

## AFFINITY PURIFICATION

# Profinia™ Protein Purification System

- Reagents and buffers are preformulated to eliminate guesswork and ensure consistent results every time
- Kits provide the ultimate convenience once a method has been chosen
- Accessories extend the flexibility of the Profinia system under a variety of working conditions and facilitate routine maintenance

## Profinia Consumables (Kits, Buffers, and Reagents) and Accessory Items Are Designed With Time in Mind

### Introduction

Profinia consumables (Figure 1) are an entire suite of purification and buffer kits (Table 1) and individually sold buffers, reagents, and accessory items that complement the Profinia protein purification system. Designed to obviate the inconsistencies and time-consuming aspects inherent in manual methods of purifying affinity-tagged proteins, Profinia consumables are dependable, replenishable elements of the Profinia system. Use them confidently for reproducible protein purification results.

Profinia consumables work seamlessly with the Profinia system to simplify the operation and execution of all steps associated with



Fig. 1. Profinia kits, buffers, and reagents.

affinity-tagged purification. Every detail — from buffer bottle label design to guide bottle placement to the ease with which the cartridges are installed to the inclusion of buffer bottle lids that protect against contaminant introduction — has been carefully thought out with high value to the researcher in mind.

Table 1. Profinia purification and buffer kit selection guide.

	Buffer Sets				Powdered Reagents		Bio-Scale™ Mini Cartridges	
	Control Lysate	Lysis and Wash	Desalting	Cleaning and Storage	Urea	Glutathione	Affinity	Desalting
<b>IMAC Kits</b>								
Native IMAC starter kit	•	•	•	•			•	•
Native IMAC purification kits		•	•	•			•	•
Denaturing IMAC purification kits*		•		•	•		•	
Native IMAC buffer kit		•	•	•				
Denaturing IMAC buffer kit*		•		•	•			
<b>GST Kits</b>								
GST starter kit	•	•	•	•		•	•	•
GST purification kits		•	•	•		•	•	•
GST buffer kit		•	•	•		•		
<b>Desalting Kits</b>								
Desalting purification kits			•	•				•
Desalting buffer kit			•	•				

\* Denaturing IMAC kits do not contain desalting cartridges or desalting buffer; dialysis is the recommended method for desalting, buffer exchange, and renaturing of proteins purified under denaturing conditions.



## Kits, Buffers, and Reagents

An extensive line of Profinia consumables is available for the most widely used purification methods, such as immobilized metal affinity chromatography (IMAC), glutathione S-transferase (GST), and desalting. Cartridges, buffers, and reagents are specially formulated to work with the instrument's preprogrammed Bio-Rad methods to carry out equilibration, binding, washing, and elution steps, as well as maintenance steps, such as cartridge cleaning and storage. Kits are available in three main configurations (Figure 2):

- Profinia purification kits
- Profinia starter kits
- Profinia buffer kits

Profinia purification kits are the most complete of the consumables kits. They contain one set of 5 ml cartridges or two sets of 1 ml Bio-Scale Mini cartridges for IMAC or GST operations, application-specific purification buffers, and cleaning and storage reagents. Purification kits for IMAC and GST applications are also supplied with desalting cartridges and buffers. Profinia starter kits facilitate system setup and initial purification; these kits contain one set of Bio-Scale Mini cartridges and application-specific buffers and reagents but are also supplied with a control protein lysate. Profinia buffer kits are an appropriate choice if only additional buffers and cleaning and storage reagents are required. All kits are optimally formulated and ready to go to minimize the time required to obtain a purified sample. The reagents are made with chromatography-grade solutions and are prefiltered through a 0.2 µm filter to certify the best possible results.

## Performance Results

Results obtained using Profinia consumables are comparable to those seen with other commercially available methods of affinity-tagged purification, such as gravity-flow kits and low-pressure chromatography instruments (Table 2). Request bulletins 5513, 5514, and 5539 for performance data. Premade buffers and reagents (see ordering information) add convenience, and help to lower concerns associated with purification procedures overall. For example, the bacterial lysis/extraction reagent

