Caution!

Please read all safety-related information before operating the Profinia system.

This instrument is for laboratory use only.

Always connect the power supply to a 3-prong, grounded AC outlet using the AC power cord provided with the Profinia instrument. Do not use an adaptor to connect to a 2-terminal outlet.

The right-side back panel is easily removable for access to user-serviceable parts. Never remove any other outer casings of the Profinia instrument. Call your local Bio-Rad office for instrument service.

Do not operate the Profinia instrument in extreme humidity (>95%), or in a condensing environment.

When taking the Profinia instrument into a cold room, the unit can be operated immediately. However, when removing the unit from the cold room, let it warm up to room temperature for a minimum of 2 hours before switching on the power. This avoids damage from possible condensation.

The Profinia instrument is not designed for use with flammable liquids. Flammable liquids should never be placed in either the buffer or sample inlets of the instrument.

This product conforms to Class A standards for Electromagnetic Emissions intended for laboratory equipment applications. It is possible that emissions from this product may interfere with some sensitive appliances when placed nearby or on the same circuit as those appliances. The user should be aware of this potential and take appropriate measures to avoid interference.

This Bio-Rad instrument is designed and certified to meet EN-61010 safety standards; EN-61010-certified products are safe to use when operated in accordance with this instruction manual.

This instrument should not be modified or altered in any way. Alteration of this instrument will void the manufacturer’s warranty, void EN-61010 certification, and create a potential safety hazard for the user.

Bio-Rad is not responsible for any injury or damage caused by the use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad or an authorized agent.
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Section 1
Profinia™ Protein Purification System Description

1.1 Profinia System Overview
The Profinia protein purification system is an automated chromatography system that provides a simple solution for the purification and desalting of milligram amounts of affinity-tagged proteins. The Profinia system is easy to operate, allowing users to concentrate on solving the questions posed by science, rather than spending time performing separation techniques. In the Profinia system, the cartridges, tubes, fittings, and Bio-Rad methods have all been optimized and are built in, so no expertise in chromatography is required to set up the instrument or perform affinity purification.

The Profinia system requires minimal user interaction; a user-friendly touch screen provides guidance through all setup, purification, and run completion procedures. The yield and purity of proteins obtained using the Profinia system are comparable to those from more complicated or labor-intensive manual methods such as gravity-flow chromatography. Users have two options for running purification methods: Bio-Rad methods with automated templates for common GST, IMAC, Protein A and G purifications, Profinity eXact purification or program methods created by editing the Bio-Rad method templates. Both Bio-Rad and program methods include automatic desalting (not all Bio-Rad methods include desalting) as well as automatic system and cartridge cleaning. Both types of methods also provide a data record of results that can either be viewed and recorded at the end of the run, exported to a computer with Profinia software for data review, or viewed in real time on a computer with Profinia software.

A variety of purification, buffer, and starter kits are available for use with the Profinia instrument. Profinia purification kits include cartridges, concentrated reagents, and buffers, and are optimized for use with Bio-Rad methods. Profinia buffer kits include all concentrated reagents necessary to run a Bio-Rad method. Profinia starter kits are available for GST and native IMAC methods. These kits include cartridges, concentrated reagents, and control lysate to perform a Bio-Rad method. All kit components are clearly labeled to show where they should be placed in the instrument, and formulations of all buffers are listed for use in reports.

Buffer and cartridge kits, optimized methods, and instrument automation ensure high quality and reproducible purification purity and yield, along with significant time savings. From setup to purification runs to exportable data files, the Profinia system is intuitive and easy to operate, allowing researchers at any level of chromatography expertise to move quickly and efficiently through purifying affinity-tagged proteins to downstream protein-based experiments.

1.2 Key Features of the Profinia System
- **Integrated system design** — no need to assemble tubing and fittings or pack chromatography columns
- **Easy instrument setup** — plug in the instrument and load reagents
- **Simple system operation** — automated purification and desalting of affinity-tagged proteins
- **Automatic calculations** — estimated purified protein yield and concentration displayed at end of run
- **Touch-screen interface** — guided instrument and method setup; displays current run information
• **Built-in cleaning and storage steps** — automated system cleaning ensures continued peak instrument performance

• **Purification kits** — affinity and desalting cartridges, buffers, and solutions optimized for Bio-Rad methods

• **Optional cooling accessory** — samples and fractions of purified samples stay cold without keeping the instrument in a cold room or using a cold box

• **Optional Profinia software** — real-time data and customized reports can be displayed, used for basic protein comparisons and analysis, and incorporated into lab notebooks and presentations

### 1.3 System Components and Optional Accessories

The Profinia protein purification system components (see Figure 1.1) are:

**Profinia instrument** — shipment box #1

**Profinia accessory kit** — shipment box #2; includes:

• **Profinia buffer bottle starter pack** — 4 x 125 ml and 4 x 250 ml empty bottles

• **Profinia buffer lids** — pack of eight lids

• **Profinia lids for 15 ml sample conical tubes** — pack of two lids

• **Profinia lids for 50 ml sample conical tubes** — pack of two lids

• **Profinia waste/diluent (water) bottle pack** — 2 x 2 L bottles

• **Profinia cleaning tray** — placed in the fraction collection area to collect liquid by products from line cleaning and system wash procedures

• **Inline filter** — 12 replacement filters

**Profinia starter kit** — shipment box #3; choice of native IMAC or GST starter kit, includes 1 ml affinity cartridge, 10 ml desalting cartridge, vial of Profinia control lysate, and a complete buffer set for native IMAC or GST purification

---

![Fig. 1.1. Profinia purification system components.](image)
The following accessories and components can be ordered separately for use with the Profinia system:

- **Profinia control lysate** — dual-tag (histidine and GST) protein that can be used to validate the performance of the Profinia system
- **Profinia software** — provides data reporting and basic analysis of run results
- **Cooling accessory** — holds two 15 ml or 50 ml conical tubes (holds two samples and/or two purified protein fraction tubes), and is designed to keep samples or purified protein cooled below 4°C when a cold room environment is not available
- **Profinia purification kits** — prepackaged cartridges, buffers, and reagents, for use with Bio-Rad methods. Bottles and accessories are clearly labeled to indicate positioning in the Profinia instrument (see Table 1.1)
- **Profinia buffer kits** — prepackaged buffer and reagents for use with Bio-Rad methods; cartridges are not included. Bottles and accessories are clearly labeled to indicate positioning in the Profinia instrument (see Table 1.1)
- **Bio-Scale™ Mini cartridges** — IMAC, GST, Protein A cartridges (1 ml and 5 ml), Profinity eXact cartridges (1 ml and 5 ml), and desalting cartridges (10 ml and 50 ml)
- **Desalting sample loops** — required for use in desalting methods only; available in 2 ml and 10 ml sizes. This accessory is not required for Bio-Rad methods that include affinity and desalting (for example, native IMAC and desalting or GST and desalting)
- **Bottle starter pack** — replacement empty buffer bottles (4 x 125 ml and 4 x 250 ml buffer bottles, eight buffer lids)
- **Waste/diluent bottle set** — replacement waste and diluent bottles with caps and tubing

Table 1.1. Types of Profinia purification and buffer kits.

<table>
<thead>
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<th>Method</th>
<th>Purification Kit</th>
<th>Buffer Kit</th>
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<td>Native IMAC*</td>
<td>1 ml or 5 ml affinity cartridge plus 10 ml or 50 ml desalting cartridge configurations</td>
<td>Buffers and reagents for native IMAC and desalting methods</td>
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<tr>
<td>Denaturing IMAC</td>
<td>1 ml or 5 ml affinity cartridge configurations; desalting does not apply to this kit</td>
<td>Buffers and reagents for denaturing IMAC methods</td>
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<tr>
<td>GST**</td>
<td>1 ml or 5 ml affinity cartridge plus 10 ml or 50 ml desalting cartridge configurations</td>
<td>Buffers and reagents for GST and desalting methods</td>
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<tr>
<td>Desalting</td>
<td>10 ml or 50 ml cartridge configurations</td>
<td>Buffers and reagents for desalting-only methods</td>
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* IMAC = immobilized metal affinity chromatography.

** GST = glutathione S-transferase.
1.4 Related Consumables and Products

The following related products are available from Bio-Rad (see Appendix B for a list of ordering information and catalog numbers):

- **Detection antibodies** — used for histidine (His) and glutathione S-transferase (GST)
- **Bacterial/Lysis/Extraction reagent** — chemical lysis buffer used to prepare sample lysates; without the need for sonication. This reagent is not used in the instrument
- **Profinia control lysate** — lyophilized sample prepared with a His- and GST-tagged protein from *E. coli*
- **Profinia eXact Antibody Reagent** — a mouse monoclonal antibody used to detect expression of the target protein

The following related products are available from other vendors:

- **Conical tubes** — 15 ml and 50 ml, for use in the sample and fraction collection positions

**Note:** Not all tubes made by all vendors will fit properly in the Profinia instrument; Bio-Rad recommends the following vendor tubes: 50 ml, VWR catalog #21008-178 and #20171-029 and BD Biosciences catalog #352098; 15 ml, VWR catalog #20171-025 and BD Biosciences catalog #352097.

1.5 About This Guide

1.5.1 Conventions

The following messages in bold are used to highlight critical information and reminders throughout this document:

- **Warning** — indicates an action that can result in an irretrievable loss of sample or data
- **Note** — serves as a reminder, repeating relevant information from previous sections or offering advice helpful to achieving desired results
- **Important** — indicates information critical to achieving optimal results

Touch-screen button commands are indicated by initial capitalization, for example: Touch the Select button.

Screen names are indicated by initial capitalization, for example: You will proceed to the Data/Utilities screen.

Functions located within menus on touch screens are indicated by initial capitalization and enclosed in double quotation marks, for example: Highlight the “System Ethanol Wash” option.
Section 2
Installation Procedures

2.1 System Installation

The Profinia™ purification system is shipped in three boxes. The largest box contains the instrument wrapped in foam-insert protective packaging; the smaller boxes contain all the additional components sold as part of the Profinia system.

Warning: Use care to follow the instrument unpacking procedures described below. Improper handling of the instrument can cause damage.

To unpack and set up the Profinia system:

1. Open the smaller boxes containing the system components and make sure all parts were received (see section 1.3 for a list of all components included with the Profinia purification system).

2. Set the larger box containing the Profinia instrument on the floor with the arrows pointing up (Figure 2.1).

3. Use scissors or wire cutters to cut the plastic straps.

4. Use a large screwdriver or similar tool to remove the staples (if present) along the sides, near the bottom of the box.

Fig. 2.1. Profinia instrument box.
5. Use the handles cut into the side of the top section of the box to lift it off the instrument (Figure 2.3).

6. Remove the protective packaging from the instrument (Figure 2.3).

**Note:** Do not grasp the instrument by the top cover; damage to the instrument can result from mishandling. Note the “No Lift” labels that hold the top cover in place.

7. Use the handles on the left and right rear of the instrument to lift it onto the benchtop (Figure 2.4).
8. Connect the power cable to the back of the instrument (see Figure 2.5 for location of power cord connection).

9. Install the diluent (water) and waste bottle tubing by unscrewing the shipping plugs from the deionized (Di) water and waste ports on the back of the instrument; connect the diluent and waste bottle tubing to these ports (Figure 2.5).

**Note:** The 2 L bottles packaged with the accessories include both “Water” and “Waste” labels. Determine which bottle will be used for which function, then use a permanent marker to cross out the other label. This will avoid confusion and possible contamination.
2.2 First-Time Use of the Profinia System

Before using the Profinia system, perform buffer, sample, and fraction line cleaning procedures to flush the system and remove any air trapped in the lines.

2.2.1 Preparing the Instrument for Cleaning

To prepare the Profinia system for cleaning prior to its first use:

1. Remove the plastic bag covering the Profinia instrument and remove the “No Lift” stickers from the top cover.

2. Turn the instrument around so the front faces you, and turn the system on using the switch on the lower right in front of the diluent (water) bottle.

3. Open the instrument top cover. Remove the plastic caps from the ends of the buffer and sample tube lines.

Fig. 2.6.a. Profinia system ports without bottles and tubes; 2.6.b. Profinia cleaning tray placement in the fraction collection area.

Note: For detailed instructions on installing reagent bottles, sample tubes, fraction tubes, and using cartridge connections, see section 6.4 and 6.5.

To prepare the Profinia system for cleaning prior to its first use:

1. Remove the plastic bag covering the Profinia instrument and remove the “No Lift” stickers from the top cover.

2. Turn the instrument around so the front faces you, and turn the system on using the switch on the lower right in front of the diluent (water) bottle.

3. Open the instrument top cover. Remove the plastic caps from the ends of the buffer and sample tube lines.
4. Fill the four 125 ml and three of the 250 ml buffer bottles provided with the instrument to capacity with deionized water, and place them in buffer positions 1 through 7.

5. Fill one buffer bottle with 100 ml of 20% ethanol and place it in buffer position 8.

6. Fill two 50 ml conical tubes with 25 ml of deionized water each, and place them in the sample 1 and 2 (S1 and S2) positions.

**Note:** Tubes containing water must be placed in both sample positions during line cleaning, even after performing a run with only one sample (line cleaning only).

7. Locate the cartridge holder fittings at the left center of the instrument. Secure the upper cartridge holder fittings to the lower cartridge holder fittings by sliding the upper fittings down until they contact the lower fittings. Tighten the black lock ring on each fitting by turning it clockwise; do not overtighten (see section 6.5 for more details).

8. Place the cleaning tray in the fraction collection position (Figure 2.6).

9. Fill the diluent bottle with deionized water and place it in the right-side bottle position, with the tube in place.

10. Make sure the waste bottle is empty and place it in the left-side bottle position, with the tube in place.

**Note:** Make sure the waste bottle is empty before running any method or cleaning procedure.

### 2.2.2 Selecting the First-Time Cleaning Procedure

To flush the system and prepare it for first use:

1. Make sure the water and ethanol solutions are placed as described in steps 4 through 6 above, and the cleaning tray is in place, as described in step 8 above.

2. Touch the Data/Utilities button in the lower toolbar of the system home screen (see section 5 for information on home screen functions).

3. Touch the arrow buttons to scroll through the list of functions and highlight "Clean All Inlet and Outlet Lines" (Figure 2.7). Select Start to perform this procedure. A new screen will display the progress toward completion. When the cleaning procedure is complete, the system will return to the Data/Utilities screen.
It is recommended that this function be performed a second time prior to first use to remove any air that might have entered the system. The system has now been flushed with ethanol and water, and is ready for use.

2.2.3 Setting the Local Time and Date

During purification runs, sample elution time is shown according to the system clock time as set by the user. To set the system time and date:

1. Touch Data/Utilities from the system home screen.
2. Touch the Diag/Maint Functions button.
3. Touch Time and Date Setup.
4. Touch the arrows adjacent date and time settings to adjust as desired (Figure 2.8); touch OK when finished to retain new settings and return to the Diag/Maint Functions screen.

![Set Time & Date screen.](image)

2.2.4 Validating the System

Performing an initial validation run of the Profinia system is recommended. This will allow you to familiarize yourself with the set up, operation, and expected results of a purification run. Use the starter kit provided with the instrument; if you need a new starter kit or additional Profinia control lysate, see Appendix B for ordering information.

The starter kit includes a His/GST dual-tag control protein lysate to be used for this validation run. The results are characterized in this section. The dual-tag control lysate can be used with both the native IMAC and GST purification methods. To prepare and use the control lysate:

1. Prepare the sample lysate by reconstituting it with 12 ml of 1x IMAC or GST lysis buffer (dilute 6 ml of IMAC or GST lysis buffer 1:1 with 6 ml of deionized water to make 12 ml of a 1x solution). This yields enough lysate for two of the 1 ml affinity runs.
2. Transfer 6 ml of resuspended sample to a 15 ml conical tube.
3. Place the tube in the sample 1 position and select the “1 Sample” option in the appropriate run set-up screen.
4. Prepare the buffers and reagents as described in the starter kit instruction manual.
5. Follow the set-up steps in section 4 (quick guide) or in section 6 (Bio-Rad methods programming) of this manual to select the method and load the buffers, sample, cartridges, and fraction tubes.
6. Compare your results to the following expected results for the IMAC or GST purification of the control lysate.

**Expected Results for IMAC Purification of the Control Lysate**

![Fig. 2.9. Typical chromatogram of the native IMAC control lysate purification with desalting.](image)

**Fig. 2.9.** Typical chromatogram of the native IMAC control lysate purification with desalting.

![Fig. 2.10. Typical gel electrophoresis results of the native IMAC control lysate purification. Lane 1, Precision Plus™ MW standard; 2, load; 3, flow-through; 4, wash 1; 5, wash 2; 6, eluted protein.](image)
Fig. 2.11. Typical Experion™ system electropherogram of the purified fraction of the native IMAC control lysate purification.

Expected Results for GST Purification of the Control Lysate

Fig. 2.12. Typical chromatogram of the GST control lysate purification with desalting.
Fig. 2.13. Typical gel result of the GST control lysate purification. Lane 1, Precision Plus™ MW standards; 2, skip; 3, load; 4, flow-through; 5, wash; 6, eluted protein.

Fig. 2.14. Typical Experion system electropherogram of the purified fraction of the GST control lysate purification.
Section 3
Key Terms and Definitions

3.1 Bio-Rad Methods

The following automated methods — Bio-Rad methods — for common affinity-tagged protein purification and desalting procedures have been programmed into the Profinia™ protein purification system:

- **Native IMAC** — native conditions with His-tagged proteins; use the Profinia native IMAC purification kit
- **Native IMAC and Desalting** — native conditions with His-tagged proteins, followed by desalting before elution to the fraction tube; use the Profinia native IMAC purification kit
- **Denaturing IMAC** — denaturing conditions with His-tagged proteins; use the Profinia denaturing IMAC purification kit
- **GST** — native conditions with GST-tagged proteins; use the Profinia GST purification kit
- **GST and Desalting** — native conditions with GST-tagged proteins, followed by desalting before elution to the fraction tube; use the Profinia GST purification kit
- **Protein A and G** — affinity purification of antibodies (typically IgG) from serum, ascites, or hybridoma cell culture supernatant using Protein A (or in some cases, Protein G) chromatography media
- **Protein A and G and Desalting** — affinity purification of antibodies (typically IgG) from serum, ascites, or hybridoma cell culture supernatant using Protein A (or in some cases, Protein G) chromatography media followed by desalting
- **Profinity eXact** — affinity purification with on column tag removal for eXact affinity tagged proteins
- **Profinity eXact and Desalting** — affinity purification with on column tag removal for eXact affinity tagged proteins followed by desalting
- **Desalting** — buffer exchange and desalting of a protein solution; use the Profinia desalting kit and desalting sample loop accessory

*IMAC = immobilized metal affinity chromatography.

**GST = glutathione S-transferase.

3.2 Program Methods

Program methods can be customized using the Bio-Rad methods as templates. Method-step parameters, such as flow rates, column volumes, and automatic peak detection settings can be edited to optimize sample-specific purification conditions. Each program method can be saved with the generic template name or can be given a unique name. Up to 35 program methods can be saved in memory for future retrieval and use.
3.3 Toolbar Navigation Buttons

Toolbar navigation buttons are located at the bottom of each touch screen (Figure 3.1).

![Toolbar Navigation Buttons](image)

**Fig. 3.1. Toolbar navigation buttons for the Select Method Type & Options screen.**

The toolbar buttons are screen-specific and include the following common button commands:

- **Back** — returns to the previous screen and allows for sequential return to the home screen
- **Home** — returns directly to the home screen. All run and sample information will be reset, except lot number tracking information. A saved method will remain stored in memory; an edited method that is not saved will revert to original settings
- **i** — retrieves quick-reference information about the current screen
- **View Method** — displays a summary of method steps, with important information. Use the up and down arrow button to scroll through all the steps. A small arrow at the bottom right-hand corner of the method step list indicates additional steps not on the screen. The following information is displayed on the View Method screen:
  - **Step** — numbered list of the primary steps that will be performed in the run
  - **Name** — brief description of the operation associated with the step
  - **Conc.** — buffer position in the instrument and buffer concentration (the instrument dilutes concentrated buffers)
  - **ml/min** — flow rate displayed in ml/min
  - **CV** — column volume based on the cartridge size (for example, for a 1 ml IMAC cartridge, 2 CV = 2 ml)
  - **Min** — duration of each step in minutes

**View Steps** — displays the details of every event in the method; the operation currently in progress will be highlighted when accessed during a run in progress (Figure 3.2):

  - **Step** — numbered list of the primary steps that will be performed in the run
  - **Name** — brief description of the operation associated with the step
- **Conc.** — buffer position in the instrument and buffer concentration (the instrument dilutes concentrated buffers)
- **ml/min** — flow rate displayed in ml/min
- **CV** — column volume based on the cartridge size (for example, for a 1 ml IMAC cartridge, 2 CV = 2 ml)
- **Min** — duration of each step in minutes

![Fig. 3.2. View Method Steps screen during a 10 ml desalting method run.](image)

### 3.4 System Status Indicator Definitions
Status indicator symbols are displayed in the top right corner of the screen. The possible indicators are C, M, A, P, and F:

- **C** — operating temperature setting. Methods including desalting steps have different optimum flow rates at room temperature than when under cold room conditions. “C” indicates that the flow rate limits are set for cold room conditions, and will display only for methods including desalting when the method temperature is set for 4°C. The method temperature setting option is located in the Data/Utilities menu (section 9.3.13).

- **M** — data memory status. The Profinia system stores up to three run data files in memory. This symbol indicates that the three stored files have not yet been exported to a computer via a portable memory device. If none of the files have been transferred, the next run data file will overwrite the oldest stored file.

- **A** — end of reagent detection status. This indicates that some or all end of reagent detectors have been disabled. Select Data/Utilities to access the end of reagent detection.

- **P** — program mode status. This indicates that the program method was selected.

- **F** — method memory status. This indicates that method memory is full.

**Note**: It is possible that the system may display more than one of these symbols as appropriate during the course of a Bio-Rad or program method run.
3.5 Alphanumeric and Numeric Keypads

3.5.1 Alphanumeric Keypad

Text is entered into the alphanumeric keypad via a text line at the top of the screen (Figure 3.3). The alphanumeric keypad has the following toolbar functions:

- **Clear All** — clears all characters in the text line
- **Backspace** — clears the character directly preceding the cursor in the text line
- **Cancel** — returns to the previous screen without saving input data
- **OK** — accepts new entries and returns to the previous screen

**Note:** When the default “UNTITLED” appears in the text line, any keystroke will automatically clear it.

![Alphanumeric keypad](image)

**Fig. 3.3. Alphanumeric keypad.**

3.5.2 Numeric Keypad

Numbers are entered into the numeric keypad in the shaded editing bar at the upper left of the screen (Figure 3.4). The numeric keypad is displayed only when numeric information is required. When customizing a program method, the step parameter limits will display immediately below the editing bar when incorrect values are entered. The following functions are displayed in the toolbar:

- **Cancel** — returns to the screen in progress without saving input data
- **OK** — accepts new entries and returns to the screen in progress
Fig. 3.4. Numeric keypad.
Section 4
Profinia System Quick Guides

4.1 Unpacking and Installing the Profinia Protein Purification System

The Profinia protein purification system is shipped in three boxes. The largest box contains the instrument enclosed in protective foam packaging. The smaller boxes contain the Profinia accessory and starter kits.

1. Profinia System Shipment

2. Remove Straps on Box Containing the Instrument
   Cut and remove plastic straps. Remove staples (if present).

3. Remove the Box Lid
   Remove upper lid of box by using side handles.

4. Unpack the Instrument
   Remove protective foam packaging covering the instrument. The Profinia system instruction manual and quick guide are packed with the instrument.

5. Remove the Instrument
   Hold lift handles and bottom of instrument to remove from box.
6 Remove Stickers and Plugs
Place instrument on lab bench. Remove plastic bag. Remove four lift instruction stickers from instrument top cover and side panels. Gently remove 16 plugs from tubing (as shown). Store plugs for later use.

7 Install Water and Waste Bottles and Connect Power Cable
Remove plugs (2) from the deionized (DI) water and waste ports at the back of the instrument. Store plugs for later use. Fill and install the DI water bottle with black fitting and long tube and the waste bottle with white cap and short tube, and connect to the labeled ports above the bottles (as shown). Connect the power cable to the back of the instrument. Components are located in the accessory kit box.

8 Turn Instrument On
Press the power button located on the lower right side of instrument to the on position. The Home screen will display. Locate stylus in accessory box. Touch the Data/Utilities button on the screen.

9 Set Time and Date
Touch the Diag/Maint Functions button. Use the arrow keys to highlight the Set Time and Date function. Touch the Select button. Set the time and date, then touch OK.

10 Clean All Inlet and Outlet Lines
Use the arrow keys to highlight the Clean All Inlet and Outlet Lines function. Touch the Select button.

11 Perform Cleaning Function Setup
Read screen instructions to guide you through the setup. See steps 12–15 for detailed instructions on completing the procedures for the Clean All Inlet and Outlet Lines function.
12 Fill and Place Buffer Bottles
Fill seven buffer bottles (in accessory kit box) with 100 ml of DI water and place in positions 1–7. Fill one buffer bottle with 100 ml of 20% ethanol and place in position 8 (see step 14). Place buffer lids on bottles to prevent dust and other contaminants from entering the system.

13 Fill and Place Sample Tubes
Fill two conical sample tubes with 50 ml of deionized water. Place lids on tubes, then place in sample positions 1 and 2 (see step 14).

14 Place Cleaning Tray Into Position
Keep cartridge holders in the closed position. Instrument is now ready for cleaning.

15 Touch the Start Button

16 Repeat Clean All Inlet and Outlet Lines
It is recommended that this function be performed a second time prior to first use to remove any air that might have entered the system.

17 Remove Components
Remove cleaning tray, sample tubes, buffer bottles (positions 1–7) before performing a purification run.

18 Perform Purification Run
Use the Profinia starter kit to validate instrument. See the Profinia system quick guide or refer to section 6 in the instruction manual for instructions on running a Bio-Rad method. (For software installation instructions, see next page.)
Installing the Profinia Software

1. Open Profinia Software Box

2. Connect USB Cable
   For real-time data collection, connect USB cable first to instrument, then to computer, before installing software (USB port is located on the back of the Profinia instrument).

3. Insert Software CD into Computer

4. Install Software
   Follow screen instructions to install Profinia software.

5. Begin Operation
   Profinia software is now installed and ready to collect real-time data or view data files from the Profinia system.

**Minimum Computer Requirements**
- Windows XP or Windows Vista operating system
- 2 GHz Pentium 4 processor
- 512 MB RAM
- 1,024 x 768 screen resolution
- 80 GB hard drive
- CD-ROM drive
- USB 2.0 port
4.2 Profinia™ System Quick Guide

To start a preprogrammed purification run, touch Bio-Rad Methods button on the home screen.

Step 1: Select Method Type and Sample Information
1. Select the method system on the Select Method System screen.
2. Select the method type on the Select Method Type & Options screen.
3. Select sample loading and washing options on the Select Sample Flow Rate & Wash Time screen.
4. Enter information on the Enter Run & Sample Information screen.

Step 2: Install Reagents, Sample Tubes, and Fraction Tubes
Install bottles and tubes as indicated on the Install Reagents, Sample & Fraction Tubes screen.

2A. Installation of Reagents/Buffer Bottles
1. Get the reagents from the Profinia purification kit designed for the selected method.
2. Add any dry materials required to the reagents and filter; see purification kit manual for details.
3. Place a green buffer lid on each buffer bottle.
4. Open the top cover of the Profinia instrument to access sipper tubes and buffer placement tray.
5. Lift the sipper tube away from the instrument.
6. With the number code on the bottle label facing out, insert the bottle onto the sipper tube for each position.
7. Fill a 250 ml bottle with 20% ethanol and place it in position 8.
8. Close the Profinia top cover during operation.
2B. Waste and Water Bottles
1. Empty the waste container, and check the diluent water bottle for sufficient volume.

2C. Sample Tube Installation
1. Add sample to 15 ml or 50 ml conical tubes.
2. Place sample tube lids on the conical tubes.
3. Lift the sipper tube away from the instrument.
4. Insert the conical tube for each position.

2D. Fraction Tube Installation
1. Insert bottom of tube into the fraction tube holder indent.
2. Tilt the top of the tube into the top holder and push into place.

Numbering of Collection Tubes

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow-through</td>
<td>1A</td>
<td>2A</td>
</tr>
<tr>
<td>Wash – 1</td>
<td>1B</td>
<td>2B</td>
</tr>
<tr>
<td>Wash – 2</td>
<td>1C</td>
<td>2C</td>
</tr>
<tr>
<td>Purified protein</td>
<td>1D</td>
<td>2D</td>
</tr>
</tbody>
</table>

Correct installation of fraction collection tubes in positions 1A through 1D. Setup shown is for one sample, as indicated on the display screen diagram above.
Step 3: Cartridge Installation

If cartridges are not already installed, touch the NO button. You will proceed to the Setup Cartridge Positions screen.

If the cartridges required for the method are already installed in the instrument, touch the YES. You will proceed directly to the Start Run screen.

3A. Cartridge Line Priming

To connect cartridge holder fittings for priming:

1. Disconnect any cartridges.
2. Join the upper cartridge holder fittings directly to the lower cartridge holder fittings in positions 1 and 2.
3. Touch the Start button on the Setup Cartridge Positions screen to begin priming the cartridge lines.
   ■ Status is shown on the Priming In Progress screen
   ■ When priming is finished, the Install Cartridges screen appears

3B. Install Cartridges

1. Disconnect the upper and lower cartridge holder fittings.
2. Raise the upper cartridge holder fitting.
3. Remove the bottom cap from the affinity cartridge.
4. Position the cartridge in the lower cartridge holder fitting and rotate the cartridge clockwise. Tighten gently.
5. Remove the top cap from the cartridge. Move the upper cartridge holder down to the cartridge.

6. Twist the black collar to tighten the upper fitting onto the cartridge until snug.

Note:
Do not overtighten fittings. These fittings are designed to seal when turned until snug. The cartridge flange may be damaged by overtightening and may then leak. If leaking occurs, replace the cartridge.

7. Repeat this procedure to install a cartridge in the cartridge 2 position, if required.

8. When cartridge installation is complete, touch the Next button on the Install Cartridges screen. You will proceed to the Start Run screen.

Step 4: Start the Run

4A. Start Run Screen
1. To view data in real time on a computer with Profinia software, connect the instrument to a computer, and start Profinia software before starting the run.

