

AFFINITY PURIFICATION

Profinia™ Buffers for Maltose Binding Protein (MBP) Chromatography

- Automated one-step affinity purification and tandem desalting for MBP-tagged proteins
- Compatibility with 1 ml and 5 ml affinity cartridges for MBP-tagged protein purification
- High reproducibility
- Easy programming with predesigned purification templates
- Large interface screen to guide purification process with minimal training

The Profinia protein purification system offers automated preprogrammed methods to purify affinity-tagged proteins and antibodies. Specific recommendations and steps for

purification of MBP-fused proteins using “Program Methods” on the Profinia system are described here in Tables 1 and 2. Typical results are shown in Figure 1.

Table 1. Recommended buffers and solutions for MBP-tagged protein purification using the Profinia affinity plus desalting method in Program methods mode.

Buffers and Solutions*	Composition	Buffer Port
2x Bind/wash buffer	40 mM Tris-HCl, 400 mM NaCl, 2 mM EDTA, pH 7.4	B1
2x Elution buffer	40 mM Tris-HCl, 400 mM NaCl, 2 mM EDTA, 20 mM maltose, pH 7.4	B3
5x Profinia desalting buffer**	685 mM NaCl, 13.5 mM KCl, 21.5 mM Na ₂ HPO ₃ , 40.5 mM KH ₂ HPO ₄ , pH 7.4	B4
Water	H ₂ O	B5
4x Cleaning solution***	0.4% SDS	B6
1x Storage solution (MBP cartridge)	20% ethanol (v/v)	B7
1x 20% Ethanol (instrument storage)	20% ethanol (v/v)	B8

* Sample should be diluted or prepared in bind/wash buffer; minimum sample load volume of 10 ml is recommended. Buffer recommendations are based on instructions included with 1 ml or 5 ml MBPTrap HP columns from GE Healthcare. Alternative buffers may be used and should meet the specifications of the column manufacturer. ** Profinia desalting buffer (catalog # 620-0216), a component of the Profinia desalting purification kit (catalog # 620-0228), is necessary for methods using integrated desalting. *** Cleaning of the desalting cartridge should be carried out separately using the Desalting Cartridge Cleaning in Place utility on the Profinia instrument.

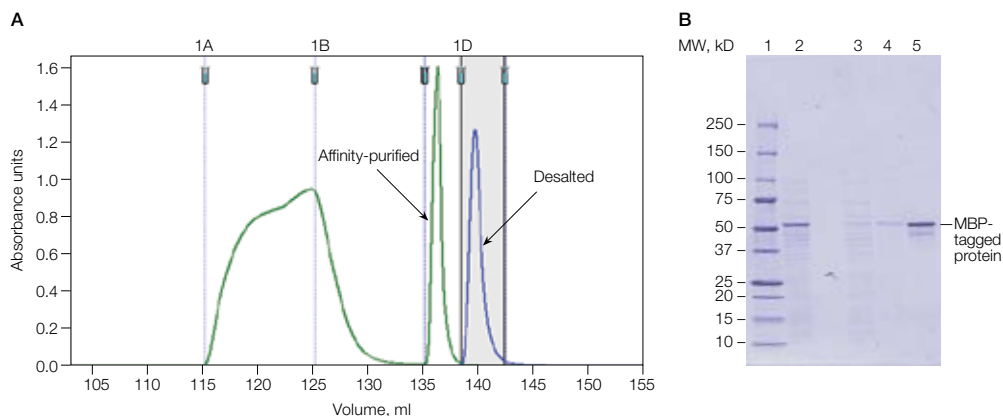


Fig. 1. Purification of MBP-tagged protein using affinity chromatography with integrated desalting on the Profinia system. MBP-tagged protein was purified from bacterial lysate using a customized affinity plus desalting method and the recommended buffers in Table 1. A 1 ml MBPTrap HP column was used for the affinity step and a 10 ml Bio-Scale™ Mini Bio-Gel® P-6 desalting cartridge for the integrated desalting. **A**, chromatogram showing different fractions; 1A, flowthrough; 1B, wash; 1D, affinity-purified and desalted elution fraction; **B**, SDS-PAGE analysis 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, elution (1D).