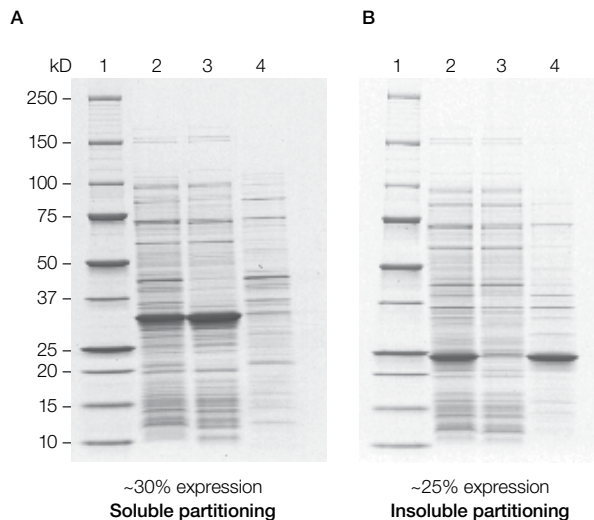


Fig. 2. Profinia kit configurations.

and premeasured urea packs simplify the steps of cell lysis, protein extraction, and solubilization. The reagent replaces mechanical lysis using sonication with a detergent-based lysis step that results in the fractionation of proteins into soluble and insoluble fractions. The gel panels in Figure 3 illustrate the partitioning profiles of two independent *E. coli* proteins. When the lysis/extraction reagent is used followed by brief centrifugation, soluble proteins are efficiently extracted into the soluble fraction and are ready for purification with the Profinia system (panel A, lane 3). Insoluble proteins found in the pellet are efficiently solubilized with buffers containing 6 M urea (panel B, lane 3) and are ready for purification using the denaturing IMAC kits.

**Table 2. Yield and purity data of the 51 kD protein.** The protein was purified and desalted with the Profinia instrument and consumables (IMAC or GST starter kit), a low-pressure chromatography system with its manufacturer's buffers, and manually using an affinity gravity-flow and desalting spin kit with appropriate buffers.

Purification System	Method	Affinity Time (min)	Desalting Time (min)	Average Yield (mg)	Average Purity (%)
Profinia system — IMAC starter kit and Profinia instrument	IMAC and desalting	34 combined		7.0	96.6
Low-pressure system — IMAC cartridges and His buffer kits	IMAC and desalting	55	20	7.3	96.7
Profinia system — GST starter kit and Profinia instrument	GST and desalting	50 combined		4.76	96.2
Manual — gravity-flow and spin column kits	GST and desalting	90		4.34	96.1

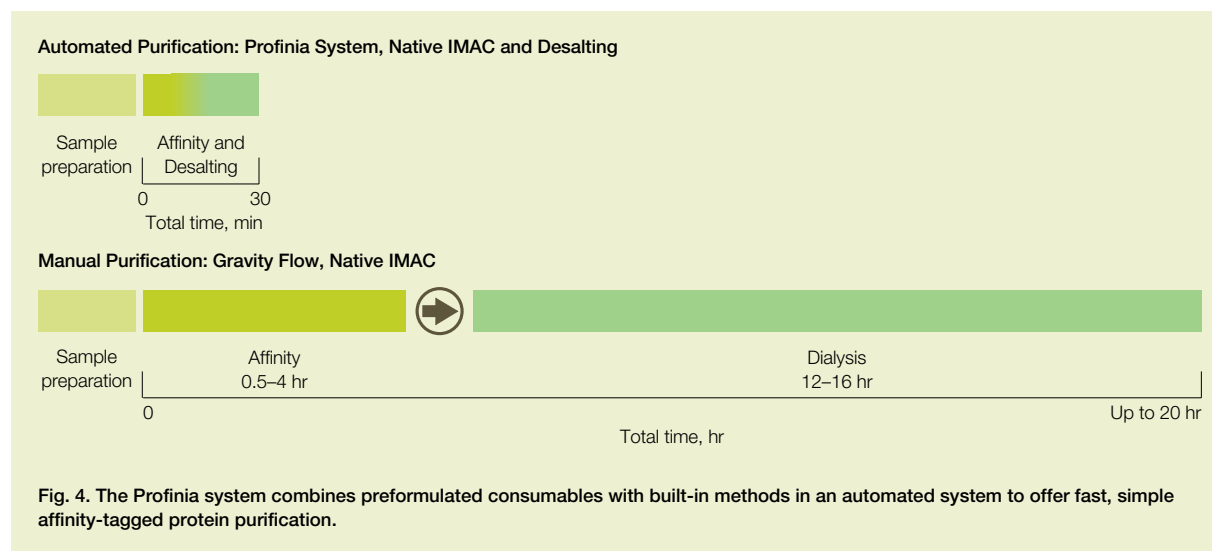


**Fig. 3. Use of Profinia chemical lysis/extraction and denaturing reagents to generate partitioning profiles.** In both gels: lane 1, Precision Plus Protein™ standards; lane 2, total protein fractions; lane 3, soluble protein fractions; lane 4, insoluble protein fractions. **A**, a 32 kD target protein partitions into the soluble fraction and can be purified using the native IMAC method. **B**, a 23 kD target protein partitions into the insoluble fraction and can be purified using the denaturing method.

