to assist in the purification of recombinant protein expressed from a gene of interest. Among the various fusion tags that have been developed, glutathione S-transferase (GST) is one of the most commonly used (Smith and Johnson 1988). Protein fused to GST can be purified from crude bacterial lysates under nondenaturing conditions by affinity chromatography on an immobilized glutathione column. The lysate is applied to the column, the GST-tagged protein binds to the immobilized glutathione, and unbound host proteins are collected from the column in the flow-through fraction. The column is then washed with several column volumes of buffer to ensure complete removal of untagged proteins. Purified protein is eluted and recovered in buffer containing reduced glutathione, which displaces the GST-tagged protein from the immobilized glutathione. This widely performed procedure is often performed under gravity-flow conditions, in which sample and buffers are applied onto an open column and fractions are collected manually.

The Profinia protein purification system is an automated liquid chromatography system designed to perform unattended affinity purification and desalting of tagged proteins. Optimized preprogrammed methods for the most common affinity applications are available and can be used together with the buffers and prepacked columns available in the Profinia purification kits. All of the parameters for routine purifications are preset, and the preplumbed system is easily maintained through automated self-cleaning protocols. Integrated collection of the flow-through, column washes, and elution fractions allows determination of the effectiveness of purification. Automated desalting of the purified protein by size exclusion chromatography is also possible without the need for user intervention between the affinity and desalting chromatography steps. When used with Profinia software, the system displays purification data in real time and allows generation and printing of publication-quality reports that include chromatograms, method steps, and data tables containing pertinent purification information, including yield, concentration, and sample application and elution volumes (Figure 1).

This study compares the performance and reproducibility of the Profinia system to traditional manual gravity-flow column purification for GST affinity purification and sequential desalting. The B-PER GST fusion protein purification kit from Pierce Biotechnology was chosen for comparison, as the column volume (1 ml) is identical to the column volume used by kits available for the Profinia protein purification system. For the comparison, identical application and elution volumes were used for the columns tested between the two systems, further allowing a valid comparison. Desalting of the gravity-flow column-purified protein requires manual application of the protein eluted from the affinity column to a second column for desalting, and this step was performed using Zeba desalt spin columns from Pierce Biotechnology. These are available with a column volume identical to the desalting column used in the Profinia system (10 ml). The two purification methods — automated and manual — were compared with respect to yield, reproducibility, electrophoretic purity, total time required, and hands-on time required.

Methods

A 51 kD GST-tagged protein expressed in *Escherichia coli* was purified using both a Profinia system method and a manual gravity-flow method for purification and desalting. Five purification runs were performed, and samples of the lysate, 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 51 kD protein. Purity of the 51 kD protein was evaluated using the Experion™ automated electrophoresis system.

Protein Purification With the Profinia System

All purification reagents, columns, and a lyophilized *E. coli* lysate containing the 51 kD GST-tagged protein were obtained from the Profinia GST starter kit (catalog #620-0230). Each vial of lysate was resuspended as directed in the kit instructions in 10 ml of Profinia lysis buffer. Purifications were performed with the Profinia method template for "GST + Desalting" and with the "low" sample flow rate. The 1 ml GST cartridge and 10 ml desalting cartridge provided in the GST starter kit were used. For each purification run, 5 ml of sample was loaded, which required placement of 6 ml of lysate in the sample tube to ensure complete loading of 5 ml of lysate. From each purification run, 4 ml of purified and desalted protein was collected. The five replicate runs were performed consecutively using the same columns. Profinia software was used for real-time monitoring of the purification runs and collection of the subsequent run data.

Protein Purification With the Pierce GST Fusion Protein Purification Kit and Zeba Desalt Spin Columns

Each vial of *E. coli* lysate containing the 51 kD GST-tagged protein from the Profinia GST starter kit was dissolved in 10 ml of the B-PER bacterial protein extraction reagent provided in the B-PER GST fusion protein purification kit (Pierce Biotechnology). Purifications were carried out according to the supplied instructions using an application volume of 5 ml and an elution volume of 4 ml. Eluted protein from each GST purification was desalted using 10 ml Zeba desalt spin columns (Pierce Biotechnology) following the manufacturer's instructions. Five purification runs were performed using new columns for each replicate.