2. Any externally connected reagents or samples should be manually primed before starting the run.

3. The View Steps button allows you to view detailed steps of the method.

4B. Run In Progress Screen
The Run In Progress screen displays progress of the run:
- S1 and S2 arrow marks indicate the approximate time for sample elution
- Expected elution time is displayed; the sample can be collected at this time
- The cleaning process is shown in the progress bar as the time portion after sample elution (S1 and S2)

Cleaning and Run Data
1. When elution is complete, proceed with instrument cleaning as indicated on the Profinia display.

2. Run data can be downloaded to a USB portable memory device through the USB port in the front of the instrument after the cleaning process.
Section 5
Home Screen Overview

The LCD touch screen on the front of the instrument is used to control Profinia™ system functions (Figure 5.1). You may use your finger or a stylus to operate the screen. The home screen displays date and time information and the UI firmware version number. The upper right corner of each set-up screen displays various status indicators (see section 3.4).

The toolbar at the bottom of the home screen displays five navigation buttons for the available user options: Bio-Rad Methods, Program Methods, Saved Methods, Data/Utilities, and i (information).

Note: The toolbar remains at the bottom of each system screen; however, button names and their respective functions will change to reflect the requirements of each screen.

![Profinia system home screen.](image)

Home screen toolbar options are:

**Bio-Rad Methods** — access preprogrammed method templates for affinity chromatography, with or without desalting steps. These methods include native and denaturing IMAC, GST purifications, Protein A and G purifications, Profinity eXact purifications, and desalting-only methods.

**Program Methods** — customize Bio-Rad method templates by editing the parameters of method steps. A program method can be saved to permanent memory.

**Saved Methods** — access saved program methods. Saved program methods (up to 35 at a time) can be retrieved and viewed by method type or as a single, alphabetical list.

**Data/Utilities** — retrieve run data files, calibrate the system, and run maintenance and diagnostic functions:

- **Data** — system memory stores up to three run data files; run data files are in .ofi format. Stored run data files are available for transfer via a USB portable memory device (USB flash drive). These run data files can then be imported into the Profinia software program (optional system accessory). Stored run data files that have been transferred to a USB portable memory device are indicated with an asterisk.
Warning: Overwritten run data files can no longer be retrieved; the data is permanently deleted.

- **Calibration** — system functions enable calibration of the touch screen, UV monitor, conductivity monitor, pH probe (optional accessory), as well as a pump flow rate check and adjustment of peak detection sensitivity (see section 9.2 for details)

- **Diagnostics/Maintenance** — display firmware version, manual operation, set time and date, clean sample lines, clean fraction lines, clean buffer lines, system ethanol wash, clean inlet and outlet lines, clean pump check valves, system NaOH wash, select end of sample/reagent detection, and select method temperature functions are built into the system (see section 9.3 for details)

i — retrieve help information for the current screen; the information button is available in the toolbar for most system screens. This information is for quick-reference purposes only. For more detailed information, refer to the appropriate section of this instruction manual.
Section 6
Bio-Rad Methods

The Bio-Rad Methods function of the Profinia™ system is used to select preprogrammed purification and desalting methods. These methods have been optimized on the Profinia instrument with Profinia purification kits to provide the highest purity and yield. Purification kits are available for all Bio-Rad methods that have been preprogrammed in the instrument. When a method type and the associated options are selected, the Profinia screen displays a method-specific diagram of the system that shows where each cartridge, bottle, and tube is placed on the instrument. Bottles and cartridges in the purification kits are labeled to match the screen diagrams.

The purification procedure used in Bio-Rad methods has the following steps:

1. Select method type and options.
2. Select sample flow rate, cartridge wash time, or incubation time.
3. Enter run and sample information.
4. Install reagents, sample, and fraction tubes.
5. Install cartridge(s).
7. Monitor run.
8. Clean sample and fraction lines.
9. Select and perform end-of-run options.
10. Shut down system or start a new method.

Instrument Operating Temperature

For Bio-Rad methods that include the desalting step, the instrument operating temperature is critical. Maximum flow rates for the desalting cartridges are lower at 4°C than at room temperature. Flow rates over maximum limits compress the bed volume and cause over-pressure errors. Make sure the operating temperature is set to match cold room or room temperature operating conditions before programming your run. Methods with desalting steps have longer run times at the cold room setting due to the lower maximum flow rates. The operating temperature setting displays as a status indicator in the upper right corner of the screen. If the cold room operating temperature is selected, “C” appears only when methods are selected that include a desalting step. If the room temperature setting is selected, “C” will not display. For affinity-only methods, the temperature setting is irrelevant and will not display. To set the operating temperature:

1. Touch the Data/Utilities button to display the system Utilities screen.
2. Touch the arrow buttons to navigate the list of system utilities; highlight “Select Method Temperature” then touch Select to display the Select Method Temperature screen (Figure 6.1).
3. Touch the arrow button to select “4°C” or “Room Temperature”, then touch the OK button to return to the system Utilities screen.

4. Touch the Home button to return to the system home screen and select the desired system programming option.

**Note:** Do not change the instrument operating temperature setting to cold room (indicated by a “C” in the upper-right of the touch screen) when using the cooling accessory. Keep the operating temperature setting at room temperature unless the entire instrument is operating in a cold room environment.
Figure 6.2 shows the screen sequence when starting a Bio-Rad method.

Fig. 6.2. Screen sequence when starting a Bio-Rad method.
To begin a Bio-Rad method purification procedure, touch the Bio-Rad Methods button in the lower toolbar of the home screen (Figure 6.3). You will proceed to the Select Method Type & Options screen.

Fig. 6.3. System home screen.

6.1 Selecting Method System
The Select Method system screen (Figure 6.4) allows you to choose:

- **Method system** — IMAC, GST, Protein A and G, Profinity eXact methods with or without desalting, or desalting only methods

6.2 Selecting Method Type and Options
The Select Method Type & Options screen (Figure 6.5) allows you to choose:

- **Method type** — The selected affinity method system or desalting only system
- **Number of samples** — program one or two samples (the desalting-only method can only be run with one sample)
- **Cartridge size** — based on sample volume and concentration
- **Number of cartridges** — available for affinity-only methods when two samples are selected. You may choose to use a single cartridge for both samples or a separate cartridge for each sample
To select the method type and options:

1. Touch the up and down arrows to select the method type from the list of options displayed on the screen.

**Note:** The name of the selected method type appears at the top of this screen and all subsequent screens.

2. Touch the arrow button to the right of the Sample list to select “1” or “2” samples.
**Note:** Two samples can be run for all methods except the desalting-only method. When two samples are selected for affinity-only methods, the number of cartridges option becomes available. You have the option to use a single cartridge for both samples or a separate cartridge for each sample. When two samples are selected for affinity with desalting methods, both samples are purified using the same cartridge set. Desalting-only methods are run with one sample that is applied to the cartridge via a sample loop placed in the cartridge 1 position. See section 6.6.5 for details on the installation and use of the desalting loop.

**Note:** When purifying two samples through the same cartridge, the method cleans and equilibrates the cartridge prior to loading the second sample.

3. Touch the arrow button to the right of the Cartridge Options list to select the appropriate cartridge size for your method. Select to install “1” or “2” cartridges when this option becomes available.

**Note:** The “1” or “2” cartridge selection option is only available with two-sample, affinity-only methods.

When finished setting the method type and options for the run, touch Next to proceed to the Select Sample parameters screen, or Select Incubation Time screen.

**Note:** Sample loading flow rate and wash time options are not required with the desalting-only method; you will proceed directly to the Enter Run & Sample Information screen (see Section 6.4).

### 6.3 Selecting Sample Options

#### 6.3.1 Selecting Sample Flow Rate and Wash Time

Each IMAC, GST, and Protein A and G Bio-Rad method is available with an option to select from two sample loading flow rates and two cartridge wash times. The Select Sample Flow Rate & Wash Time screen (Figure 6.6) is used to select the sample loading flow rate and wash time for optimum protein yields and purity (for example, with a high molecular weight protein, slower flow rates may improve protein binding efficiency and an extended wash time may improve protein purity). Flow rates and wash options can be selected separately for each sample (up to two) used in the run.

![Fig. 6.6. Select Sample Flow Rate & Wash Time screen with options displayed for a two-sample method.](image)
The Bio-Rad methods function provides two sample loading flow rate options:

- **Standard** — recommended for normal- to high-expression proteins (≤100 kD)
- **Low** — may improve the yield of low-expression proteins, high MW proteins (≥100 kD) or samples containing detergents

The Bio-Rad methods function provides two cartridge wash options:

- **Standard** — recommended for most purification conditions
- **Extended** — may improve the purity of product for some sample types

Parameters for each type of method are provided in Table 6.1.

**Table 6.1. Sample loading Flow rates and wash volumes by cartridge size for each method type option.**

<table>
<thead>
<tr>
<th>Method Type</th>
<th>Cartridge Size, ml</th>
<th>Sample Loading Flow Rate, ml/min</th>
<th>Wash-1 Column Volume (CV)</th>
<th>Standard</th>
<th>Low</th>
<th>Standard</th>
<th>Extended</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMAC, Native</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>6</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>2.5</td>
<td>6</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMAC, Denaturing</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>6</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10</td>
<td>2.5</td>
<td>6</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST, Protein A and G</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>12</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>2.5</td>
<td>12</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eXact</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>14</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>5</td>
<td>5</td>
<td>–</td>
<td>10</td>
<td>–</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To select the flow rate(s) and wash time(s) for your sample(s):

1. Touch the arrow button to select either the “Standard” or “Low” flow rate for each sample.
2. Touch the arrow button to select either the “Standard” or “Extended” wash option for each sample.

**Note:** If a method will be performed with two samples, make flow rate and wash time selections separately for each sample.

When the method options are selected, touch the Next button to proceed to the Enter Run & Sample Information screen. A View Method button will be available in the lower toolbar to view the individual method steps for the sample(s).

6.3.2 Selecting Sample Incubation Time

Each eXact Bio-Rad method is available with an option to select from three different incubation times, 0.5 hrs, 2.0 hrs, or 15 hrs (Figure 6.7).
Elution of target protein is typically conducted by incubating the resin in the appropriate buffer at room temperature (see Appendix J for buffer formulation details) for 0.5 hrs. Incubation times may need to be empirically determined, and for some proteins the incubation time may be longer. For elution of slower-cleaving proteins, the 2 hour incubation time may be selected for room temperature cleavage reactions. However, if elution at 4°C is desired, the 15 hr incubation time may be selected to ensure complete cleavage. If the Profinia system is operated in a cold environment, the method temperature should be set to the 4°C setting in the Utilities section of the user interface. See Section 9.3.14 for a detailed description of this setting.

During the programmed incubation period, the Stop Incubation button may be used to prematurely terminate the incubation and resume the elution and collection steps of the method.

**Note:** If incubation times are needed other than the available options in the Bio-Rad Mode, the user can access this method in the Program Mode and program incubation times from 0–999 hrs.

When the sample incubation time(s) are selected, touch the Next button to proceed to the Enter Run and Sample information screen.

![Select Incubation Time screen](image)

Fig. 6.7. Select Incubation Time screen.

### 6.4 Entering Run and Sample Information

The Enter Run & Sample Information screen (Figure 6.8) allows you to enter identification information for the run. You can enter a run name, lot number information related to the components used in this run, and specific sample details, such as name, volume, and the A₂₈₀ extinction coefficient. This information is stored with the exportable run data file. This screen also allows access to view the individual method steps and the step parameters via the View Method button in the lower toolbar.

**Note:** If you are running a method with two samples, you can enter separate information for both samples.
Fig. 6.8. Enter Run & Sample Information screen.

**Note:** When two samples are selected for the method, press the View Method button in the lower toolbar to view the method steps and the step parameters for each sample. A View Sample button is displayed above the arrows in the View Method screen.

The following run-specific information can be entered or selected:

- **Username** — optional; user-assigned name 3–15 characters in length to document the operator of the run
- **Run name** — user-designated file name for the run, up to 30 characters in length
- **(External fc)** — external fraction collection option; currently not available for operation
- **Lot # tracking** — optional; lot number information for the buffers, cartridges, and kits used for the run (“BLANK” indicates no information has been entered). The word “PRESENT” indicates that lot number information was entered. The lot number information remains in memory as long as the instrument power remains on. When cycling the power, the lot number information reverts to “BLANK”

The following sample-specific information can be entered or selected:

- **Sample name** — optional; user-assigned name for the sample, up to 15 characters in length (“UNTITLED” indicates no information has been entered). If you have chosen to perform a run with two samples, you can assign a name for each sample
- **Volume** — user-entered sample volume between 2 ml and 50 ml. If you have chosen to perform a run with two samples, a volume must be entered for each sample. If the sample volume is greater than 50 ml, see section 7 for information on program methods. If the sample volume is less than 2 ml, diluting the sample with 1x lysis buffer to a total volume of >10 ml is recommended to obtain optimum yield

**Note:** When the desalting-only method is selected, the sample volume is fixed at 2 ml for the 10 ml cartridge and 10 ml for the 50 ml cartridge. The sample volume is 20% of the cartridge volume, the optimized volume for buffer exchange.
• **A<sub>280</sub> of 1 mg/ml** — extinction coefficient for the protein expressed as the absorbance of a 1 mg/ml solution of the protein of interest at 280 nm for a 1 cm UV pathlength. The default value for this parameter is 1.00. An extinction coefficient can be assigned for each sample. This value is used to calculate the estimated total protein and protein concentration at the end of the run. An extinction coefficient converts absorbance units (AU or OD) to concentration units (mg/ml). For example, if a protein has an AU of 1.38, and an extinction coefficient of 1.25 AU/mg/ml, the concentration of that protein solution is calculated as 1.38 AU/1.25 AU/mg/ml = 1.1 mg/ml. The Profinia firmware uses this algorithm to calculate protein concentration and yield from the absorbance units under the purified peak.

**Note**: If you measure the absorbance of your protein with a short-pathlength cuvette, you must multiply the absorbance value to normalize to 1 cm. For example, the absorbance measured in a 2 mm cuvette must be multiplied by 5. This value is then entered for A<sub>280</sub> of 1 mg/ml.

• **Home** — returns to the home screen. All entered run and sample information, except lot number tracking information, will be cleared when you select the Home button. The entered lot number tracking information remains in temporary memory as long as instrument power is on. When cycling power, lot number tracking information defaults to “BLANK”.

### 6.4.1 Adding or Editing Run and Sample Information

To add or edit sample and run information:

1. Touch the arrow buttons to the right of the information text box to scroll through the list of options and highlight the parameter for which you’d like to add information.

2. Touch the Edit button to access the alphanumeric or numeric keypad. The appropriate keypad displays.

3. Enter or edit information for the selected parameter in the text line at the top of the screen.

**Note**: If values entered exceed the system-set limits, the value limits appear immediately below the text line of the numeric keypad and the system overrides the entered value with the maximum or minimum value allowed.

4. Touch OK to accept all changes or Cancel to delete any entered data and return to the Enter Run & Sample Information screen.

5. Repeat this process for all information parameters. For details on entering lot number tracking, see section 6.4.2 below.

**Note**: Touch the Clear All button to delete the text or data entered into the text line. The default “UNTITLED” text can be cleared with any keystroke.

### 6.4.2 Adding Lot Number Tracking Information

To add lot number information for any of the reagents or components used in your method:

1. Use the arrow buttons to the right of the information text box to scroll through the list of options and highlight “Lot # Tracking”.

2. Touch the Edit button. You will proceed to the Edit Lot Number Information screen (Figure 6.9).
Fig. 6.9. Edit Lot Number Information screen.

3. Touch the arrow buttons to the right of the lot number information text box to scroll through the list of kit information (buffers, solutions, and cartridges) relevant to the run. Highlight the item for which you want to add lot number information.

4. Touch the Edit button to retrieve the alphanumeric keypad.

5. Enter desired information into the keypad text line.

6. Touch OK to save input data and return to the Edit Lot Number Information screen, or Cancel to return to the Edit Lot Number Information screen without saving changes.

7. Repeat this process for each item that requires lot number information.

8. Touch Clear All to delete all information added, or OK to accept changes and return to the Enter Run & Sample Information screen.

When all run and sample information has been satisfactorily updated, touch the Next button. You will proceed to the Install Reagents, Sample & Fraction Tubes screen.

6.5 Installing Reagents, Sample Tubes, and Fraction Tubes

The Install Reagents, Sample & Fraction Tube screen (Figure 6.10) displays a diagram showing where buffer, diluent, sample, and waste containers should be placed in the Profinia instrument (cartridge installation is described in Section 6.6). **This diagram is specific to the method type and options selected for the run.** The volumes indicated for the buffer and water bottles are the minimum volumes required. Volumes indicated for the fractions are those that will be delivered to the fraction tubes. An “X” in any position indicates that no reagent or container is required in that position.

**Note:** If the reagent volumes exceed the container capacity, a larger container can be connected to the instrument externally. When reagents are connected externally make sure to prime the lines manually immediately prior to starting the run. The manual priming function is available in the Start Run screen.
Fig. 6.10. Install Reagents, Sample & Fraction Tubes screen (left) with arrows demonstrating the correlation between the diagram and placement of reagents in the Profinia instrument (right).

The installation screen diagram displays:

- **Buffer bottles** — eight buffer bottle positions indicated by labels B1 through B8 on the touch screen (top of the screen) and 1 through 8 on the instrument (upper tray). The touch-screen diagram also displays the minimum buffer volumes required for the selected method; an “X” indicates no reagent is required at that location.

  **Note:** Profinia purification kit reagent bottles are labeled with the instrument buffer position numbers, which are also displayed on the instrument Install Reagents, Sample & Fraction Tubes screen.

- **Waste bottle** — left side of the touch-screen diagram and positioned at the lower left of the instrument. On the touch screen, the waste bottle is displayed without a volume as a reminder to make sure the waste bottle is emptied frequently.

- **Diluent (water) bottle** — right side of the touch-screen diagram and positioned at the lower right of the instrument. On the touch screen, the diagram is labeled with the minimum volume of water required for the selected method run.

- **Sample tubes** — indicated by S1 and S2 labels on the touch screen, the sample tubes are placed beneath the buffer bottles toward the right of the instrument. The touch screen displays one or two sample tubes with the sample volumes, depending on the number of samples and sample volumes that have been entered by the user on the Enter Run & Sample Information screen.

  **Note:** Each run primes the sample line(s) with about 1 ml of sample. To ensure that the entered sample volume is loaded onto the cartridge, filling the sample tubes with 1 ml more than the entered volume is recommended. If the exact entered volume is added to the sample tube, the end of sample detection, if activated, applies the complete sample to the system. In this case, the total amount of sample added to the cartridge will be approximately 0.5 ml less than the entered volume. To maximize protein yields, 10 ml is the minimum recommended sample load.
• **Fraction collection tubes** — indicated on the touch screen by a set of four tubes on the left labeled 1A through 1D for sample 1 collection, and four tubes on the right labeled 2A through 2D for sample 2 collection. Fraction tubes are placed at the bottom of the instrument. Each fraction tube shows the volume that will be collected in the tube. Only the required fraction tubes are represented on the Install Reagent, Sample & Fraction Tubes screen. Before beginning your run, make sure the fraction tube capacities match the volumes indicated on the screen.

**Note:** The fraction positions are numbered 1A through 1D from left to right, and 2A through 2D are ordered right to left. This places the purified protein in the center two positions for easiest access and for use with the cooling accessory.

• **View Details** — lower toolbar button; proceeds to a screen that displays the name for each of the components shown in the system setup screen (Figure 6.11). Touch OK to return to the Install Reagents, Sample & Fraction Tubes screen.

![Image of Buffer & Fraction Details screen](image)

Fig. 6.11. Review Buffer & Fraction Details screen displayed when the View Details button is selected.

### 6.5.1 Loading Buffers

The Bio-Rad Methods function of the Profinia system is designed to operate with Profinia purification kits that contain concentrated buffers and cartridges for each preprogrammed method type. To prepare reagents for the method run:

**Note:** See Appendix I and J for recommended reagents for the Protein A and G and Profinity eXact methods.

1. Remove reagents from the Profinia purification kit designed for your selected method type (see section 10).

2. Prepare reagents as specified in the purification kit instruction manual. Some of the buffers require the addition of dry reagents followed by filtration, such as those for denaturing IMAC and GST methods.

3. When reagents are ready for loading into the Profinia instrument, place a buffer lid on each buffer bottle (Figure 6.12).
Fig. 6.12. Replace buffer caps with buffer lids before installation.

4. Open the lid on the Profinia instrument to access buffer sipper tubes and the placement tray.

5. In the position where the buffer bottle is being installed, lift the sipper tube away from the instrument.

6. With the number code on the bottle label facing out and graduated marks visible, insert the sipper tube in the bottle (Figure 6.13).

Fig. 6.13. Loading buffer bottle onto sipper tube in buffer position 1.
**Important**: It is important to orient the buffer bottles properly in the instrument; the graduated marks on the buffer bottles are used to determine how much buffer is available for the purification run, so you must be able to see them (Figure 6.14).

7. Close the Profinia lid to protect the buffer bottles and sipper tubes during operation (Figure 6.15).

![Fig. 6.14. Proper placement of buffer bottles in the instrument, with orientation marks visible; buffer caps are stored above respective bottle positions.](image1)

![Fig. 6.15. Buffer bottles properly placed in all positions and lid closed in preparation for instrument operation. The native IMAC plus desalting buffer setup is shown.](image2)
Note: Some kits do not require a reagent bottle in every position. If a position does not require a bottle (indicated by an "X" on the installation screen), leave that position empty, or place a bottle with water in that position.

6.5.2 Installing Sample Tubes

The sample loading area is on the front right side of the instrument (Figure 6.16).

Fig. 6.16. Instrument sample loading area.

To load sample(s):

1. Add the required amount of sample to the sample tubes as indicated on the touch screen. If you are running more than one sample, place sample 1 in the sample 1 position, and sample 2 in the sample 2 position (S1 and S2 on the installation screen, respectively).

Warning: If end of sample detection has been disabled, enter a sample volume approximately 0.5 ml less than the actual sample volume to prevent air from entering the instrument (see Section 9.3.12 for more information).

2. Place sample lids on the sample tubes.

3. For each sample tube position, lift the sipper tube away from the instrument and insert the sample tube into the sample sipper tube. If only one sample is needed (S1), the second sample position (S2) can remain empty, or place a tube with water in the S2 position.
6.5.3 Installing Fraction Collection Tubes

The fraction collection area is at the bottom of the Profinia instrument (Figure 6.17). Fractions are collected into standard laboratory conical tubes. The Profinia instrument can accommodate both 15 ml and 50 ml conical tubes (see Section 1.4 for conical tube recommendations). When one sample has been installed for a purification run, place fraction collection tubes as indicated in the system installation screen in positions 1A through 1D. For two-sample purification runs, place fraction collection tubes in positions 1A through 1D and 2A through 2D (the number of tubes is method-dependent; not all methods require four tubes per sample).

Operations performed or product obtained by position:

- 1A and 2A: flow-through
- 1B and 2B: wash 1
- 1C and 2C: wash 2
- 1D and 2D: purified protein

Fig. 6.17. Fraction tube collection area with tube positions marked 1A through 1D (for runs performed with one sample) and 2A through 2D (for runs performed with two samples).

To install the fraction collection tubes:

1. For each fraction collection position, insert the bottom of a conical tube into the indented spot on the bottom of the fraction collection area.

2. Push the top of the conical tube into the top tube holder (Figure 6.18).

3. Insert the tubes for each position required in your purification run.
When all fraction tubes required in the run have been installed, touch the Next button to begin the cartridge installation procedure.

6.6 Installing Cartridges

The cartridge installation process includes preparatory steps such as priming the system cartridge lines to prevent introduction of air bubbles into the cartridge. The cartridge installation routine is carried out through four consecutive screens:

- **Select Cartridge Status** — includes the option to bypass the installation procedure when cartridges are already installed (for example, from a previous run)
- **Setup Cartridge Positions for Priming** — directs connection of the cartridge 1 and 2 position fittings
- **Priming in Progress** — displays the priming time progress bar
- **Install Cartridges** — displays the required cartridges and their respective positions

**Warning**: When the desalting-only method is selected DO NOT fill the sample loop with sample until the sample loop and cartridge installation is completed. Inject the sample immediately prior to starting the run. (See Section 6.6.5 for sample loop installation instructions.)

6.6.1 Select Cartridge Status

Two options are available on the Prepare for Cartridge Installation screen (Figure 6.19):

- **No** — cartridges are not installed and the system cartridge lines need to be primed; touch the No button to proceed to the Prepare for Cartridge Line Priming screen
- **Yes** — cartridges are already installed and priming is not needed; touch the Yes button to bypass priming and proceed directly to the Start Run screen (Section 6.7)
6.6.2 Setting Up Cartridge Positions for Priming

Follow the instructions on the screen to prepare both cartridge positions for priming. Both positions need to be closed as indicated on the screen (Figure 6.20).

**Note:** Make sure the luer fittings are connected properly. Do not overtighten. Overtightening can lead to stripped threads which, over time, can lead to leaking.

When both cartridge positions are closed, touch the Start button to begin the priming process.

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**Fig. 6.19.** Select cartridge status screen.

**Fig. 6.20.** Prepare for Cartridge Line Priming screen showing instructions for connecting cartridge positions (left); upper and lower fittings connected (closed) for both cartridge positions (right).
6.6.3 Priming the Cartridge Lines

The Priming in Progress screen (Figure 6.21) displays a progress bar that indicates the percentage of priming time completed. Priming is completed in 30 seconds.

![Fig. 6.21. Priming in Progress screen.](image)

If the system leaks from the cartridge positions during priming, the fittings are not properly connected. To correct this problem:

1. Touch the Pause button in the lower right corner of the screen to stop the priming procedure.
2. If necessary, reconnect the upper and lower cartridge fittings for each position (1 and 2).
3. Touch Start to resume the priming procedure, or touch the Back button to return to the previous screen and restart the priming process.

When priming is finished, you will automatically proceed to the Install Cartridges screen.

6.6.4 Installing Cartridges

The Install Bio-Scale Mini Cartridges screen (Figure 6.22) shows the required Bio-Scale™ Mini cartridges and their respective positions. It also displays instructions for installing the cartridges into the Profinia system.
To install new cartridges:

1. Disconnect the upper and lower cartridge fittings (Figure 6.23).

2. Remove the bottom cap from the Bio-Scale Mini cartridge.

3. Place the cartridge in the desired cartridge position and tighten the lower fittings by twisting the cartridge in a clockwise direction (Figure 6.24).
Fig. 6.24. Inserting the cartridge into the cartridge 1 position; twist the cartridge in a clockwise direction as indicated by the arrow to fit the bottom part of the cartridge into position.

4. Remove the top cap from the cartridge.

5. Lower the cartridge arm and twist the black collar clockwise to tighten the upper fitting and connect it to the cartridge (Figure 6.25). Do not use excessive force.
6. Repeat this procedure to install a Bio-Scale Mini cartridge into the cartridge 2 position.

**Note**: If no cartridge will be placed in the cartridge 2 position, keep the system cartridge fittings in the closed position (Figure 6.26).

**Note**: Do not overtighten fittings. Luer lock fittings are designed to seal when turned to snug. The cartridge flange may be damaged by overtightening and cause leakage. In this case, replace the cartridge.
Fig. 6.26. Lower fitting tightened into place (left) and upper cartridge fitting tightened into place (right); cartridge 2 position remains closed.

When cartridge installation is complete, touch the Next button in the lower right corner of the screen. You will proceed to the Start Run screen.

6.6.5 Installing the Sample Loop (Desalting-Only Methods)

Warning: When the desalting-only method is selected, DO NOT fill the sample loop with sample until the sample loop and cartridge installation is completed. Inject the sample immediately prior to starting the run.

Sample loading for a desalting-only method is achieved via a sample loop. Installation is directed by the Install Bio-Scale Mini Cartridges screen (Figure 6.27). The desalting sample loop accessory connects to the instrument in the cartridge 1 position and the desalting cartridge in the cartridge 2 position (Figure 6.29).
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. Install the desalting cartridge in the cartridge 2 position. Touch the Next button to proceed to the Start Run screen (Figure 6.28).
4. 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 up (toward the top inlet fitting), and inject the sample into the loop.

5. 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.

![Figure 6.29. Sample loop installed in the cartridge 1 position.](image)

**Note:** 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, since it will result in increased dilution of the sample and reduced desalting resolution. If desired, small volume samples can be diluted to 2 or 10 ml for buffer exchange applications.

### 6.7 Starting the Run

The Start Run screen (Figure 6.30) displays a summary of the sample information. A reminder prompts you to connect the instrument to a computer running Profinia software if you would like to view the chromatogram in real time (see section 12 for more information on Profinia software). The Profinia system does not require software to operate the instrument; current data points appear on the run screen.

The lower toolbar contains buttons for viewing the methods steps and for optional manual priming of any extra tubing attached to the sample, buffer, diluent, or waste lines to accommodate larger containers in these instrument ports (see section 11.2 for details).
6.7.1 Viewing Steps

Touch the View Steps button (available on the Start Run screen at any point during the run) to display the View Method Steps screen (Figure 6.31). During a run, the step that is currently in progress is highlighted.

The following columns of information are displayed on the View Method Steps screen:

- **Step** — numbered list of the primary steps that will be performed in the run
- **Name** — brief description of the operation associated with the step
- **Conc.** — buffer position in the instrument and buffer concentration (the instrument dilutes concentrated buffers)
- **ml/min** — flow rate displayed in ml/min
- **CV** — column volume based on the cartridge size (for example, for a 1 ml IMAC cartridge, 2 CV = 2 ml)
- **Min** — duration of each step in minutes

During a run, the step currently in progress is highlighted.
6.7.2 Viewing Real-Time Data with Profinia Software

The Profinia system does not require software and a computer to operate the instrument; individual data points are displayed on the run screen. However, with Profinia software (optional) installed on a computer and connected directly to the instrument through the USB port on the back of the instrument (Figure 6.32), the run chromatogram can be viewed in real time. The USB cable for instrument-to-computer connection is included with the software.

Note: The front USB port does not output real-time data. The front port is used to download the run data files onto a portable memory device when the run is completed, or from system memory using the Export Data function in the Data/Utilities menu (section 9.1).

To collect real-time data:

1. Connect the USB cable to the USB port on the back of the instrument and to the computer.
2. Open Profinia software on your computer by double-clicking on the desktop Profinia software icon. Look for the “Waiting for data” message in the lower left corner of the software screen. This indicates the instrument is communicating with the software and a run can be started (Figure 6.33).

Fig. 6.33. “Waiting for data” message on the bottom of the Profinia software screen.

At this point, the run can be started and the chromatogram appears on the computer screen.

Important: The real-time USB data connection cannot be established once the Start button has been touched and a run has been started. However, the run data file can be exported to a USB portable memory device and transferred to Profinia software after the completion of the run.