### Time Savings

The Profinia system, including consumables, is designed to remove guesswork and save time in purifying affinity-tagged proteins. The Profinia system delivers purified protein faster than traditional methods, which can take hours to complete (Figure 4). A representative listing of run times on the Profinia system using its complementary consumables follows:

- Native IMAC: ≥10 mg of protein in about 30 min
- Denaturing IMAC: ≥10 mg of protein in about 20 min
- GST purification: ≥11 mg of protein in about 50 min
- Desalting: ≥90% recovery in about 15 min



**Fig. 4. The Profinia system combines preformulated consumables with built-in methods in an automated system to offer fast, simple affinity-tagged protein purification.**

Individually packaged reagent-based products are also available for use with the Profinia system. While designed specifically to work in accord with the Profinia system, these reagent-based products also have general applicability within all stages of a purification workflow, from sample preparation to protein detection.



#### **Profinia Bacterial Lysis/Extraction Reagent**

The bacterial lysis/extraction reagent contains a proprietary, mild, nonionic detergent to lyse cells chemically and is used for soluble protein extraction or inclusion body purification.



#### **Profinia Bottled Buffers**

For added convenience, preformulated buffers are available for native IMAC, denaturing IMAC, GST, and desalting purifications.



#### **Profinia Control Lysate**

The Profinia control lysate is a lyophilized, dual-tagged 51 kD target protein that is meant to eliminate concerns over variability in the purification buffer solutions or in the purification matrix itself. The control lysate facilitates system setup and initial purification and is included in every Profinia IMAC and GST starter kit.



#### **Urea and Glutathione Reagents**

Powdered reagents such as urea and glutathione are available for use with denaturing IMAC and GST applications, respectively. The urea packs (2 x 45 g or 2 x 90 g) are ideal for solubilizing and denaturing proteins in *E. coli* extracts. The glutathione pack (1.23 g) is essential for eluting GST fusion proteins from immobilized glutathione resins.



#### **Histidine (His) and GST Antibodies**

His-tagged and GST-tagged monoclonal antibodies are used to detect target protein expression of overexpressed 6x His and GST fusion proteins, respectively. They are supplied at a concentration of 1 mg/ml in phosphate buffered saline, pH 7.4 with 0.05% NaN<sub>3</sub>.

## Profinia Accessories

A collection of supporting products are available for use with the Profinia system. Profinia system accessories include the instrument accessory kit, pH monitor kit, instrument cooling accessory, desalting sample loops, sipper tube replacement kit, and inline filter pack. Some of these items are supplied with the Profinia instrument, and the others are available to support routine maintenance operations.

### Instrument Accessory Kit

Included with every Profinia instrument, the instrument accessory kit has the necessary plasticware to operate and complete purification runs. The kit includes buffer bottles (125 ml and 250 ml) with lids, waste and diluent bottles, a cleaning tray, an inline filter pack, and 15 ml and 50 ml sample tube lids.

### pH Monitor Kit

The pH monitor kit includes the pH probe, flow cell, and all tubing and accessories required to monitor pH conditions during a sample run. pH monitoring is fully supported by the Profinia system and optional Profinia software, and data are automatically captured during each purification run.

### Instrument Cooling Accessory

Temperature-sensitive proteins can be purified on the benchtop using the cooling accessory, which will maintain samples and fractions at 2–8°C for 5–6 hours at ambient temperature. The cooling accessory is filled with water and frozen.

### Desalting Sample Loops

Desalting methods are designed for rapid desalting or buffer exchange applications and require that a desalting loop accessory be used in conjunction with a desalting cartridge. A syringe and three-way stopcock combination is used to fill the desalting loop, which is available in 2 ml and 10 ml sizes. Its luer fittings allow easy installation onto the Profinia instrument.

### Sipper Tube Replacement Kit

The sipper tube replacement kit consists of ten pieces of precut, chemically compatible polypropylene tubing to be used during general maintenance of the Profinia system. When the instrument is running, these tubes serve to pull liquid out of either the sample tubes or the buffer bottles.