SDS-PAGE and Experion Pro260 Analysis

SDS-PAGE analysis 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 10-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.

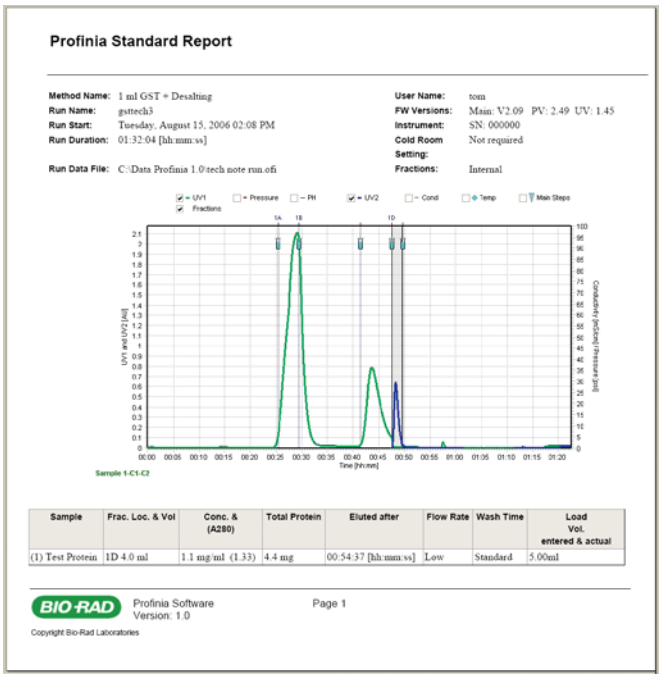

Fig. 1. Example of a Profinia standard report. Profinia software prepares a report containing chromatograms, continuous records of various method parameters, protein yield, sample application and elution volumes, and purification conditions.

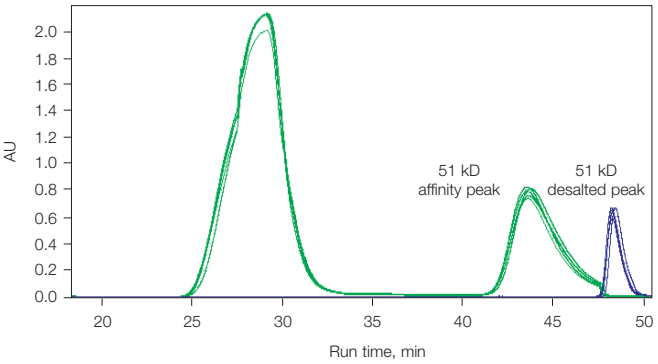

Fig. 2. Superimposed UV absorbance profiles from replicate GST + Desalting purifications of the 51 kD protein on the Profinia system. The UV absorbance of samples at 280 nm (AU) was collected over time for five consecutive purifications, and the data are shown on the same pair of axes. ( ), absorbance at the output of the affinity column; ( ), absorbance at the output of the desalting column.

Results and Discussion

UV absorbance data from the Profinia purifications demonstrate the performance of the automated GST + Desalting method (Figure 2). The profiles of the UV traces at the output of either column were virtually indistinguishable from one another when compared using Profinia software. The elution times for both the affinity and desalting peaks varied by no more than 10 sec.

