

AFFINITY PURIFICATION SYSTEMS

Bio-Scale™ Mini Profinity™ Cartridges

- Prepacked affinity chromatography cartridges for reproducible results
- Equivalent or better yield and purity compared to other prepacked columns
- Optimized buffer kits
- Integrated cleaning and maintenance
- Ease of use

Comparison of Bio-Scale Mini Profinity Cartridges to Other Affinity Columns on the Profinia™ Protein Purification System

Introduction

Affinity-tagged purification of recombinant proteins is a common procedure in many laboratories. Two commonly used affinity tags are glutathione-S-transferase (GST) and polyhistidine (His). The Profinia protein purification system simplifies the process by automating the purification and desalting of these tagged proteins in a single step.

The Profinia system features:

- Preprogrammed, optimized methods for the most common affinity purifications
- Convenient, prepackaged chromatography buffers and cartridges
- Large, user-friendly touch-screen interface for selecting or customizing methods for specific applications
- Automatic instrument and cartridge cleaning routines, performed after purifications to ensure optimal performance
- Ability to produce similar quality target protein by multiple users

Bio-Scale Mini Profinity cartridges are designed for use on the Profinia system and offer a number of advantages, including ease of use and reproducibility. Here we compare the performance and reproducibility of other commercially available affinity columns to Bio-Scale Mini Profinity cartridges when used on the Profinia protein purification system.

Methods

Protein purifications were carried out at room temperature using the Profinia protein purification system. His- and GST-tagged proteins of seven different molecular weights were purified using 1 ml Bio-Scale Mini Profinity GST and IMAC cartridges. Protein purifications were also performed using 1 ml HisTrap FF, GSTrap FF, HisTrap HP, and GSTrap HP columns (GE Healthcare) under identical chromatography conditions. Purified proteins were analyzed by SDS-PAGE and the Experion™ automated electrophoresis system.

The Profinia GST purification kit was used to purify the GST-tagged proteins. This kit contains all the necessary buffers and reagents to perform purifications on the Profinia system, including binding, washing, and elution buffers. Frozen bacterial cell pellets containing the target proteins C1bβII (36 kD), aldolase (65 kD), and 101Thr (101 kD) were resuspended in 1x GST lysis buffer (1:10 w/v) and lysed by sonication. Lysates were clarified by centrifugation and filtered through a 0.22 μm filter prior to GST purification. Identical volumes of sample were purified using Bio-Scale Mini Profinity GST cartridges and GSTrap FF and GSTrap HP columns. Yield of purified proteins was determined by measuring the absorbance at 280 nm (A_{280}) using a spectrophotometer. Each purification was carried out in triplicate using the preprogrammed GST affinity-only method on the Profinia system.

BIO-RAD

Chromatograms

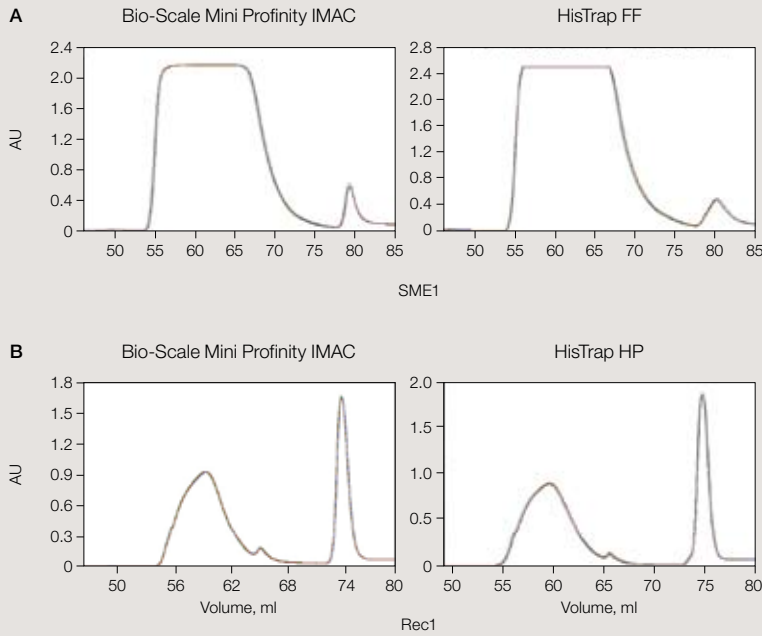


Fig. 1. Comparison of native IMAC purifications using the Profinia system. Chromatograms indicate similar yields when using Bio-Scale Mini Profinity IMAC cartridges compared to HisTrap FF and HP columns. **A**, purification of SME1 protein (11 kD). Overlaid traces show three purifications for each cartridge type; **B**, purification of Rec1 protein (75 kD). Overlaid traces show three purifications for each cartridge type.