6.7.3 Clearing System Memory to Start the Run

To start your purification run:

Touch the Start button. The Preparing Data Memory Storage screen displays a progress bar indicating temporary memory data clearance. The system can store up to three run data files in memory. An “M” status indicator displayed in the upper right corner of the screen indicates none of the run data files stored in system memory have been transferred. If system memory is full (indicated by “F” in the status position), the oldest transferred or stored data file will be overwritten with the new file when a purification run is started.

Fig. 6.34. Touch Start on the Start Run screen to begin a purification run.
Once memory has cleared, the method starts and the Run in Progress screen displays.

### 6.8 Monitoring Run Progress

#### 6.8.1 Run in Progress Screen

The Run in Progress screen (Figure 6.35) displays an overview of the current run, as well as basic details about the current step in progress and expected sample elution times.

![Fig. 6.35. Run in Progress screen.](image)

**Note:** The View Steps screen can be accessed from both the Start Run screen and the Run in Progress screen at any time while the run is in progress. To return to the Run in Progress screen, touch the OK button.

The Run in Progress screen displays the following information:

- **Elapsed/total run time** — elapsed time versus the total run time for the method. The total run time indicated refers to the time necessary to complete the protein purification and cartridge cleaning.

- **Progress bar** — percent of total run time that has elapsed. The arrows underneath the progress bar indicate the point at which the sample will be eluted. If you have chosen to perform a run with one sample, there will be one arrow linked to an S1 symbol; for two-sample procedures, there will be S1 and S2 arrow indicators.

- **Current step** — name of the current step in progress.

- **Step time remaining** — duration of time left to complete the current step in progress.

- **Expected elution times** — time at which each programmed sample is expected to elute. As soon as the protein is eluted, the actual time of elution and estimated protein yield and concentration are displayed.

**Note:** Make sure the time and date are properly set in the Utilities menu. The sample elution time is shown in system clock time as set by the user (see section 2.2.3 or 9.3.3 for instructions on setting the system clock).
The lower part of the Run in Progress screen shows the real-time data:

- **Real-time data at** — system clock time. The date and time setting is available in the Data/Utilities section accessed from the home screen.
- **Flow rate** — flow rate of the current step (displayed in ml/min).
- **Collection** — in which fraction tube the system is diverting output from the current step.
- **UV cartridge** — appears as UV cartridge 1 when fluid goes through cartridge 1, and switches to UV cartridge 2 when fluid goes through cartridge 2, displayed in absorbance units (AU).

**Note:** Automatic peak detection is activated for specific elution steps. When Profinia firmware is actively looking for the peak, a light bulb icon is displayed immediately in front of the UV cartridge line. For details on automatic peak detection, see section 6.8.2.

- **Conductivity** — value is given in mS/cm.
- **pH** — value appears when a pH monitor (optional) is connected to the Profinia system.

6.8.2 Peak Detection

Affinity and desalting protein peaks are identified using an automatic peak detection algorithm that is active during elution steps on the Profinia instrument. In addition to automatic peak detection, the option to manually detect and divert the protein peak is also available during affinity and desalting elution step(s). When manual peak detection is available during affinity and desalting elution steps, the manual peak detection button displays on the Run in Progress screen. Once a peak is detected either automatically or manually and the next elution step is started, the manual peak divert button displays. For the vast majority of proteins, automatic peak detection is the preferred and most robust route for peak collection.

**Automatic Peak Detection**

Automatic peak detection is an essential feature for identification, diversion, and collection of the eluted protein peaks on the Profinia instrument. Automatic peak detection includes several parameters that may be changed from the default settings, depending on the method:

- **Peak detect delay** — at the start of the elution step, the automatic peak detection algorithm is activated after a set time delay. The programmed default setting for peak detect delay is based on the fluidics of the Profinia instrument and is optimized for Bio-Rad methods. After the specified peak detect delay, the Profinia instrument starts actively monitoring for the expected affinity or desalting peak, and a light bulb icon displays on the screen immediately in front of the UV cartridge line. Following peak detection, the method proceeds to the next step and the light bulb icon disappears. The peak detect delay parameter is intended to prevent premature activation of the automatic peak detection algorithm, which could be caused by late eluting contaminants on wash 2 steps.

- **Max peak volume** — maximum volume the system pumps before overriding automatic peak detection. Once this volume is reached and a peak was not detected, the method proceeds to the next step. The programmed default setting for max peak volume is based on the fluidics of the Profinia instrument and is optimized for Bio-Rad methods. The max peak volume parameter is intended to prevent loss of sample in the event the automatic peak detection algorithm does not recognize a peak during the specified time window.
Note: The peak detect delay and max peak volume parameters can only be edited in the program methods mode (see section 7.3.1).

- **Peak detection sensitivity** — settings range between 0.1 (most sensitive) and 10 (least sensitive); the default setting is 1. Peak detection sensitivity is a global setting accessible in the Data/Utilities menu for both Bio-Rad methods and program methods modes. The peak detection sensitivity parameter programs the Profinia instrument to adjust the automatic peak detection algorithm to various peak shapes and sizes in the event the default setting does not recognize a peak during the specified time window. In general, use lower numbers (more sensitive) if a small protein peak is expected.

Note: Using more sensitive settings for peak detection may lead to activation of automatic peak detection from small UV deviations not associated with the protein peak.

### Manual Peak Detection

In addition to automatic peak detection, the instrument provides the option to manually detect and divert the protein peak during affinity and desalting elution steps. Manual peak detection can be used for:

- Purifications where the target protein is in a very low concentration (<1 mg/ml) or has a very low extinction coefficient. Cold room purification and using program methods with the protein elution at a flow rate lower than 0.5 ml/min may generate UV trace slopes not steep enough to be detected by the Profinia peak detection algorithm. If these types of sample and running conditions are typical, then it is recommended to adjust the peak detection setting to the highest sensitivity: 0.1. However, the Bio-Rad methods default value for the max peak volume is intended as a safety for cases where the peak is not automatically detected, for example, for very low expression proteins. When this value is reached the method will advance to the next step to ensure that the region where the protein is eluting will be collected.

- Overriding the automatic peak detection to collect the highest possible protein concentration by manually selecting the peak section with the highest absorbance values. To override the automatic peak detection, set the peak detect delay time and max peak volume to values well beyond the point the peak is expected to elute. Editing these values is possible in program methods mode only (see section 7.3.1 for more details).

The manual peak detect and manual peak divert buttons will display on the screen during the appropriate protein elution steps only.

**Using manual peak detect:**

When the protein peak starts to elute, touch the Manual Peak Detect button to immediately advance to the next step without waiting for automatic peak detection or the max peak volume setting.

**Using manual peak divert:**

You have the option to minimize the volume in which the purified protein is collected. Diverting fraction collection before the peak trailing ends decreases the total yield and increases protein concentration.
When the protein peak has eluted (Figure 6.36), you can touch the Manual Peak Divert button (Figure 6.37) to automatically advance to the next step without waiting for the completion of that step.

**6.8.3 Collecting the Purified Sample**

The purified sample is collected in fraction tube position 1D (sample 1) or 2D (sample 2). When the purified protein is collected, the Run in Progress screen displays the actual elution times and tubes 1D and 2D can be removed. After the target protein has eluted, the Profinia system automatically continues to run the cartridge cleaning and storage procedures and purify the second sample if selected.
6.8.4 Canceling or Pausing a Run

To cancel the run (when you cancel a run, you proceed to the Prepare System to Clean Lines screen):

1. Touch the Cancel Run button in the lower right of the screen. A confirmation screen appears. The run continues until the cancel confirmation is made.

2. Touch OK to terminate the run, or Cancel to return to the Run in Progress screen and continue the run.

To pause the run:

1. Touch the Pause button in the lower right of the screen to proceed to the Pause screen.

2. To resume the run, touch the Run button.

3. To cancel the run, touch the Cancel button.

6.8.5 Completing the Run

Run results display as soon as the end-of-run cleaning procedures are completed.

6.9 Preparing System for End-of-Run Cleaning

System cleaning is an automatic feature that occurs upon completion of each method run and is required to prevent cross-contamination between runs and to maintain the system in optimum condition. The two sample inlet lines and the fraction line valve (all eight fraction lines) are cleaned as part of the end-of-run cleaning process. The pump seals are also washed as the final step in this process and the internal system lines are stored with 20% ethanol from buffer position 8 (Figure 6.38).

![PREPARE SYSTEM TO CLEAN LINES](image)

Fig. 6.38. Prepare System to Clean Lines screen.

6.9.1 Preparing for Line Cleaning

To prepare the system for end-of-run cleaning:

1. Remove all sample tubes from the instrument.
2. Place tubes containing 10 ml of deionized water in both sample 1 and sample 2 positions; place water in both sample positions even if only one sample was used in the run.

3. Fill a buffer bottle with 20% ethanol and place it in buffer position 8.

4. Remove all fraction collection tubes from the instrument.

5. Place the cleaning tray (provided with the instrument) in the fraction collection area.

6. Touch the Start button to begin the cleaning procedure.

You will proceed to the System Cleaning in Progress screen.

### 6.9.2 System Cleaning in Progress

When system cleaning starts, the System Cleaning in Progress screen (Figure 6.39) displays the four system cleaning procedures: Clean Sample Lines, Clean Fraction Lines, Clean Buffer Lines, and System EtOH Wash. The cleaning steps that will be performed after a run is completed are indicated by darkened circles. A checkmark adjacent to a darkened circle indicates the cleaning step has been completed. When the cleaning process is completed, you will proceed to the Run Completed screen where the run results are displayed.

![Figure 6.39. System Cleaning in Progress screen showing fraction line cleaning is in progress. The actual cleaning step, indicated by a number code and brief description, is displayed below the progress bar.](image)

**Note:** The Clean Buffer Lines and System EtOH Wash options are available as short-term or long-term cleaning methods, respectively, in the Run Completed screen. All cleaning options are also accessible via the Data/Utilities menu accessible from the home screen.

### 6.10 Performing End-of-Run Procedures

The Run Completed screen (Figure 6.40) is displayed upon completion of all purification run steps, including system cleaning. Run details for each sample purified are displayed in the run data table. The total estimated protein (yield) and protein concentration have been calculated and are displayed for each sample.
6.10.1 Exporting Data to a Computer

The run data file can be transferred to a portable memory device as an .ofi file, which can be imported into Profinia software (optional) for viewing data in tabular form or in a chromatogram. If Profinia software has not been purchased, the data file can be viewed in any compatible spreadsheet software (for example, Microsoft Excel). To export the run data file:

1. Insert a USB portable memory device into the USB port directly below the touch screen. The Export Data button is initially displayed with an “X” across it, indicating that the button is not yet functional. As soon as the system recognizes the portable memory device, the “X” disappears and the Export Data button is available. The time required for recognition depends on the portable device used and can take up to 15 sec. (See troubleshooting in Appendix C if the memory device is not recognized).

2. Touch the Export Data button in the lower right corner of the End of Run screen. A progress bar displays indicating that the transfer is in progress; typical transfer times are approximately 60 sec. The actual transfer time depends on the amount of data in the run data file and the portable memory device.

Upon completion of data export, you will automatically return to the Run Completed screen.

6.10.2 Performing Final Run Procedures

The toolbar at the bottom of the Run Completed screen displays final steps to shut down the system or prepare for a new run. Choose one of the following options:

- **Re-Run Method** — immediately begin a new sample run with the same method
- **Run New Method** — immediately begin another purification run using a different method type, or new buffers for the run that was just performed
- **Short-Term Wash** — if the system will not be used again on the same day, the buffer lines and valves should be rinsed with water
- **Long-Term Wash** — if the system will not be used in the next 2 days, it should be rinsed with water and ethanol or other sanitizer to maintain optimum mechanical performance and prevent bacterial growth
Re-Running a Method

To repeat the method just performed with the same buffers, touch the Re-Run Method button in the toolbar at the bottom of the End of Run screen. You will return to the Enter Run & Sample Information screen to repeat the Bio-Rad methods set-up and run process.

Running a New Method

Two options are available when the Run New Method button is selected upon completion of a run: select a new method utilizing the same buffers, or select a new method that requires a different buffer set. When the same buffers are used you will proceed directly to the Select Method Type & Options screen to follow the procedures for starting a new run. When different buffers are required, you can rinse the buffer lines prior to proceeding to the home screen.

To run a new method:

1. Touch the Run New Method button in the toolbar at the bottom of the End of Run screen. You will proceed to the Prepare System to Clean Lines screen (Figure 6.41).

```
Fig. 6.41. Prepare System to Clean Lines screen displays when running a new method to clean buffer lines if a new buffer set is being used.
```

2. Touch the Home button in the toolbar if a new method with the same buffers will be run. You will proceed directly to the home screen.

3. Touch the Back button to return to the End of Run screen to select a different option.

4. To select the wash buffer line cleaning procedure, follow the onscreen directions and touch the Start button to begin the cleaning procedure. When the buffer line cleaning procedure is complete, you will automatically proceed to the Select Method Type & Options screen.

Cleaning the System for Short-Term Storage

If the Profinia system will not be used again on the same day, performing the short-term wash procedure (buffer lines and pump seal wash) to maintain the system in optimum condition is recommended. To perform a wash for short-term storage, touch the Short-Term Wash button in the toolbar at the bottom of the End of Run screen. You will proceed to the Prepare System to Clean Lines, short-term wash screen (Figure 6.42).
To prepare the system for the short-term wash, follow the onscreen instructions:

1. Place bottles with deionized water in buffer positions 1 through 7.
2. Fill a buffer bottle with 20% ethanol and place it in buffer position 8.
3. Remove all cartridges and directly connect the upper and lower cartridge fittings.
4. Place the Profinia cleaning tray in the fraction collection port.
5. Touch the Start button to begin short-term storage cleaning; the cleaning progress screen displays. Immediately below the progress bar, the current line cleaning is indicated.

When the cleaning process is completed, you will automatically return to the home screen.

**Cleaning the System for Long-Term Storage**

If the Profinia system will not be used within the next 2 days, it should be rinsed with water and 20% ethanol or other sanitizer to maintain optimum mechanical performance and prevent bacterial growth. To perform a long-term storage wash, touch the Long-Term Wash button in the toolbar. You will proceed to the Prepare System to Clean Lines, long-term wash screen (Figure 6.43).
To prepare the system for the long-term wash:

1. Place bottles of deionized water, 20% ethanol, or other sanitizer in buffer positions 1 through 7.

2. Place a bottle with 20% ethanol (or other sanitizer) in buffer position 8.

3. Place tubes containing 10 ml of deionized water, 20% ethanol, or other sanitizer in both sample 1 and sample 2 positions.

4. Remove all cartridges and directly connect the upper and lower cartridge fittings.

5. Place the cleaning tray in the fraction collection area.

6. Touch the Start button to begin long-term storage cleaning; the cleaning progress screen displays. Immediately below the progress bar, the current line cleaning is indicated.

When long-term cleaning is finished, a message on the touch screen appears prompting you to cap all system lines and turn the instrument off.

**Important:** When the Profinia system is used in a cold environment, it is best to leave instrument power on when not in use; the slight amount of heat generated helps control condensation. The UV lamp automatically turns off one hour after the last separation run, and turns back on when instrument operation resumes. If the instrument will be stored long term in a cold environment, it is acceptable to turn the power off. When the instrument is removed from a cold environment, the unit must be equilibrated to room temperature before turning on the instrument. This prevents condensation from forming which could possibly be damaging to internal components.
Section 7
Program Methods

The program methods function of the Profinia™ system is used to create methods by editing the Bio-Rad purification and desalting method templates. The method template remains the same in terms of number and sequence of steps; however, step parameters such as flow rate, column volume, and peak detection can be edited to create a program method. Once a method is programmed it can be stored in memory for future access and reuse.

The purification procedure used in program methods has the following steps:

1. Select method type and options.
2. Enter run and sample information.
3. Edit method; save method.
4. Install reagents, sample, and fraction tubes.
5. Install cartridge(s).
7. Monitor run.
8. Clean sample and fraction lines.
9. Select and perform end-of-run procedures.
10. Shut down system.

Instrument Operating Temperature

For program methods that include the desalting step, the instrument operating temperature is critical. Maximum flow rates for the desalting cartridges are lower at 4°C than at room temperature. Flow rates over maximum limits compress the bed volume and cause over-pressure errors. Make sure the operating temperature is set to match cold room or room temperature operating conditions before programming your run. Methods with desalting steps have longer run times at the cold room setting due to the lower maximum flow rates. The operating temperature setting displays as a status indicator in the upper right corner of the screen. If the cold room operating temperature is selected, “C” appears only when methods are selected that include a desalting step. If the room temperature setting is selected, “C” will not display. For affinity-only methods, the temperature setting is irrelevant and will not display. See section 6.2 for flow rate details, or section 9.3.13 for information on setting the operating temperature.

Warning: When operating at 4°C, make sure that selected methods that include desalting are programmed for 4°C. Methods created using room temperature operating parameters could have flow rates that are too high for 4°C and damage to the cartridges can occur.

Note: Do not change the instrument operating temperature setting to cold room (indicated by a “C” in the upper-right of the touch screen) when using the cooling accessory. Keep the operating temperature setting at room temperature unless the entire instrument is operating in a cold room environment.

Figure 7.1 shows the screen sequence when starting a program method.
Fig. 7.1. Screen sequence when starting a program method run.

To create a method, touch the Program Methods button in the lower toolbar of the home screen. You will proceed to the Select Method System screen.

### 7.1 Selecting Method System

The Select Method System screen (Figure 7.2) allows you to choose:

- **Method system** — IMAC, GST, Protein A and G, Profinity eXact, with or without desalting, or desalting-only methods
7.2 Selecting Method Type and Options

The Select Method Type & Options screen (Figure 7.3) allows you to choose:

- **Method type** — Affinity-tagged methods with or without a desalting step
- **Number of samples** — program one or two samples (the desalting-only method can only be run with one sample)
- **Cartridge size** — based on sample volume and concentration
- **Number of cartridges** — available for affinity-only methods when two samples are selected. You may choose to use a single cartridge for both samples or a separate cartridge for each sample

Fig. 7.3. Select Method Type & Options screen.
To select the method type and options:

1. Touch the up and down arrows to select the method template from the list of options displayed on the screen.

**Note:** The name of the selected method type appears at the top of this screen and all subsequent screens. (The method name can be edited in the Enter Run & Sample Information screen.)

2. Touch the arrow button to the right of the Sample list to select “1” or “2” samples.

**Note:** Two samples can be run for all methods, except the desalting-only method. When two samples are selected for affinity-only methods, the number of cartridges option becomes available. You have the option to use a single cartridge for both samples or a separate cartridge for each sample. When two samples are selected for affinity with desalting methods, both samples are purified using the same cartridge. Desalting-only methods are run with one sample that is applied to the cartridge via a sample loop placed in the cartridge 1 position. See section 6.5.5 for details on the installation and use of the desalting loop.

**Note:** When processing two samples through the same cartridge, the method cleans and equilibrates the cartridge prior to processing the second sample.

3. Touch the arrow button to the right of the Cartridge Options list to select the appropriate cartridge size for your method (see section 6.2 for details on cartridge capacity and recommended sample loads). Select “1” or “2” cartridges when the option is available.

**Note:** The “1” or “2” cartridge selection option is only available with two-sample, affinity-only methods.

When finished setting the method type and options for the run, touch the Next button to proceed to the Enter Run & Sample Information screen. (The Select Sample Flow Rate and Wash Time screen is not available in the program method mode. The default values will be the standard sample loading flow rate and standard wash time. These values can be edited.)

### 7.3 Entering Run and Sample Information

The Enter Run & Sample Information screen (Figure 7.4) allows you to enter identification information for the purification run. You can enter a method name, a run name, lot number information related to the components used in this run, and specific sample details such as name, volume, and the $A_{280}$ extinction coefficient. This screen also allows access to edit the method step parameters to customize the method for your run.

**Note:** If you are running a method with two samples, you can enter separate information and edit method steps for both samples.
The following method-specific information can be entered or selected:

- **Method name** — name for the method that can be stored in the system (the system stores up to 35 program methods) and retrieved for later use. Enter the method name before editing and saving the method parameters.

- **Username** — optional user-designated name 3–15 characters in length to document the operator of the run; default is “Anonymous”. The entered username remains on the user interface until a new user edits the name or when cycling the instrument power. In the latter case the username defaults to “Anonymous”.

**Note:** The programmed method is saved with the date it was created and the username.

The information below the text box line on the screen relates only to the run and will not be saved with the method. This information will be stored with the run data file.

The following run-specific information can be entered:

- **Run name** — user-designated file name for the run, up to 30 characters in length.

- **(External fc)** — external fraction collection option; currently not available for use.

- **Lot # tracking** — optional; lot number information for the buffers, cartridges, and kits used for the run (“BLANK” indicates no information has been entered). The word “PRESENT” indicates that lot number information was entered. The lot number information remains in memory as long as the instrument power remains on. When cycling the power, the lot number information reverts to “BLANK”.

The following sample-specific information can be entered or selected:

- **Sample name** — optional; user-assigned name for the sample, up to 15 characters in length (“UNTITLED” indicates no information has been entered). If you have chosen to perform a run with two samples, you can assign a name for each sample.
• **Volume** — user-entered sample volume between 2 and 999 ml. If you have chosen to perform a run with two samples, a volume must be entered for each sample. If the sample volume is greater than 50 ml, the instrument sample line(s) can be connected directly to an external sample container (see section 11.2 for details on external connections). Make sure to follow the manual priming protocol at the Start Run screen if using external containers. See Appendix E for sample preparation details.

• **A$_{280}$ of 1 mg/ml** — extinction coefficient for the protein is expressed as the absorbance of a 1 mg/ml solution of the protein of interest at 280 nm for a 1 cm UV pathlength. The default value for this parameter is 1.00. An extinction coefficient can be assigned for each sample. This value is used to calculate the estimated total protein and protein concentration at the end of the run.

• **Home** — returns to the home screen. All entered run and sample information, except lot number tracking information, will be cleared when you touch the Home button. The entered lot number tracking information remains in temporary memory as long as instrument power is on. When cycling power, the lot number tracking information defaults to “BLANK”.

### 7.3.1 Adding or Editing Run and Sample Information

To add or edit sample and run information:

1. Touch the arrow buttons to the right of the information text box to scroll through the list of options and highlight the parameter for which you’d like to add information.

2. Touch the Edit button to access the alphanumeric or numeric keypad. The appropriate keypad displays.

3. Enter or edit information for the selected parameter in the text line at the top of the screen.

**Note**: If values entered exceed the system-set limits, the value limits appear immediately below the text line of the numeric keypad and the system overrides the entered value with the maximum or minimum value allowed.

4. Touch OK to accept all changes or Cancel to delete any entered data and return to the Enter Run & Sample Information screen.

5. Repeat this process for all information parameters. For details on entering lot number tracking, see section 7.3.2 below.

6. The Save button displays when the method name and method parameters have been edited. Touch the Save button to save the method in memory.

**Note**: Editing the name of a saved method file does not delete the original file. If the method with the edited name is saved, both files will be accessible in the list of saved methods (see section 8 for information on retrieving saved methods and renaming existing methods).

### 7.3.2 Adding Lot Number Tracking Information

To add lot number information for any of the reagents or components used in your method run:

1. Touch the arrow buttons to scroll through the list of options and highlight “Lot # Tracking”.

2. Touch the Edit button. You will proceed to the Edit Lot Number Information screen.
3. Touch the arrow buttons to the right of the lot number information text box to scroll through the list of buffers, solutions, and kit information relevant to the run. Highlight the item for which you want to add lot number information.

**Note:** In this screen only, the highlighted area will automatically advance to the next line each time lot number information is added.

4. Touch the Edit button to retrieve the alphanumeric keypad.

5. Add desired information into the keypad text line.

6. Touch OK to save input data and return to the Edit Lot Number Information screen, or Cancel to return to the Edit Lot Number Information screen without saving changes.

7. Repeat this process for each item that requires lot number information.

8. Touch Clear All to delete all information added, or OK to accept changes and return to the Enter Run & Sample Information screen.

**Note:** When lot number tracking information is entered, the word “PRESENT” is displayed. If no lot number tracking information is entered, the word “BLANK” is displayed. All entered run and sample information, except lot number tracking information will be cleared when you press the Home button. The entered lot number tracking remains in temporary memory as long as instrument power is on. When cycling power, the lot number tracking information defaults to “BLANK”.

### 7.4 Editing and Saving a Program Method

The method type that was selected in the Select Method Type & Options screen serves as a template for the program method. The sequence of the individual steps of the template cannot be changed; however, individual parameters of the steps such as flow rate, column volume, buffer concentration, and peak detection thresholds can be edited. See Appendix I for method-specific planning worksheets to help guide individual method step programming.

Touching the Edit Method button in the lower toolbar of the Enter Run & Sample Information screen displays the Method Information screen, which contains a table listing the major steps of the run. Step parameters are edited on the Select Parameters to Edit screen.

**Important:** Make sure the correct method temperature is selected for methods that include the desalting step.

#### 7.4.1 Editing a Program Method

To edit method parameters for your run:

1. Touch the Edit Method button in the Enter Run & Sample Information screen toolbar. You will proceed to the Method Information screen (Figure 7.5), where the first editable step will be highlighted.
Fig. 7.5. Method Information screen. The edit button displays only if the highlighted step has editable parameters. The View Sample button appears for two-sample methods and is used to toggle between the method steps for each sample.

**Note:** When two samples are selected for the method, the View Sample button is displayed above the arrow buttons. The View Sample button is used to toggle between the method steps for sample 1 and 2. The sample relevant to the displayed method is shown in the upper right corner, immediately above the information text box.

2. Touch the arrows to scroll through the list of method steps. The Edit button is only available for those steps that can be edited.

3. Highlight the step you want to edit and touch the Edit button. You will proceed to the Select Step Parameter to Edit screen (Figure 7.6). The step name and all parameters associated with that step are listed on the screen.

Fig. 7.6. Select Step Parameter to Edit screen with typical parameters for most editable steps displayed.
Most steps show the following information and parameters:

- **Method name** — editable in the Enter Run & Sample Information screen
- **Step name** — cannot be edited
- **Flow rate and column volume** — limited parameters; dependent on method type, cartridge type and size, and operating temperature (for desalting cartridges only). The numeric keypad displays the value limits immediately below the text line if the entered values exceed the limits
- **Step time** — calculated from the entered values for flow rate and column volume; cannot be edited
- **Concentration** — buffer position in the instrument and the concentration of that buffer. The instrument dilutes the concentrated buffer to 1x. Concentration values allowed are whole integers between 1 and 5
- **Frac** — fraction number indicates the fraction designation for that step and cannot be edited

4. Touch the arrow button to scroll through step parameters. The Edit button is available only for those parameters that can be edited.

5. Highlight the parameter you want to edit and touch the Edit button to retrieve the numeric keypad.

6. Enter new values for the parameter in the text line.

7. In the numeric keypad, touch the OK button to accept the new value or Cancel to revert to the previous value. You will return to the Select Step Parameter to Edit screen.

8. When finished editing parameters for the step, touch the OK button to return to the Method Information screen.

9. Repeat this procedure to edit all desired step parameters for your method, then touch the Save button to store the method in memory. When the method is saved in the Method Information screen it will be saved with the name as shown in that screen. If a different name is needed, return to the Enter Run & Sample Information screen and edit the method name.

10. Touch the OK button at the bottom of the Method Information screen to return to the Enter Run & Sample Information screen.

**Editing Peak Detection Parameters**

Peak detection parameters are available for the protein elution steps when the bound protein is eluted from either the affinity cartridge to the desalting cartridge or directly from the affinity or desalting cartridge to a fraction tube.
Fig. 7.7. Select Step Parameter to Edit screen with parameters for protein elution steps displayed.

The peak detection parameters that can be edited for these steps are:

- **Peak detect delay** — time value indicates the amount of time that occurs in this step before automatic peak detection is activated. (The run screen displays a light bulb icon immediately in front of the UV cartridge line when the automatic peak detection is activated)

- **Max peak volume** — peak detect threshold. If the peak is not detected by the time this volume is reached, the method will advance to the next step. The default setting is intended as a safety setting for when the peak is not automatically detected, for example, with very low expression proteins. When this value is reached, the method will advance to the next step to ensure that the region where the protein is eluting is collected

Note: If manual peak detection is preferred, you can enter a large peak detect delay time so that peak detection is activated after the protein peak starts to elute. In this case, press the Manual Peak Detect button on the run screen to advance to the next step when the protein starts to elute. (The UV value, displayed on the run screen, starts to increase when the protein begins to elute). The next step also has a Manual Peak Divert button to manually determine the end of the peak and advance to the next step.

Flow rate, step time, concentration, and frac are not editable.

7.4.2 Saving a Program Method

The Save button displays both in the Enter Run & Sample Information screen when the method name has been edited and in the Method Information screen when edits are made. When the method name is edited, touch Save to store the method in memory. When all edits are completed, touch the Save button to save and store the edited method to memory and proceed to the Install Reagents, Sample & Fraction Tubes screen to set up the instrument for a run. The programmed method is saved with the date it was created and the username. You can also proceed with the programmed method without saving the method. When the name of a saved method is edited in the Enter Run & Sample Information screen, the Save feature then acts as a “save as” function, and the new method is also stored in memory. (See section 8, Saved Methods, for additional topics, such as deleting and renaming methods and full memory).
When all edits are completed, touch the Save button in the Enter Run & Sample Information screen to store your method in system memory. As your method is written to memory, a popup screen appears with a “Working — Please Wait” message.

**Note:** You can also run the edited method without saving it to memory.

If all run and sample information has been satisfactorily updated, touch the Next button. You will proceed to the Install Reagents, Sample & Fraction Tubes screen.

### 7.5 Installing Reagents, Sample Tubes, and Fraction Tubes

The Install Reagents, Sample & Fraction Tubes screen (Figure 7.8) displays a diagram showing where buffer, diluent, sample, fraction, and waste containers should be placed in the Profinia instrument (cartridge installation is described in section 6.6). **This diagram is specific to the method type and options selected for the run.** The volumes indicated for the buffer, diluent, and waste bottles are the minimum volumes required. Volumes indicated for the fractions are those that will be delivered to the fraction tubes. An “X” in any position indicates that no reagent or container is required in that position. The empty waste container displayed on the diagram serves as a reminder to empty the waste container prior to starting a run.

**Note:** Adjustments made to methods during programming may result in buffer, diluent, or waste bottle and sample or fraction tube volume requirements greater than the capacities of the standard bottles and tubes provided with the instrument. For instructions on modifying the system to accommodate larger bottles and tubes, see section 11.2.