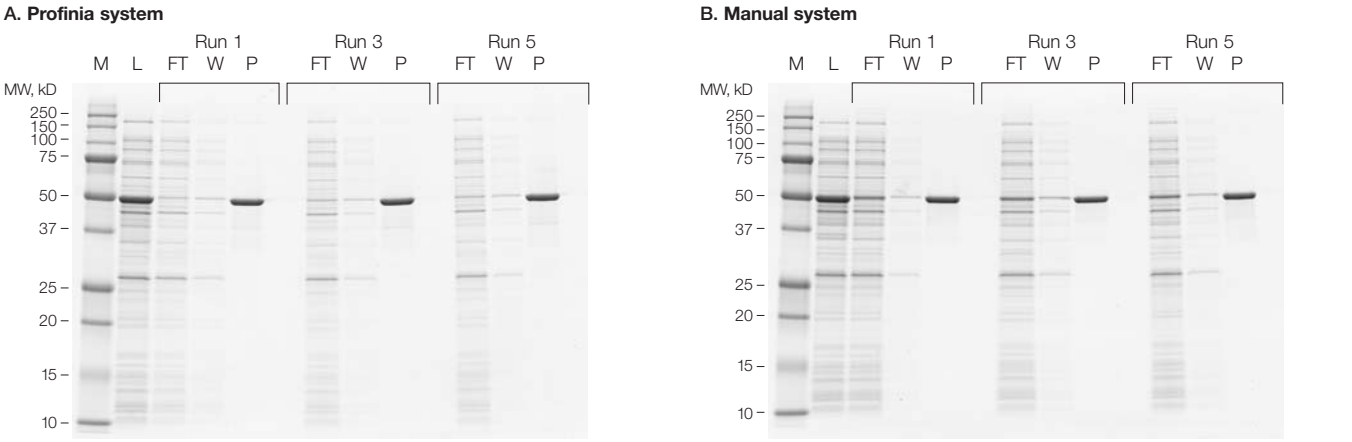

Fig. 3. SDS-PAGE analysis of purification fractions. Samples were separated by SDS-PAGE on 8–16% Tris-HCl Criterion precast gels, and gels were stained with Bio-Safe Coomassie stain. **A**, samples from the Profinia separations; **B**, samples from the manual separations. Lane designations are as follows: M, markers (Precision Plus Protein™ dual color standards); L, lysate (unfractionated); FT, flow-through fraction; W, wash fraction; P, purified protein.

Both the automated and manual methods compared in this study are capable of purifying the 51 kD protein to apparent homogeneity, as determined by SDS-PAGE and Coomassie Blue staining (Figure 3). Both the Bio-Scale™ Mini GST cartridges used with the Profinia system and the gravity-flow affinity columns used with the manual method bound most of the tagged fusion protein applied, as judged by comparing the profiles of the flow-through fractions to those of the unfractionated lysate. The protein profile in the flow-through fraction and the purity of the protein product were consistent from run to run with both methods tested (Figure 3).

Though both systems delivered 51 kD GST-tagged protein of comparable purity, the Profinia system yielded slightly more purified protein with less variability in yield than did the manual system (Table 1). This increased reproducibility may be due to the fact that the Profinia system reduces sample handling.

Table 1. Yield and purity of the 51 kD protein. Protein was purified and desalted either with the Profinia system and GST starter kit, or manually with the B-PER GST fusion protein purification kit and Zeba desalt spin columns. Average yield of the five runs performed using each method is shown ± standard deviation.

Purification Method	Average Yield (mg)	Average Purity (%)
Manual system	4.34 ± 0.24	96.1
Profinia system	4.76 ± 0.14	96.2

Both purification methods required approximately 90 min to complete. With the manual method, purified and desalted protein was available in 90 min. However, the purified protein from the Profinia system was available and ready for use in downstream applications within 55 min for all five purifications. The remaining time required for the Profinia system is accounted for by the automated cycle for column cleaning and regeneration, a step not carried out using the manual method. The manual method required continuous user presence in order to apply buffer and collect fractions, while the Profinia system ran unattended and only required approximately 5 min of hands-on time to initiate and complete the purification. The Profinia system automatically collected the sample from the affinity column and applied it to the desalting column. This must be performed manually in the gravity-flow method in a step that also requires the use of a centrifuge. In contrast to the Profinia system, the length of time using the gravity-based purification varied by as much as 30 min due to flow rate differences between the columns used in the manual method.

Conclusions

The Profinia protein purification system has been tested against a manual gravity-flow purification system for purification of a GST-tagged protein. The manual system used in this comparison matched the Profinia system in application volume, column volumes, and elution volume.

The Profinia system is fully automated and requires no user handling between application of the sample and conclusion of both the affinity purification and desalting steps. While reduced sample handling and hands-on time are the principal benefits of the Profinia system over manual purification, the Profinia system appeared superior when compared to the manual method tested in terms of protein yield and reproducibility, and equivalent in terms of protein purity.

A key feature of the Profinia system is automated collection of purification data, an option not available with the manual system, and this simplifies evaluation of the effectiveness of purification. In addition, a report is prepared that contains chromatograms, continuous records of various method parameters, yield, volumes, and purification conditions (Figure 1).

Reference

Smith DB and Johnson KS, Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione S-transferase, Gene 67, 31–40 (1988)

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