Profinia™ Purification and Buffer Kits

Instruction Manual

For technical support, call your local Bio-Rad office or, in the U.S., call **1-800-4BIORAD** (1-800-424-6723)

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Section 1
Introduction

1.1 Background

The Profinia™ protein purification system provides a complete line of consumables that interface with an automated, user-friendly instrument for the purification of polyhistidine- and GST- tagged proteins. The system has been designed to take standard chromatography buffers, resins, reagents, and instrumentation and package them into a complete, kit-based, easy-to-use platform. Four main types of applications currently used in the Profinia system have kits: native IMAC, denaturing IMAC, GST, and desalting. The reagents and consumables available for each of these applications include prepackaged buffers, IMAC and GST affinity media in prepacked cartridges, and antibody detection tools. Other methods such as antibody, Profinity eXact™ tag, MBP tag, and Strep-tag purifications, can also be performed on the Profinia system without kits. Refer to bulletins 5701, 5725, 5741, and 5744 for buffer formula. See the Ordering Information section for a complete list of Profinia products.

1.2 Product Information

Two types of kits can be used for the purification of affinity-tagged proteins: Profinia purification kits and Profinia buffer kits. The all-inclusive Profinia purification kits include two 1 ml affinity and two 10 ml desalting cartridges, in addition to a complete set of buffers solutions (affinity, desalting*, cleaning, and storage solutions), and reagents (glutathione) required for affinity and desalting applications. Profinia buffer kits are an appropriate choice for those users requiring additional buffers and cleaning reagents, but are not supplied with the affinity or desalting cartridges. The desalting and cartridge cleaning buffer kit contains solutions and cartridges specific for desalting or buffer exchange. Each kit contains sufficient reagents to perform 10 ml of affinity purifications, followed by 100 ml of desalting separations. This can be broken up into ten individual 1 ml affinity cartridge runs, two 5 ml affinity cartridge runs, or a single 5 ml and five 1 ml cartridge runs. The cartridges for affinity/desalting applications are provided as 1 ml/10 ml pairs. Larger 5 ml/50 ml affinity/desalting cartridges can be purchased separately for larger-scale purification runs. Because each ml of IMAC or GST resin typically yields ≥10 mg of purified protein, each buffer or purification kit typically yields ≥100 mg of purified protein. Table 1 summarizes the complete line of buffer and purification kits available for the Profinia system.

*** For denaturing IMAC applications, stepwise dialysis is the suggested method for desalting and buffer exchange and for renaturing proteins.**

Important note: Do not use GST lysis and wash buffer for IMAC purification because GST lysis buffer and wash buffer contain a high concentration (10 mm) of EDTA that will damage the IMAC cartridge.

Table 1. Profinia purification and buffer kits.

Kit	Catalog #	Solutions			Powdered Reagent	Cartridges	
		Purification Buffers	Desalting Buffer	Storage Solutions	Glutathione	Affinity	Desalting
Profinia Native IMAC Kits							
Native IMAC purification kit	620-0225 620-0235	✔ ✔	✔ ✔	✔ ✔		✔ ✔	✔ ✔
Native IMAC buffer kit	620-0221	✔	✔	✔			
Profinia GST Kits							
GST purification kit	620-0226 620-0236	✔ ✔	✔ ✔	✔ ✔	✔ ✔	✔ ✔	✔ ✔
GST buffer kit	620-0223	✔	✔	✔	✔		
Profinia Desalting Kits							
Desalting kit	620-0228 620-0238		✔ ✔	✔ ✔			✔ ✔
Desalting and cartridge cleaning buffer kit	620-0224		✔	✔			

1.3 Equipment and Materials Required

Profinia purification and buffer kits are specifically designed to be used with the Profinia protein purification instrument. All of the buffers and solutions come formulated as concentrates that are made with chromatography-grade solutions, and have been prefiltered to remove particulates. The buffers that require the addition of urea (not provided) or glutathione require filtration through a 0.2 µm filter prior to use with the system.

Accessories available for the Profinia instrument include Profinia software, cooling blocks, and desalting sample loops, buffer bottles, and tubing. Consumables that can be purchased separately include His- and GST- detection antibodies, and a dual-tagged lyophilized control lysate containing GST- and His-tagged proteins. Complete catalog and ordering information can be found in Section 9.

1.4 Storage Conditions

All unopened Profinia IMAC purification and buffer kits can be stored between 4 and 22°C. The GST kits can be stored at 22°C for up to two weeks. For long-term storage, the GST kits should be stored at 4°C. The labels on the outside of the kit box provide exact expiration dates.

Section 2
Native IMAC Purification and Buffer Kits

2.1 General Buffer and Cartridge Information

The buffers provided in the native IMAC kit are formulated from potassium salts and buffers, and contain increasing concentrations of imidazole for optimized binding, washing, and eluting of histidine (His)-tagged proteins. Native IMAC buffers* are used for proteins that partition into the soluble fraction of *Escherichia coli* lysates and that have accessible His sequence tags. Table 2 provides a list of buffer compositions. The native lysis buffer, the same as wash buffer 1, is used for sample preparations (see Section 6), and is not used in any of the instrument ports.