![Fig. 7.8. Install Reagents, Sample & Fraction Tubes screen (left) with arrows demonstrating the correlation between the diagram and placement of reagents in the Profinia instrument (right).](image)

The installation screen diagram displays:

- **Buffer bottles** — eight buffer bottle positions indicated by labels B1 through B8 on the touch screen (top of the screen) and 1 through 8 on the instrument (upper tray). The touch-screen diagram also displays the minimum buffer volumes required for the selected run; an “X” indicates no reagent is required at that location. Three dashes (---) followed by “ml volume” indicates the required volume exceeds 999 ml

**Note:** If the reagent volumes exceed the container capacity, a larger container can be connected to the instrument externally. When reagents are connected externally make sure to prime the lines manually immediately prior to starting the run. The manual priming function is available in the Start Run screen.
• **Waste bottle** — left side of the touch screen and positioned at the lower left of the instrument. On the touch screen, the waste bottle is displayed without a volume as a reminder to make sure the waste bottle is emptied frequently.

• **Diluent (water) bottle** — right side of the touch-screen diagram and positioned at the lower right of the instrument. On the touch screen, the diagram is labeled with the minimum volume of water required for the selected method run.

• **Sample tubes** — indicated by S1 and S2 labels on the touch screen, the sample tubes are placed beneath the buffer bottles toward the right of the instrument. The touch screen displays one or two sample tubes with the sample volumes, depending on the number of samples and sample volumes that have been entered by the user on the Enter Run & Sample Information screen.

**Note:** Each run primes the sample line(s) with approximately 1 ml of sample. To ensure the entered sample volume is loaded on the cartridge, filling the sample tubes with 1 ml more than the stated volume is recommended. If the exact entered volume is added to the sample tube, the end of the sample detection, if activated, applies the complete sample to the system. In this case, the total amount of sample added to the cartridge will be about 0.5 ml less than the entered volume. To maximize protein yields, 10 ml is recommended sample load.

• **Fraction collection tubes** — indicated on the touch screen by a set of four tubes on the left labeled 1A through 1D for sample 1 collection, and four tubes on the right labeled 2A through 2D for sample 2 collection. Fraction tubes are placed at the bottom of the instrument. Each fraction tube shows the volume that will be collected in the tube. Only the required fraction tubes are represented in the run method installation diagram. Before beginning your run, make sure the fraction tube capacities match the volumes indicated.

**Note:** The fraction positions are numbered 1A through 1D from left to right, and 2A through 2D are ordered right to left. This places the purified protein in the center two positions, for easiest access and for use with the cooling accessory.

• **View Details** — lower toolbar button; proceeds to a screen (Figure 7.9) that displays the name for each of the components shown in the system setup screen. Touch OK to return to the Install Reagents, Sample & Fraction Tube screen.

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**Fig. 7.9. Review Buffer & Fraction Details screen displayed when the View Details button is selected.**
Note: Reagent volumes that exceed system container sizes can be used in external containers connected to the instrument via extra tubing. Make sure to manually prime these lines prior to starting the run. The Start Run screen has a Manually Prime button in the lower toolbar (see section 7.8.2).

7.5.1 Preparing and Installing Reagents and Samples for the Purification Run

To install program method reagents:

1. Fill each buffer bottle with the minimum volume as indicated on the screen.
2. Place a buffer lid on each buffer bottle and install each buffer bottle as indicated on the screen.

Note: Buffer lids help keep contaminants out of the buffer bottles and guide sipper tubes into the bottles.

3. Position the buffer bottles with the graduated marks facing out, making it easier to check buffer volume during the method (see section 6.5.1 for proper buffer bottle orientation).
4. Add sample to the sample tube(s).
5. Place sample lids on the sample tubes and position the sample(s) in the instrument.

Important: Each run primes the sample line with about 1 ml of sample. To ensure that the full amount is loaded onto the cartridge, fill the sample tubes with an additional 1 ml than the entered volume. If the exact entered volume is added to the sample tube, the end of sample detection, if activated, applies the complete sample to the system. In this case, the total amount of sample added to the cartridge will be approximately 0.5 ml less than the entered volume. The Run Completed screen displays the actual amount of sample that was loaded onto the cartridge.

6. Make sure the diluent bottle is filled with sufficient deionized water and the waste container is empty or has sufficient space to hold the generated waste.

7. Install the fraction tubes as indicated on the screen diagram. If the volume required exceeds the instrument capacity, connect external collection containers using extra tubing (see section 11.2).

When all the reagents, sample(s), and fraction tubes have been installed according to the installation diagram, touch the Next button to proceed to the Prepare for Cartridge Installation screen.

7.6 Installing Cartridges

Prior to installing the required cartridges, the instrument cartridge lines must be primed to prevent introduction of air bubbles into the cartridge. The cartridge installation routine consists of four consecutive screens (see section 6.5 for complete cartridge installation instructions):

- Select Cartridge Status — includes the option to bypass the installation procedure when the cartridges are already installed (for example, from a previous run) and proceed directly to the Start Run screen
- Setup Cartridge Positions for Priming — directs connection of the cartridge system fittings at positions 1 and 2
- Priming in Progress — displays the priming time progress bar
• **Install Cartridges** — displays the required cartridges and their respective positions

When cartridge installation is complete, touch the Next button to proceed to the Start Run screen.

### 7.6.1 Installing the Sample Loop (Desalting-Only Methods)

**Warning:** When the desalting-only method is selected, DO NOT fill the sample loop with sample until the sample loop and cartridge installation is completed. Inject the sample immediately prior to starting the run.

Sample loading for a desalting-only method is achieved via a sample loop. Installation is directed by the Install Bio-Scale Mini Cartridges screen (Figure 7.10). The desalting sample loop accessory connects to the instrument in the cartridge 1 position and the desalting cartridge in the cartridge 2 position (Figure 7.12).

![Install Bio-Scale Mini Cartridges screen](image)

**Fig. 7.10.** Install Bio-Scale Mini Cartridges screen showing sample loop installation in the cartridge 1 position.

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. Install the desalting cartridge in the cartridge 2 position. Touch the Next button to proceed to the Start Run screen (Figure 7.10).
4. 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 toward the loop and fill the loop with sample, avoiding bubbles as much as possible.

5. 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.

Fig. 7.11. Start Run screen directs application of the sample to the sample loop (desalting-only method).

Fig. 7.12. Sample loop installed in the cartridge 1 position.

**Note:** 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, since it will result in increased dilution of the sample and reduced desalting resolution.
7.7 Viewing Real-Time Data With Profinia Software

To collect real-time data:

1. Connect the USB cable provided with the Profinia software package to the USB port on the back of the instrument and to the computer.

**Important**: The front USB port does not output real-time data.

2. Open Profinia software on your computer by double-clicking on the desktop Profinia software icon. Look for the connection icon in the Profinia software in the lower left corner of the software screen (see section 12 for more information on using Profinia software).

At this point, the run can be started and the chromatogram displays on the computer screen.

7.8 Starting the Run

The Start Run screen (Figure 7.13) displays a summary of the sample information. It also displays a reminder to connect the instrument to a computer running Profinia software if you would like to view the chromatogram in real time. The Profinia system does not require software and a computer to operate the instrument; current data points appear on the running screen.

The lower toolbar contains buttons for viewing the methods steps and for optional manual priming of any extra tubing attached to the sample, buffer, diluent, or waste lines to accommodate larger containers in these ports.

**Note**: Touching the Home button returns the user directly to the home screen and all the run and sample information resets, except the lot number tracking information. A saved method remains stored in memory. An edited method that is not saved reverts to the original settings.

Fig. 7.13. Start Run screen.
7.8.1 Viewing Steps

Touch the View Steps button (available on the Start Run screen, and at any point during the run) to retrieve the View Method Steps screen. During a run, the step that is currently in progress is highlighted.

7.8.2 Running the Manual Prime Function

**Note:** The manual prime function is needed to prime any extra tubing up to the point of connection to original inlets.

To prime extra tubing attached to buffer, sample, fraction tube, diluent, or waste outlets to accommodate larger containers, touch the Manually Prime button at the bottom of the Start Run screen. The Select Item to Prime Manually screen displays (Figure 7.14).

![Fig. 7.14. Select Item to Prime Manually screen.](image)

1. Touch the arrow buttons to scroll through the list of reagent lines and highlight the line that requires manual priming.
2. Touch the Prime button to start priming. The pump continues priming until you touch the Stop button.
3. Select the next reagent line for manual priming (if needed), and press Prime.
4. Continue until all reagent lines connected with extra tubing have been primed.

**Note:** Prime lines until no air is visible in the tubing or when fluid is seen entering the waste container.

**Priming Multiple Lines**

To prime multiple lines, either follow the procedure outlined in the previous section, or:

1. Touch the arrow buttons to scroll through the list of reagent lines, highlight the desired line, then touch the Prime button.
2. While the system is priming the first reagent line, use the arrow buttons to select the next reagent line (priming of the first line continues).
3. Touch the Prime button again to immediately start priming the next selected reagent line upon completion of the first selected line.

4. Repeat until all the required reagent lines are primed, then touch the Stop button to stop the pump.

When all manual priming is completed, touch the Back button in the toolbar to return to the Start Run screen.

7.8.3 Clearing System Memory to Start Run

To start your purification run:

Touch the Start button in the lower right of the screen. The Preparing Data Memory Storage screen displays with a progress bar indicating temporary memory data clearance.

**Note:** The system can store up to three run data files in memory. An “M” status indicator displayed in the upper right corner of the screen indicates none of the run data files stored in system memory have been transferred. If system memory is full, the oldest transferred or stored data file will be overwritten with the new file when a purification run is started.

Once memory has cleared, the method starts and the Run in Progress screen displays.

7.9 Monitoring Run Progress

The Run in Progress screen (Figure 7.15) displays an overview of the progress of the current run, as well as basic details about the current step in progress and expected sample elution times (see section 6.8.1 for a complete list of screen features).

![Fig. 7.15. Run in Progress screen.](image)

7.9.1 Peak Detection

Affinity and desalting protein peaks are identified using an automatic peak detection algorithm that is active during elution steps on the Profinia instrument. In addition to automatic peak detection, the option to manually detect and divert the protein peak is also available during affinity and desalting elution step(s). When manual peak detection is available, the manual peak detect/divert button displays on the Run In Progress screen. See section 6.8.2 for details on automatic and manual peak detection.
7.9.2 Collecting Purified Sample

The purified sample is collected in fraction tube position 1D or 2D. When the purified protein is collected, the Run in Progress screen displays the actual elution times and the tube can be removed. After the target protein has eluted, the Profinia system will automatically continue to run the cartridge cleaning and storage procedures and process the second sample if selected.

7.9.3 Completing the Run

Run results display as soon as the end-of-run cleaning procedures are completed.

7.10 Preparing System for End-of-Run Cleaning

System cleaning is an automatic feature that occurs upon completion of each method run and is required to prevent cross-contamination between runs and to maintain the system for optimum operation. The two sample lines and the fraction line valve (all eight fraction lines) will be cleaned as part of the end-of-run cleaning process. The pump seals are also washed as the final step in this process and the internal system lines are cleaned with 20% ethanol from buffer position 8.

7.10.1 Preparing for Line Cleaning

To prepare the system for end-of-run cleaning:

1. Remove all sample tubes from the instrument.
2. Place tubes containing 10 ml of deionized water in both sample 1 and sample 2 positions; place water in both sample positions even if only one sample was used in the run.
3. Fill a buffer bottle with 20% ethanol and place it in buffer position 8.
4. Remove all fraction collection tubes from the instrument.
5. Place the cleaning tray (provided with the instrument) in the fraction collection area.
6. Touch the Start button to begin the cleaning procedure.

You will proceed to the System Cleaning in Progress screen.

7.10.2 System Cleaning in Progress Screen

When system cleaning starts, the System Cleaning in Progress screen displays the four system cleaning procedures: Clean Sample Lines, Clean Fraction Lines, Clean Buffer Lines, and System EtOH Wash. The cleaning steps that will be performed after a run is completed are indicated by darkened circles. A checkmark adjacent to a darkened circle indicates the cleaning step has been completed. When the cleaning process is completed, you will proceed to the Run Completed screen and the run results are displayed.

7.11 Performing End-of-Run Procedures

The Run Completed screen (Figure 7.16) is displayed on completion of all purification run steps, including system cleaning. Run details for each sample purified are displayed in the run data table. The total estimated protein (yield) and protein concentration have been calculated and are displayed for each sample.
7.11.1 Exporting Data to a Computer

The run data file can be transferred to a portable memory device as an .ofi file, which can be imported into Profinia software (optional) for viewing data in tabular form and in a chromatogram. If Profinia software has not been purchased, the data file can be viewed in any compatible spreadsheet software (for example, Microsoft Excel). To export the run data file:

1. Insert a USB portable memory device into the USB port directly below the touch screen. The Export Data button is initially displayed with an "X" across it, indicating that the button is not yet functional. As soon as the system recognizes the portable memory device, the "X" disappears and the Export Data button is available. The time required for recognition depends on the portable memory device used and can take up to 15 sec. (See the troubleshooting details in Appendix C if the memory device is not recognized.)

2. Touch the Export Data button. A progress bar displays indicating that the transfer is in progress; typical transfer time is approximately 60 sec. The actual transfer time depends on the amount of data in the run data file and the portable drive.

Upon completion of data export, you will automatically return to the Run Completed screen.

7.11.2 Performing Final Run Procedures

The toolbar at the bottom of the Run Completed screen displays the final steps to shut down the system or prepare for a new run. Choose one of the following options:

- **Re-Run Method** — immediately begin a new sample run with the same method
- **Run New Method** — immediately begin performing another purification run using a different method type, or new buffers for the run that was just performed
- **Short-Term Wash** — if the system will not be used again on the same day, the buffer lines and valves should be rinsed with water
- **Long-Term Wash** — if the system will not be used in the next 2 days, it should be rinsed with water and ethanol or other sanitizer to maintain optimum mechanical performance and prevent bacterial growth

See section 6.10.2 for details on performing final run procedures.
**Important:** When the Profinia system is used in a cold environment, it is best to leave instrument power on; the slight amount of heat generated helps control condensation. The UV lamp automatically turns off one hour after the last separation run, and turns back on when instrument operation resumes. If the instrument will be stored long term in a cold environment, it is acceptable to turn the power off. When the instrument is removed from a cold environment, the unit must be equilibrated to room temperature. This prevents condensation from forming which could possibly be damaging to internal components.
Section 8
Saved Methods

The Profinia™ instrument can store up to 35 program methods in memory. Saved methods can be retrieved alphabetically according to the method name assigned by the user during programming, by method type, or as a single list of all stored methods. Program methods can be retrieved from memory for subsequent runs or used as method templates for additional customizing. Saved methods are retrieved directly from the home screen (Figure 8.1) through the Saved Methods menu.

Run data and parameters retained in saved program methods include:

- Method name
- Username
- Date method created
- Flow rate
- Column volume
- Buffer concentration
- Peak detection thresholds

Sample volumes and other run-specific information are not retained with saved program methods and need to be entered when running a saved method.

Note: Make sure the correct method temperature is selected for saved methods that include the desalting step.

8.1 Retrieving Saved Methods

To retrieve methods saved in system memory:

1. Touch the Saved Methods button in the toolbar on the home screen. You will proceed to the Select Saved Method System to View screen.
2. Use the arrows to select the viewing mode by: “Method System” or “All Method Systems”. Touch the Next button to proceed to the Select Saved Method screen.

8.2 Selecting Saved Methods

In the Select Saved Method screen (Figure 8.3), immediately above the list of saved methods, the date the method was created, the username (person who created the method), and the method type display for the highlighted method.

**Note:** To ensure that you are listed as the person who created your method, make sure you enter your name in the Username field of the Enter Run & Sample Information screen when programming a method.

**Note:** System date and time settings can be modified under the Data/Utilities menu; see section 9.3.3 for more information.

![Fig. 8.2. Select Saved Method screen with username, creation date, and method type displayed for the highlighted stored method.](image)

The following options are available in the Select Saved Method screen:

- **Delete** — deletes the selected method only; a popup message appears, requesting confirmation
- **Rename** — renames the selected method; the old name will be overwritten with the new name. To create a duplicate method with a different name press Select, edit the method name in the Enter Run & Sample Information screen, and press Save; this is the equivalent to a “save as” function
- **Select** — opens the highlighted method and proceeds to the Enter Run & Sample Information screen
- **Back** — returns to the Select Method Type to View screen
- **i** — retrieves help information about the screen

8.3 Running Saved Methods

To run a saved method, highlight the desired method from the Select Saved Method screen and touch the Select button to proceed to the Enter Run & Sample Information
screen (Figure 8.3). See section 7.3 for details on editing the method and entering run and sample information.

![Enter Run & Sample Information screen](image)

**Fig. 8.3.** Enter Run & Sample Information screen retrieved when saved method is selected.

The following options are available in the Enter Run & Sample Information screen:

- **Save** — saves revised information (button displays only when edits have been made). Touch the Save button to store the edited method to memory.

**Note:** The Save button in this screen is programmed as a “save as” function. When the name of a saved method is edited in the Enter Run & Sample Information screen, the newly named method is stored in memory in addition to the original saved method. A popup message displays, indicating method memory is full when 35 methods are stored. You will need to delete a method prior to saving a new method. The new method can be run without saving it to memory. When memory is full, the upper right status corner of the screen displays “F”.

- **Edit** — accesses the alphanumeric or numeric keypad to edit method name, username, and run and sample information.
- **Edit method** — accesses the method steps for editing the individual step parameters.
- **Back** — returns to the Select Method Type & Options screen; allows editing of the method options.
- **Save — Methods** — returns to the “Select saved Method System to View” screen.

For details on setting up and starting the run, see section 6.

**Important note for Program Methods users:**

The saved methods using older version of firmware may not work properly using newer versions of firmware. The best practice is to record the parameters saved in the old methods and reprogram it as a new method on the new firmware. Refer to Appendix K for Profinia planning worksheets to record parameter changes.
Section 9
Data and Utilities

The Data/Utilities screen on the Profinia™ purification system provides access to maintenance, diagnostic, and calibration functions that are used to ensure optimum instrument performance; it also provides options for managing data generated from purification runs.

9.1 Exporting Data & Real-Time Data Acquisition

Profinia system run data files include information about sample protein yield, concentrations, elution times, and the main steps of a purification method. Profinia system memory can store up to three data files. If three data files are already in system memory when a new run is started, the most recent file overwrites the oldest file.

9.1.1 Exporting Data to USB Portable Memory Device

To transfer a stored run data file to a computer running Profinia software or a spreadsheet-based software program:

1. Insert a portable memory device into the USB port on the front of the Profinia instrument (Figure 9.1).

2. Touch the Data/Utilities button in the toolbar at the bottom of the home screen.

3. On the Utilities screen, touch the Data button to proceed to the Select a Stored Run Data File screen.

4. Touch the arrow buttons to scroll through the list of stored run data files; highlight the file you would like to transfer.

5. Touch the Export Data button to begin file transfer. The screen displays a progress bar during file transfer.

6. When transfer is complete, remove the USB portable memory device.
**Warning:** Most USB portable memory devices display a flashing light when data is being transferred. Wait until the light stops flashing, an indication that the file transfer is complete, before removing the portable memory device from the instrument.

**Note:** An asterisk appears next to run data files that have been transferred to a USB portable memory device.

### 9.1.2 Real-Time Data Transfer to Profinia Software

To transfer a run data file from the Profinia system to a computer in real time:

1. Before starting your purification run, use the USB cable provided with Profinia software to connect the instrument (using the USB port on the back of the instrument) to a computer that has Profinia software installed.

   **Important:** To capture data in real time, the USB cable connection must be established prior to starting a purification run.

2. Open Profinia software on the computer. A connection between the software and the instrument will be indicated by a “Waiting for data” message at the bottom of the Profinia software screen on your computer (Figure 9.2). This message indicates that a run can be started.

   ![Fig. 9.2. “Waiting for data” message on the bottom of the Profinia software screen.](image)

3. Use the directory section of Profinia software in the upper left corner of the screen to determine where you want your data file stored on your computer (see section 12 for general information on running Profinia software).

4. Select Default from the software Options menu, and use Browse to select the file or create a new file in the Profinia instrument for your run data information.

5. Touch the Start button on the Start Run screen.

   Data from your purification displays in real time on your computer screen as the run progresses.

### 9.2 System Utilities

The following list of calibration functions is accessible from the system menu on the Utilities screen:

- Calibrate Touch Screen
- Pump Flow Rate Check
- Calibrate UV Monitors
- Calibrate Conductivity
- Adjust Peak Detection Sensitivity
- Calibrate pH Probe
- Calibrate Air Detection System
• **Calibrate Pressure Sensor**

9.2.1 **Calibrating Touch Screen**

To calibrate the system touch screen:

1. Touch the Data/Utilities button from the system home screen, then touch the Diag/Maint Functions button.

2. Touch the up and down arrows to scroll through the list of functions and highlight “Calibrate Touch Screen”, then touch the Select button.

3. Use the stylus to tap the center of five consecutive screen targets (Figure 9.3). When you touch the center of the last confirmation target in the center of the screen, the system returns to the Home screen.

![Touch screen calibration screen.](image)

9.2.2 **Checking Pump Flow Rate**

The Profinia instrument’s pump does not require calibration. If the fraction size is not as expected or other symptoms are seen, check the flow rate. If the flow rate is not within specifications, clean the system check valves and replace the inline filter disk (see section 13.2 for more details). The following items are required to check the pump:

• **Graduated cylinder** — suitable for measuring the volume of water delivered

• **Deionized water** — Profinia diluent (water) bottle filled to 200 ml

To check the pump flow rate:

1. Touch the Data/Utilities button in the toolbar at the bottom of the home screen, then touch Calibration Functions.

2. Touch the up and down arrows to scroll through the list of calibration functions and highlight “Pump Flow Rate Check”, then touch Select; an information screen appears.

3. Touch the Next button to proceed to the Calibration screen.

4. Touch the Edit button to choose the flow rate, then touch the Edit button again to set the time for the flow rate test using the alphanumeric keypad.
5. Touch the Start button; the system pumps water from the water bottle to the waste outlet to remove air from the system and the delivery tube. When the step is finished, the screen displays “Priming Complete” and additional instructions.

6. Remove the waste line from the waste bottle and place the waste line in an empty graduated cylinder.

7. Touch Start to pump deionized water through the system at the flow rate and for the duration set in step 4.

8. When the test is complete the system displays “Flow Rate Check Completed”. Note the expected quantity on the screen. The actual quantity delivered when bypassing the cartridge during the flow rate check can be up to 7% over the expected value if the system is working optimally. The additional restriction from directing the flow through the cartridges during the method will result in the expected quantity displayed on the screen.

If the quantity is less than the displayed expected quantity, change the inline filter disk and make sure that all the air has been purged from the system before repeating the test procedure.

If you continue to have problems with system flow rates, clean or replace the pump check valves (Section 13.2.1) or replace the pump seals (Section 13.2.2). If the system still does not operate normally, contact your local Bio-Rad office for assistance.

9.2.3 Calibrating UV Monitors

You may wish to calibrate the UV monitors using a protein similar to your protein of interest for improved quantitation. The following materials are required to calibrate the UV monitors:

**Protein solution of known absorbance** — prepare a protein solution and measure its absorbance in a spectrophotometer at 280 nm. The absorbance value for a 1 cm pathlength will be entered prior to the calibration process. Note:

- Choose a solution that has an absorbance reading less than 2 AU at 280 nm, for a 1 cm pathlength on the spectrophotometer
- If you use a cuvette that is less than 1 cm pathlength, correct the absorbance reading to 1 cm. For example, if you use a 2 mm cuvette, multiply the reading by 5 and enter this value in the calibration screen
- Choose a solution that has an absorbance reading less than 1 unit on the UV monitor (the instrument has a 2 mm pathlength)
- A protein concentration of 1–2 mg/ml is a suggested starting point

**Deionized water** — 45 ml in a 50 ml sample tube; 500 ml in the diluent (water) bottle for placement in the system’s Di water port

**20% ethanol solution** — 100 ml in a Profinia 125 ml buffer bottle

To calibrate the UV monitors:

1. Fill a 125 ml Profinia buffer bottle with 20% ethanol and place it in buffer position 8.
2. Prepare the standard protein solution and measure its absorbance at 280 nm. Correct the absorbance value to 1 cm pathlength.
3. Fill a 50 ml sample tube with 45 ml of this UV absorbance standard solution, and place it in sample position 1.
4. Fill a 50 ml sample tube with 45 ml of deionized water, and reserve it for later use (to wash sample line 1 when calibration is complete).

5. Fill the diluent (water) bottle to 500 ml with deionized water, and place it in the Di water port.

**Note:** Make sure to empty the waste bottle before performing this procedure to prevent overflow.

6. Turn the Profinia instrument on.

7. Touch the Data/Utilities button on the system home screen, then touch Calibration Functions.

8. Touch the up and down arrows to scroll through the list of functions and highlight “Calibrate UV Monitors”, then touch Select; the UV Calibration Information screen appears.

9. Touch the Next button; you will proceed to the Calibrate UV Monitors screen.

**Note:** The screen displays the UV-1 and UV-2 values, as well as the C (correction) factors for each of the two monitors.

10. Touch the Edit button to enter the absorbance of your standard solution (in absorbance units per centimeter) into the alphanumeric keypad. Touch the OK button to confirm.

11. Touch the Start button. The instrument will:
   a) Pump ethanol through both UV flow cells.
   b) Rinse the UV flow cells with water.
   c) Zero the UV monitors on water.
   d) Fill the UV flow cells with the protein solution.
   e) Set the UV absorbance value for both UV monitors to the absorbance value entered in step 10.

12. Upon completion of these procedures, the instrument displays “UV Calibration complete — Place tube of DI water at sample position 1”. Place the tube filled with deionized water in sample position 1, then touch the Start button. The system washes with water, then returns to the Calibration Functions screen.

**9.2.4 Calibrating Conductivity**

The Profinia system’s conductivity monitor is calibrated during instrument manufacturing, and generally does not require calibration unless the conductivity flow cell is replaced. The following materials are required to calibrate the conductivity monitor:

- **Conductivity standard solution** — conductivity 10 millisiemens per centimeter (mS/cm); 45 ml of solution is required
- **Deionized water** — 45 ml in a 50 ml sample tube, 500 ml in the diluent (water) bottle for placement in the system’s Di water port

To calibrate the conductivity monitor:

1. Turn the instrument on; the system should warm up for 20 min before calibrating the conductivity monitor.

2. Fill a 50 ml sample tube with 45 ml of conductivity standard solution, then place it in sample position 1.
3. Fill the system’s water bottle with at least 500 ml of deionized water. Empty the waste bottle if needed.

4. Touch the Data/Utilities button on the system’s home screen, then touch Calibration Functions.

5. Touch the up and down arrows to scroll through the list of functions and highlight “Calibrate Conductivity Monitor”, then touch Select to display the Calibrate Conductivity screen.

6. Touch Next to proceed to the Calibrate Conductivity Flow Cell screen, which displays calibration instructions.

7. Touch the Start button. The system pumps conductivity standard solution through the conductivity flow cell, then set conductivity to 10 mS/cm. When finished with this step, the system will pause and display “Calibration Complete”.

8. Place the tube of water in sample position 1, then touch the OK button. The system flushes the sample line with water and when complete, returns to the Calibration Functions screen.

9.2.5 Adjusting Peak Detection Sensitivity

Peak detection sensitivity settings range between 0.1 (most sensitive) and 10 (least sensitive); the default setting is 1. Peak detection sensitivity is a global setting for both the Bio-Rad methods and the program methods modes. The peak detection sensitivity parameter allows the instrument to adjust the automatic peak detection algorithm to various peak shapes and sizes in the event the default setting does not recognize a peak during the specified time window. In general, use lower numbers (more sensitive) if a small protein peak is expected.

**Note:** Using more sensitive settings for peak detection may lead to activation of automatic peak detection from small deviations in the UV that are not associated with the protein peak.

To adjust peak detection sensitivity settings:

1. Touch the Data/Utilities button on the system home screen, then touch Calibration Functions.

2. Touch the up and down arrows to scroll through the list of functions and highlight “Adjust Peak Detection Sensitivity”, then touch the Select button; the numeric keypad displays.

3. Enter a value between 0.1 and 10, then press OK to return to the Calibration Functions screen.

9.2.6 Calibrating pH Probe

The Profinia pH monitor is calibrated at pH 4 and pH 10. The following materials are required to calibrate the pH probe:

- **Standard pH calibration buffers** — available from scientific supply companies; approximately 50 ml of each buffer is required to calibrate the system. Using colorless standards is recommended, since certain dye additives require additional wash steps for removal

- **Deionized water** — diluent (water) bottle filled to 500 ml, and two sample tubes to 50 ml to flush the system after calibration
To calibrate the pH probe:

1. Touch the Data/Utilities button on the system home screen, then touch Calibration Functions.
2. Touch the up and down arrows to scroll through the list of functions and highlight “Calibrate pH Probe”, then touch the Select button. An information screen appears.
3. Touch the Next button; the Calibrate pH Probe screen displays with a list of detailed instructions.
4. Pour 50 ml of each pH buffer (pH 4 and pH 10) in separate 50 ml sample tubes.
5. Place the bottle containing the pH 4 buffer in sample position 1.
6. Place the bottle containing the pH 10 buffer in sample position 2.
7. Touch the Start button. The system will:
   a) Flush the pH probe with water.
   b) Pump pH 4 buffer to prime sample line 1 and the pH probe. The system reduces the flow rate and sets the pH value to 4.
   c) Pump pH 10 buffer to prime sample line 2 and the pH probe. The system reduces the flow rate and sets the pH value to 10. The system pauses and displays “pH Probe Calibration Complete”.
8. Replace the tubes of pH buffer (sample positions 1 and 2) with 50 ml tubes filled with deionized water.
9. Touch the Start button. The system flushes the lines with water, then returns to the Calibration Functions screen.

9.2.7 Calibrating Air Detection System

Calibration of the three air sensors is required for optimum performance. The calibration process measures electronic signals of air and liquid to establish the thresholds for end of sample/reagent detection. To calibrate the air detection system, follow the on screen instructions.

9.2.8 Calibrating Pressure Sensor

The Pressure Sensor Calibration process contains the re-calibration for the zero point of the pressure measuring system. Calibration of the zero pressure point is needed if the pressure reading during non-operation of the system does not return to zero. Access the Manual mode from the Data/Utilities section to observe the pressure reading.

To calibrate the zero pressure point, access the Calibrating Pressure Transducer from Home/Data-Utilities/Calibration Functions and follow the on-screen directions.

