

AFFINITY PURIFICATION

Profinity eXact™ Purification Methods on the Profinia™ System

- Preprogrammed methods to automate Profinity eXact fusion-tag purification
- On-column cleavage and immediate desalting for ready-to-use, tag-free proteins
- Compatibility with 1 or 5 ml Bio-Scale™ Mini Profinity eXact cartridges
- Automated, integrated cartridge cleaning and regeneration
- Purified tag-free proteins for applications where native proteins are preferred

The Profinity eXact fusion-tag system is a novel protein expression and purification system that addresses the key bottleneck of recombinant tag technology. The Profinity eXact system improves the efficiency of tag removal and generates a highly purified protein containing only its native amino acid sequences — all with one simple elution step.

Protein purification with the Profinity eXact fusion-tag system is even more powerful when combined with the Profinia protein purification system (Figure 1). The Profinia system automates

the purification steps, including on-column cleavage of the tag and/or desalting after affinity purification, using the preprogrammed Profinity eXact purification methods. Figure 2 shows the purification of interferon- α 2a (IFN- α 2a) from *E. coli* periplasm after the protein was expressed as a secreted Profinity eXact fusion-tagged protein. The purification, cleavage, and desalting process were completed in 70 minutes of unattended running time, resulting in highly purified, tag-free protein that is ready to use in downstream assays.

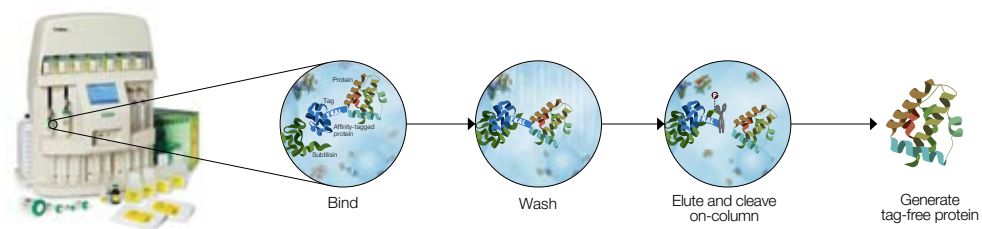


Fig. 1. Affinity purification of proteins using the Profinity eXact purification methods on the Profinia system.

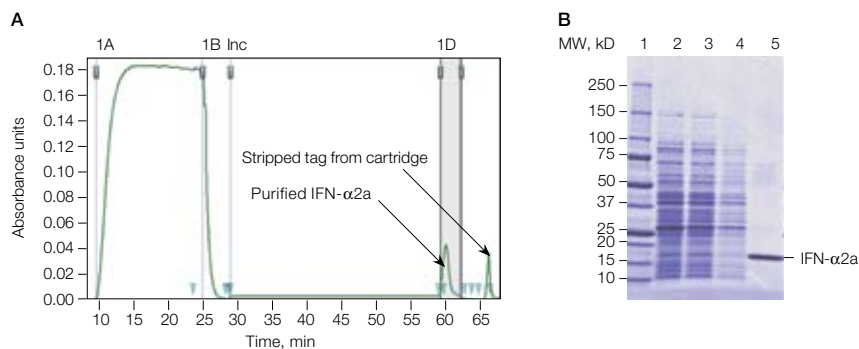


Fig. 2. Purification of IFN- α 2a. The Profinity eXact tag-IFN- α 2a fusion gene was cloned downstream of the coding region of the OmpA signal sequence, and the fusion protein was expressed and secreted into the periplasm of *E. coli* BL21(DE3) cells. The 18 ml periplasmic lysate containing Profinity eXact fusion-tagged IFN- α 2a was loaded on the Profinia system and purified with a 1 ml Bio-Scale Mini Profinity eXact cartridge, using the Profinity eXact purification method. **A**, chromatogram showing the different chromatography fractions; 1A, flowthrough; 1B, wash; Inc, incubation step; 1D, purified IFN- α 2a; **B**, SDS-PAGE analysis of chromatography fractions shown in panel A. Lane 1, Precision Plus Protein™ standards; lane 2, load; lane 3, flowthrough (1A); lane 4, wash (1B); lane 5, elution (1D). The incubation time for cleavage was 30 min. Data kindly provided by Natalia Oganesyana and Philip Bryan from Potomac Affinity Proteins, LLC.

BIO-RAD

Many proteins have been successfully expressed and purified with the Profinity eXact fusion-tag system (see Table 1). Different lysate buffers and detergents commonly used in affinity purifications can be applied in the purification process (see Profinity eXact fusion-tag system instruction manual). Use of Profinity eXact fusion-tag technology on the Profinia system offers an easy way to automate the purification of proteins,

especially in situations where native proteins are preferred for downstream applications, such as in structural biology, and for in vitro reconstitution of protein complexes.

For more information on the Profinity eXact fusion-tag system, see bulletins 5652 and 5626. For more information on the Profinia protein purification system, see bulletin 5548.

Table 1. Proteins purified with the Profinity eXact fusion-tag system.

Protein	Source	Molecular Mass, kD	Purity [†] , %
β-galactosidase	Bacteria	116	88
iTaq™ DNA polymerase	Bacteria	94	87
Poly ADP-ribose polymerase	Human	83	99
YGR211W ^{**}	Yeast	55	91
51 kD protein	Bacteria	51	88
Apiose synthase	Plant	44	92
MBP	Bacteria	40	99
Chimeric G-alpha protein subunits ^{***}	Bovine	40	95
YGL221C [†]	Yeast	32	79
YDR428 ^{††}	Yeast	30	81
Green Fluorescent Protein	Jellyfish	25	97
25 kD protein	Bacteria	25	99
Dihydrofolate reductase	Mouse	22	95
IL-1β	Human	17	99
GA (HSA-binding domain)	<i>Streptococcus</i> group G	6	95

[†] Determined by SDS-PAGE. ^{**} gb/EDN61799.1. ^{***} Courtesy of Kevin Ridge, University of Texas Health Science Center, Houston, Texas.

[†] gb/AA193235.1. ^{††} AAB64885.1.

Ordering Information

Catalog # Description

Profinia Systems

620-1010 ProfiniaProtein Purification System With Native IMAC Starter Kit

620-1011 ProfiniaProtein Purification System With GST Starter Kit

Profinia Instruments

620-1005 ProfinialInstrument With Accessory Kit and Native IMAC Starter Kit

620-1006 ProfinialInstrument With Accessory Kit and GST Starter Kit

Profinia Consumables

620-0216 5x Profinia Desalting Buffer, 200 ml

620-0217 2x Profinia Cleaning Solution 1, 125 ml

732-4646 Bio-Scale Mini Profinity eXact Cartridges, 2 x 1 ml

732-4647 Bio-Scale Mini Profinity eXact Cartridges, 4 x 1 ml

732-4648 Bio-Scale Mini Profinity eXact Cartridge, 1 x 5 ml

732-5304 Bio-Scale Mini Bio-Gel® P-6 Desalting Cartridges, 5 x 10 ml

732-5312 Bio-Scale Mini Bio-Gel P-6 Desalting Cartridge, 1 x 50 ml

732-5314 Bio-Scale Mini Bio-Gel P-6 Desalting Cartridges, 5 x 50 ml

156-3001 Profinity eXact pPAL RIC-Ready Expression Vector Kit

156-3002 Profinity eXact pPAL Supercoiled Expression Vector Kit

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