The IMAC cartridges are shipped in 20% ethanol and can be stored between 4 and 22°C prior to use. The cartridges are packed with 1 ml of Bio-Rad's Profinity™ IMAC resin, and typically yield 10 mg of purified protein per run (dependent upon protein expression level and culture volume loaded). To minimize any possibility of cross-contamination, it is suggested that individual cartridges be dedicated to the purification of a unique His-tagged protein. The desalting cartridges are provided in 20 mM Bis-Tris, pH 6.5 with 0.05% azide as a preservative and can be stored between 4 and 22°C prior to use.

*** IMAC buffers made with potassium salts do not form precipitates with long-term storage. However, to prevent the formation of insoluble potassium-SDS complexes in Laemmli buffer, native IMAC samples and fractions (lysate load, flowthrough, wash 1, and wash 2 samples) should be diluted at least 1:7 in Laemmli buffer prior to electrophoresis. The desalted and purified fraction is collected in a PBS-based buffer and only needs dilution 1:2 prior to electrophoresis.**

Table 2. Formulations for buffers and solutions provided in the Native IMAC Kits.

Profinia Solution	Supplied As	Concentrated Formulation	Working 1x Formulation	Volume	Position #
Native IMAC lysis buffer	2x	600 mM KCl, 100 mM KH_2PO_4, 10 mM imidazole, pH 8.0	300 mM KCl, 50 mM KH_2PO_4, 5 mM imidazole, pH 8.0	125 ml	N/A
Native IMAC wash buffer 1	2x	600 mM KCl, 100 mM KH_2PO_4, 10 mM imidazole, pH 8.0	300 mM KCl, 50 mM KH_2PO_4, 5 mM imidazole, pH 8.0	125 ml	1
Native IMAC wash buffer 2	2x	600 mM KCl, 100 mM KH_2PO_4, 20 mM imidazole, pH 8.0	300 mM KCl, 50 mM KH_2PO_4, 10 mM imidazole, pH 8.0	100 ml	2
Native IMAC elution buffer	2x	600 mM KCl, 100 mM KH_2PO_4, 500 mM imidazole, pH 8.0	300 mM KCl, 50 mM KH_2PO_4, 250 mM imidazole, pH 8.0	100 ml	3
Desalting buffer	5x	685 mM NaCl, 13.5 mM KCl, 21.5 mM Na_2HPO_4, 40.5 mM KH_2HPO_4, pH 7.0 (pH 7.4 upon dilution)	137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 8.1 mM KH_2PO_4, pH 7.4	200 ml	4
Cleaning solution 1	2x	1,000 mM NaCl, 100 mM Tris, pH 8.0	500 mM NaCl, 50 mM Tris, pH 8.0	125 ml	5
Cleaning solution 2	4x	2,000 mM NaCl, 400 mM NaOAc, pH 4.5	500 mM NaCl, 100 mM NaOAc, pH 4.5	125 ml	6
Storage solution	2x	4% $C_6H_5CH_2OH$ (benzyl alcohol)	2% $C_6H_5CH_2OH$ (benzyl alcohol)	200 ml	7

2.2 Instructions for Use

The buffers are provided as concentrates and are automatically proportioned to a 1x formulation by the Profinia instrument when using the preprogrammed methods. Each bottle is clearly labeled with a white strip near the graduations, which provides the buffer name and the buffer position number for insertion (1–7). The position number on the bottle must match the position number on the instrument to properly run the preprogrammed methods. Prior to inserting buffers into the instrument, the following steps must be performed. Remove cap closures from buffer bottles and rest closures on top of instrument, directly above appropriate buffer positions. Rest a green buffer lid on the opening of each bottle and carefully guide the tubing into the bottle. Place buffer bottle in appropriate numbered position. Buffer lids help prevent buffers from being contaminated with particulates and minimize evaporation during purification runs. Refer to Figure 1 for a diagram of proper bottle installation and placement. If fewer than 10 runs are used for a single kit, the remaining buffers can be capped and stored at 4°C for up to 3 months.

1.

1. Replace buffer caps with buffer lids prior to installation into the instrument.

2.

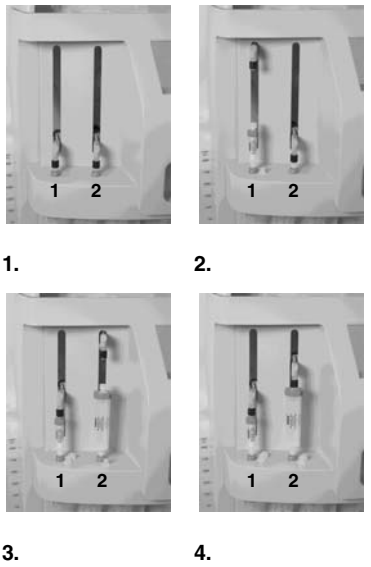
2. For easy identification, buffer caps can be placed directly above buffer bottle positions. Guide tubing from the instrument through the buffer lid while placing bottle into proper position.