SDS-PAGE

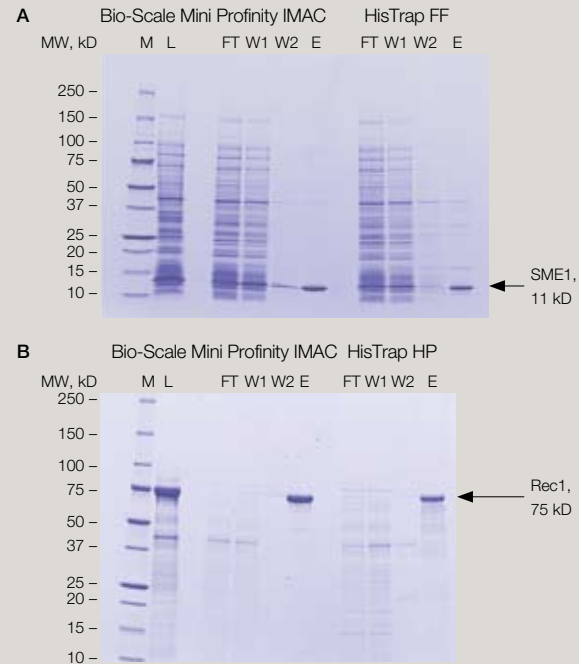


Fig. 2. SDS-PAGE analysis of collected fractions from IMAC purifications. **A**, SME1 protein purification using Bio-Scale Mini Profinity IMAC cartridges and HisTrap FF columns; **B**, Rec1 protein purification using Bio-Scale Mini IMAC cartridges and HisTrap HP columns. M, Precision Plus Protein™ unstained standards; L, lysate; FT, flowthrough; W1, wash 1; W2, wash 2; E, elution.

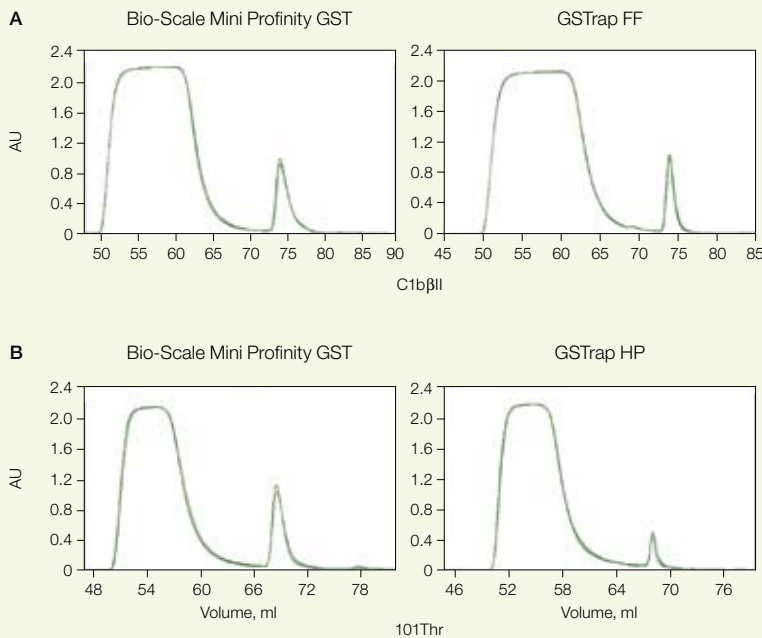


Fig. 5. Comparison of GST purifications using the Profinia system. Chromatograms indicate an increase in yield when using Bio-Scale Mini Profinity GST cartridges compared to GSTrap FF and HP columns. **A**, purification of C1bβII protein (36 kD). Overlaid traces show three purifications for each cartridge type; **B**, purification of 101Thr protein (101 kD). Overlaid traces show three purifications for each cartridge type.