9.3 Diagnostic/Maintenance Functions

The following list of diagnostic and maintenance functions is accessible from the system menu on the Diag/Maint Functions screen (Figure 9.4):

- Display Firmware Version
- Manual Operation
- Set Time and Date
- Clean Sample Lines
- Clean Fraction Lines
- Clean Buffer Lines
- System Ethanol Wash
- Clean All Inlet and Outlet Lines
- Clean Pump Check Valves
- System NaOH Wash
- Clean Inline Check Valve
- Affinity Cartridge Cleaning in Place
- Desalting Cartridge Cleaning in Place
- Select End of Sample/Reagent Detection
- Select Method Temperature
- Adjust Display Contrast

![SELECT FUNCTION OR FUNCTION CATEGORY](image)

Fig. 9.4. Diag/Maint Functions screen.

9.3.1 Displaying Firmware Versions

To retrieve the version numbers for the system’s main firmware components:

1. Touch the Data/Utilities button on the system home screen.
2. On the Utilities screen, touch the Diag/Maint Functions button.
3. Touch the up and down arrows to scroll through the list of functions and highlight “Display Firmware Versions”, then touch the Select button. The screen displays the firmware versions for the main board, pump/valve controls, and UV monitor.
4. Note the version numbers for the firmware, then touch OK to return to the Diag/Maint Functions screen.

9.3.2 Setting and Operating Instrument Manually

Manual operation allows you to set the system valves in a desired position and manually run the system pump. Refer to the schematic diagram below (Figure 9.5) to identify which component you want to control.
The adjustable components are:

**Pump flow rate** — set in ml/min. After a flow rate is entered into the Edit menu and you touch the Update button, the pump starts. To stop the pump, press the Stop Pump button on the bottom of the screen.

**Inlet select valve (ISV)** — rotary valve (10 port) that selects the buffer or sample. After you enter a valve position into the Edit menu and touch the Update button, the valve moves:

- Positions 1 through 8 are buffers 1 through 8
- Positions 9 and 10 are samples 1 and 2
Outlet select valve (OSV) — rotary valve (10 port) that directs system output. After a valve position is entered in the Edit menu and you touch the Update button on the Manual Operation screen, the valve moves

- Positions 1 through 4 are fractions 1A through 1D
- Positions 5 through 8 are fractions 2A through 2D
- Position 9 is the fraction collector outlet
- Position 10 is the system waste outlet

Column valve 1 (CV1) — rotary valve (6 port) that directs the flow for cartridge 1. After a valve position is entered in the Edit menu and the Update button is pressed on the Manual Operation screen, the valve will move

- Position 1 directs flow to the system waste outlet
- Position 2 directs flow to flush behind the pump pistons
- Position 5 directs flow to column valve 2
- Position 6 directs flow to cartridge 1
- All other valve ports are plugged

Column valve 2 (CV2) — rotary valve (6 port) that directs the flow for cartridge 1. After a valve position is entered in the Edit menu and the Update button is pressed on the Manual Operation screen, the valve will move

- Position 4 directs flow to the system waste outlet
- Position 5 directs flow to the conductivity monitor, pH monitor (optional) and to the outlet select valve
- Position 6 directs flow to cartridge 2
- All other valve ports are plugged

Proportioning valve (PV) — solenoid valve (3 port, 2 position). The proportioning valve mixes concentrated buffer from the inlet select valve with water from the system water bottle. Position settings are 0x, and 1x through 5x:

- 0x draws 100% water from the water bottle
- 1x draws 100% buffer from the inlet select valve (ISV)
- 2x makes a 1:1 concentrate/water mixture
- 3x makes a 1:2 concentrate/water mixture
- 4x makes a 1:3 concentrate/water mixture
- 5x makes a 1:4 concentrate/water mixture

The proportioning valve operates only when the pump is running. The timing of the valve depends on the pump flow rate

Warning: Use care when controlling components manually. Improper settings may damage the cartridges or system components.
Manual control toolbar — buttons (5) at the bottom of the Manual Operations screen (Figure 9.6) that control:

- **Toggle lamp** — switches the UV lamp on or off: a light bulb icon in the upper left corner of the screen indicates that the UV lamp is on. Normally the Profinia lamp will turn off one hour after the last run is finished. If the lamp is manually switched on, it will remain on until it is manually switched off
- **Zero UV Detector 1** — sets the absorbance of UV monitor 1 to zero
- **Zero UV Detector 2** — sets the absorbance of UV monitor 2 to zero
- **Stop Pump** — stops the system buffer pump, if running
- **OK** — returns to the Diag/Maint Functions screen

Fig. 9.6. Manual Operation of Hardware screen.

To access the manual operation features:

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. Touch the up and down arrows to scroll through the list of functions and highlight "Manual Operation", then touch the Select button.

The Manual Operation screen has three parts — the status screen, the manual operation screen, and the toolbar (near the bottom of the screen):

**Status screen** — top of the Manual Operation screen, includes information regarding:

- **PSI** — system pressure value
- **pH** — value for pH. If pH probe is not connected the value shown is ~25
- **T** — system temperature value
- **AU1 and AU2** — absorbance values for UV monitor 1 and UV monitor 2
- **VR1 and VR2** — lamp operation indicator; must be greater than 0.05 volts
- **VS1 and VS2** — flow cell operation indicator; must be greater than 65% of the VR value
- **C** — conductivity value in mS
**Manual control screen** — center section of the screen, for manually operating system components

**Toolbar** — lower section of the screen, includes command button options described in section 3.3

To change the system component settings prior to starting a run:
1. On the Manual Operation screen, touch the up and down arrows to scroll through the list of components and highlight a component to manually operate.
2. Touch the Edit button to retrieve the numeric keypad.
3. Enter the value (for example, flow rate, valve position, proportioning percent) desired.
4. Touch OK to confirm and return to the main screen.
5. When all values on the screen are set correctly, touch Update to start the system as described for each component in the list of adjustable components above.

To change the system component settings when a run is in progress:
1. On the Manual Operation screen, touch the arrow buttons to highlight the desired component.
2. Touch the Edit button to retrieve the numeric keypad and enter the new values; touch OK to return to the Manual Operation screen.
3. Touch Update to continue the run with the new settings.
9.3.3 Setting Time and Date

To change the system date and time settings (Figure 9.7):
1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight “Set Time and Date”, then touch the Select button.
4. Touch the up and down arrows next to each date and time setting to adjust for local settings; press OK to save selections and return to the Diag/Maint Functions screen.

Fig. 9.7. Set Time & Date screen.
9.3.4 Cleaning Sample Lines

This procedure is used to clean the two sample lines; buffer position 8 holds the 20% ethanol solution and is used as a final system purge:

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight “Clean Sample Lines”, then touch the Select button.
4. Follow the directions displayed on the screen (Figure 9.8).
5. Touch the Start button. The system washes the sample lines with water followed by a system purge with ethanol from buffer position 8. A progress bar displays while cleaning is in process.

When cleaning is complete, the system returns to the Diag/Maint Functions screen.

![Prepare system to clean sample lines screen.](image)
9.3.5 Cleaning Fraction Lines

This procedure is used to clean the eight fraction lines; buffer position 8 holds the 20% ethanol solution and is used as a final system purge:

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight “Clean Fraction Lines”, then touch the Select button.
4. Follow the directions displayed on the screen (Figure 9.9).
5. Touch the Start button. The system purges the fraction lines with water followed by a system purge with ethanol from buffer position 8. A progress bar displays while cleaning is in process.

**Note:** This procedure always cleans all eight fraction lines.

When cleaning is complete, the system returns to the Diag/Maint Functions screen.

---

**Fig. 9.9.** Prepare system to clean fraction lines screen.
9.3.6 Cleaning Buffer Lines

This procedure is used to clean buffer lines 1 through 7; buffer position 8 holds the 20% ethanol solution and is used as a final system purge:

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight “Clean Buffer Lines”, then touch the Select button.
4. Follow the directions displayed on the screen (Figure 9.10).
5. Touch the Start button. The system purges buffer lines 1 through 7 with water, followed by a system purge with ethanol from buffer position 8. A progress bar displays while cleaning is in process.

When cleaning is complete, the system returns to the Diag/Maint Functions screen.

Fig. 9.10. Prepare system to clean buffer lines screen.
9.3.7 Washing System With Ethanol

This procedure is used to clean the complete system with ethanol; all buffer lines, sample lines, fraction lines, cartridge position lines, and valve positions are purged with ethanol:

**Note:** If the buffer and sample inlet lines and the fraction outlet lines are stored with ethanol, make sure to wash these lines with water prior to starting a new method using the “Clean All Inlet and Outlet Lines” procedure (section 9.3.8).

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight “System Ethanol Wash”, then touch the Select button.
4. Follow the reagent placement instructions displayed on the screen (Figure 9.11).
5. Remove both cartridges and connect the cartridge fittings.
6. Remove all fraction tubes and replace them with the cleaning tray or empty fraction tubes.
7. Touch the Start button; the system flushes the internal flow paths. A progress bar displays while cleaning is in process.
8. When system flush is complete, touch OK to return to the Diag/Maint Functions screen.

![PREPARE SYSTEM TO CLEAN LINES](image)

*You MUST remove ALL cartridges and directly connect the upper and lower cartridge fittings.*
*Place bottle with 100 ml 20% ETOH in buffer position B8.*
*Place 50 ml 20% ETOH at buffer positions B1-B7.*
*Place 100 ml 20% ETOH at DI water inlet.*
*Place tubes with 25 ml 20% ETOH in positions S1 & S2.*
*Place cleaning tray in the fraction collection area.*
*When ready, press "Start"!

**REMINDER!** If ethanol was used to clean the buffer and sample inlet lines and the fraction outlet lines, use the “Clean All Inlet and Outlet Lines” function to flush the ethanol from these lines prior to running the next method.

Fig. 9.11. Prepare System to Clean Lines, system ethanol wash screen.
9.3.8 Cleaning Inlet and Outlet Lines

This procedure cleans all buffer and sample inlet lines and fraction outlet lines, followed by a system purge with ethanol from buffer position 8:

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight “Clean All Inlet and Outlet Lines”, then touch the Select button.
4. Follow reagent placement instructions displayed on the screen (Figure 9.12).
5. Touch the Start button; the system flushes the lines with water, followed by a system purge with ethanol from buffer position 8. A progress bar displays while cleaning is in progress.

When cleaning is complete, the system will return to the Diag/Maint Functions screen.

Fig. 9.12. Clean All Inlet and Outlet Lines screen.
9.3.9 Cleaning Pump Check Valves

Use this procedure to pump 0.5 M sodium hydroxide (NaOH) solution through the pump to clean the check valves:

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight “Clean Pump Check Valves”, then touch the Select button.
4. Follow instructions displayed on the screen (Figure 9.13).
5. Fill one buffer bottle with 50 ml of NaOH solution (0.5 M) and place it in buffer position 8.
6. Touch the Start button. The system slowly pumps NaOH through the pump for 10 min, followed by a water wash at which point the system pauses. A progress bar displays while cleaning is in process.
7. Follow screen instructions for the two available options: 1) Repeat the NaOH wash, or 2) Complete the wash (wash the buffer 8 position with water).
8. Touch the Repeat button to repeat the NaOH wash, or touch the Continue button to complete the procedure. A progress bar displays while cleaning is in process.

When cleaning is complete, the system returns to the Diag/Maint Functions screen.

![Figure 9.13. Pump Check Valve Cleaning screen.](image-url)
9.3.10 Washing System With NaOH

Use this procedure to wash the internal system lines (including the cartridge lines) with NaOH solution:

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight “System NaOH Wash”, then touch the Select button.
4. Follow instructions displayed on the screen (Figure 9.14).
5. Touch the Start button. The system slowly pumps NaOH through the system for 15 min, followed by a system water wash at which point the system pauses. A progress bar displays while cleaning is in process.
6. Follow screen instructions for the two available options: 1) Repeat the NaOH wash, or 2) Complete the wash (wash the buffer 8 position with water).
7. Touch the Repeat button to repeat the NaOH wash, or touch the Continue button to complete the procedure. A progress bar will display while cleaning is in process.

When cleaning is complete, the system will return to the Diag/Maint Functions screen.

Fig. 9.14. System NaOH wash screen.
9.3.11 Cleaning Inline Check Valve

Use this procedure to manually clean the inline check valve positioned between the cartridge 2 outlet and the fraction valve (outlet selector valve); cleaning the inline check valve manually with a syringe allows removal of residue as well as small particles:

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight “Clean Inline Check Valve”, then touch the Select button.
4. Follow instructions displayed on the screen (Figure 9.15).
5. When the manual procedure is complete, touch the Finish button to wash the system with water from the diluent bottle. A progress bar displays while cleaning is in process.

When cleaning is complete, the system returns to the Diag/Maint Functions screen.

9.3.12 Affinity Cartridge Cleaning in Place

A convenient cleaning in place method for prepacked 1 ml and 5 ml affinity chromatography cartridges can be accessed through the Profinia Data/Utilities menu. This cleaning in place method uses a wash buffer, a cleaning buffer, and a storage buffer. The formulation of these buffers depends upon the specific type of affinity cartridge to be cleaned and the nature of the contaminants to be removed. The instruction manual for prepacked affinity chromatography cartridges contains pertinent information on compatible and recommended cleaning reagents. Note that the formulation for the buffers should not be diluted and needs to be supplied at the working concentration (1x).

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight “Affinity Cartridge Cleaning in Place”, then touch the Select button.

4. Select the size of the cartridge to be cleaned (1 ml or 5 ml).

5. Follow instructions displayed on the screen (Figure 9.16).

6. After the cartridge and reagents have been installed press the Start button.

7. At the end of the cleaning method the user has the option to end or repeat the method. (Repeating the method will subject the cartridge to another cleaning cycle).

The cleaning in place method steps are listed in Table 9.1. Note that 20% aqueous ethanol needs to be supplied at buffer position 8. This is for system cleaning only and is not part of the cleaning in place method. The flow rate for 1 ml and 5 ml cartridges is 1 ml/min and 5 ml/min, respectively.

### Table 9.1. Steps of the Affinity Cleaning in Place Method

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Buffer Port</th>
<th>Buffer Conc.</th>
<th>Column Volumes</th>
<th>Flow Rate (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equilibration</td>
<td>B5</td>
<td>1x</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Cleaning</td>
<td>B6</td>
<td>1x</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Equilibration</td>
<td>B5</td>
<td>1x</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Storage</td>
<td>B7</td>
<td>1x</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>20% Ethanol</td>
<td>B8</td>
<td>1x</td>
<td>–</td>
<td>1</td>
</tr>
</tbody>
</table>

### Fig. 9.16. Affinity Cartridge Cleaning in Place screen.

#### 9.3.13 Desalting Cartridge Cleaning in Place

A cleaning in place method for 10 ml and 50 ml Bio-Gel P6 desalting cartridges is available through the Profinia Data/Utilities Menu for storage or cleaning with user defined reagents. This method uses 1x buffer concentrates at predefined flow rates of 4 and 20 ml/min for 10 ml and 50 ml cartridges respectively. For Profinia coldroom setting the flow rates are reduced to 2 and 10 ml/min to prevent overpressure (Table 9.2 and Figure 9.17).
**Fig. 9.17. Desalting Cartridge Cleaning in Place screen.**

**Table 9.2 Steps for Desalting Cartridge Cleaning in Place**

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Buffer Port</th>
<th>Buffer Conc.</th>
<th>Column Volumes</th>
<th>RT</th>
<th>4°C</th>
<th>RT</th>
<th>4°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean-1</td>
<td>B5</td>
<td>1X</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Clean-2</td>
<td>B6</td>
<td>1X</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Water</td>
<td>DI</td>
<td>—</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Storage</td>
<td>B7</td>
<td>1X</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>20% Ethanol</td>
<td>B8</td>
<td>1X</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

**9.3.14 Selecting End of Sample/Reagent Detection (Air Sensors)**

The Profinia instrument monitors its fluid stream for air at three locations. A general system reagent sensor monitors depletion of reagents, and the system will pause and generate an error message if air is detected. After replenishing the reagents, the system purges the lines, then continues operation. In addition, each sample line has a separate air sensor to detect the end of the sample. When the end of the sample is detected, the system stops loading the sample and automatically continues with the next method step.

*Note:* This feature is not activated during initial method priming when sample/reagent detection is enabled.

To disable (or enable) the sample/reagent sensor monitors:

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight “Select Sample/Reagent Detection”, then touch the Select button.
4. Touch the up and down arrows to enable or disable the air sensors at the system pump inlet and at both sample inlets (Figure 9.16).
5. Select the OK button to confirm selections and return to the Diag/Maint Functions screen.

![Select End of Sample/Reagent Detection screen.](image)

**Fig. 9.18.** Select End of Sample/Reagent Detection screen.

### 9.3.15 Selecting Method Temperature

For methods that include the desalting step, the instrument operating temperature is critical. Maximum flow rates for the desalting cartridges are lower at 4°C than at room temperature. Flow rates over maximum limits compress the bed volume and cause over-pressure errors. Make sure the operating temperature is set to match cold room or room temperature operating conditions before programming your run. Methods with desalting steps have longer run times at the cold room setting due to the lower maximum flow rates. The operating temperature setting displays as a status indicator in the upper right corner of the screen. If the cold room operating temperature is selected, "C" appears only when methods are selected that include a desalting step. If the room temperature setting is selected, "C" will not display. For affinity-only methods, the temperature setting is irrelevant and will not display. See Table 6.1 (section 6) for flow rate details. To set the operating temperature:

1. Touch the Data/Utilities button on the system home screen.
2. Touch the Diag/Maint Functions button.
3. On the Diag/Maint Functions screen, use the up and down arrows to scroll through the list of functions and highlight "Select Method Temperature", then touch the Select button. The Select Method Temperature screen will display (Figure 9.18).
4. Use the up and down arrow buttons to select room temperature or cold room (4°C) operation.
5. Touch the OK button to return to the Diag/Maint Function screen.
9.3.16 Select Display Contrast

Follow the on-screen instructions to increase or decrease the contrast.

If the screen becomes unreadable, touch the lower left-hand corner of the screen.
Section 10
Profinia™ Purification Kits

10.1 Introduction

10.1.1 Background
The Profinia protein purification system includes a complete line of consumables that interface with the instrument for the purification of affinity-tagged proteins. The consumables consist of standard chromatography buffers, resins, reagents, and accessories that have been packaged into a complete, kit-based, and easy-to-use platform. Reagents and purification kits have been developed for the four main types of system applications — native IMAC, denaturing IMAC, GST, and desalting — and include bacterial lysis reagents, prepackaged buffers, IMAC and GST affinity media in prepacked cartridges, and antibody detection tools. See Appendix B (Ordering Information) for a complete list of Profinia products.

10.1.2 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 and solutions (affinity, desalting*, cleaning, and storage solutions) and reagents (urea and 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 buffer and separations kits contain 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 10.1 summarizes the complete line of purification and buffer kits available for the Profinia system.

* Denaturing IMAC kits do not contain desalting cartridges or desalting buffer; stepwise dialysis is the suggested method for desalting and buffer exchange and for renaturing proteins purified under denaturing conditions.
Table 10.1. Profinia purification and buffer kits.

<table>
<thead>
<tr>
<th>Kit</th>
<th>Catalog #</th>
<th>Solutions</th>
<th>Powdered Reagents</th>
<th>Cartridges</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Purification Buffers</td>
<td>Desalting Buffer</td>
<td>Storage Solutions</td>
</tr>
<tr>
<td>Profinia Native IMAC Kits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native IMAC purification kit</td>
<td>620-0225</td>
<td>✔✔✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Native IMAC buffer kit</td>
<td>620-0221</td>
<td>✔✔✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Profinia Denaturing IMAC Kits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Denaturing IMAC purification kit</td>
<td>620-0227</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Denaturing IMAC buffer kit</td>
<td>620-0222</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Profinia GST Kits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GST purification kit</td>
<td>620-0226</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>GST buffer kit</td>
<td>620-0223</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Profinia Desalting Kits</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desalting kit</td>
<td>620-0228</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Desalting buffer kit</td>
<td>620-0224</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>

10.1.3 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 prefilled to remove particulates. The buffers that require the addition of urea or glutathione also require filtration through a 0.2 µm filter prior to use with the system. Accessories to the Profinia instrument include Profinia software, cooling accessory units, and desalting sample loops. Consumables that can be purchased separately include Profinia bacterial lysis and extraction reagents, His and GST detection antibodies, and a dual-tagged lyophilized control lysate containing GST and histidine residues. Complete catalog number and ordering information can be found in Appendix B.

10.1.4 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, and each kit is guaranteed for ~1 year when stored at the proper temperature. Once opened, the kits and reagents should be stored at 4°C.

10.2 Native IMAC Purification and Buffer Kits

The buffers provided in the native IMAC kit are formulated from potassium salts and buffers, and contain increasing concentrations of imidazole for optimum lysing, binding, washing, and elution of histidine (His)-tagged proteins.
Native IMAC buffers* are used for proteins that partition into the soluble fraction of bacterial lysates and that have accessible His sequence tags. Table 10.2 provides a list of buffer compositions. The native lysis buffer is used for sample preparation (see Appendix E), and is not used in any of the instrument ports.

The IMAC cartridges are provided 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 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 water 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 are more stable than sodium salt-based buffers, and do not form precipitates with long-term storage. However, to prevent the formation and precipitation of 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 10.2. Formulations for buffers and solutions provided in the Profinia native IMAC kits.

<table>
<thead>
<tr>
<th>Profinia Solution</th>
<th>Supplied</th>
<th>Concentrated Formulation</th>
<th>Working 1x Formulation</th>
<th>Vol, ml</th>
<th>Buffer Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native IMAC lysis buffer</td>
<td>2x</td>
<td>600 mM KCl, 100 mM KH₂PO₄, 10 mM imidazole, pH 8.0</td>
<td>300 mM KCl, 50 mM KH₂PO₄, 5 mM imidazole, pH 8.0</td>
<td>125</td>
<td>N/A</td>
</tr>
<tr>
<td>Native IMAC wash buffer 1</td>
<td>2x</td>
<td>600 mM KCl, 100 mM KH₂PO₄, 10 mM imidazole, pH 8.0</td>
<td>300 mM KCl, 50 mM KH₂PO₄, 5 mM imidazole, pH 8.0</td>
<td>125</td>
<td>1</td>
</tr>
<tr>
<td>Native IMAC wash buffer 2</td>
<td>2x</td>
<td>600 mM KCl, 100 mM KH₂PO₄, 20 mM imidazole, pH 8.0</td>
<td>300 mM KCl, 50 mM KH₂PO₄, 10 mM imidazole, pH 8.0</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>Native IMAC elution buffer</td>
<td>2x</td>
<td>600 mM KCl, 100 mM KH₂PO₄, 500 mM imidazole, pH 8.0</td>
<td>300 mM KCl, 50 mM KH₂PO₄, 250 mM imidazole, pH 8.0</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Desalting buffer</td>
<td>5x</td>
<td>685 mM NaCl, 13.5 mM KCl, 21.5 mM Na₂HPO₄, 40.5 mM KH₂PO₄, pH 7.0 (pH 7.4 upon dilution)</td>
<td>137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 8.1 mM KH₂PO₄, pH 7.4</td>
<td>200</td>
<td>4</td>
</tr>
<tr>
<td>Cleaning solution 1</td>
<td>2x</td>
<td>1,000 mM NaCl, 100 mM Tris, pH 8.0</td>
<td>500 mM NaCl, 50 mM Tris, pH 8.0</td>
<td>125</td>
<td>5</td>
</tr>
<tr>
<td>Cleaning solution 2</td>
<td>4x</td>
<td>2,000 mM NaCl, 400 mM NaOAc, pH 4.5</td>
<td>500 mM NaCl, 100 mM NaOAc, pH 4.5</td>
<td>125</td>
<td>6</td>
</tr>
<tr>
<td>Storage solution</td>
<td>2x</td>
<td>4% C₆H₅CH₂OH (benzyl alcohol)</td>
<td>2% C₆H₅CH₂OH (benzyl alcohol)</td>
<td>200</td>
<td>7</td>
</tr>
</tbody>
</table>
10.3 Denaturing IMAC Purification and Buffer Kits

The buffers provided in the denaturing IMAC kit are formulated with identical salts and buffers as the native IMAC solutions, but require the addition of urea from the urea packs provided with each kit. Denaturing IMAC buffers are used for proteins that partition into the insoluble fraction of bacterial lysates or have inaccessible His sequence tags. The denaturing IMAC buffers can also be used to solubilize the entire bacterial pellet without separation of the soluble and insoluble fractions. Purifications from unfractionated bacterial pellets may result in slightly less pure proteins. Table 10.3 provides a list of buffer compositions. The lysis buffer is used for sample preparation (see Appendix E), and is not used in any of the instrument ports. The IMAC cartridges are identical to the cartridges described in section 10.2, and all specifications apply to denaturing IMAC purifications.
Table 10.3. Formulations for buffers and solutions provided in the Profinia denaturing IMAC kits.

<table>
<thead>
<tr>
<th>Profinia Solution</th>
<th>Supplied Concentrated Formulation</th>
<th>Working 1x Formulation</th>
<th>Supplied Vol, ml</th>
<th>Vol*, ml</th>
<th>Buffer Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Denaturing IMAC lysis buffer</td>
<td>1.4x 420 mM KCl, 70 mM KH₂PO₄, 7 mM imidazole, pH 8.0</td>
<td>6 M urea, 300 mM KCl, 50 mM KH₂PO₄, 5 mM imidazole, pH 8.0</td>
<td>176</td>
<td>250</td>
<td>N/A</td>
</tr>
<tr>
<td>Denaturing IMAC wash buffer 1</td>
<td>1.4x 420 mM KCl, 70 mM KH₂PO₄, 7 mM imidazole, pH 8.0</td>
<td>6 M urea, 300 mM KCl, 50 mM KH₂PO₄, 5 mM imidazole, pH 8.0</td>
<td>176</td>
<td>250</td>
<td>1</td>
</tr>
<tr>
<td>Denaturing IMAC wash buffer 2</td>
<td>1.4x 420 mM KCl, 70 mM KH₂PO₄, 14 mM imidazole, pH 8.0</td>
<td>6 M urea, 300 mM KCl, 50 mM KH₂PO₄, 10 mM imidazole, pH 8.0</td>
<td>88</td>
<td>125</td>
<td>2</td>
</tr>
<tr>
<td>Denaturing IMAC elution buffer</td>
<td>1.4x 420 mM KCl, 70 mM KH₂PO₄, 350 mM imidazole, pH 8.0</td>
<td>6 M urea, 300 mM KCl, 50 mM KH₂PO₄, 250 mM imidazole, pH 8.0</td>
<td>88</td>
<td>125</td>
<td>3</td>
</tr>
<tr>
<td>Cleaning solution 1</td>
<td>2x 1,000 mM NaCl, 100 mM Tris, pH 8.0</td>
<td>500 mM NaCl, 50 mM Tris, pH 8.0</td>
<td>125</td>
<td>N/A</td>
<td>5</td>
</tr>
<tr>
<td>Cleaning solution 2</td>
<td>4x 2,000 mM NaCl, 400 mM NaOAc, pH 4.5</td>
<td>500 mM NaCl, 100 mM NaOAc, pH 4.5</td>
<td>125</td>
<td>N/A</td>
<td>6</td>
</tr>
<tr>
<td>Storage solution</td>
<td>2x 4% C₆H₅CH₂OH (benzyl alcohol)</td>
<td>2% C₆H₅CH₂OH (benzyl alcohol)</td>
<td>200</td>
<td>N/A</td>
<td>7</td>
</tr>
</tbody>
</table>

*After addition of urea.
Figure 10.2 shows proper placement of denaturing IMAC buffers in the Profinia instrument.

Fig. 10.2. Denaturing IMAC buffers properly positioned with instrument lid closed.

10.4 GST Purification and Buffer Kits

The lysis and wash buffers contained in the GST kit are formulated from sodium salts, phosphate buffers, and EDTA, and provide optimum lysing, binding, washing, and elution of GST-tagged proteins. EDTA is included as a chelating compound and protects against metalloproteases, a class of proteases that can be present in bacterial 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 bacterial lysates and that have accessible, and biologically active, GST sequence tags. Table 10.4 provides a list of buffer compositions. The lysis buffer is used for sample preparation (see Appendix E), 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 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 a PBS/0.05% azide buffer and can be stored at 4–22°C prior to use.
Table 10.4. Formulations for buffers and solutions provided in the GST kits.

<table>
<thead>
<tr>
<th>Profinia Solution</th>
<th>Supplied</th>
<th>Concentrated Formulation</th>
<th>Working 1x Formulation</th>
<th>Vol, ml</th>
<th>Buffer Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST lysis buffer</td>
<td>2x</td>
<td>300 mM NaCl, 20 mM Na₂HPO₄, 10 mM EDTA, pH 7.4</td>
<td>150 mM NaCl, 10 mM Na₂HPO₄, 5 mM EDTA, pH 7.4</td>
<td>125</td>
<td>N/A</td>
</tr>
<tr>
<td>GST wash buffer</td>
<td>2x</td>
<td>300 mM NaCl, 20 mM Na₂HPO₄, 10 mM EDTA, pH 7.4</td>
<td>150 mM NaCl, 10 mM Na₂HPO₄, 5 mM EDTA, pH 7.4</td>
<td>200</td>
<td>1</td>
</tr>
<tr>
<td>GST elution buffer</td>
<td>2x</td>
<td>40 mM glutathione, 200 mM Tris, 10 mM EDTA, pH 8.0</td>
<td>20 mM glutathione, 100 mM Tris, 5 mM EDTA, pH 8.0</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Desalting buffer</td>
<td>5x</td>
<td>685 mM NaCl, 13.5 mM KCl, 21.5 mM Na₂HPO₄, 40.5 mM KH₂PO₄, pH 7.0 (pH 7.4 upon dilution)</td>
<td>137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄, 8.1 mM KH₂PO₄, pH 7.4</td>
<td>200</td>
<td>4</td>
</tr>
<tr>
<td>Cleaning solution 1</td>
<td>2x</td>
<td>1,000 mM NaCl, 100 mM Tris, pH 8.0</td>
<td>500 mM NaCl, 50 mM Tris, pH 8.0</td>
<td>125</td>
<td>5</td>
</tr>
<tr>
<td>Cleaning solution 2</td>
<td>4x</td>
<td>2,000 mM NaCl, 400 mM NaOAc, pH 4.5</td>
<td>500 mM NaCl, 100 mM NaOAc, pH 4.5</td>
<td>125</td>
<td>6</td>
</tr>
<tr>
<td>Storage Solution</td>
<td>2x</td>
<td>4% C₆H₅CH₂OH (benzyl alcohol)</td>
<td>2% C₆H₅CH₂OH (benzyl alcohol)</td>
<td>200</td>
<td>7</td>
</tr>
</tbody>
</table>

Figure 10.3 shows proper placement of GST buffers in the Profinia instrument.