3.

3. Shown is proper native IMAC buffer bottle positioning, with instrument lid in closed position.

Fig. 1. Native IMAC buffer installation procedure.

After placement of the buffer bottles and the cartridge lines have been automatically primed, the cartridges are ready to insert into the instrument. They are automatically equilibrated by the buffers while running a preprogrammed method. Remove the luer caps and screw cartridges into the cartridge holders on the instrument. Be careful not to overtighten cartridges. Refer to Figure 2 for the proper connection of the cartridges in the Profinia system. The female luer inlet (top) and the male luer outlet (bottom) simplify the orientation and connection. At the end of the run, the cartridges are equilibrated into storage solution, and should be recapped with the luer fittings. After use, the cartridges should be stored at 4°C and be reused within 6 months.



1. Prior to runs, the cartridge holders are maintained in the connected position for storage.
2. Unscrew and separate column connection holders so that they are ready to receive purification cartridges. Remove the bottom luer cap of an affinity cartridge and insert it into cartridge position #1 (left).
3. Pull the top portion of the column holder down and screw closed to create a seal for cartridge #1. Be careful not to overtighten cartridges.
4. Desalting applications for native IMAC and GST require the addition of the desalting cartridge in cartridge position #2 (right).

Fig. 2. Bio-Scale™ Mini cartridge installation procedure.

Section 3
Denaturing IMAC Purification

3.1 General Buffer and Cartridge Information

The buffers used in the denaturing IMAC purification are identical to the native IMAC solutions, but require the addition of urea. We do not provide ready-to-use denaturing IMAC purification kits. Denaturing IMAC buffers are used for proteins that partition into the insoluble fraction of *E. coli* lysates or have inaccessible His sequence tags. The denaturing IMAC buffers can also be used to solubilize the entire *E. coli* pellet without separation of the soluble and insoluble fractions. Purifications from unfractionated *E. coli* pellets may result in slightly less pure proteins.

Table 3 provides a list of denaturing IMAC buffer compositions. All buffers are 1x working buffer and placed on positions 1–7. The lysis buffer is used for sample preparations (see Section 6), and is not used in any of the instrument ports.

The IMAC cartridges are identical to the cartridges described in Section 2.1, and all specifications apply to denaturing IMAC purifications.

Table 3. Formulations for buffers and solutions for denaturing IMAC purification.

Profinia Solution	Supplied As	Other Components Needed	Working 1x Formulation, for Denaturing IMAC Purification	Final Volume aAfter Urea Addition*	Position #
Native IMAC lysis buffer	2x	Add 90 g urea and H_2O to 250 ml	6 M urea, 300 mM KCl, 50 mM KH_2PO_4 5 mM imidazole, pH 8.0	250 ml	N/A
Native IMAC wash buffer 1	2x	Add 90 g urea and H_2O to 250 ml	6 M urea, 300 mM KCl, 50 mM KH_2PO_4 5 mM imidazole, pH 8.0	250 ml	1
Native IMAC wash buffer 2	2x	Add 72 g urea and H_2O to 200 ml	6 M urea, 300 mM KCl, 50 mM KH_2PO_4 10 mM imidazole, pH 8.0	200 ml	2
Native IMAC elution buffer	2x	Add 72 g urea and H_2O to 200 ml	6 M urea, 300 mM KCl, 50 mM KH_2PO_4 250 mM imidazole, pH 8.0	200 ml	3
Cleaning solution 1	2x		500 mM NaCl, 50 mM Tris, pH 8.0	125 ml	5
Cleaning solution 2	4x		500 mM NaCl, 100 mM NaOAc, pH 4.5	125 ml	6
Storage solution	2x		2% $C_6H_5CH_2OH$ (benzyl alcohol)	200 ml	7

*For bigger bottle to fit the buffer volume, order catalog #620-0231.

3.2 Instructions for Use

After urea addition, the solutions containing completely dissolved urea should be filtered through 0.2 µm filters to remove particulates. The position labeling of the bottles and insertion into the instrument are identical to that described in Section 2.2. **Note: Denaturing IMAC methods do not use desalting buffers (or cartridges) and position 4 is left empty or filled with water**. Refer to Figure 3 for proper positioning of the denaturing kit bottles. In this figure, the bottles are installed and the instrument lid is in the closed position. If less than 10 ml is needed for a purification, the denaturing buffers containing urea can be frozen and used within 3 months. The stored buffers should only be thawed once and discarded after use.

