

AFFINITY PURIFICATION

Profinia™ Buffers for Profinity eXact™ Chromatography

- Preprogrammed methods for tag-free affinity purification
- Integrated desalting for buffer exchange after elution
- High reproducibility
- Built-in incubation and tag-cleavage steps
- Large interface screen to guide purification process with minimal training
- 1 ml or 5 ml Bio-Scale Mini Profinity eXact cartridges for a wide range of sample volumes

The Profinia protein purification system has preprogrammed purification methods specific for use with the Profinity eXact fusion-tag technology. These methods combine automated chromatography with Bio-Scale™ Mini Profinity eXact cartridges, making it easy to generate milligram quantities of recombinant, tag-free proteins within as little as one hour.

Users can customize the methods and buffers on the Profinia system according to individual needs, but specific buffers for achieving optimal purification results are recommended in Table 1 and typical results are shown in Figure 1.

Table 1. Recommended buffers and solutions for Profinity eXact purification methods.

Buffer and Solution*	Composition	Buffer Port
Bind/equilibration/wash buffer	100 mM sodium phosphate, pH 7.2	B1
Wash buffer 2 (optional)**	100 mM sodium phosphate, 300 mM sodium acetate, pH 7.2	B2
Elution buffer (recommended)	100 mM sodium phosphate, 100 mM sodium fluoride, pH 7.2	B3
Elution buffer (optional)	100 mM sodium phosphate, 10 mM sodium azide, pH 7.2	
Desalting buffer (PBS)***	137 mM NaCl, 2.7 mM KCl, 4.3 mM Na ₂ HPO ₄ , 8.1 mM KH ₂ PO ₄ , pH 7.4	B4
Cleaning solution***	500 mM NaCl, 50 mM Tris, pH 8.0	B5
Regeneration solution	100 mM H ₃ PO ₄	B6
Storage solution	100 mM sodium phosphate, 0.02% sodium azide, pH 7.2	B7
20% ethanol (instrument storage solution)	20% ethanol (v/v)	B8

* 250 ml of buffer is sufficient for single sample methods; sample should be diluted with bind/wash buffer; minimum sample volumes of 10 ml are recommended. ** Methods include an optional second wash step with sodium acetate (0.3–3.0 M) or binding buffer containing compatible additives. *** 5x Profinia desalting buffer (catalog #620-0216) and 2x Profinia cleaning solution 1 (catalog #620-0217) are available individually or as part of the Profinia desalting purification kit (catalog #620-0228) and should be diluted to 1x for use with Profinity eXact purification methods.

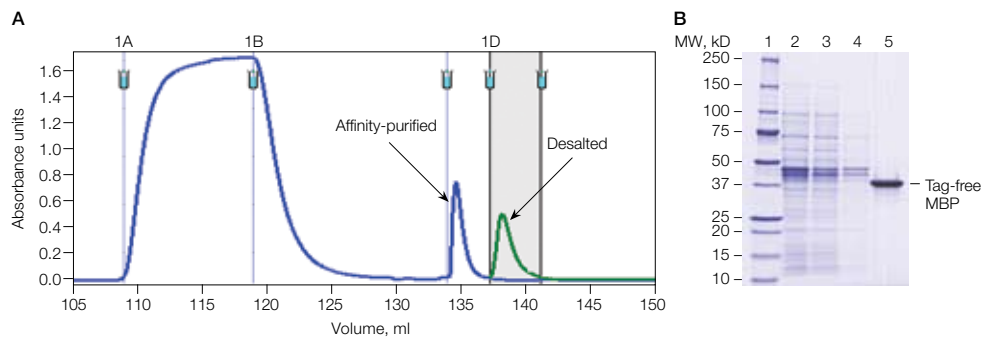


Fig. 1. Purification of maltose-binding protein (MBP) using the Profinity eXact plus desalting method on the Profinia system. Profinity eXact fusion-tagged MBP was purified from 10 ml *E. coli* lysate using a 1 ml Bio-Scale Mini Profinity eXact cartridge and a 10 ml Bio-Scale™ Mini Bio-Gel® P-6 desalting cartridge with recommended buffers. **A**, chromatogram showing different fractions; 1A, flowthrough; 1B, wash; 1D, affinity-purified plus desalted MBP; **B**, SDS-PAGE of chromatography fractions shown in panel A. Lane 1, Precision Plus Protein™ standards; lane 2, lysate (load); lane 3, flowthrough (1A), lane 4, wash (1B); lane 5, eluted tag-free MBP (1D).

BIO-RAD

The recommended buffers for preprogrammed “Bio-Rad methods” on the Profinia system are all 1x working solutions. However, concentrated buffers can also be used in “Program methods” mode.

After sample loading and wash, tag-free protein is generated by triggering a specific cleavage reaction to remove the Profinity eXact tag. Several options for triggering anions are available (Table 2). Triggering anions should be avoided in buffers used in crude protein lysate preparations and in the binding and wash steps.

Table 2. Triggering anions to activate tag cleavage activity.

Anion	Compound	Cleavage	
		Fast	Moderate
F ⁻	Na, KF	100 mM	5 mM
N ₃ ⁻	NaN ₃	10 mM	1 mM
NO ₂ ⁻	NaNO ₂	5 mM	1 mM
HCO ₂ ⁻	NaHCO ₃	1,000 mM	25 mM
Cl ⁻	NaCl, KCl	>1,000 mM	75 mM

Many buffers, detergents, and protease inhibitors have been tested and shown to be compatible with the Profinity eXact fusion-tag system. For detailed information, please refer to the Profinity eXact fusion-tag system instruction manual.

With preprogrammed methods, users can choose from different incubation times (0.5, 2, or 15 hr) for cleavage, or incubation times can be customized. Using the Profinity eXact purification plus desalting method, the tag-free protein can be eluted and desalted (buffer exchanged) in a single purification step.

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.

Ordering Information

Catalog # Description

Profinia Instruments

620-1005	Profinia Instrument With Accessory Kit and Native IMAC Starter Kit
620-1006	Profinia Instrument With Accessory Kit and GST Starter Kit

Profinia Systems

620-1010	Profinia Protein Purification System With Native IMAC Starter Kit
620-1011	Profinia Protein Purification System With GST Starter Kit

Profinia Consumables

620-0216	5x Profinia Desalting Buffer, 200 ml
620-0217	2x Profinia Cleaning Solution 1, 125 ml
620-0228	Profinia Desalting Purification Kit, 10 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 Cartridges, 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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