10.5 Desalting Buffer and Separation Kits

The buffers and solutions provided in the desalting kit consist of a PBS-based desalting buffer, cleaning buffers, and a storage solution. The PBS solution provides an optimum 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 buffers are already included in native IMAC and GST purification and buffer kits. Optimized desalting and storage solutions for unique proteins 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 10.5 provides a list of buffer compositions.

The desalting cartridges are provided in water 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 P6 size exclusion resin, and typically yield ~80% or more recovery of applied target protein. The resin in the P6 desalting cartridges interacts minimally with proteins. When used with the cleaning buffers, different proteins can safely be applied to the same desalting cartridge.

### Table 10.5. Formulations for buffers and solutions provided in the Profinia desalting kits.

<table>
<thead>
<tr>
<th>Profinia Solution</th>
<th>Supplied</th>
<th>Concentrated Formulation</th>
<th>Working 1x Formulation</th>
<th>Vol, ml</th>
<th>Buffer Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Desalting buffer</td>
<td>5x</td>
<td>685 mM NaCl, 13.5 mM KCl, 21.5 mM Na₂HPo₄, 40.5 mM KH₂PO₄, pH 7.0 (pH 7.4 upon dilution)</td>
<td>137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPo₄, 8.1 mM KH₂PO₄, pH 7.4</td>
<td>200</td>
<td>4</td>
</tr>
<tr>
<td>Cleaning solution 1</td>
<td>2x</td>
<td>1,000 mM NaCl, 100 mM Tris, pH 8.0</td>
<td>500 mM NaCl, 50 mM Tris, pH 8.0</td>
<td>125</td>
<td>5</td>
</tr>
<tr>
<td>Cleaning solution 2</td>
<td>4x</td>
<td>2,000 mM NaCl, 400 mM NaOAc, pH 4.5</td>
<td>500 mM NaCl, 100 mM NaOAc, pH 4.5</td>
<td>125</td>
<td>6</td>
</tr>
<tr>
<td>Storage solution</td>
<td>2x</td>
<td>4% C₆H₅CH₂OH (benzyl alcohol)</td>
<td>2% C₆H₅CH₂OH (benzyl alcohol)</td>
<td>200</td>
<td>7</td>
</tr>
</tbody>
</table>

Figure 10.4 shows proper placement of desalting buffers in the Profinia instrument.

![Profinia instrument with desalting buffers](image)

Fig. 10.4. Desalting buffers properly positioned with instrument lid closed. Also shown, the desalting loop installed in the cartridge 1 position.
Section 11  
Connecting External Components

Functionality of the Profinia™ protein purification system can be enhanced by add-on components. The instrument can be connected to a pH monitor, larger buffer bottles (standard sizes with Bio-Rad methods are 125 ml and 250 ml), larger sample and fraction tubes (standard sizes with Bio-Rad methods are 15 ml and 50 ml), and the Profinia cooling accessory.

11.1 pH Monitor

The optional pH monitor (catalog #620-0411) allows the Profinia system to monitor the pH of the fluid stream of a protein sample. This pH information is displayed on the instrument’s screen, stored in the run data record, and can be displayed in real time on the computer screen if the system is connected to a computer running Profinia software. Run data can also be exported for later viewing and analysis.

The pH monitor kit (Figure 11.1) includes:

- Bio-Rad pH probe — calomel pH probe, sealed for maintenance-free operation
- Profinia pH flow cell — minimum swept volume for best response
- Tubing assembly — connects the flow cell to the instrument
- Mounting bracket and screws
- Instructions

Fig. 11.1. Profinia pH monitor kit.

Additional materials required are standard pH calibration buffers, pH 4.0 and 10.0, available from laboratory supply companies.

To install the pH flow cell and probe:

1. Use the power switch to turn the instrument off and unplug the power cord.
2. Use the handles on the back of the unit to turn it so the back of the unit is facing you.

Important: Use care not to spill any fluids when turning the unit.

3. Disconnect and remove the waste bottle (right side of the instrument).
4. Remove the thumbscrews connecting the rear access panel to the instrument; there are two thumbscrews at the top of the panel, and two near the center.
5. Lift off the access panel by pulling it straight toward you (Figure 11.2).

![Fig. 11.2. Rear view of instrument with access panel removed.](image)

6. Locate the two holes provided for mounting the pH flow cell.

7. Install the pH flow cell bracket using two Phillips head screws (Figure 11.3).

![Fig. 11.3. Bracket mounted in the correct location in the instrument fluidics area.](image)

8. Find the tubing connecting the conductivity flow cell to the center of the outlet select valve. Unscrew the fitting at the outlet select valve.
9. Connect the fitting you disconnected in step 8 to the inlet of the pH flow cell.

10. Find the length of tubing supplied with the pH monitor kit. Connect one end to the outlet fitting of the pH flow cell.

11. Connect the other end of the tubing to the center port of the outlet select valve.

12. Add a small amount of water (2 ml) to the pH flow cell.

13. Connect the pH probe’s electrical connector to the receptacle on the instrument (Figure 11.4).

![Fig. 11.4. Connecting the pH probe electrical connector.](image)

14. Remove the plastic cap from the pH probe and install it on the pH flow cell; tighten the black collar until it is snug. If a small amount of liquid drips from the flow cell during this operation, wipe it off with a paper towel.

15. Replace the user access panel, four thumbscrews, and the waste bottle, and turn the unit around so it is facing you.

16. Reconnect the waste bottle tube and power and USB cables to prepare the instrument for operation.

17. Turn the instrument power on.

18. On the home screen, touch the Data/Utilities button, then touch the Calibration Functions button.

19. Touch the arrow buttons to scroll through the list of calibration functions and highlight “Calibrate pH”, then touch the Select button.

20. Follow the instructions displayed on the screen to calibrate the pH probe.
11.2 Larger Buffer, Sample, and Fraction Containers

The Profinia system is designed to operate with built-in components (buffer bottles and fraction and sample tubes), but can accommodate larger bottles and tubes for large-sample runs or program methods that require buffer volumes greater than those accommodated by the Profinia 250 ml buffer bottle.

**Important**: Prior to starting a method, all the externally connected reagent tubes must be primed manually. Use the manual priming function on the Start Run screen to do this.

11.2.1 External Buffer Containers

To connect an external buffer container to the Profinia instrument, all that is required is 1/8" OD PTFE tubing (catalog #750-0603 or similar), sufficient in length to connect the external bottle(s) to the buffer inlet port on the instrument. To use buffer bottles that do not fit in the system buffer bottle tray:

1. Place buffer bottle(s) to be used in your purification run next to the instrument.
2. Locate the bottom section of tubing that is connected to each buffer inlet valve.
3. Use one hand to push up on the silicone coupler, and the other to disconnect the short piece of tubing by pulling it off.
4. Install the longer piece of 1/8" OD PTFE tubing by pushing one end into the silicone coupling (Figure 11.6).
5. Place the other end of the tubing into the external buffer bottle.

**Note:** If connecting more than one external buffer bottle for a run, it is helpful to use stickers to label each tube with the Profinia buffer position number marked on the buffer tray.

Once the longer tubes have been installed and other standard setup procedures are complete, the system is ready for a purification run.

**Note:** The inlet tube must be primed before starting a run. This can be done using the manual priming function in the method Start Run screen.

### 11.2.2 External Sample Containers

To connect an external sample container to the Profinia instrument, all that is required is PTFE tubing, 1/8" OD, sufficient in length to connect the external container(s) to the sample inlet port(s) on the instrument. You need a length of tubing for each port that accommodates the external sample containers. To use sample tubes that do not fit in the system sample inlet tray:

1. Place external sample container(s) next to the instrument.
2. Locate the bottom section of tubing that is connected to each sample inlet valve.
3. Use one hand to push up on the silicone coupler, and the other to disconnect the tubing by pulling it off.
4. Install the 1/8" OD PTFE tubing by pushing one end into the silicone coupling.
5. Place the other end of the tubing in the external sample container.

**Note:** If connecting more than one external sample container for a run, it is helpful to use stickers to label each tube with the Profinia sample position number marked on the buffer tray.
Once the longer tubes have been installed and other standard setup procedures are complete, the system is ready for a purification run.

**11.2.3 External Fraction Collection Containers**

To connect an external fraction collection container to the Profinia instrument, all that is required is 1.6 mm ID, 0.8 mm wall silicone tubing (catalog #731-8211 or similar) sufficient in length to connect the external container(s) to the fraction outlet port(s) on the instrument. It is best to keep tubing length as short as possible. To use fraction containers that do not fit in the system fraction collection tray:

1. Place fraction container(s) to be used in your purification run next to the instrument.

**Note:** One-sample runs require four fraction collection containers; two-sample runs require eight fraction collection containers.

2. Locate the bottom section of tubing that is connected to each fraction outlet valve (Figure 11.7).

![Fig. 11.7. Fraction outlet valves. 1, White support tubing; 2, clear outlet tubing.](image)

3. Slip the 1.6 mm ID tubing over the clear outlet tubing, then over the white support tubing.

4. Place the other end of the tubing into the external fraction collection container.

**Note:** If connecting more than one external fraction collection container for a run, it is helpful to use stickers to label each tube with the Profinia fraction tube position number marked on the instrument.

Once the longer tubes are installed and other standard setup procedures are complete, the system is ready for a purification run.

**11.3 Profinia Cooling Accessory**

The Profinia cooling accessory (Figure 11.8) is designed for users that need to keep protein sample and fraction temperatures below 4°C, but don’t have access to a cold room. This accessory holds either two 15 ml, two 50 ml, or one of each size conical tubes. The cooling accessory cools the two samples or the two purified protein fractions.
Fig. 11.8. Profinia cooling accessory. The instrument comes with two cooling units as shown.

To install the cooling accessory on the instrument:

1. Fill the cooling accessory with water to the inside chamber fill line.

**Warning:** Use tap water when filling the cooling accessory with water; do not use deionized water because it will expand in the unit during freezing. Do not fill the cooling accessory past the top of the vertical rib located inside the unit.

2. Chill the cooling accessory in a freezer.

3. Place the sample or fraction tubes in the cooling accessory.

4. Place the tubes and the cooling accessory on the instrument in the desired sample and/or fraction collection position.

Fig. 11.9. Placing sample tubes in the cooling accessory on instrument sample lines (left); cooling accessory units installed in both the sample and purified protein fraction collection tube positions (right).
11.4 Connecting non-Bio-Rad Columns

Columns from other suppliers can be connected to the Profinia instrument. Figure 11.10 displays other commercially available affinity columns connected in cartridge position 1 on the Profinia instrument. For instructions on how to connect non-Bio-Rad columns to the Profinia instrument, please request bulletin 5326, Chromatography Column Connection Guide.

Fig. 11.10. Examples of non-Bio-Rad columns connected to the Profinia instrument.


Section 12
The Profinia Software

The Profinia software runs on a PC platform, and allows viewing and reporting of run data; it is an optional accessory to the Profinia protein purification instrument and does not control instrument functions. This section contains basic instructions for operating the software. For complete software instructions, see the Profinia Software User’s Guide on the Profinia software CD.

12.1 Operating Requirements
The following are minimum system requirements for running Profinia software:

- **Operating system** — Windows XP or Windows Vista
- **CPU** — Pentium 4 processor at 2 GHz or higher
- **RAM** — 512 MB minimum
- **Screen resolution** — 1,024 x 768
- **Hard drive space** — 80 GB minimum
- **Other driver** — CD-ROM
- **USB port** — 2.0

12.2 Software Structure
The default opening screen of Profinia software is divided into five main areas (Figure 12.1):

- **Menu options** — top portion of the screen includes pull-down menus and button commands for navigating software functionality
- **Directory menu** — upper left window displays folders located on the computer’s internal and external drives; click on the plus symbol located next to the drive names to expand directory information for that drive
- **File menu** — lower left window displays any Profinia data files (.ofi file extension) located in the folder highlighted in the directory menu window
- **Chromatogram window(s)** — upper right section displays chromatographic data from the purification profile; the y-axis displays absorbance units (AU) and the x-axis displays run time (hh:mm). A chromatogram is displayed for each sample (up to two) purified in the run
- **Data table** — lower right section displays a data table of run information and results; click on the tabs at the bottom of the screen to view data for different parameters of the run
12.3 Transferring Data to Profinia Software

There are two ways to transfer Profinia instrument data files to Profinia software:

- **Real-time data transfer** — transfer in-progress run data via USB cable connection between a computer operating Profinia software and the instrument
- **Portable memory device data transfer** — transfer files via USB portable memory device at the end of a run

12.3.1 Real-Time Data Transfer

To import data into Profinia software via real-time data transfer:

1. Connect the instrument to your computer using the USB cable provided with the software.
2. Open Profinia software on your computer.
3. Make sure communication between the instrument and computer has been established. This is indicated by a “Waiting for data” message displayed at the bottom of the Profinia software screen (Figure 12.2).
4. Highlight the directory in your computer to which you’d like the run data transferred.

5. Start the purification run according to the procedure outlined in section 6.6. As the run progresses, data appears in real time in the software chromatogram(s) and data tables. At the end of the run, the completed data file will be stored in the directory highlighted in step 4.

**Note:** The run data filename format consists of the run name assigned by the user on the Enter Run & Sample Information screen on the instrument (see section 6.3) and an .ofi file extension.

### 12.3.2 Portable Memory Device Data Transfer

After the file has been transferred from the Profinia instrument to the portable USB portable memory device (see section 9.1.1 for information on transferring run data files to a portable memory device):

1. Plug the portable memory device into any available USB port on your computer.

2. Open the folder for the USB portable memory device.

3. Choose the folder on the computer in which you would like to place the run data file, or create a new folder in which to store the data file. Profinia automatically creates a “Data” folder in the software system files created upon installation (for example, if you installed the software program in the “C: Program Files” location, there will be a “Data” folder automatically created in the same location).

4. Copy and paste or drag and drop the run file from the portable USB portable device folder to the desired data folder.

### 12.4 Viewing Run Data Files

#### 12.4.1 Opening Files in Profinia Software

1. Open Profinia software.

2. Using the directory on the left side of the screen, locate the folder that contains the desired run data file. All run data files contained in the selected folder will be displayed in the lower file menu portion of the screen.

3. Double-click the run data file to be viewed. The file chromatogram(s) and data tables launch in the right side of the screen.
12.4.2 Chromatogram Overview

The following run parameters are displayed in the software chromatogram (Figure 12.3):

- **UV-1** — data trace from the UV-1 detector
- **Pressure** — data trace from the pressure monitor
- **pH** — data trace from the pH monitor (optional accessory)
- **UV-2** — data trace from the UV-2 detector
- **Cond** — data trace from the conductivity monitor
- **Temp** — data trace from the temperature monitor
- **Main Steps** — indicated by a downward-facing arrow symbol that marks the time at which each main step occurred during the course of the run
- **Fraction** — indicated by “collection tube” symbol that marks the points at which collection began during the course of the run

![Check boxes for run parameters](image)

Fig. 12.3. Run parameters displayed at the top of a Profinia software chromatogram.

12.4.3 Eliminating Run Parameters Displayed

The top of each chromatogram displays all possible run parameters. Checked boxes indicate the parameters displayed in the chromatogram. To remove any parameter from the chromatogram, click on the box to remove the checkmark. The trace for that parameter as well as any parameter-specific axis information will be removed from the screen.

12.4.4 Customizing Chromatogram Display Options

Customize the way information is displayed on your chromatogram by changing line colors and widths, choosing whether UV-1 and UV-2 information is displayed on separate axes, determining the major steps that are included in the chromatogram, and whether a sound is heard at sample elution (real-time data display only).

**Changing Chromatogram Background and Trace Colors**

There are two categories of data for which you can customize the colors that are displayed:

- **Chromatogram background** — color of the entire chromatogram background
- **Traces** — color of each line that indicates UV, pressure, pH, conductivity, and fraction information from the run

To change the colors that are used to distinguish information in your chromatogram:

1. Click “Options” > “Colors” from the software main menu.
2. Click on the bubble next to the parameter to change.
3. Click the Color button located at the bottom of the list of parameters.
4. Choose from Named or System Colors, or customize a color using the screen tabs. Once a color has been selected, click the OK button to return to the Chromatogram Option screen.

**Note:** Changes appear on the chromatogram as you make adjustments, but won’t be saved until the entire procedure is completed.
5. Repeat this procedure for each data category for which you’d like to implement a color change. When finished, click Save to retain changes and return to the main software screen, or click Close to return to the screen without implementing changes.

Fig. 12.4. Changing the color of the UV-1 trace line by selecting from the “Named” color list; it is also possible to create custom colors.

Changing Trace Line Width

To change the thickness of trace lines:

1. Click “Options” > “Trace Options” from the software main menu.
2. Use the up and down arrows located in the “Width” box next to the parameter to increase or decrease the width of its associated trace.

Note: Changes appear on the chromatogram as you make adjustments, but won’t be saved until the entire procedure is completed.

3. Repeat this procedure for each data category for which you’d like to implement a trace width change. When finished, click Save to retain changes and return to the main software screen, or click Close to return to the screen without implementing changes.

Changing UV Axis Display

The software automatically generates separate axes for each UV detector. To merge UV axis information into one y-axis:

1. Click “Options” from the software main menu.
2. Locate the “Separate Axis for UV-1 and UV-2 (Top)” and “Separate Axis for UV-1 and UV-2 (Bottom)” menu options. A checkmark next to these menu options indicates the function is in effect.

Note: Only two-sample runs have top and bottom chromatograms.

3. Click on the option for which you’d like to merge y-axis labels. The checkmark next to the menu option disappears and UV-1 and UV-2 axis information displays together.
4. Repeat this procedure to separate UV-1 and UV-2 axis information.
Changing Major Steps Displayed

Major steps are regions between main steps such as sample load begin/end and wash-1 begin/end. The following major steps are displayed on the chromatogram and indicated by downward-facing arrows:

- Sample load
- Wash-1
- Wash-2
- Elute affinity
- Elute desalting

To add highlights to the screen indicating the time during which these steps occurred:

1. Click “Options” > “Major Steps”.
2. Click the box of the main step you’d like highlighted.

Note: Changes appear on the chromatogram as you make adjustments, but won’t be saved until the entire procedure is completed.

3. Repeat this procedure for each major step for which you’d like to implement a change in display.

4. Adjust the transparency of the highlights using the up and down arrows next to the Transparency command.

5. When finished, click Save to retain changes and return to the main software screen, or click Close to return to the screen without implementing changes.

Changing Chromatogram Default Options

The following default information can be adjusted to change the way all chromatograms display:

- Trace visibility — turn off trace displays for checked items: UV-1, UV-2, Pressure, Conductivity, pH, Temperature, Fraction, and Main Steps

Note: UV-1 and UV-2 are not applicable for all methods and will be turned off automatically.

- UI configuration — remove file browsing or data table windows from the main screen
- X-axis — choose whether the x-axis displays data related to “Time” or “Volume”
- Standard report y-scales — choose whether the y-axis displays “Y-axes according to Sample 1”, “Y-axes according to Sample 2”, or “Y-axes as is”

To adjust default information:

1. Click “Options” > “Defaults”.
2. Click the items you’d like to add or remove from the default displays.

Note: Changes appear on the chromatogram as you make adjustments, but won’t be saved until the entire procedure is completed.

3. When finished, click Save to retain changes and return to the main software screen, or click Close to return to the screen without implementing changes.
12.5 Reports
Profinia software enables users to create and print reports for use in presentations or laboratory recordkeeping.

12.5.1 Standard Reports
Standard reports display:

- **Method and run information** — method name, run name, run start (day, date, and time), run duration (hh:mm:ss), run data file (directory and file name), user name, FW (firmware) version, and instrument serial number

- **Chromatogram(s)** — sample 1 and sample 2 (two-sample runs) as they appear on the screen. Adjust the zoom and other settings to the image you want, then create the report to include the chromatogram as you have specified

- **Data tables** — default displays sample-related data including concentration, elution time, load volume, flow rates, and wash times for the run

- **Footer** — default information is the day, date, and time the report was printed

To create a standard report:
1. Click “Reports”, then highlight “Standard Report”.
2. Choose to “Print to Default Printer” or “Preview”.

This procedure can also be accomplished by clicking the Print Std Reports button located at the top of the software screen. You cannot preview a report before printing it using this shortcut command.

12.5.2 Custom Reports
The following parameters can be adjusted to customize reports:

- **Chromatogram(s) size** — choose Small, Medium, Large, or Custom; custom sizes are created using the up and down arrows to specify chromatogram height and width

- **Information to include** — select each type of information to include in the report; also use this list to specify the order in which information is to appear in the report

- **Select a report** — choose any previously customized report

- **Add report comments** — insert any details you’d like included in the report

- **Name your custom report** — create a name for your report to simplify future identification and retrieval

To create a custom report:
1. Click “Reports” > “Custom Reports” to open the Define or Select a Custom Report screen.
2. Select the chromatogram size and information to include in the report by clicking on the bubbles or boxes next to the list of options.
3. Change the order of information included in the report by highlighting an option in the “Information to Include” list and using the up and down arrows to the right to move the selection. The order of the information listed in the menu is the order in which it appears on the report.
4. Type any desired notes into the “Add Report Comments” box.
5. Type a name for the report into the “Name your custom report” text line.

6. Click the Save button to save the customized report version. The name of the report appears in the “Select a Report” list.

7. If desired, click the Make Favorite button to identify this custom report as the preferred version. For future run data files, simply click the Print Favorite button located at the top of the screen to print a report with these options selected.

8. Click Preview to see how the report will print, Print to print the report, or Close to close the window and return to the software’s main screen.

**12.6 Exporting Chromatograms**
Profinia chromatogram images can be exported in many different image files, including .jpg and .tif file formats. To export chromatograms:

1. Click “File” > “Export Chromatograms”.

2. Select the desired image file format.

3. Select to “Copy to Clipboard” for immediate use, or “Save to File” for future use.

**12.7 Exporting Data Files**
Profinia run data files can be exported in either text or Excel file format. To export run data:

1. Click “File” > “Export Run Data” to open the Export Run Data window.

2. If a run is currently open in Profinia software, that filename appears as the data to export. To export a different data file, click the Browse button to navigate computer drives and directories.

3. Select the information to be exported with the file by marking the bubble or boxes next to each option.

4. Choose “Text” or “Excel” file format from the “Select File Format” pull-down menu.

5. Choose whether you’d like the data delimited by tab, comma, space, or colon using the “Select Delimiter” pull-down menu.

6. Click Save to proceed with exporting data with the options selected or Close to abandon the procedure and return to the main screen.

7. If proceeding with data export, the Save As window opens. Select a folder and file name for the data export, then click Save to finish data export or Cancel to return to the Export Run Data screen.

**12.8 Help Information**
You can find complete instructions on software functionality in the Profinia Software User’s Guide, or use the software Help screen for more information on how to perform software procedures:

1. Click “Help” from the software pull-down menu.

2. Click “Contents and Index” to retrieve a list of help topics or to search the help index, or “About” to retrieve information about the software version.
Section 13
Care and Maintenance

Proper care and maintenance of Profinia™ system parts help keep the instrument operating at optimum performance levels.

13.1 Cleaning the Instrument

The Profinia instrument automatically flushes system lines at the end of each purification run. To keep the system clean, there are several automated cleaning and storage procedures:

- **Daily cleaning** — buffer, sample, and fraction line cleaning
- **Weekly cleaning** — system NaOH and ethanol washes
- **Storage**

The procedures are selected from the Diag/Maint Functions menu, which is accessible via the Data/Utilities menu. The procedures outlined below are designed to use water, ethanol, or sodium hydroxide (NaOH). Other cleaning solutions, such as 0.5 M hydrochloric acid (HCl), or 6 M guanidine HCl, may also be used. When using multiple cleaning solutions (for example, 0.1 M NaOH followed by 0.5 M HCl), flush the system with water between steps to prevent interactions between the cleaning chemicals.

Before running the cleaning procedures, remove the cartridges and connect the cartridge inlet and outlet fittings by turning the plastic collars clockwise. Remove all fraction tubes and place the cleaning tray in the fraction collection area. To run each cleaning procedure:

1. Touch the Data/Utilities button, then the Diag/Maint Functions button on the lower toolbar to retrieve the menu for cleaning functions.
2. On the Diag/Maint Functions screen, touch the up and down arrows to scroll through the list of functions and highlight the desired cleaning procedure.
3. Touch Select, then follow the touch screen instructions.

13.1.1 Daily Cleaning

When finished using the system for the day, perform buffer, sample, and fraction line cleaning procedures as described in section 13.1.4.

13.1.2 Weekly Cleaning

Once a week, run the system NaOH wash, then the system ethanol wash as described in section 13.1.4.

13.1.3 Storage

If the system will not be used for more than a week:

1. Run the system NaOH wash, then the system ethanol wash as described in section 13.1.4.
2. Empty the diluent and waste bottles.
3. Cap the ends of the buffer and sample inlet tubes and fraction outlet tubes with the caps the instrument was shipped with (replacement Catalog #620-0444) to prevent the entry of dust or foreign objects.
4. If system power will be left on, go to the Manual Operation screen (Data/Utilities > Diag/Maint Functions > Manual Operation) and turn the lamp off, using the Toggle Lamp button.

**Note:** When the Profinia instrument is switched on, the UV lamp does not start until a method starts. The UV lamp shuts off automatically one hour after a method is finished. If you use the Toggle Lamp button to switch the lamp on, it will stay on until the machine is powered down or until you toggle the lamp off. The one hour shutoff does not function when the lamp is turned on manually.

### 13.1.4 System Cleaning Procedures

**Cleaning Sample Lines**

Use this procedure to clean the lines between the inlet select valve and the sample tubes:

1. Remove all sample tubes from the instrument.
2. Fill conical tubes with 25 ml of deionized water and place them in sample positions 1 and 2.
3. Fill a buffer bottle with 100 ml of 20% ethanol and place it in buffer position 8.
4. Touch the Start button. A progress bar displays, tracking the duration of the procedure.

**Cleaning Fraction Lines**

Use this procedure to clean the lines between the outlet select valve and the fraction tubes:

1. Remove all fraction tubes from the instrument.
2. Place the cleaning tray in the fraction collection area.
3. Place a bottle with 100 ml of 20% ethanol in buffer position 8.
4. Touch the Start button. A progress bar displays, tracking the duration of the procedure.

**Cleaning Buffer Lines**

Use this procedure to clean the lines between the buffer containers and the inlet select valve:

1. Fill seven buffer bottles with 50 ml of deionized water each, and place them in buffer positions 1 through 7.
2. Fill one buffer bottle with 100 ml of 20% ethanol and place it in buffer position 8.
3. Touch the Start button. A progress bar displays, tracking the duration of the procedure.

**System Ethanol Wash**

Use this procedure to pump ethanol solution through the system:

1. Remove both cartridges from the system, and connect the inlet and outlet cartridge fittings together.
2. Fill seven buffer bottles with 50 ml of 20% ethanol each, and place them in buffer positions 1 through 7.
3. Fill one buffer bottle with 100 ml of 20% ethanol and place it in buffer position 8.
4. Fill two conical tubes with 25 ml of 20% ethanol each, and place them in sample positions 1 and 2.
5. Place the cleaning tray in the fraction collection area.
6. Touch the Start button. A progress bar displays, tracking the duration of the procedure.

**Cleaning Inlet and Outlet Lines**

Use this procedure to clean all buffer and sample inlets and fraction outlets:

1. Fill diluent bottle with deionized water.
2. Fill seven buffer bottles with 50 ml of deionized water each, and place them in buffer positions 1 through 7.
3. Fill one buffer bottle with 100 ml of 20% ethanol and place it in buffer position 8.
4. Fill two conical tubes with 25 ml of deionized water each, and place them in sample positions 1 and 2.
5. Place the cleaning tray in the fraction collection area.
6. Touch the Start button. A progress bar displays, tracking the duration of the procedure.

**Cleaning Pump Check Valves**

Use this procedure to pump NaOH solution through the pump and clean the piston and check valves:

1. Fill the diluent bottle with deionized water.
2. Fill one buffer bottle with 50 ml of NaOH solution (0.1 to 0.5 M) and place it in buffer position 8.
3. Touch the Start button. A progress bar displays, tracking the duration of the procedure.

**System NaOH Wash**

Use this procedure to wash the system (except the buffer inlets) with NaOH solution:

1. Fill the diluent bottle with deionized water.
2. Fill one buffer bottle with 50 ml of NaOH solution (0.1 to 0.5 M) and place it in buffer position 8.
3. Remove both cartridges from the system, and connect the inlet and outlet cartridge fittings together.
4. Touch the Start button. A progress bar displays, tracking the duration of the procedure.

**13.1.5 Cleaning Touch Screen**

To clean the system touch screen:

1. Apply a neutral pH cleaner, such as ethanol or 3% or less isopropyl alcohol, to a soft, lint-free cloth.

**Note:** Do not apply cleaning solution directly to the surface of the touch screen.

2. Wipe the touch screen gently with the cloth without allowing fluid or compressed air to enter the small 1 mm vent hole along the edge of the touch screen.

**Warning:** Never use acidic or alkaline cleaners or organic chemicals, such as ammonia, phosphates, ethylene glycol, paint thinner, acetone, toluene, xylene, propyl alcohol, or kerosene to clean the Profinia touch screen.
13.2 Maintenance

Regular maintenance helps keep the system performing optimally. Figure 13.1 is a diagram of the instrument's main parts and connections.

Fig. 13.1. Schematic diagram of the Profinia system.

The following internal components require regular maintenance and may need to be repaired or replaced occasionally (for a checklist of annual maintenance procedures, see Appendix G):

- Pump check valves
- Pump seals
- UV monitor lamp
- UV monitor flow cells
- Buffer and sample inlet tubes
- Tubing and fittings
- Inline filter
Use the following procedure to access all internal components, then locate the part-specific procedure for servicing or replacing the hardware in the sections that follow:

1. Turn instrument power off and unplug the power cord.
2. Turn the instrument around so the rear of the instrument is facing you.
   **Important:** Use care not to spill any fluids when turning the unit.
3. Unplug the USB cable if connected to the instrument.
4. Disconnect the waste tube where it threads into the rear of the unit (right side of the instrument) and remove the waste bottle; set it aside.
5. The fluidics access panel is on the right side, held in place by four thumbscrews. Remove the thumbscrews connecting the rear access panel to the instrument; there are two screws located at the top of the panel and two near the center. Do not remove the electronics access panel, held in place by Phillips head screws.
6. Lift off the access panel by pulling it straight toward you (Figure 13.2). Set the access panel and thumbscrews aside.