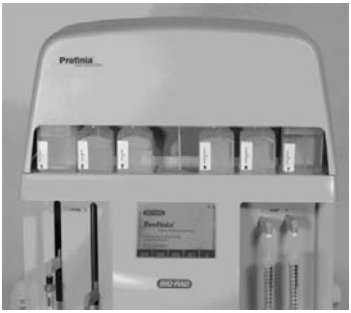


Fig. 3. Placement and positioning of denaturing IMAC buffers.

After buffer bottle insertion, and the cartridge lines are automatically primed, the cartridges are ready to insert into the instrument. They are automatically equilibrated using the buffers and the preprogrammed methods. Insert the cartridges as described in Section 2.2 and as shown in Figure 2. After use, the cartridges should be stored at 4°C and used within 6 months.

Section 4
GST Purification and Buffer Kits

4.1 General Buffer and Cartridge Information

The lysis and wash buffers contained in the GST kit are formulated from sodium salts, phosphate buffers, and EDTA, and provide optimized binding, washing, and eluting of GST-tagged proteins. EDTA is included as a chelating compound and protects against metalloproteases, a class of proteases that can be present in *E. coli* lysates. GST fusion proteins must be enzymatically active prior to purification. The buffers used in this system are designed for the purification of proteins that partition into the soluble fraction of *E. coli* lysates and that have accessible, and biologically active, GST sequence tags. Table 4 provides a list of buffer compositions. The lysis buffer is used for sample preparations (see Section 6) and is not used in any of the buffer bottle positions.

The GST cartridges are provided in 20% ethanol and should be stored at 4°C prior to use. The cartridges are packed with 1 ml of Bio-Rad's Profinity™ GST resin, and typically yield ≥10 mg of purified protein per run (dependent upon protein expression level and culture volume loaded). To minimize any possibility of cross-contamination, it is suggested that individual cartridges be dedicated to the purification of a unique GST-tagged protein. The desalting cartridges are provided in 20 mM Bis-Tris, pH 6.5 with 0.05% azide and can be stored at 4–22°C prior to use.

Table 4. Formulations for buffers and solutions provided in the GST kits.

Profinia Solution	Supplied As	Concentrated Formulation	Working 1x Formulation	Volume	Position #
GST lysis buffer	2x	300 mM NaCl, 20 mM Na_2HP0_4, 10 mM EDTA, pH 7.4	150 mM NaCl, 10 mM Na_2HP0_4, 5 mM EDTA, pH 7.4	125 ml	N/A
GST wash buffer	2x	300 mM NaCl, 20 mM Na_2HP0_4, 10 mM EDTA, pH 7.4	150 mM NaCl, 10 mM Na_2HP0_4, 5 mM EDTA, pH 7.4	200 ml	1
GST elution buffer	2x	40 mM glutathione, 200 mM Tris, 10 mM EDTA, pH 8.0	20 mM glutathione, 100 mM Tris, 5 mM EDTA, pH 8.0	100 ml	3
Desalting buffer	5x	685 mM NaCl, 13.5 mM KCl, 21.5 mM Na_2HP0_4, 40.5 mM KH_2HP0_4, pH 7.0 (pH 7.4 upon dilution)	137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HP0_4, 8.1 mM KH_2P0_4, pH 7.4	200 ml	4
Cleaning solution 1	2x	1,000 mM NaCl, 100 mM Tris, pH 8.0	500 mM NaCl, 50 mM Tris, pH 8.0	125 ml	5
Cleaning solution 2	4x	2,000 mM NaCl, 400 mM NaOAc, pH 4.5	500 mM NaCl, 100 mM NaOAc, pH 4.5	125 ml	6
Storage solution	2x	4% $C_6H_5CH_2OH$ (benzyl alcohol)	2% $C_6H_5CH_2OH$ (benzyl alcohol)	200 ml	7

4.2 Instructions for Use

The buffers are provided as concentrates and are diluted to a 1x formulation by the instrument using the preprogrammed methods. The entire contents (1.23 g) of the glutathione pack need to be added to the GST elution buffer prior to a purification. Add 5 ml of the elution buffer to the vial of glutathione and mix to completely resuspend. Either pipet or pour the glutathione solution into the elution buffer bottle. Filter the solution through a 0.2 µm filter to remove particulates. The position labeling of the bottles and insertion into the instrument is identical to that described in Section 2.2. **Note: GST methods do not use a second wash buffer and position 2 is left empty or filled with water**. Refer to Figure 4 for proper placement of the GST buffer bottles. If less than 10 ml of purifications are performed, the glutathione-containing elution buffer can be frozen in aliquots and used within 3 months. The stored elution buffer should only be thawed once. The other GST buffers can be capped and stored at 4°C for up to 3 months.

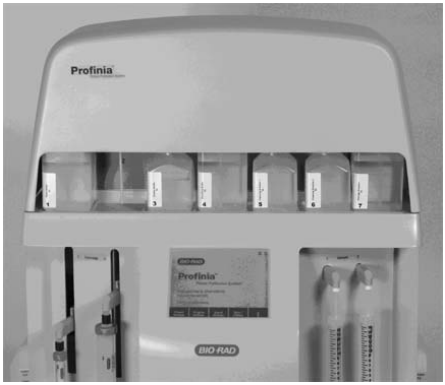


Fig. 4. Placement and positioning of GST buffers.