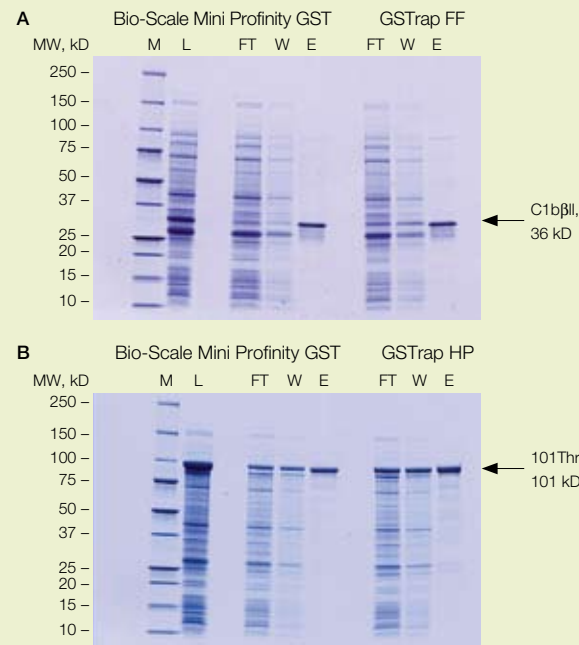


Fig. 6. SDS-PAGE analysis of collected fractions from GST purifications. **A**, C1bβII protein purification using Bio-Scale Mini Profinity GST cartridges and GSTrap FF columns; **B**, 101Thr protein purification using Bio-Scale Mini Profinity GST cartridges and GSTrap HP columns. M, Precision Plus Protein unstained standards; L, lysate; FT, flowthrough; W, wash; E, elution.

Experion Analysis

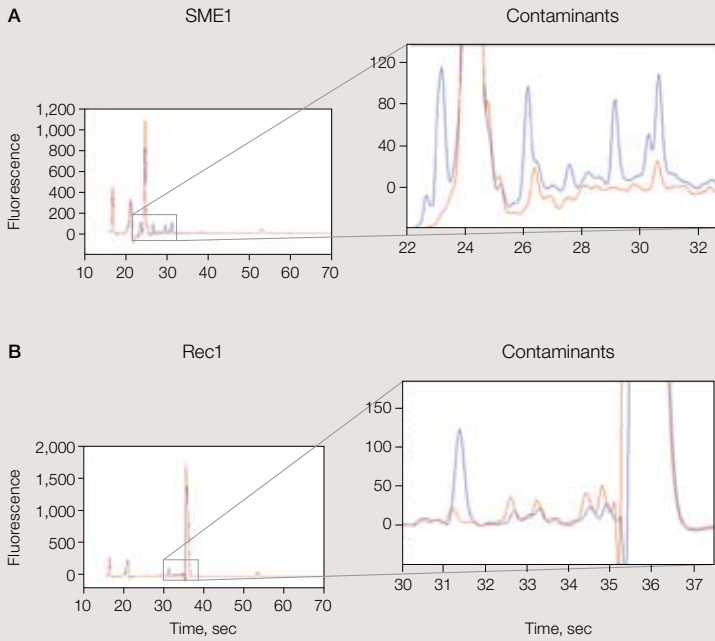


Fig. 3. Electropherograms of purified protein fractions from Bio-Scale Mini Profinity IMAC cartridges (■) and HisTrap columns (■) analyzed on the Experion electrophoresis system. Bio-Scale Mini Profinity IMAC cartridges provide higher purity than HisTrap columns when used on the Profina system. **A**, purity analysis of SME1 protein (11 kD) on a Bio-Scale Mini Profinity cartridge compared to a HisTrap FF column; **B**, purity analysis of Rec1 protein (75 kD) on a Bio-Scale Mini Profinity cartridge compared to a HisTrap HP column.