![Fig. 13.2. Rear view of instrument with access panel removed.](image)

**13.2.1 Pump Check Valves**

The Profinia system automatically cleans the flow path after every run, so the check valves should only require service during preventive maintenance. If pump flow rates are not as expected or sample volumes are too small, the check valves may not be working properly.

The system uses two different types of check valves:

- **Disk check valve** — inlet (two valves on the bottom of the pump body)
- **Button check valve** — outlet (two valves on the top of the pump body)

Removal, inspection, cleaning, and replacement procedures for both types of check valves are similar, but not identical. Materials required to service the check valves are:
Diluent bottle — filled with deionized water

Wrenches:
- Open end 7/16"
- Open end 1/2"
- Open end 9/16"

Replacement check valves (if required):
- Inlet — catalog #620-0413
- Outlet — catalog #620-0414

To access the system check valves:
1. Use the procedure in section 13.2 to remove the instrument access panel.
2. Locate the pump assembly; the inlet check valves are located at the bottom of the pump, and the outlet check valves are located at the top (Figure 13.3).

![Fig. 13.3. Location of the inlet (bottom) and outlet (top) check valves on the pump assembly.](image)

Note: The inlet and outlet check valves look similar, but they are different parts. Assembly and disassembly procedures for the valves are not identical; be sure to return them to their correct locations (inlet valves on the bottom and outlet valves on the top). The instrument will not operate properly if the check valves are replaced in the incorrect positions.
Cleaning and Replacing Inlet (Bottom) Check Valves

1. Remove the two tubing fittings from the bottom of the inlet flow cell.
2. Using your fingers or a 9/16" open-end wrench, unscrew the inlet flow cells from the pump body.
3. Hold the check valve body with a 9/16" open-end wrench, and loosen the threaded insert with a ½" open-end wrench.
4. Unscrew the insert (male threaded part) from the valve body (female threaded part). Note the black rubber disk on the insert, and look into the cavity of the valve body; you will see a tan-colored plastic disk. This disk has a groove machined into one side (the side facing you) and three small, drilled holes.
5. Turn the check valve body over and tap it on the benchtop to remove the plastic disk.
6. Carefully pry the rubber disk from the insert.
7. Rinse the check valve parts thoroughly in water and examine for damage; replace if damaged.
8. Replace the plastic disk in the check valve body, with the groove facing toward you.
9. Replace the rubber disk in the threaded insert — it is the same on both sides.
10. Thread the check valve insert into the check valve body and tighten, using the two wrenches as described in step 3 above.

**Note:** Do not overtighten the insert.
11. Thread the assembled check valve into the pump body, and tighten with a 9/16" open-end wrench; do not overtighten.
12. Reconnect the tubes and fittings to the top of the check valves.
Cleaning and Replacing Outlet (Top) Check Valves

To clean or replace the outlet check valve:

1. Remove the tubing fittings from the top of the outlet check valve.
2. Using your fingers or a 9/16" open-end wrench, unscrew the outlet check valve from the pump body.
3. Hold the check valve body with a 9/16" open-end wrench, and loosen the threaded insert with a 7/16" open-end wrench.
4. Unscrew the threaded insert from the valve body. The insert has a concave surface with a hole in it. Set the insert aside in a safe place.
5. Look into the cavity of the check valve body; you will see a tan-colored disk with a rubber “button” facing toward you. Turn the check valve body over and tap it on the benchtop to remove the plastic disk and button from the valve body.
6. Gently pull the black button from the disk, using your fingertips.
7. Examine parts for damage; replace the check valve if necessary.
8. Using your fingers, gently replace the rubber button in the plastic disk. Take care to center the head in the disk. The plastic disk is the same on both sides.
9. Replace the disk in the check valve body, with the black rubber button toward you.
10. Thread the insert into the check valve body and tighten, using the two wrenches as described in step 3 above.

**Note:** Do not overtighten the insert.

11. Thread the assembled check valve into the pump body, and tighten with a 9/16" open-end wrench.
12. Reconnect the tubing to the check valves.
Note: Do not overtighten the fittings.

Finishing Pump Check Valve Maintenance Procedures

When finished with check valve cleaning or replacement procedures:

1. Replace the access panel. It is best to thread all four thumbscrews loosely into the instrument body, then tighten.
2. Reconnect the power cord and tubing.
3. Replace the waste bottle and reconnect the waste tube to the instrument.
4. Turn the unit around so the front is facing outward.

13.2.2 Pump Seals

The Profinia system automatically cleans the flow path after every run, including the area behind the pump seals. The Profinia pump has four seals, two main seals located in the pump body, and two piston guide seals located in the piston guides. Pump seals do not normally require replacement, except during periodic preventative maintenance. If flow rates are not as expected or sample volumes are too small, the pump seals may be leaking. The Profinia pump seals are located in the instrument cabinet, inside the pump assembly.

Materials required to clean or replace the pump seals:

- **Pump seal kit** — catalog #620-0412, includes four pump seals, pump seal tool, and instruction sheet
- **9/64" Allen wrench**
- **Wrench for tubing fittings**
- **Profinia 125 ml bottle** — filled with 100 ml of 50% alcohol (methanol, ethanol, or isopropanol)
- **Shallow tray and alcohol** — for soaking pump parts

To clean or replace the system pump seals:

1. Use the procedure in section 13.2 to remove the instrument access panel.
2. Locate the pump head assembly.
3. Use your fingers or the tubing wrench to remove the 1/8" inlet tube from the front of the pump body (Figure 13.6).
4. Use your fingers to remove the 1/16" outlet tube from the front of the pump body; you may leave the tubes in place on the top and bottom of the valve body.

5. Use a standard 9/64" Allen wrench to remove the four screws that hold the pump head to the drive assembly (Figure 13.7).

6. Pull the pump head away gently (Figure 13.8); the pump wash tubing will still be connected.
7. Using your fingers, remove the pump wash fittings from the top of the pump head.

8. Remove both pistons and piston carriers from the pump head by pulling them out with your fingers.

9. Use the piston seal tool to remove both main seals located at the bottom of the recess where the piston carriers were removed from by inserting the tip of the tool in the seal, and gently prying sideways until the seal pops loose from the recess (Figure 13.9).

Note: Once a seal is removed, it cannot be reused.

10. Rinse the pump head in deionized water.

11. It is best to soak the new seals in 50% alcohol (methanol, isopropanol, or ethanol). Sonicate in alcohol if possible. This removes any air that might be trapped in the seal.
12. Press the new seals into the recesses in the pump body with your fingers, then use the piston seal tool to snap them into place. Soak the pump body in 50% alcohol, sonicate in alcohol if possible to remove any trapped air.

13. To replace the piston carrier seals, pull the pistons from the piston carriers, and wash all parts with deionized water.

Fig. 13.10. Piston and piston carrier.

14. Use the piston seal tool to remove both piston carrier seals from the piston guide by inserting the tip of the tool in the seal and gently prying sideways until the seal pops loose from the recess (Figure 13.11).

Note: Each piston guide has seals at one end; these are identical to the main seals.

Fig. 13.11. Removing the piston from the piston carrier (left); and removing the piston carrier seal, using the piston seal tool (right).

15. Rinse the piston carrier in water, and press the new seal into place with your finger. Do not use the seal tool to install the new seal.

16. Reassemble the pistons, springs, and carriers.

17. Push the piston carriers into the pump head recess.

Note: The flats on the piston carriers must be straight up and down (Figure 13.12).

16. Wipe off the four screws and place a small amount of Vaseline or stopcock grease on the last 1/8" of the screws. Reconnect the piston wash tubing to the top of the pump head (2 fittings).

17. Place the screws in the pump head, and put the pump head back in the hole in the carrier. If you have trouble getting the pump head inserted, check to see that the flats on the guides align with the flats on the carrier assembly (Figure 13.12).
18. Use the 9/64" Allen wrench to tighten the screws. Start the inside screws first, alternating between screws until they are finger-tight, then install the outside screws and tighten all four.

19. Connect the 1/8" inlet tube to the pump head, and tighten the fitting with the fitting wrench; do not overtighten the fitting.

20. Connect the 1/16" outlet tube to the pump head, and tighten the fitting with the fitting wrench; do not overtighten the fitting.

21. Connect the 1/8" pump seal wash tubes to the top of the pump body.

22. Replace the access cover, reconnect the power cord and tubing, and turn the unit around to face you.

23. Turn the instrument power on.

24. Place a bottle of 20% ethanol in buffer position 8.

25. Touch the Data/Utilities button on the system home screen.

26. Touch the Diag/Maint Functions button.

27. Touch the up and down arrows to select “System Ethanol Wash”, then press the Start button. When the ethanol wash is complete, the system returns to the Diag/Maint Functions screen.

28. Check the pump flow rate after changing piston seals (see section 9.2.2).

### 13.2.3 UV Monitor Lamp

When the UV lamp has passed its service life, it must be replaced. The most common symptom of an expired lamp is a noisy UV baseline. Use the UV monitor utility outlined below to check the lamp intensity.

#### Checking the UV Lamp Intensity

The UV monitor measures absorbance by comparing a reference voltage from a detector that looks directly at the UV lamp, with a sample voltage from a detector that looks through...
the UV monitor cuvette. If the reference voltage is less than 0.05 volts, the lamp intensity is low and the lamp should be replaced. If the sample voltage is less than 65% of the reference voltage, the flow cell may be dirty. Either of these conditions may cause an unstable UV baseline.

To check voltage:

1. Switch on power to the Profinia instrument.
2. From the system home screen, touch the Data/Utilities button.
3. Touch the Diag/Maint Functions button.
4. Select the Manual Operation button.
5. Touch the “Toggle Lamp” button, at the bottom of the screen. The “lamp” icon should appear at the upper left hand corner of the display.
6. Wait 10 minutes for the lamp to warm up.
7. The Sample and Reference values will be displayed in the upper left corner of the Manual Operation of hardware screen.

Replacing the UV Monitor Lamp

Safety warnings:

- Do not remove the UV lamp when the Profinia instrument is turned on; the UV light can injure your eyes or skin
- The UV lamp gets hot during operation. Allow 15 min to cool before touching the lamp assembly to avoid burns
- Do not touch the glass surface of the lamp. If this happens, clean with methanol and a clean cloth and allow it to dry before installing
- The lamp contains a small amount of mercury vapor — dispose of the lamp in accordance with local regulations. Do not break or incinerate the lamp

The Profinia instrument’s UV lamp is located inside the instrument cabinet. The Profinia UV lamp replacement kit (catalog #620-0406) is required to replace the lamp. To replace the Profinia UV lamp:

1. Use the procedure in section 13.2 to remove the instrument access panel.
2. Locate the UV monitor assembly (Figure 13.13).
3. Follow the lamp’s power cable to the metal panel, and unplug it (Figure 13.14).

**Note:** Depress the locking tab before pulling the connector out.

4. Reach behind the black UV monitor assembly, and remove the two screws holding the lamp assembly using the 9/64" Allen wrench provided with the new lamp. Pull the lamp carrier and filter holder gently from the UV monitor body (Figure 13.15). Set the filter holder and screws aside in a safe place.
5. Using the 5/64" Allen wrench provided with the new lamp, remove the set screw holding the UV lamp in place (Figure 13.16).

6. Slide the UV lamp out of its carrier (Figure 13.17).
7. Remove the new UV lamp from its package. Note the round window for the UV light (Figure 13.18), and the flat for the set screw.

8. Slide the new UV lamp into the lamp carrier, and align the hole in the lamp with the window in the carrier.

9. Using the 5/64" Allen wrench, tighten the set screw that holds the lamp in place.

10. Find the filter carrier; place this on top of the UV lamp carrier with the silver side of the filter facing toward the lamp. Align the mounting holes in the filter carrier with the mounting holes in the lamp carrier (Figure 13.19).
Fig. 13.19. Filter carrier and UV lamp carrier.

11. Place the lamp and filter carriers on the UV module body and secure with the screws using the 9/64" Allen wrench provided with the new lamp.

12. Plug the lamp's cord into its receptacle.

13. Replace the access panel and waste bottle, reconnect the power cord, and turn the unit around to face you. The system is now ready to operate.

13.2.4 UV Monitor Flow Cells

The Profinia system automatically cleans the flow path after every run, so the flow cells do not generally require service. If the flow cells should become dirty or clogged, the most common symptom is a noisy UV baseline. To check the flow cells, check the UV signal and reference voltages, as described in section 13.2.3. With the flow cell flushed with clean water, the sample voltage should be at least 65% of the reference voltage.

Removing and Replacing the UV Monitor Flow Cells

The Profinia UV flow cells are located inside the instrument cabinet, in the UV monitor assembly (Figure 13.13). To inspect or replace the flow cell:

1. Use the procedure in section 13.2 to remove the instrument access panel.

2. Locate the UV monitor assembly.

3. Remove the two tubing fittings from the top of the flow cells; mark them so they can be reconnected to the same flow cell (Figure 13.20).
Fig. 13.20. Removing the two tubing fittings from the top of the flow cells; follow this procedure for each flow cell.

4. Using a 9/64" Allen wrench, remove the two Allen head screws holding the UV monitor assembly to its bracket.

5. Turn the UV monitor on its side and remove the two fittings from the bottom (Figure 13.21); mark these so they can be reconnected to the proper flow cell.

Fig. 13.21. Removing the two bottom fittings of the flow cells.

6. Temporarily replace the Allen screws holding the UV monitor assembly to the bracket; do not allow the monitor assembly to hang from the tubing.

7. Remove the two screws holding the flow cell assembly using a small flat-blade screwdriver (Figure 13.22).
8. Gently pull the flow cell assembly from its recess.

9. Look through the light passages (Figure 13.23) to inspect the flow cell; there should be no debris or obstruction.

10. Reinstall the flow cell assembly using a small flat-blade screwdriver.

11. Remove the UV monitor assembly from its bracket and reconnect the tubing bottom of the flow cell assembly. Reattach the UV monitor assembly to its bracket and tighten the screws.

12. Reattach the two tubing fittings to the top of the UV flow cell.

13. Replace the access panel and effluent bottle, reconnect the power cord, and turn the unit around to face you.
14. Turn on the unit and wait 5 min.

15. Check the sample and reference voltages, as described above.

13.2.5 Buffer and Sample Inlet Tubes

If the buffer or sample inlet tubes become worn or damaged, replace them with 1/8" OD Tefzel tubing. Precut pieces of tubing are available (catalog #620-0405, Profinia sipper tube replacement kit). You may also use tubing from a roll. Cut this tubing to 13.46 cm (5.3") long, and straighten it; heat it with a hair dryer if straightening is difficult. Pull the existing tubing from the silicone tubing coupler and replace with the new tubing.

13.2.6 Tubings and Fittings

Under normal circumstances, the internal tubing and fittings for the Profinia system should last indefinitely; they are protected from damage by the instrument’s cabinet and the system’s automatic cleaning functions. Should the tubing require replacement, however, you may order the Profinia instrument tubing kit (catalog #620-0415), which contains tubing and fittings. It is important that the replacement tubing is the same diameter as the original, and the length must be within ±3 mm, because method programming is based on the volume of the tubing.

You may also make a replacement tube from bulk tubing and fittings. Tubing fittings are ¼-28 flat bottom, using lock rings and ferrules to make a seal. The small tubing is 1/16" Tefzel (Bio-Rad catalog #750-0602). Fittings for the 1/16" tubing are nut/ferrule/lock ring sets (catalog #750-0554). The large tubing is 1/8" Tefzel (Bio-Rad catalog #750-0603). Fittings for the 1/8" tubing are nut/ferrule/lock ring sets (catalog #750-0553).

To make a replacement tube:

Note where each end of the tubing connects (Table 13.1). Refer to the diagram on the inside of the fluidic access panel or to the diagram below (Figure 13.24) to find the connection inlets and outlets.
Table 13.1. Diameters and lengths required for Profinia system tubing connections.

<table>
<thead>
<tr>
<th>System Connection</th>
<th>System Label</th>
<th>Tubing OD, Inches</th>
<th>Tubing Length, Inches</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer bottle to inlet select valve port</td>
<td>B1 to ISV 1; B2 to ISV 2; B3 to ISV 3; B4 to ISV 4; B5 to ISV 5; B6 to ISV 6; B7 to ISV 7; B8 to ISV 8</td>
<td>1/8</td>
<td>21</td>
</tr>
<tr>
<td>Inlet select valve common to proportioning valve NC port</td>
<td>ISV Com to PV (NC)</td>
<td>1/8</td>
<td>8</td>
</tr>
<tr>
<td>Diluent bottle inlet to proportioning valve NO port</td>
<td>Water to PV (NO)</td>
<td>1/8</td>
<td>17</td>
</tr>
<tr>
<td>Proportioning valve common to inline filter</td>
<td>PV (COM) to PUMP IN</td>
<td>1/8</td>
<td>12</td>
</tr>
<tr>
<td>Pump inlet tee (in pump block) to inlet check valve (below pump block), 2 each</td>
<td>Pump in to check VLV</td>
<td>1/8</td>
<td>5</td>
</tr>
<tr>
<td>Pump outlet tee (in pump block) to inlet check valve (above pump block), 2 each</td>
<td>Pump out to check VLV</td>
<td>1/16</td>
<td>5</td>
</tr>
<tr>
<td>Pump outlet to pressure sensor inlet</td>
<td>Pump out to pressure</td>
<td>1/16</td>
<td>8</td>
</tr>
<tr>
<td>Pump wash loop (top of pump block, near sheet metal)</td>
<td>PWash loop</td>
<td>1/8</td>
<td>4</td>
</tr>
<tr>
<td>Pump wash outlet to waste tee (bottom pump block)</td>
<td>PWash out to waste</td>
<td>1/8</td>
<td>8</td>
</tr>
<tr>
<td>Inline filter outlet (above buffer bottles) to pump inlet</td>
<td>Filter out to pump in</td>
<td>1/8</td>
<td>18</td>
</tr>
<tr>
<td>Column valve 1 port 2 to pump wash in (bottom pump block)</td>
<td>CV1 P2 to PWash in</td>
<td>1/8</td>
<td>8</td>
</tr>
<tr>
<td>Pressure sensor to column valve 1 common (center) port</td>
<td>Pressure to CV1 COM</td>
<td>1/16</td>
<td>8</td>
</tr>
<tr>
<td>Column 1 tee to UV monitor 1 flow cell (below UV monitor)</td>
<td>Tee to UV1 IN</td>
<td>1/16</td>
<td>8</td>
</tr>
<tr>
<td>UV monitor 1 flow cell outlet (above UV monitor) to column valve 2 common (center) port</td>
<td>UV1 to CV2 COM</td>
<td>1/16</td>
<td>8</td>
</tr>
<tr>
<td>Column valve 2 port 5 to column valve 2 tee</td>
<td>CV2 P5 to tee</td>
<td>1/16</td>
<td>5</td>
</tr>
<tr>
<td>UV monitor 2 flow cell outlet to column valve 2 tee</td>
<td>UV2 out to tee</td>
<td>1/16</td>
<td>5</td>
</tr>
<tr>
<td>Column 2 tee to conductivity monitor inlet (or pH flow cell inlet)</td>
<td>Tee to COND IN</td>
<td>1/16</td>
<td>5</td>
</tr>
<tr>
<td>(Optional) pH flow cell to outlet selector valve common (center) port</td>
<td>pH to OSV</td>
<td>1/16</td>
<td>6</td>
</tr>
<tr>
<td>Sample inlet short 1/8” tube with air sensor (inside electronics cabinet)</td>
<td>S1 or S2</td>
<td>1/8</td>
<td>2.5</td>
</tr>
<tr>
<td>Sample inlet 1/16” tube from union (inside electronics cabinet) to inlet select valve</td>
<td>S1 to ISV P9; S2 to ISV P10</td>
<td>1/16</td>
<td>16</td>
</tr>
<tr>
<td>Column valve 1 port 5 to column 1 tee</td>
<td>CV1 P6 to COL1 IN</td>
<td>1/16</td>
<td>9</td>
</tr>
<tr>
<td>Column valve 1 port 6 to column 2 Inlet</td>
<td>CV2 P6 to COL2 IN</td>
<td>1/16</td>
<td>6</td>
</tr>
<tr>
<td>Column 1 out to column 1 tee</td>
<td>COL1 out to TEE</td>
<td>1/16</td>
<td>27</td>
</tr>
<tr>
<td>Column 1 tee outlet to UV monitor flow cell 1</td>
<td>COL1 TEE to UV1 IN</td>
<td>1/16</td>
<td>27</td>
</tr>
<tr>
<td>Column 2 outlet to UV monitor 2 inlet (under UV monitor)</td>
<td>COL2 out to UV2 IN</td>
<td>1/16</td>
<td>9</td>
</tr>
<tr>
<td>Waste manifold to waste outlet</td>
<td>Manifold to out</td>
<td>1/8</td>
<td>12</td>
</tr>
<tr>
<td>System Connection</td>
<td>System Label</td>
<td>Tubing OD, Inches</td>
<td>Tubing Length, Inches</td>
</tr>
<tr>
<td>-------------------------------------------------------</td>
<td>-------------------------------------------</td>
<td>-------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Outlet select valve port 10 to waste manifold</td>
<td>OSV P10 to waste</td>
<td>1/8</td>
<td>8</td>
</tr>
<tr>
<td>Column valve 2 port 4 to waste manifold</td>
<td>CV2 P4 to waste</td>
<td>1/8</td>
<td>8</td>
</tr>
<tr>
<td>Column valve 1 port 1 to waste</td>
<td>CV1 P1 to waste</td>
<td>1/8</td>
<td>9</td>
</tr>
<tr>
<td>Conductivity monitor out to outlet select valve common (center) port</td>
<td>COND out to OSV COM</td>
<td>1/16</td>
<td>8</td>
</tr>
<tr>
<td>Outlet select valve port 9 to fraction collector</td>
<td>OSV P9 to FC</td>
<td>1/16</td>
<td>18</td>
</tr>
<tr>
<td>Outlet select valve ports 1–3 to fraction tube 1A–1C</td>
<td>OSV P1, EFF 1A; OSV P2, EFF 1B; OSV P3, EFF 1C</td>
<td>1/16</td>
<td>23</td>
</tr>
<tr>
<td>Outlet select valve port 4 to fraction tube 1D</td>
<td>OSV P4, EFF 1D</td>
<td>1/16</td>
<td>18</td>
</tr>
<tr>
<td>Outlet select valve ports 5, 6, 1 to fraction tubes 2A–2C</td>
<td>OSV P5, EFF 2A; OSV P6, EFF 2B; OSV P7, EFF 2C</td>
<td>1/16</td>
<td>23</td>
</tr>
<tr>
<td>Outlet select valve port 1 to fraction tube 2D</td>
<td>OSV P8, EFF 2D</td>
<td>1/16</td>
<td>18</td>
</tr>
<tr>
<td>Short tube from buffer bottle to inlet tube (straighten), 1/buffer bottle</td>
<td>(Unlabeled) buffer inlet (short) tube, 8 ea.</td>
<td>1/8</td>
<td>5.3</td>
</tr>
<tr>
<td>Waste outlet to waste bottle (rear of cabinet)</td>
<td>(Unlabeled) waste outlet to waste bottle</td>
<td>1/8</td>
<td>17</td>
</tr>
<tr>
<td>Diluent inlet to diluent bottle (rear of cabinet)</td>
<td>(Unlabeled) diluent bottle to diluent inlet</td>
<td>1/8</td>
<td>10</td>
</tr>
</tbody>
</table>
Fig. 13.24. Schematic diagram of the Profinia system.

To install the replacement tube:

1. Remove the damaged tube and measure it, or refer to the list of tubing lengths in Table 13.1.

2. Look at the nut, ferrule, and lock ring on the tubing ends to determine how to install the new tubing.

3. Cut the new tubing to the proper length, using a tubing cutter or razor blade.

Note: The replacement tubing must be within ±3 mm of the specified length.

4. Slip the nut over the tubing with the small end of the tubing toward the cut end of the tube.
5. Slip the lock ring over the tubing.

**Note:** The lock ring is tapered on the inside; the wide end of the lock ring faces the cut end of the tubing.

6. Slip the ferrule over the tubing, with the flat end of the tubing toward the cut end of the tube (Figure 13.25).

![Fig. 13.25. Ferrule and lock ring properly slipped over the tubing.](image)

7. Thread the nut into a \(\frac{1}{4}-28\) fitting; you may wish to use the external fraction collector connection on the back panel of the instrument. This compresses the ferrule and locks it onto the tube (Figure 13.26).

![Fig. 13.26. Ferrule locked onto the tube.](image)

8. Repeat the steps above for the other end of the tube.

9. Using tape, label the new tubing assembly with the same words as the tubing it replaces.

10. Install the new tubing and tighten the fittings.
13.2.7 Inline Filter

The inline filter is a 6 µm polyester membrane filter, which protects the system from particles in the inlet fluid stream. The inline filter is located under the top cover of the instrument above the buffer bottles, near the left side of the instrument. The inline filter should be replaced every month, or after 20 purification runs.

Preparing the filter for installation:

1. Fill the Luer-Lok syringe with 30 ml of 20% ethanol and connect to the inline filter (Figure 13.27).
2. Push the ethanol through the inline filter in approximately 30 sec.
3. Remove the inline filter from the syringe and follow installation instructions.

Fig. 13.27. Connecting the syringe to the inline filter.

Installing the filter:

1. Open the top cover and locate the filter (Figure 13.28).
2. Twist both luer fittings counterclockwise to unlock them, then remove the filter.
3. Install the new filter, and tighten the fittings.

Fig. 13.28. Location of the inline filter under the instrument top cover.
13.2.8 Power Conditioning

The Profinia system is designed to operate in a laboratory environment with other instruments nearby; it complies with the CE standard for immunity from interference. During operation, the Profinia system draws a maximum of 40 volt-amps (VA). This is 0.33 amps at 120 volts and 0.17 amps at 240 volts. As with any electronic device, it is desirable to use a surge suppressor or uninterruptible power supply (UPS) to avoid damage caused by voltage surges or other severe power line problems:

- **Surge suppressors** — designed to prevent damage due to surges or spikes in power line voltage. Select a surge suppressor that is rated to deliver enough current for the Profinia (40 VA) and any accessories (such as a computer). Avoid plugging motor-driven items, such as vacuum pumps, into the same surge suppressor as the Profinia system — this may cause interference.