After the buffer bottles are installed, the cartridges are ready to insert into the instrument, and are automatically equilibrated by the preprogrammed methods. Insert the cartridges as described in Section 2.2 and as shown in Figure 2. After use, the cartridges should be stored at 4°C and be reused within 6 months.

Section 5
Desalting and Cartridge Cleaning Buffer Kit

5.1 General Buffer and Cartridge Information

The buffers and solutions provided in the desalting and cartridge cleaning buffer kit consist of a PBS-based protein desalting buffer, cartridge cleaning buffers, and a cartridge storage solution. The PBS solution provides an optimized ionic strength to prevent nonspecific interactions between proteins and the matrix, and is a commonly used buffer for the storage of a wide variety of proteins. **Note: desalting and dartridge dleaning buffers are already included in Profinia™ native IMAC and GST purification and buffer kits**. It can be used to purify other proteins with MBP- or Strep-tag, using the Profinia system (see Bulletin 5741 and 5744.) Optimized desalting and storage solutions for unique proteins will need to be determined empirically. The desalting solutions and cartridges can be used to remove or exchange salts, buffers, and small molecules from any protein sample. Table 5 provides a list of buffer compositions.

The desalting cartridges are provided in 20 mM Bis-Tris, pH 6.5 with 0.05% sodium azide as a preservative, and can be stored between 4 and 22°C prior to use. The cartridges are packed with 10 ml of Bio-Rad's

Bio-Gel® P-6 size exclusion resin, and typically yield ≥80% recovery of applied target protein. The resin in the Bio-Gel P-6 desalting cartridges exhibits minimal interaction with proteins. When used with the cleaning buffers, different proteins can safely be applied to the same desalting cartridge.

Table 5. Formulations for buffers and solutions provided in the desalting kits.

Profinia Solution	Supplied As	Concentrated Formulation	Working 1x Formulation	Volume	Position #
Desalting buffer	5x	685 mM NaCl, 13.5 mM KCl, 21.5 mM Na_2HP0_4, 40.5 mM KH_2HP0_4, pH 7.0 (pH 7.4 upon dilution)	137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HP0_4, 8.1 mM KH_2P0_4, pH 7.4	200 ml	4
Cleaning solution 1	2x	1,000 mM NaCl, 100 mM Tris, pH 8.0	500 mM NaCl, 50 mM Tris, pH 8.0	125 ml	5
Cleaning solution 2	4x	2,000 mM NaCl, 400 mM NaOAc, pH 4.5	500 mM NaCl, 100 mM NaOAc, pH 4.5	125 ml	6
Storage solution	2x	4% $C_6H_5CH_2OH$ (benzyl alcohol)	2% $C_6H_5CH_2OH$ (benzyl alcohol)	200 ml	7

5.2 Instructions for Use

The buffers are provided as concentrates and are diluted to a 1x formulation by the instrument using the preprogrammed methods. The position labeling of the bottles and insertion into the instrument is identical to that described in Section 2.2. Because desalting does not use any of the affinity buffers, positions 1–3 are not used. Refer to Figure 5 for the proper placement of the desalting buffer bottles. For desalting applications using affinity purification as a first step, refer to Sections 2 and 4 — Native IMAC Purification and Buffer Kits, and GST Purification and Buffer Kits.

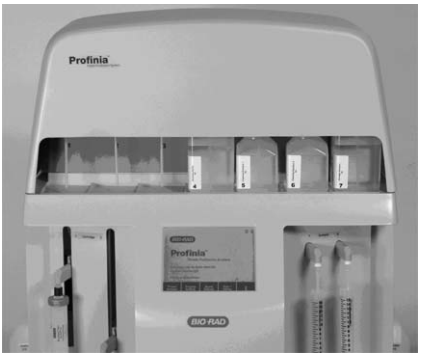


Fig. 5. Desalting and cartridge cleaning buffer bottle positioning.

The desalting methods are designed for rapid desalting or buffer exchange and require that a desalting loop accessory be used in

conjunction with a desalting cartridge. A syringe and a 3-way stopcock combination are used to fill the desalting loop. The desalting loop accessory comes ready to use in two sizes, 2 ml and 10 ml. For ordering information, refer to Section 6.

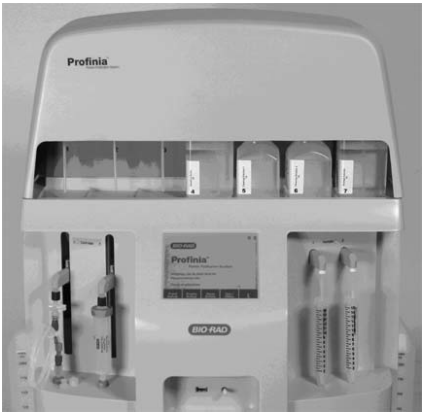


Fig. 6. Desalting sample loop and fittings.