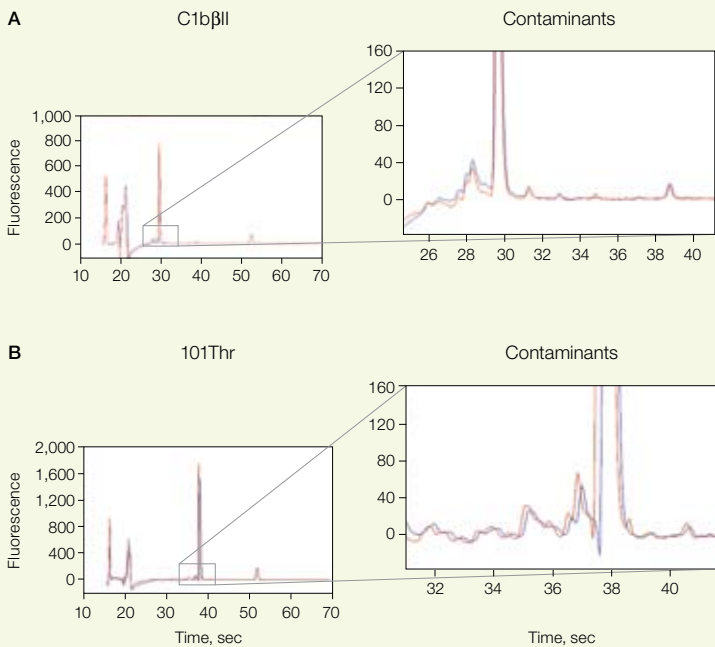


Fig. 7. Electropherograms of purified protein fractions from Bio-Scale Mini Profinity GST cartridges (■) and GSTrap columns (■) analyzed on the Experion electrophoresis system. Bio-Scale Mini Profinity GST cartridges provide similar purity as the GSTrap columns when used on the Profina system. **A**, purity analysis of C1bβII protein (36 kD) on a Bio-Scale Mini Profinity cartridge compared to a GSTrap FF column; **B**, purity analysis of 101Thr protein (101 kD) on a Bio-Scale Mini Profinity cartridge compared to a GSTrap HP column.

Profina Cartridge Comparison

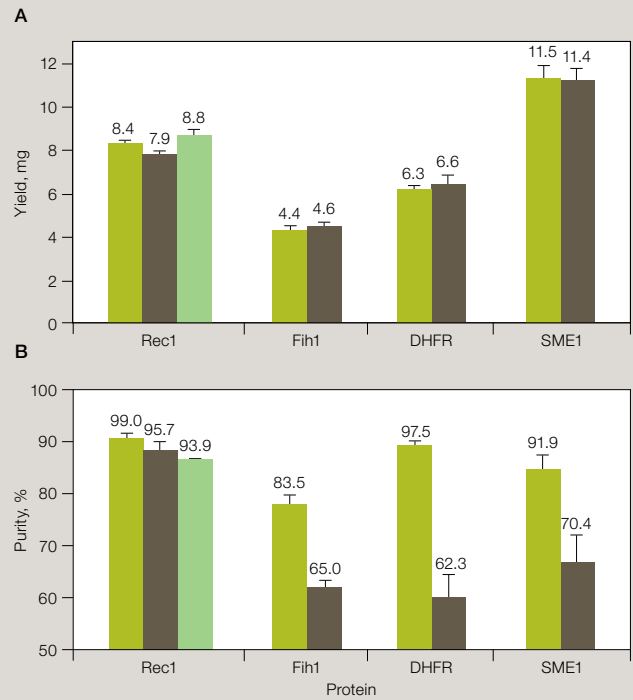


Fig. 4. Yield (A) and purity (B) of various proteins purified using either Bio-Scale Mini Profinity IMAC cartridges (■) or commercially available prepacked columns HisTrap FF (■) or HisTrap HP (■) on the Profina system. Rec1, 75 kD; Fih1, 37 kD; DHFR, 25 kD; SME1, 11 kD.

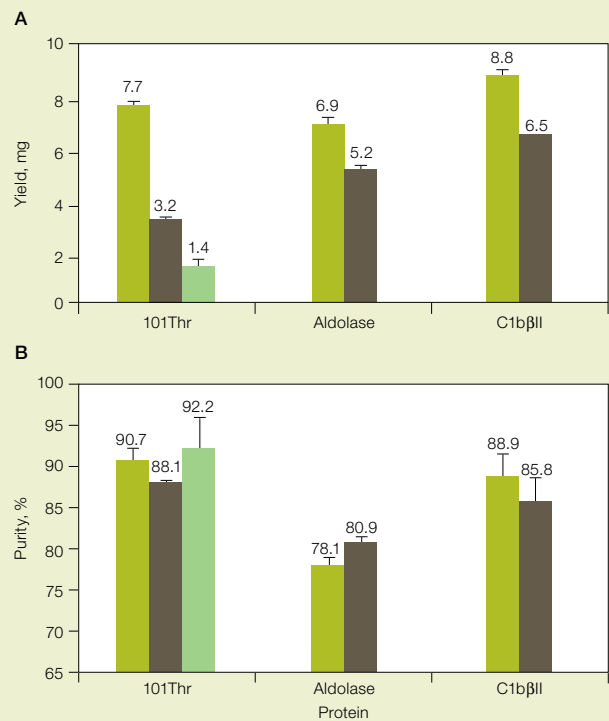


Fig. 8. Yield (A) and purity (B) of various proteins purified using either Bio-Scale Mini Profinity GST cartridges (■) or commercially available prepacked columns GSTrap FF (■) or GSTrap HP (■) on the Profina system. 101Thr, 101 kD; aldolase, 65 kD; C1bβII, 36 kD.