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It is important to note that the recommended buffers in Table 1 should be used at the concentrations indicated. The Profinia system automatically dilutes buffers to 1x working solutions. Other buffer formulations may be used, as desired.

Users can select the Affinity Method template in Program methods mode to customize settings. Use the following steps and the values in Table 2 to modify the generic affinity method or the affinity plus desalting method for purifications using 1 ml or 5 ml MBP cartridges available from a number of commercial vendors.

1. In Program methods (accessible from the home screen), select **AFFINITY** in the Select Method System screen.
2. Choose **Affinity** or **Affinity + Desalting** in the Select Method Type & Options screen.

3. In the Enter Run & Sample Information screen, select **Edit** to access the Method Information Screen.
4. Following the information in Table 2, use the **Edit** button on a highlighted step to view the Select Step Parameters To Edit screen for that step. Select the parameter to be edited and select **Edit** to change the default value to those listed in the table. Select **OK** to save the change.
5. Once all of the steps requiring modification have been edited, select **OK** in the Method Information Screen to return to the Enter Run & Sample Information screen. Rename the method (for example, 1 ml MBP method) and select **Save** to save the modified method. This modified method can now be accessed from the home screen in Saved Methods.

Table 2. Modifications to the Profinia affinity and affinity plus desalting methods in Program methods mode for MBP-tagged protein purification.

Step	Buffer Concentration	Modified Column Volumes		Flow Rate (1 ml/5 ml Cartridges)
		MBP Affinity Method	MBP Affinity Plus Desalting Method	
Equilibrate Di-C1	—*	5	5	—
Equilibrate C1	—	—	—	1/5
Load C1	—	—	—	1/5
Wash-1 C1	—	10	10	1/5
Wash-2 C1	—	0**	0	1/5***
Elute 1-C1	—	—	—	1/5
Elute 2-C1	—	4	3	1/5
Elute 3-C2	—	—	2	—
Clean 1-C2	1x	—	0.4	—
Clean 2-C2	4x	—	0	—
Clean 3-C2	—	—	0	—
Clean 1-C1	1x	3	3	—
Clean 2-C1	4x	3	3	—
Clean 3-C1	—	3	3	—
Store C1 (Aff)	1x	8	8	—
Store C2 (DS)	1x	—	0	—

* —, no change is needed or step is not present in the selected method. ** Value of 0 indicates that step will be skipped during execution of the method. *** Flow rates should be as indicated if a second wash step is used.

Note: Use the Desalting Cartridge Cleaning in Place utility (see section 9.3.13 of the instrument manual) after completion of the method to properly clean and store the desalting cartridge. Recommended cleaning buffers are listed in Table 3.

Table 3. Buffers for the Desalting Cartridge Cleaning in Place utility for 10 ml or 50 ml desalting cartridges.

Buffers and Solutions*	Composition	Volume**	Buffer Port
1x Cleaning solution 1	500 mM NaCl, 50 mM Tris, pH 8.0	200	B5
1x Cleaning solution 2	500 mM NaCl, 100 mM NaOAc, pH 4.5	200	B6
1x Storage solution	2% benzyl alcohol	200	B7
1x 20% ethanol (instrument storage solution)	20% ethanol (v/v)	100	B8

* These buffers are available as part of the Profinia desalting purification kit (catalog #620-0228) or Profinia desalting buffer kit (catalog #620-0224). They should be diluted from stock concentrations to 1x for use with the Desalting Cartridge Cleaning in Place utility. ** Volumes shown are sufficient for cleaning 4 x 10 ml desalting cartridges.

Program Method Planning Worksheet – Profinia Affinity Purification Method

Use this worksheet to plan an affinity-only method. General method step and reagent descriptions are on the left side of the worksheet; specific programming parameters are on the right. An "X" in any parameter indicates it is not an editable setting for the corresponding method step.