Determine the appropriate combination of desalting loop and desalting cartridge for the protein sample volume to be applied. For a 2 ml sample, choose the 2 ml desalting loop and the 10 ml desalting cartridge. For a 10 ml sample, choose the 10 ml desalting loop and the 50 ml desalting cartridge. Application of a sample volume smaller than the desalting loop volume is not recommended, as it will result in increased dilution of the sample and reduced desalting resolution.

After cartridge lines are automatically primed, the sample loop and desalting cartridge can be installed. The desalting loop accessory is designed to connect to the instrument in the cartridge 1 position, while the desalting cartridge is placed in the cartridge 2 position. Refer to Figure 6 for the proper installation of a 2 ml desalting loop and 10 ml desalting cartridge.

To connect and fill the desalting loop:

1. Insert the male luer fitting from cartridge holder 1 into the top female luer fitting of the 3-way stopcock. Ensure that the second female luer fitting of the 3-way stopcock is facing out so the syringe may be easily inserted.
2. Connect the male luer of the end of the desalting loop to the female luer fitting of cartridge holder 1.
3. To apply sample, fill the syringe and insert it into the open female luer of the 3-way stopcock. Turn the 3-way stopcock lever down, towards the loop, and fill the loop with sample, avoiding bubbles as much as possible.

4. Return the 3-way stopcock lever to the middle position, facing toward the syringe. The desalting loop is now filled with sample and ready for the desalting method to begin.

Once installed, the cartridges are automatically equilibrated by the preprogrammed methods. After use, the cartridges should be stored at 4°C and be used within 6 months.

Section 6
Sample Preparation

6.1 Preparing Lysates Prior to Purification

Lysates from *E. coli* cultures can be prepared using conventional sonication procedures with the lysis buffers supplied in each kit, or using compatible chemical lysis reagents. For *E. coli* cultures expressing medium to high levels of fusion proteins (≥10% of total protein), 200 ml of culture will normally yield sufficient material for a 1 ml cartridge purification, and 1,000 ml of culture will yield sufficient material for a 5 ml cartridge purification run. For cultures expressing protein at low levels (≤10% of total protein), the culture volumes will need to be determined empirically for each protein. Bacterial cultures can be grown in advance and centrifuged. The pellets can be stored at –70°C for several months and lysed at a convenient date for sample preparation.

Native Lysate Preparation (Profinia™ Native IMAC or GST Kits)

1. Harvest cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine weight of pellet and resuspend in 10 volumes of Profinia native IMAC lysis buffer or Profinia GST lysis buffer depending on the application (200 ml of culture typically yields 0.8–1.0 g of paste or 8–10 ml of lysate). Thoroughly resuspend the pellet by pipetting or vortexing.
3. As an optional step and to decrease the viscosity, add a nuclease solution (DNase at 100 units/ml or Benzonase at 25 units/ml).
4. Sonicate the lysate (on ice, using 25% output) 4 times at 1 min intervals.
5. Centrifuge at 16,000 x g for 20 min at 4°C to clarify the lysate.
6. Filter clarified lysate supernatant through a 0.45 µm filter to remove particulates.
7. Transfer the filtered lysate to a 15 ml or 50 ml sample tube and insert into the Profinia instrument.

8. If the lysate is not going to be used immediately, it can be frozen at –20°C and thawed once to be purified at a later date. However, proteolysis or protein degradation can occur upon freezing and thawing, and the quality of the purified product may be compromised. This will have to be determined empirically for individual proteins. Upon thawing, refilter through a 0.45 µm filter, as precipitates often form after freezing.

Denaturing Lysate Preparation

1. Harvest cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine weight of pellet and resuspend in 10 volumes of Profinia denaturing IMAC lysis buffer containing urea (see Table 3) (200 ml of culture typically yields 0.8–1.0 g of paste or 8–10 ml of lysate). Thoroughly resuspend the pellet by pipetting or vortexing.
3. Sonicate the lysate (on ice, using 25% output) 4 times at 1 min intervals.
4. Centrifuge at 16,000 x g for 20 min at 4°C to clarify lysate.
5. Filter clarified lysate through a 0.45 µm filter to remove particulates. Transfer the filtered lysate to a 15 ml or 50 ml sample tube and insert into the Profinia instrument.
6. If the lysate is not going to be used immediately, it can be frozen at –20°C and thawed once to be purified at a later date. See the description under the native lysate prep for treatment upon freezing/thawing.

Native Lysate Preparation Using Bacterial Lysis/Extraction Reagent
(Recommended for IMAC procedures; binding capacities of GST fusion proteins will be decreased ~30% using chemical lysis methodologies.)