Native IMAC purifications were performed using the Profinia native IMAC buffer kit, which includes all necessary buffers and reagents for purification. Native *E. coli* SME1 (11 kD), DHFR (25 kD), and Rec1 (75 kD) proteins were produced using the Profinia bacterial lysis/extraction reagent. Lysates were centrifuged and filtered through a 0.22 µm filter before being loaded into the Profinia instrument. The preprogrammed native IMAC affinity-only method was used to purify proteins on Bio-Scale Mini Profinity IMAC cartridges and HisTrap FF and HisTrap HP columns. Denatured *E. coli* lysate, Fih1 (37 kD), was produced by sonication of the cell paste in Profinia denaturing IMAC lysis buffer containing 6 M urea. The lysate was centrifuged, filtered, loaded into the Profinia instrument, and purified using Bio-Scale Mini Profinity IMAC cartridges or HisTrap FF columns and the preprogrammed denaturing IMAC method. Each protein was purified in triplicate under the same preprogrammed method and operating conditions. The yield from each purification was determined by measuring A_{280} of the eluted product using a spectrophotometer.

Elution fractions containing purified proteins in GST, native IMAC, and denaturing IMAC elution buffers from triplicate purification runs of each protein were prepared and analyzed using the Experion Pro260 analysis kit according to instructions provided in the kit. Purity (% of lane total area) was determined automatically by Experion software.

Compared to commercially available columns, purifications performed with Bio-Scale Mini Profinity IMAC cartridges resulted in proteins with higher purity and comparable yield (Figures 1–4). With GST-tagged proteins of various sizes, there was a marked increase in binding capacity (1.3- to 2.3-fold) when using Bio-Scale Mini Profinity GST cartridges compared with using GSTrap FF and GSTrap HP columns, without sacrificing target protein purity (Figures 5–8).

Conclusions

Bio-Scale Mini Profinity cartridges, when used on the Profinia system, provide an automated solution for affinity protein purification. Not only does this system offer prepacked affinity cartridges, but it also contains preprogrammed optimized methods, resulting in increased efficiency and production in the laboratory. The Bio-Scale Mini Profinity cartridges provide comparable, or better, yield and purity to other commercially available prepacked affinity columns.

Ordering Information

| Catalog # | Description |
|--|---|
| Bio-Scale Mini Profinity Cartridges | |
| 732-4610 | Bio-Scale Mini Profinity IMAC Cartridges, 5 x 1 ml |
| 732-4612 | Bio-Scale Mini Profinity IMAC Cartridge, 1 x 5 ml |
| 732-4614 | Bio-Scale Mini Profinity IMAC Cartridges, 5 x 5 ml |
| 732-4620 | Bio-Scale Mini Profinity GST Cartridges, 5 x 1 ml |
| 732-4622 | Bio-Scale Mini Profinity GST Cartridge, 1 x 5 ml |
| 732-4624 | Bio-Scale Mini Profinity GST Cartridges, 5 x 5 ml |
| Profinia Purification Kits | |
| 620-0225 | Profinia Native IMAC Purification Kit, 1 ml, includes Profinia native IMAC buffer kit, 2 x 1 ml IMAC and 2 x 10 ml desalting cartridges |
| 620-0226 | Profinia GST Purification Kit, 1 ml, includes Profinia GST buffer kit, 2 x 1 ml GST and 2 x 10 ml desalting cartridges |
| 620-0227 | Profinia Denaturing IMAC Purification Kit, 1 ml, includes Profinia denaturing IMAC buffer kit, 2 x 1 ml IMAC cartridges |
| 620-0228 | Profinia Desalting Purification Kit, 10 ml, includes Profinia desalting buffer kit, 2 x 10 ml desalting cartridges |
| 620-0235 | Profinia Native IMAC Purification Kit, 5 ml, includes 2 Profinia native IMAC buffer kits, 1 x 5 ml IMAC and 1 x 50 ml desalting cartridge |
| 620-0236 | Profinia GST Purification Kit, 5 ml, includes 2 Profinia GST buffer kits, 1 x 5 ml GST and 1 x 50 ml desalting cartridge |
| 620-0220 | Profinia Bacterial Lysis/Extraction Reagent |
| Profinia Instruments and Systems | |
| 620-1005 | Profinia Instrument With Accessory Kit and Native IMAC Starter Kit, 100–240 V |
| 620-1006 | Profinia Instrument With Accessory Kit and GST Starter Kit, 100–240 V |
| 620-1010 | Profinia Protein Purification System With Native IMAC Starter Kit, 100–240 V, with Profinia software |
| 620-1011 | Profinia Protein Purification System With GST Starter Kit, 100–240 V, with Profinia software |
| 620-1015 | Profinia Protein Purification System With Computer and Native IMAC Starter Kit, 100–240 V |
| 620-1016 | Profinia Protein Purification System With Computer and GST Starter Kit, 100–240 V |

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