### Inline Filter Pack

The inline filter pack consists of 12 filters and is also part of the Profinia system's general maintenance regimen. The filters help to reduce clogged lines and minimize high backpressure. The filters should be replaced at regular intervals to keep the system running at its optimal level.

### Storage, Shelf Life, and Stability

Profinia consumables should be stored at the temperatures specified on the product labels of each of the kits or reagents. Outer packaging and instruction manuals also provide exact expiration dates for buffers and reagents. All Profinia consumables are guaranteed for up to one year after the date of manufacture. Use Table 3 as a guide to ensure reproducible results.

**Table 3. Storage and use conditions for Profinia system consumables.**

Consumable	Storage	Use/Comments
<b>Purification and Buffer Kits</b>		
IMAC (native and denaturing)	4–22°C	Room temperature
GST	4°C	Recommended for long-term storage
Desalting	4–22°C	Room temperature
<b>Starter Kits</b>		
IMAC and GST	4°C	Recommended for long-term storage
<b>Profinia Bottled Buffers</b>		
IMAC (native and denaturing), GST, and desalting	4–22°C	Room temperature
<b>Reagents</b>		
Bacterial lysis/extraction reagent	4–22°C	Room temperature
His antibody	–20°C	Working solutions can be stored at 4°C for up to 3 months
GST antibody	–20°C	Working solutions can be stored at 4°C for up to 3 months
Control lysate	4°C	Rehydrated lysate provides materials for two 1 ml affinity runs. The prepared lysate can be stored at –20°C for up to 6 months and thawed once prior to use
Urea packs	4–22°C	When added to denaturing IMAC buffers, urea-based solutions should be stored at 4°C and used within 7 days, or stored at –20°C for up to 6 months and thawed once prior to use
Glutathione	4°C	Fresh glutathione solutions should be prepared and used the day of chromatography. Unused solutions should be stored at 4°C and used within 7 days, or stored at –20°C for up to 6 months and thawed once prior to use