1. Harvest cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine weight of pellet and resuspend in 10 volumes of bacterial lysis/extraction reagent (Pierce catalog #78243 or 78266) (200 ml of culture typically yields 0.8–1.0 g of paste or 8–10 ml of lysate). Thoroughly resuspend the pellet by pipetting or vortexing.
3. As an optional step and to decrease the viscosity, add a nuclease solution (DNase at 100 units/ml or Benzonase at 25 units/ml) and incubate for 10 min at room temperature.
4. Centrifuge at 16,000 x g for 20 min to clarify the lysate.
5. Filter the clarified lysate through a 0.45 µm filter to remove particulates. Transfer the clarified lysate to a 15 ml or 50 ml sample tube and insert into the Profinia instrument.

6. If the lysate is not going to be used immediately, it can be frozen at –20°C and thawed once to be purified at a later date. See description under the native lysate preparation (Profinia native IMAC or GST kits) for treatment upon freezing/thawing.

Denaturing IMAC Lysate Preparation Using Bacterial Lysis/Extraction Reagent

1. Harvest cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine weight of pellet and resuspend in 10 volumes of Profinia bacterial lysis/extraction reagent (Pierce catalog #78243 or 78266) (200 ml of culture typically yields 0.8–1.0 g of paste or 8–10 ml of lysate).
3. To decrease the viscosity, add a nuclease (DNase at 100 units/ml or Benzonase at 25 units/ml) to the suspension, and thoroughly resuspend by pipetting or vortexing. Let the solution incubate with gentle shaking 10 min at room temperature.
4. Centrifuge at 16,000 x g for 20 min at 4°C.
5. **Discard** the supernatant and add 10 volumes of Profinia denaturing IMAC lysis buffer containing urea (see Table 3) to the inclusion body pellet.
6. Vortex or mix well to thoroughly resuspend the pellet (may take ~10 min).
7. Centrifuge at 16,000 x g for 20 min at 4°C to clarify lysate.
8. Filter the clarified lysate through a 0.45 µm filter to remove particulates. Transfer the clarified lysate to a 15 ml or 50 ml sample tube and insert into the Profinia instrument.
9. If the lysate is not going to be used immediately, it can be frozen at –20°C and thawed once to be purified at a later date. See description under the native lysate preparation (Profinia native IMAC or GST kits) for treatment upon freezing/thawing.

Section 7
Frequently Asked Questions and Troubleshooting

For an extensive and comprehensive list of troubleshooting tips for protein expression, sample preparation, purification and analysis, refer to the Profinia™ Protein Purification Instrument Instruction Manual. Questions and tips below focus on the use of the buffer kits and solutions. More information on Profinia applications can be found in the Profinia FAQs on www.bio-rad.com.

Proper Storage of Solutions and Kits

- All of the kits can be stored at 22°C (room temperature) for short periods of time, either upon receipt or during normal usage. For the kits that have labile reagents (glutathione in GST kits), 4°C is the recommended long-term storage temperature
- Once opened and used with the instrument, all reagents should be stored at 4°C for up to a period of 3 months. After insertion into the instrument, the solutions are no longer sterile and require 4°C storage
- Once opened and used with the instrument, check the solutions for particulates and clarity before reusing. If there is any indication of particulates, contamination, or microbial growth, the solution should discarded

Addition of Additives or Reagents to the Solutions

- The solutions are provided as concentrates, and are diluted by the preprogrammed methods in the instrument. If it is desired to add an additional component to one of the solutions (such as, a protease inhibitor), it should be added as a concentrate and brought to the final concentration of the solution (such as, final 2x concentration for the affinity buffers). The volume to be added should be minimized so that it does not dramatically alter the final concentration of the solution labeled on the bottle

Addition of Glutathione

- Urea (not provided) and glutathione should be added to the buffers on the day purification starts. Any unused solution can be stored at 4°C for up to 7 days
- For longer-term storage, any unused buffer can be stored at –20°C and used within a 3 month period. Upon thawing, check for any particulates, and if necessary, heat in a 37°C waterbath to dissolve precipitates. Always refilter solutions through a 0.2 µm filter if particulates are present

Leftover Buffer After Ten Runs

- Each kit contains sufficient reagents for ten purification runs (equivalent to 10 ml of resin). Depending upon which method is used (standard vs. extended wash, rerun of same method, insertion of new cartridge vs. using the same cartridge, etc.), some bottles may not be completely depleted, while others may be nearly empty. To minimize the risk of contamination, it is recommended to discard any unused buffers rather than pool multiple old lots of buffer to refill bottles

Viscous Lysates

- The lysis buffers that are supplied in each kit are optimized for lysis by sonication. A ratio of 10 parts 1x buffer to 1 part cell pellet (v/w) is ideal for lysing cell pellets. Depending upon the density of the culture, the power of the sonicator, and the condition of the sonication tip, the final viscosity of the lysate may vary. Extremely viscous lysates create system backpressure and run the risk of slowing down the flow during sample loads
- To decrease viscosity, a nuclease should be added prior to sonication. Benzonase (Novagen) or DNase are commonly used nucleases

Large Sample Volumes

- When low protein expression requires large culture volumes (>2 L of starting culture), the lysate loading volume can exceed 45 ml, which exceeds the volume that can be used in a standard sample tube. In these instances, bacterial pellets can be lysed at 5:1 (v/w) ratios. When concentrating starting samples at 5:1 ratios, it is **strongly recommended** to add a nuclease to minimize sample viscosity

Section 8
Legal Notices

Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US patent 5,284,933 and US patent 5,310,663, including foreign patents (assignee Hoffman-LaRoche).