Method type: _____

Number of cartridges: _____

Affinity cartridge size: _____

Number of samples: _____

Sample 1 name and volume: _____

Sample 2 name and volume: _____

Method name: _____

Method Step	Step Description	Position/ Reagent	Sample 1 & 2 Settings		Sample 1 Setting				Sample 2 Setting			
			Buffer Conc. 1-5x	Frac. # (S1/S2)	Flow Rate, ml/min	CV	Peak Detection Parameters		Flow Rate, ml/min	CV	Peak Detection Parameters	
							Peak Detect. Delay	Max. Peak Detect. Vol.			Peak Detect. Delay	Max. Peak Detect. Vol.
Priming	Not editable		X	W/W	X	X	X	X	X	X	X	X
Equilibrating C1/C2 (equilibrating affinity cartridge)												
Equilibrate C1/C2	Wash cartridge with water	DI/deionized water		W/W			X	X			X	X
Equilibrate C1/C2	Equilibrate with equilibration wash-1 buffer	B1/equilibration wash-1 buffer		W/W			X	X			X	X
Loading Sample												
Load C1	Load sample to affinity cartridge C1		X	1A/2A		X	X	X		X	X	X
Load C2	Load sample to affinity cartridge C2		X	2A						X	X	X
Washing												
Wash-1 C1/C2	Wash affinity cartridge with equilibration/wash-1 buffer	B1/equilibration wash-1 buffer		1B/2B			X	X			X	X
Wash-2 C1/C2	Wash affinity cartridge with wash-2 buffer	B2/wash-2 buffer		1C/2C			X	X			X	X
Eluting												
Elute 1-C1/C2	Start elution until purified protein peak is detected	B3/elution buffer		W/W		X				X		
Elute 2-C1/C2	Collect purified protein peak in fraction tube	B3/elution buffer		1D/2D			X	X			X	X
Cartridge Cleaning												
Clean 1-C1/C2	Clean affinity cartridge	B5/cleaning solution 1		W/W			X	X			X	X
Clean 2-C1/C2	Clean affinity cartridge	B6/cleaning solution 2		W/W			X	X			X	X
Clean 3-C1/C2	Clean affinity cartridge	DI/deionized water		W/W			X	X			X	X
Storing												
Store C1/C2	Storage buffer affinity cartridge	B7/storage solution		W/W			X	X			X	X
End of Run Cleaning Steps	Not editable		X	W/W	X	X	X	X	X	X	X	X

W, waste; 1A/2A, flowthrough fraction; 1B/2B, wash 1 fraction; 1C/2C, wash 2 fraction; 1D/2D, elution fraction; CV, column volumes.

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,
100–240 V, includes same as 620-1005 with Profinia software
620-1011 Profinia Protein Purification System With GST Starter Kit,
100–240 V, includes same as 620-1006 with Profinia software

Profinia Consumables

620-0216 5x Profinia Desalting Buffer, 200 ml
620-0217 2x Profinia Cleaning Solution 1, 125 ml
620-0224 Profinia Desalting Buffer Kit, includes purification buffers, cleaning
and storage solutions; sufficient for 10 applications
620-0228 Profinia Desalting Purification Kit, 10 ml, includes Profinia
desalting buffer kit, 2 x 10 ml desalting cartridges
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

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Canada 905 364 3435 China 86 21 6426 0808 Czech Republic 420 241 430 532 Denmark 44 52 10 00 Finland 09 804 22 00 France 01 47 95 69 65
Germany 089 318 84 0 Greece 30 210 777 4396 Hong Kong 852 2789 3300 Hungary 36 1 455 8800 India 91 124 4029300 Israel 03 963 6050
Italy 39 02 216091 Japan 03 6361 7000 Korea 82 2 3473 4460 Mexico 52 555 488 7670 The Netherlands 0318 540666 New Zealand 0508 805 500
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