## Ordering Information

Catalog #	Description	Catalog #	Description
<b>Profinia Instruments</b>			
620-1005	<b>ProfiniaInstrument With Accessory Kit and Native IMAC Starter Kit</b> , 100–240 V	620-0221	<b>ProfiniaNative IMAC Buffer Kit</b> , includes purification buffers, cleaning and storage solutions; sufficient for 10 applications
620-1006	<b>ProfiniaInstrument With Accessory Kit and GST Starter Kit</b> , 100–240 V	620-0222	<b>ProfiniaDenaturing IMAC Buffer Kit</b> , includes purification buffers, cleaning and storage solutions, urea reagent; sufficient for 10 applications
<b>Profinia Systems</b>			
620-1010	<b>ProfiniaProtein Purification System With Native IMAC Starter Kit</b> , 100–240 V, includes same as 620-1005 with Profinia software	620-0223	<b>ProfiniaGST Buffer Kit</b> , includes purification buffers, cleaning and storage solutions, glutathione reagent; sufficient for 10 applications
620-1011	<b>ProfiniaProtein Purification System With GST Starter Kit</b> , 100–240 V, includes same as 620-1006 with Profinia software	620-0224	<b>Profinia Desalting Buffer Kit</b> , includes purification buffers, cleaning and storage solutions; sufficient for 10 applications
<b>Profinia Systems With Computers</b>			
620-1015	<b>ProfiniaProtein Purification System With Computer and Native IMAC Starter Kit</b> , 100–240 V, includes same as 620-1010 with computer	<b>Profinia Starter Kits</b>	
620-1016	<b>ProfiniaProtein Purification System With Computer and GST Starter Kit</b> , 100–240 V, includes same as 620-1011 with computer	620-0229	<b>Profinia Native IMAC Starter Kit</b> , includes Profinia native IMAC buffer kit, 1 x 1 ml IMAC and 1 x 10 ml desalting cartridge, <i>E. coli</i> lysate
<b>Profinia Accessories</b>			
620-0010	<b>ProfiniaSoftware</b> , includes USB cable	620-0230	<b>Profinia GST Starter Kit</b> , includes Profinia GST buffer kit, 1 x 1 ml GST and 1 x 10 ml desalting cartridge, <i>E. coli</i> lysate, glutathione reagent
620-0401	<b>ProfiniaInstrument Cooling Accessory</b> , includes 2 cooling units	<b>Profinia Reagents</b>	
620-0402	<b>Profinia Desalting Sample Loop</b> , 2 ml	620-0220	<b>Profinia Bacterial Lysis/Extraction Reagent</b>
620-0403	<b>Profinia Desalting Sample Loop</b> , 10 ml	620-0203	<b>Profinia His Antibody</b>
620-0404	<b>Profinia Instrument Inline Filter Pack</b> , includes 12 filters	620-0204	<b>Profinia GST Antibody</b>
620-0405	<b>ProfiniaSipper Tube Replacement Kit</b> , includes 10 pieces of precut tubing	620-0233	<b>Profinia Control Lysate</b>
620-0410	<b>ProfiniaInstrument Accessory Kit</b> , includes cleaning tray, inline filter pack, 2 x 50 ml sample lids, 2 x 15 ml sample lids, bottle starter pack, waste/diluent bottle set	620-0200	<b>Profinia Small Urea Pack</b> , 2 x 45 g
620-0231	<b>BottleStarter Pack</b> , includes 4 x 125 ml buffer bottles, 4 x 250 ml buffer bottles, 8 buffer bottle lids	620-0201	<b>Profinia Large Urea Pack</b> , 2 x 90 g
620-0411	<b>ProfiniapH Monitor Kit</b> , includes pH electrode, flow cell, mounting accessories	620-0202	<b>Profinia Glutathione Pack</b> , 1.23 g
<b>Profinia Purification Kits</b>			
620-0225	<b>ProfiniaNative IMAC Purification Kit</b> , 1 ml, includes Profinia native IMAC buffer kit, 2 x 1 ml IMAC and 2 x 10 ml desalting cartridges	<b>Bio-Scale Mini Affinity and Desalting Cartridges</b>	
620-0235	<b>ProfiniaNative IMAC Purification Kit</b> , 5 ml, includes 2 Profinia native IMAC buffer kits, 1 x 5 ml IMAC and 1 x 50 ml desalting cartridge	732-4610	<b>Bio-Scale Mini Profinity™ IMAC Cartridges</b> , 5 x 1 ml
620-0227	<b>Profinia Denaturing IMAC Purification Kit</b> , 1 ml, includes Profinia denaturing IMAC buffer kit, 2 x 1 ml IMAC cartridges	732-4612	<b>Bio-Scale Mini Profinity IMAC Cartridge</b> , 1 x 5 ml
620-0237	<b>ProfiniaDenaturing IMAC Purification Kit</b> , 5 ml, includes 2 Profinia denaturing IMAC buffer kits, 1 x 5 ml IMAC cartridge	732-4614	<b>Bio-Scale Mini Profinity IMAC Cartridges</b> , 5 x 5 ml
620-0226	<b>ProfiniaGST Purification Kit</b> , 1 ml, includes Profinia GST buffer kit, 2 x 1 ml GST and 2 x 10 ml desalting cartridges	732-4620	<b>Bio-Scale Mini Profinity GST Cartridges</b> , 5 x 1 ml
620-0236	<b>ProfiniaGST Purification Kit</b> , 5 ml, includes 2 Profinia GST buffer kits, 1 x 5 ml GST and 1 x 50 ml desalting cartridge	732-4622	<b>Bio-Scale Mini Profinity GST Cartridge</b> , 1 x 5 ml
620-0228	<b>ProfiniaDesalting Purification Kit</b> , 10 ml, includes Profinia desalting buffer kit, 2 x 10 ml desalting cartridges	732-4624	<b>Bio-Scale Mini Profinity GST Cartridges</b> , 5 x 5 ml
620-0238	<b>ProfiniaDesalting Purification Kit</b> , 50 ml, includes 2 Profinia desalting buffer kits, 1 x 50 ml desalting cartridge	732-4502	<b>Bio-ScaleMini Bio-Gel® P-6 Desalting Cartridge</b> , 1 x 5 ml
		732-4504	<b>Bio-ScaleMini Bio-Gel P-6 Desalting Cartridges</b> , 5 x 5 ml
		732-5304	<b>Bio-ScaleMini Bio-Gel P-6 Desalting Cartridges</b> , 5 x 10 ml
		732-5312	<b>Bio-ScaleMini Bio-Gel P-6 Desalting Cartridge</b> , 1 x 50 ml
		732-5314	<b>Bio-ScaleMini Bio-Gel P-6 Desalting Cartridges</b> , 5 x 50 ml

Purification and preparation of fusion proteins and affinity peptides containing 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: Hoffmann-La Roche).

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

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