Expression and purification of GST fusion proteins may require a license under US patent 5,654,176 (assignee Chemicon International).

Benzonase is a trademark of Merck KGaA.

Section 9
Ordering Information

Catalog #	Description
Profinia™ 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 System With Software, Accessary Kit and Native IMAC Starter Kit**, 100–240 V
620-1011	**Profinia System With Software Accessory Kit and GST Starter Kit**, 100–240 V
Profinia Accessories	
620-0010	**Profinia Software With USB Cable**
620-0401	**Profinia Cooling Accessory**
620-0402	**Profinia 2.0 ml Desalting Sample Loop**
620-0403	**Profinia 10.0 ml Desalting Sample Loop**
620-0404	**Profinia Instrument Inline Filter Assembly Replacement**
620-0410	**Profinia Instrument Accessory Kit**, includes cleaning tray, inline filter assembly replacement, 8 buffer lids, 2 x 50 ml sample lids, 2 x 15 ml lids, bottle starter pack, waste/diluent bottle set
620-0231	**Bottle Starter Pack**, 4 x 125 ml and 4 x 250 ml bottles and 8 buffer lids
620-0411	**Profinia pH Monitor With Mounting Accessory Kit**, includes pH electrode, flow cell, mounting accessories
732-0112	**Adaptor Kit,** allows connection to GE Healthcare and Pierce prepacked cartridges at bottom
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
620-0235	**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
620-0226	**Profinia GST Purification Kit**, 1 ml, includes Profinia GST buffer kit, 2 x 1 ml GST and 2 x 10 ml desalting cartridges
620-0236	**Profinia GST Purification Kit**, 5 ml, includes 2 Profinia GST buffer kits, 1 x 5 ml GST and 1 x 50 ml desalting cartridge

Catalog #	Description
620-0228	**Profinia Desalting Purification Kit**, 10 ml, includes Desalting and Cartridge Cleaning Buffer Kit, 2 x 10 ml desalting cartridges
620-0238	**Profinia Desalting Purification Kit**, 50 ml, includes 2 Desalting and Cartridge Cleaning Buffer Kit, 1 x 50 ml desalting cartridge

Profinia Buffer Kits

620-0221	**Profinia Native IMAC Buffer Kit**, includes purification buffers, cleaning and storage solutions; sufficient for 10 applications for 1 ml cartridge
620-0223	**Profinia GST Buffer Kit**, includes purification buffers, cleaning and storage solutions, glutathione reagent; sufficient for 10 applications for 1 ml cartridge
620-0224	**Desalting and Cartridge Cleaning Buffer Kit**, includes desalting buffer, cleaning and storage solutions; sufficient for 10 applications for 10 ml cartridge

Profinia Starter Kits

620-0229	**Profinia IMAC Starter Kit**, includes Profinia native IMAC buffer kit, 1 x 1 ml IMAC and 1 x 10 ml desalting cartridge, *E. coli* lysate
620-0230	**Profinia GST Starter Kit**, includes Profinia GST buffer kit, 1 x 1 ml GST and 1 x 10 ml desalting cartridge, *E. coli* lysate, glutathione reagent

Profinia Reagents

620-0203	**His Antibody**, 100 µl, 1 mg/ml
620-0204	**GST Antibody**, 100 µl, 1 mg/ml
620-0233	**His and GST Purification *E. coli* Control Lysate**, Lyophilized
620-0202	**Glutathione Pack**, 1.23 g

Catalog #	Description

Bio-Scale™ Mini Affinity and Desalting 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
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
732-4600	**Bio-Scale Mini Affi-Prep Protein A Cartridges**, 5 x 1 ml
732-4602	**Bio-Scale Mini Affi-Prep Protein A Cartridge**, 1 x 5 ml
732-4200	**Bio-Scale Mini UNOsphere SUPrA Cartridge**, 1 x 1 ml
732-4201	**Bio-Scale Mini UNOsphere SUPrA Cartridges**, 5 x 1 ml
732-4202	**Bio-Scale Mini UNOsphere SUPrA Cartridge**, 1 x 5 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

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Web site *www.bio-rad.com* ***USA*** *800 4BIORAD*
Australia *61 02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11*
Brazil *55 21 3237 9400* ***Canada*** *905 364 3435* ***China*** *86 21 6426 0808*
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