- **Uninterruptible power supplies (UPS)** — act as surge suppressors but also maintain power when the main power fails. When selecting a UPS, first consider the current capacity of the unit; a UPS should have a rating of at least 80 VA (twice the draw of a Profinia system). When selecting a UPS, remember to add the current requirements of any computer, monitor, or other accessory that will be used with the system. Avoid plugging motor-driven items, such as vacuum pumps, into the same UPS as the Profinia system — this may cause interference and shortens the amount of time the UPS supplies power. The manufacturer’s runtime chart defines how long the system runs after the power fails. Select a UPS that supplies power through the longest expected power outage, with a safety margin of 20%.
Appendix A
Profinia™ System Specifications

Operating limits
Flow rate range 0.2–20 ml/min
Maximum back pressure 45 psi (3.4 bar)

Detection
UV detection 280 nm
Conductivity monitor range 0–500 mS/cm
pH monitor pH range 1–14
Sample and Fraction 15 or 50 ml conical tubes and ability to use
Collection containers external reservoirs connected by tubing
Buffer selection Rotary selector valve for up to 8 buffers
Solvent compatibility All commonly used chromatographic solvents*
Operating temperature 4–30°C; cold room compatible

Data output USB mass storage device
Direct USB cable to PC

Dimensions (W x D x H) 58 x 33 x 67 cm (22.7 x 13 x 26.4 in)
Weight 28 kg (62 lb)

Optional accessories**
Cooling accessory
Optional Profinia PC software
Desalting sample loops
pH monitor

*Chemical Compatibility (Chemical/Buffer Concentration)
8 M Urea 1 M Hydrochloric acid
6 M Guanidine hydrochloride 3 M Ammonium sulfate or saturated solution
30% Ethanol 30 mM β-mercaptoethanol
100% Methanol 10% SDS in PBS
5 M Sodium chloride 5% Triton in PBS
2 M Sodium citrate Aqueous buffers, pH 2–12
30% Glycerol
2 M Sodium hydroxide
3 M Sodium acetate

** Sold separately
## Appendix B
### Profinia™ System Ordering Information

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>156-3004</td>
<td>Profinity eXact Antibody Reagent</td>
</tr>
<tr>
<td>620-0010</td>
<td>Profinia Software, includes USB cable</td>
</tr>
<tr>
<td>620-0200</td>
<td>Profinia Small Urea Pack, 2 x 45 g</td>
</tr>
<tr>
<td>620-0201</td>
<td>Profinia Large Urea Pack, 2 x 90 g</td>
</tr>
<tr>
<td>620-0202</td>
<td>Profinia Glutathione Pack, 1.23 g</td>
</tr>
<tr>
<td>620-0203</td>
<td>Profinia His Antibody</td>
</tr>
<tr>
<td>620-0204</td>
<td>Profinia GST Antibody</td>
</tr>
<tr>
<td>620-0205</td>
<td>2x Profinia Native IMAC Lysis/Bind Buffer, 125 ml</td>
</tr>
<tr>
<td>620-0206</td>
<td>2x Profinia Native IMAC Equilibration/Wash Buffer 1, 125 ml</td>
</tr>
<tr>
<td>620-0207</td>
<td>2x Profinia Native IMAC Wash Buffer 2, 100 ml</td>
</tr>
<tr>
<td>620-0208</td>
<td>2x Profinia Native IMAC Elution Buffer, 100 ml</td>
</tr>
<tr>
<td>620-0209</td>
<td>1.4x Profinia Denaturing IMAC Lysis/Bind Buffer, 176 ml</td>
</tr>
<tr>
<td>620-0210</td>
<td>1.4x Profinia Denaturing IMAC Equilibration/Wash Buffer 1, 176 ml</td>
</tr>
<tr>
<td>620-0211</td>
<td>1.4x Profinia Denaturing IMAC Wash Buffer 2, 88 ml</td>
</tr>
<tr>
<td>620-0212</td>
<td>1.4x Profinia Denaturing IMAC Elution Buffer, 88 ml</td>
</tr>
<tr>
<td>620-0213</td>
<td>2x Profinia GST Lysis/Bind Buffer, 125 ml</td>
</tr>
<tr>
<td>620-0214</td>
<td>2x Profinia GST Equilibration/Wash Buffer, 200 ml</td>
</tr>
<tr>
<td>620-0215</td>
<td>2x Profinia GST Elution Buffer, 100 ml</td>
</tr>
<tr>
<td>620-0216</td>
<td>5x Profinia Desalting Buffer, 200 ml</td>
</tr>
<tr>
<td>620-0217</td>
<td>2x Profinia Cleaning Solution 1, 125 ml</td>
</tr>
<tr>
<td>620-0218</td>
<td>4x Profinia Cleaning Solution 2, 125 ml</td>
</tr>
<tr>
<td>620-0219</td>
<td>2x Profinia Storage Solution, 200 ml</td>
</tr>
<tr>
<td>620-0221</td>
<td>Profinia Native IMAC Buffer Kit, includes purification buffers, cleaning and storage solutions; sufficient for 10 applications</td>
</tr>
<tr>
<td>620-0222</td>
<td>Profinia Denaturing IMAC Buffer Kit, includes purification buffers, cleaning and storage solutions, urea reagent; sufficient for 10 applications</td>
</tr>
<tr>
<td>620-0223</td>
<td>Profinia GST Buffer Kit, includes purification buffers, cleaning and storage solutions, glutathione reagent; sufficient for 10 applications</td>
</tr>
<tr>
<td>620-0224</td>
<td>Profinia Desalting Buffer Kit, includes purification buffers, cleaning and storage solutions; sufficient for 10 applications</td>
</tr>
<tr>
<td>620-0225</td>
<td>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</td>
</tr>
<tr>
<td>Catalog #</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>620-0226</td>
<td><strong>Profinia GST Purification Kit</strong>, 1 ml, includes Profinia GST buffer kit, 2 x 1 ml GST and 2 x 10 ml desalting cartridges</td>
</tr>
<tr>
<td>620-0227</td>
<td><strong>Profinia Denaturing IMAC Purification Kit</strong>, 1 ml, includes Profinia denaturing IMAC buffer kit, 2 x 1 ml IMAC cartridges</td>
</tr>
<tr>
<td>620-0228</td>
<td><strong>Profinia Desalting Purification Kit</strong>, 10 ml, includes Profinia desalting buffer kit, 2 x 10 ml desalting cartridges</td>
</tr>
<tr>
<td>620-0229</td>
<td><strong>Profinia Native IMAC Starter Kit</strong>, includes Profinia native IMAC buffer kit, 1 x 1 ml IMAC and 1 x 10 ml desalting cartridge, <em>E. coli</em> lysate</td>
</tr>
<tr>
<td>620-0230</td>
<td><strong>Profinia GST Starter Kit</strong>, includes Profinia GST buffer kit, 1 x 1 ml GST and 1 x 10 ml desalting cartridge, <em>E. coli</em> lysate, glutathione reagent</td>
</tr>
<tr>
<td>620-0231</td>
<td><strong>Bottle Starter Pack</strong>, includes 4 x 125 ml buffer bottles, 4 x 250 ml buffer bottles, 8 buffer bottle lids</td>
</tr>
<tr>
<td>620-0232</td>
<td><strong>Waste/Diluent Bottle Set</strong>, includes 2 graduated bottles with caps, tubing</td>
</tr>
<tr>
<td>620-0233</td>
<td><strong>Profinia Control Lysate</strong></td>
</tr>
<tr>
<td>620-0235</td>
<td><strong>Profinia Native IMAC Purification Kit</strong>, 5 ml, includes 2 Profinia native IMAC buffer kits, 1 x 5 ml IMAC and 1 x 50 ml desalting cartridge</td>
</tr>
<tr>
<td>620-0236</td>
<td><strong>Profinia GST Purification Kit</strong>, 5 ml, includes 2 Profinia GST buffer kits, 1 x 5 ml GST and 1 x 50 ml desalting cartridge</td>
</tr>
<tr>
<td>620-0237</td>
<td><strong>Profinia Denaturing IMAC Purification Kit</strong>, 5 ml, includes 2 Profinia denaturing IMAC buffer kits, 2 x 1 ml IMAC cartridges</td>
</tr>
<tr>
<td>620-0238</td>
<td><strong>Profinia Desalting Purification Kit</strong>, 50 ml, includes 2 Profinia desalting buffer kits, 1 x 50 ml desalting cartridge</td>
</tr>
<tr>
<td>620-0400</td>
<td><strong>Profinia Cleaning Tray</strong>, includes 1 cleaning tray</td>
</tr>
<tr>
<td>620-0401</td>
<td><strong>Profinia Instrument Cooling Accessory</strong>, includes 2 cooling units</td>
</tr>
<tr>
<td>620-0402</td>
<td><strong>Profinia Desalting Sample Loop</strong>, 2 ml</td>
</tr>
<tr>
<td>620-0403</td>
<td><strong>Profinia Desalting Sample Loop</strong>, 10 ml</td>
</tr>
<tr>
<td>620-0404</td>
<td><strong>Profinia Inline Filter Replacement Kit</strong>, includes 12 inline filters</td>
</tr>
<tr>
<td>620-0405</td>
<td><strong>Profinia Sipper Tube Replacement Kit</strong>, includes 10 pieces of precut tubing</td>
</tr>
<tr>
<td>620-0406</td>
<td><strong>Profinia Lamp Replacement Kit</strong>, includes 1 lamp</td>
</tr>
<tr>
<td>620-0407</td>
<td><strong>Profinia Buffer Lids</strong>, includes 8 lids</td>
</tr>
<tr>
<td>620-0408</td>
<td><strong>Profinia 50 ml Sample Lids</strong>, includes 2 lids</td>
</tr>
<tr>
<td>620-0409</td>
<td><strong>Profinia 15 ml Sample Lids</strong>, includes 2 lids</td>
</tr>
<tr>
<td>620-0410</td>
<td><strong>Profinia Instrument Accessory Kit</strong>, 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</td>
</tr>
<tr>
<td>620-0411</td>
<td><strong>Profinia pH Monitor Kit</strong>, includes pH electrode, flow cell, mounting accessories</td>
</tr>
<tr>
<td>620-0412</td>
<td><strong>Profinia Pump Seal Kit</strong>, includes 4 pump seals and seal insertion tool</td>
</tr>
<tr>
<td>620-0413</td>
<td><strong>Profinia Inlet Check Valve Kit</strong>, includes 2 check valves</td>
</tr>
<tr>
<td>620-0414</td>
<td><strong>Profinia Outlet Check Valve Kit</strong>, includes 2 check valves</td>
</tr>
<tr>
<td>Catalog #</td>
<td>Description</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>620-0415</td>
<td><strong>Profinia Instrument Tubing Kit</strong>, includes assembled system tubing and fittings</td>
</tr>
<tr>
<td>620-0416</td>
<td><strong>Profinia UV Flow Cell Replacement Kit</strong>, includes flow cell and fittings</td>
</tr>
<tr>
<td>620-1004</td>
<td><strong>Profinia Instrument With Accessory Kit</strong>, 100–240 V, 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</td>
</tr>
<tr>
<td>620-1005</td>
<td><strong>Profinia Instrument With Accessory Kit and Native IMAC Starter Kit</strong>, 100–240 V, 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, Profinia native IMAC buffer kit, 1 x 1 ml IMAC and 1 x 10 ml desalting cartridge, <em>E. coli</em> lysate</td>
</tr>
<tr>
<td>620-1006</td>
<td><strong>Profinia Instrument With Accessory Kit and GST Starter Kit</strong>, 100–240 V, 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, Profinia GST buffer kit, 1 x 1 ml GST and 1 x 10 ml desalting cartridge, <em>E. coli</em> lysate, glutathione reagent</td>
</tr>
<tr>
<td>620-1009</td>
<td><strong>Profinia Protein Purification System</strong>, 100–240 V, includes same as 620-1004 with Profinia software</td>
</tr>
<tr>
<td>620-1010</td>
<td><strong>Profinia Protein Purification System With Native IMAC Starter Kit</strong>, 100–240 V, includes same as 620-1005 with Profinia software</td>
</tr>
<tr>
<td>620-1011</td>
<td><strong>Profinia Protein Purification System With GST Starter Kit</strong>, 100–240 V, includes same as 620-1006 with Profinia software</td>
</tr>
<tr>
<td>620-1014</td>
<td><strong>Profinia Protein Purification System With Computer</strong>, 100–240 V, includes same as 620-1009 with computer</td>
</tr>
<tr>
<td>620-1015</td>
<td><strong>Profinia Protein Purification System With Computer and Native IMAC Starter Kit</strong>, 100–240 V, includes same as 620-1010 with computer</td>
</tr>
<tr>
<td>620-1016</td>
<td><strong>Profinia Protein Purification System With Computer and GST Starter Kit</strong>, 100–240 V, includes same as 620-1011 with computer</td>
</tr>
<tr>
<td>732-4600</td>
<td><strong>Bio-Scale Mini Affi-Prep Protein A Cartridges</strong>, 5 x 1 ml</td>
</tr>
<tr>
<td>732-4602</td>
<td><strong>Bio-Scale Mini Affi-Prep Protein A Cartridges</strong>, 1 x 5 ml</td>
</tr>
<tr>
<td>732-4646</td>
<td><strong>Bio-Scale Mini Profinity eXact Cartridges</strong>, 2 x 1 ml</td>
</tr>
<tr>
<td>732-4647</td>
<td><strong>Bio-Scale Mini Profinity eXact Cartridges</strong>, 4 x 1 ml</td>
</tr>
<tr>
<td>732-4648</td>
<td><strong>Bio-Scale Mini Profinity eXact Cartridges</strong>, 1 x 5 ml</td>
</tr>
<tr>
<td>732-5312</td>
<td><strong>Bio-Scale Mini Bio-Gel P-6 Desalting Cartridge</strong>, 1 x 50 ml</td>
</tr>
<tr>
<td>732-5314</td>
<td><strong>Bio-Scale Mini Bio-Gel P-6 Desalting Cartridges</strong>, 5 x 50 ml</td>
</tr>
</tbody>
</table>
## Appendix C
### Profinia™ System Troubleshooting

## Problems and Solutions

<table>
<thead>
<tr>
<th>Problem</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No power to system</td>
<td>If the instrument will not start, check that the system is plugged in to a live outlet and that the power cord is inserted into the rear panel receptacle. Make sure the switch is in the on position. The Profinia system is compatible with 100–240 volt mains power without adjustments, and there are no fuses or other user serviceable power protection devices required.</td>
</tr>
<tr>
<td>Air in system</td>
<td>The most common cause of air in the system is running out of water or buffer. When this happens, the system will display “Air in line detected!”. To solve this problem:</td>
</tr>
<tr>
<td></td>
<td>1. Examine all buffer and water containers and refill as necessary.</td>
</tr>
<tr>
<td></td>
<td>2. Make sure all tubes are submerged in the liquid.</td>
</tr>
<tr>
<td></td>
<td>3. Touch the Start button. The system will purge the air, and continue the separation run.</td>
</tr>
<tr>
<td></td>
<td>If all fluids are present, lift the instrument’s top cover to check the inline filter fittings and the small pieces of tubing that connect the sample and buffer inlet tubes. If necessary, remove the fluidics compartment access cover and check for loose fittings or damaged tubing.</td>
</tr>
<tr>
<td>System fails to detect end of sample, or air in system</td>
<td>The air detectors can be switched on and off manually. To make sure the end of sample/reagent detection feature is on:</td>
</tr>
<tr>
<td></td>
<td>1. Touch the Data/Utilities button.</td>
</tr>
<tr>
<td></td>
<td>2. Touch the up and down arrows to scroll through the list of functions and highlight “End of Sample/Regent Detection”.</td>
</tr>
<tr>
<td></td>
<td>3. Select this function to make sure the air detectors are switched on.</td>
</tr>
<tr>
<td>Clogged inline filter</td>
<td>A clogged inline filter will reduce the system flow rate or cause bubbles to appear in the flow stream. If the filter clogs frequently, be sure to filter your sample with a 0.45 micron or smaller membrane filter immediately before use. Use the specified 10 micron membrane filter only; other filters may restrict the flow or clog prematurely.</td>
</tr>
<tr>
<td>Fraction size small, or flow rate low</td>
<td>Examine the sample tubes and verify that all of the sample volume that was programmed has been injected into the system.</td>
</tr>
<tr>
<td>Problem</td>
<td>Solution</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>If there aren't problems noted</td>
<td>Use the “Check Flow Rate” utility (section 9.2.2) to check if the flow rate is correct. If the flow rate is low, possible causes are:</td>
</tr>
<tr>
<td>with sample application,</td>
<td>• <strong>Inline filter</strong> — replace the inline filter, and repeat the flow rate check</td>
</tr>
<tr>
<td>use the “Check Flow Rate”</td>
<td>• <strong>Pump check valves</strong> — run the system NaOH wash to clean any contamination from the check valves. Check the flow rate again. If the flow</td>
</tr>
<tr>
<td>utility (section 9.2.2) to</td>
<td>rate is still low, disassemble and clean the check valves</td>
</tr>
<tr>
<td>check if the flow rate is</td>
<td>• <strong>Pump seals</strong> — if cleaning the check valves does not resolve the problem, replace the pump seals</td>
</tr>
<tr>
<td>correct. If the flow rate is</td>
<td></td>
</tr>
<tr>
<td>low, possible causes are:</td>
<td></td>
</tr>
<tr>
<td>Method runs longer than</td>
<td>Check the method temperature setting (section 9.3.13). If the system is set for low temperature (4°C), flow rates are slower and the method</td>
</tr>
<tr>
<td>expected</td>
<td>will take longer to run.</td>
</tr>
<tr>
<td>Leaks at cartridges</td>
<td>If you see leakage at the cartridge inlets or outlets, first tighten the locking collars. If this does not stop the leakage, replace the</td>
</tr>
<tr>
<td></td>
<td>cartridges.</td>
</tr>
<tr>
<td>Leaks to the benchtop</td>
<td>The most common cause of leakage to the benchtop is an overflowing waste bottle or fraction tube. If this is not the cause, remove the</td>
</tr>
<tr>
<td></td>
<td>cover of the fluidics compartment (section 13.2) and check for leaks caused by loose fittings or damaged tubing. When replacing tubing,</td>
</tr>
<tr>
<td></td>
<td>use the same size and length tubing (section 13.2.5).</td>
</tr>
<tr>
<td>Uneven UV baseline</td>
<td>If the UV trace wavers excessively, first check and replace the system inline filter (a clogged filter may cause bubbles in the fluid). If</td>
</tr>
<tr>
<td></td>
<td>this does not resolve the problem, go to the Manual Operation screen (section 9.3.2) and check the intensity of the UV lamp and the</td>
</tr>
<tr>
<td></td>
<td>signal/reference voltage ratio. It may be necessary to replace the UV lamp, or clean the UV flow cell.</td>
</tr>
<tr>
<td>Different readings between</td>
<td>Check UV lamp intensity and signal/reference voltage ratio (section 13.2.3), clean the flow cell, or change the UV lamp if necessary. You</td>
</tr>
<tr>
<td>UV monitors</td>
<td>may calibrate the UV monitors with a protein solution to match the readings.</td>
</tr>
<tr>
<td>Protein estimates are</td>
<td>Protein absorbances vary; use the UV monitor calibration utility (section 9.2.3) to calibrate the system with a protein similar to your</td>
</tr>
<tr>
<td>not as expected</td>
<td>samples.</td>
</tr>
<tr>
<td>Incorrect conductivity reading</td>
<td>If the conductivity monitor does not display the proper reading, use conductivity standard solution (10 mS) to calibrate the</td>
</tr>
<tr>
<td></td>
<td>conductivity meter.</td>
</tr>
<tr>
<td>Incorrect pH reading</td>
<td>Recalibrate the pH probe using the pH calibration utility (section 9.2.6).</td>
</tr>
<tr>
<td>Problem</td>
<td>Solution</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Data does not display on a computer running Profinia software during a separation run</td>
<td>The Profinia system must be connected to the computer via USB cable, and Profinia software must be running before the method is started. It is not possible to start a real-time computer display after a run has started.</td>
</tr>
<tr>
<td>System does not recognize a USB portable memory device</td>
<td>If the system does not recognize the USB portable memory device, remove and reinsert the device. If this does not solve the problem, try another USB device. The Profinia system is compatible with most, but not all, USB portable memory devices (memory sticks).</td>
</tr>
<tr>
<td>System error messages</td>
<td>See Appendix D.</td>
</tr>
</tbody>
</table>
### Instrument Error Codes and Solutions

<table>
<thead>
<tr>
<th>ERROR CODE</th>
<th>ERROR MESSAGES</th>
<th>Possible Cause(s) and Solution(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CANNOT TALK TO I2C DEVICE XX. CHECK INTERNAL CONNECTIONS AND TRY AGAIN CONNECTIONS</td>
<td>Inter-processor communication problems, possibly due to loose.</td>
</tr>
<tr>
<td>4</td>
<td>VALVE CONFIGURATION ERROR BAD VALVE: XXX. (and one of the following messages): POSITION ERROR: VALVE XXX. CAN'T FIND HOME VALVE XXX. CAN'T FIND PROP VALVE.</td>
<td>Dirty valves, defective valves, or defective cables.</td>
</tr>
<tr>
<td>5</td>
<td>INTERNAL ERROR: XX. POWER CYCLE UNIT. CONTACT BIO-RAD IF ERROR PERSISTS.</td>
<td>Power cycle the unit; contact Bio-Rad if error persists.</td>
</tr>
<tr>
<td>7,14,19</td>
<td>AIR IN BUFFER LINE DETECTED! REPLACE OR ADD REAGENT. CHECK ALL REAGENTS AND DI WATER. PRESS START TO RE-PRIME THE LINE AND CONTINUE METHOD. PRESS STOP TO TERMINATE.</td>
<td>Check all reagents and DI water. Press Start to re-prime the lines and continue the method.</td>
</tr>
<tr>
<td>8</td>
<td>PUMP ERROR – CANNOT FIND HOME POSITION. IF ERROR PERSISTS, CONTACT BIO-RAD.</td>
<td>Cycle power, contact Bio-Rad if problem persists.</td>
</tr>
<tr>
<td>10,11,18</td>
<td>OVERPRESSURE DETECTED @60PSI. OPERATIONS SUSPENDED. CORRECT AND CONTINUE.</td>
<td>Correct problem and continue. • Check cartridge and method • Check temperature setting • Check zero pressure point</td>
</tr>
<tr>
<td>12</td>
<td>METHOD STORAGE IS FULL. DELETE A STORED METHOD OR PROCEED WITHOUT SAVING.</td>
<td>Saved method memory is full. Delete a stored method or proceed without saving the new method.</td>
</tr>
<tr>
<td>13</td>
<td>INTERNAL HARDWARE ERROR. DISCONNECTED COMPONENTS. CHECK DEVICE XX.</td>
<td>Check connections. Contact Bio-Rad if problem persists.</td>
</tr>
<tr>
<td>15</td>
<td>TROUBLE WITH PV BOARD. ERROR =XX. CHECK CONNECTIONS.</td>
<td>Check connections. Contact Bio-Rad if problem persists.</td>
</tr>
<tr>
<td>16,17</td>
<td>VALVE MOVE WHILE PUMPING RUN PAUSED. IF ERROR PERSIST CONTACT BIO-RAD.</td>
<td>Contact Bio-Rad if problem persists.</td>
</tr>
<tr>
<td></td>
<td>There is a problem using your memory stick. Please re-insert it and try again. If problem persists power-cycle unit and try again (or try a different memory stick).</td>
<td>Failed memory stick – use a different memory stick.</td>
</tr>
</tbody>
</table>

*X* represent an internal device address. Please contact Bio-Rad technical support (1-800-4BIO-RAD) for assistance.
**Appendix D**

**Profinia™ System Error Messages**

The error messages listed below are displayed on the Profinia touch screen when instrument operation errors occur.

<table>
<thead>
<tr>
<th>Message</th>
<th>Possible Cause(s) and Solutions</th>
</tr>
</thead>
</table>
| **Cannot Talk to I2C device X**              | Interprocessor communication problems; possibly due to loose connections.  
Check internal connections and try again. |
| **Valve configuration error**                | Dirty valves, defective valves, or defective cables.                |
| **Bad valve X**                             |                                                                     |
| **Position error: valve X**                  |                                                                     |
| **Can’t home: valve X**                      |                                                                     |
| **Can’t find prop valve**                    |                                                                     |
| **Internal error: X**                        | Power cycle the unit; contact Bio-Rad if error persists.            |
| **Air in line detected!**                    | Replace or add reagent. Check all reagents and deionized water.     
Press Start to re-prime the line and continue the method.  
Press Stop to terminate run.                 |
| **Pump Error**                               | Contact Bio-Rad if error persists.                                   |
| **Over pressure detected @60 psi**           | Correct and continue.                                               |
| **operations suspended**                     |                                                                     |
| **Over pressure detected-paused**            | Correct and continue.                                               |
| **check cartridges and method**              |                                                                     |
| **Over pressure-did you remove the waste line shipping plug?** | Correct and cycle power |
| **Method storage is full saving**            | Delete a stored method or proceed without (35 methods can be stored; user can continue without saving methods). |
| **Internal hardware error, disconnected components, check device X** | Check connections; contact Bio-Rad if error persists.              |
| **Trouble with PV board, error X**           | Check connections.                                                   |
| **Valve move while pumping, run paused**     | Contact Bio-Rad if error persists.                                   |
| **There is a problem using your memory stick. Please re-insert it and try again.** | If problem persists, power-cycle the unit and retry. Use a different portable memory device. |

*X* represents an internal device address. Please contact Bio-Rad technical support (1-800-4BIORAD) for assistance.
Appendix E
Sample Preparation

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 chemical lysis methods and the Profinia bacterial lysis/extraction reagent. 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 the 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 wash buffer 1 or Profinia GST wash buffer (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 U/ml or benzonase at 25 U/ml).
4. Sonicate the lysate (on ice, using 25% output) four 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 through a 0.45 µm filter to remove particulates.
7. Transfer the clarified lysate supernatant 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 (Profinia Denaturing IMAC Kits)

1. Harvest the 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 wash buffer 1 (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) four 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 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 the description under the native lysate prep for treatment upon freezing/thawing.
Native Lysate Preparation Using Profinia Bacterial Lysis/Extraction Reagent

(Recommended for IMAC procedures; binding capacities of GST fusion proteins will be decreased ~30% using chemical lysis methodologies.)

1. Harvest the 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 (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 U/ml or benzonase at 25 U/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 Profinia Bacterial Lysis/Extraction Reagent

1. Harvest the cell pellet by centrifugation at 8,000 x g for 10 min at 4°C.
2. Determine weight of the pellet and resuspend in 10 volumes of Profinia bacterial lysis/extraction reagent (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 U/ml or benzonase at 25 U/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 wash buffer 1 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.

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# Appendix F
## Sample Preparation, Application, and Analysis Tips

<table>
<thead>
<tr>
<th>Topic</th>
<th>Tips and Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample Preparation</strong></td>
<td></td>
</tr>
<tr>
<td>Sample resuspension and volumes</td>
<td>Resuspend harvested bacterial cells (wet or frozen) at a 1:10–1:20 (w:v) ratio of cell (g) to lysis buffer (ml). For example, resuspend 1 g of cell in 10–20 ml of lysis buffer. Use a minimum of 10 ml of lysis buffer for cell weights &lt;1 g to facilitate cell lysis and to minimize sample loss during preparation and handling.</td>
</tr>
<tr>
<td>Sample lysis — sonication</td>
<td>Use volumes 10 ml, even for small amounts of harvested cells. Adequately immerse the sonication horn to avoid frothing the cell suspension. Select a sonication vessel that allows the tip of the sonication horn to remain immersed during bursts. Use short (30 sec) sonication bursts interspersed with frequent cooling of the sample on ice. Excessive sample heating from prolonged sonication times or high output settings can lead to fragmentation of proteins and poor recovery of the protein of interest. Monitor the viscosity of cell suspension during sonication to ensure adequate disruption of cell walls and nucleic acid fragments. Highly viscous samples may benefit from pretreatment of the cell suspension with DNase.</td>
</tr>
<tr>
<td>Sample lysis — chemical disruption</td>
<td>Chemical lysis of bacterial cell pellets with buffers containing detergents can result in decreased binding of GST fusion proteins to the cartridge compared to samples prepared by mechanical disruption (sonication). Yields can be 20–40% lower, depending on the protein characteristics. To minimize lysate viscosity, add a nuclease (such as benzonase) to the resuspended pellet. Add benzonase at 25 U/ml (or DNase at 100 U/ml), and incubate for 10 min at room temperature.</td>
</tr>
<tr>
<td>Sample clarification</td>
<td>Clarify and filter the prepared cell lysate prior to application on the Profinia system. Following cell disruption, centrifuge the cell suspension for 15–30 min at 6,000 x g or greater. After obtaining the soluble or insoluble fraction containing the protein of interest, use a low-protein binding 0.45–1.0 μm filtration device to clear any particulates not removed by centrifugation. For small sample volumes (&lt;50 ml), a 22–32 mm syringe filter is generally sufficient. For larger sample volumes, it may be necessary to use a vacuum or pressure-driven filtration device with sufficient surface area to efficiently remove suspended particulates.</td>
</tr>
</tbody>
</table>
### Topic Tips and Recommendations

#### Sample Screening — Solubility Profile

Before choosing a native or denaturing purification protocol, approximate expression level and determine if the overexpressed target protein partitions into the soluble or insoluble fractions. Soluble proteins are typically purified with a native purification procedure, while insoluble proteins must be solubilized in stringent denaturants (up to 8 M urea) and are purified with the denaturing procedure. To determine the solubility profile:

1. Pellet ~ 2 ml of *E. coli* culture by centrifugation at 4,000 x g for 10 min at 4°C.
2. Resuspend the pellet in 500 µl of PBS, and sonicate for 60 sec on ice, in 10 sec pulses. Remove 50 µl of the sonicate and label as the “total” sample. Centrifuge the lysate at 12,000 x g for 20 min at 4°C. Transfer the supernatant to a clean tube. Remove 50 µl of the supernatant, and label as the “soluble” sample.
3. Resuspend the insoluble pellet in 500 µl of 6 M urea in 1x PBS, and sonicate for 60 sec on ice, in 10 sec pulses. Centrifuge the lysate at 12,000 x g for 20 min at 4°C. Remove 50 µl of the supernatant, and label “insoluble” sample.
4. To each of the 50 µl samples, add 150 µl of Laemmli buffer, and boil for 5 min at 95°C.
5. Load 10 µl of each sample on an SDS-PAGE gel. Examine the soluble and insoluble fractions for the target protein. Approximate the expression level and determine the portioning of the target protein.

#### Sample Degradation

Once cell lysis has begun, work quickly to get the purification started. The longer a complex lysate sits, the greater the chance it will degrade. Performing lysis at 4°C can also help minimize degradation.

The GST lysis/wash buffer contains 5 mM EDTA and is an effective inhibitor of metalloproteases. However, additional protease inhibitors or the addition of a protease inhibitor cocktail to the sample immediately prior to the cell lysis step may improve the yield of target protein. Minimize the time between cell lysis and application of the cleared lysate to the column. Keep the sample on ice.

#### General Sample Preparation Tips

Keep cell suspensions on ice to avoid precipitation and degradation of the proteins of interest.

Freeze harvested bacterial cells at −20°C or below. Prepare cell suspensions fresh and avoid freeze-thawing the cells or the prepared lysate.

Keep samples of each fractionation step used during sample preparation for analysis by SDS-PAGE or similar in order to monitor the efficiency of lysis and recovery of the protein of interest. For SDS-PAGE samples, preparing a 1:10 dilution of the sample in sample loading buffer and loading 5–10 µl of the preparation is generally sufficient for detection of the bands of interest.
<table>
<thead>
<tr>
<th>Topic</th>
<th>Tips and Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Application</td>
<td>The yield of the target protein is dependent on the flow rate at which the sample is applied to the column. Lowering the flow rate may significantly improve yield.</td>
</tr>
<tr>
<td>Load and Flow Rate Dependency</td>
<td>For viscous samples, choose the low flow sample application parameter in order to avoid exceeding the pressure limits of the Profinia system. It may be necessary to improve the efficiency of cell lysis of highly viscous samples by: • Increasing sonication time • Clarifying and filtering the sample prior to use in the Profinia system • Adding nucleases or trying a different lysis method Slowing down the flow rate of the sample loading step allows the target protein more time to diffuse within the column and find a binding site. This is especially true when purifying protein by denaturing IMAC. Denaturing IMAC buffers are very viscous, so slowing down the flow rate for the sample loading and wash-1 steps can greatly increase yield.</td>
</tr>
<tr>
<td>Load Volumes</td>
<td>For optimal recovery on the Profinia system, use load volumes 5 ml or greater. Ideally, dilute small/concentrated samples from &lt;5 ml to ≥10 ml to maximize yield.</td>
</tr>
<tr>
<td>Large Sample Volumes</td>
<td>When low protein expression requires large culture volumes (&gt;2 L of starting culture), bacterial pellets can be lysed at 5:1 (v/w) ratio to help keep the total sample volume equal to the maximum load volume allowable in the preset Bio-Rad Methods (50 ml). Alternatively, a custom Program Method can be used along with an external sample container (see Section 11.2.2 on pg. 128) to load volumes up to a maximum of 999 liters.</td>
</tr>
<tr>
<td>Proper Load Volume Not Applied</td>
<td>Ensure the sample is free from particulate matter by filtering it through a 0.22 or 0.45 μm filter immediately prior to chromatography.</td>
</tr>
<tr>
<td>Sample Analysis</td>
<td>Check/change the inline filter. Change the inline filter on a regular basis if running large sample volumes.</td>
</tr>
<tr>
<td>Low Target Protein Yield</td>
<td>Periodically run the short-term system wash with 20% ethanol to ensure optimal system performance and flow rates.</td>
</tr>
</tbody>
</table>

**Notes:**
- Load volumes should be 5 ml or greater for optimal recovery.
- Volumes should be <5 ml to ≥10 ml for small/concentrated samples.
- Large sample volumes can be handled by adjusting the lysis ratio or using custom methods.
- Ensure the sample is free from particulate matter by filtering it prior to chromatography.
- Change the inline filter regularly to maintain system performance.
- Periodically run the short-term system wash with 20% ethanol.
<table>
<thead>
<tr>
<th>Topic</th>
<th>Tips and Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precipitation of Elution Peak</td>
<td>Precipitation of the elution peak can occur when the eluted protein is too highly concentrated. Load a lesser volume of sample or add protein stabilization reagents such as glycerol (up to 10%), reducing agents, and detergents to help prevent precipitation of the eluted protein.</td>
</tr>
<tr>
<td>Target Protein in Flow Through</td>
<td>Check the pH of the sample after lysis and ensure that it is 7.0. His-tagged proteins will not bind under acidic conditions.</td>
</tr>
<tr>
<td></td>
<td>Keep the concentration of chelating agents, such as EDTA, to 1 mM. EDTA will strip the nickel-charged resin if used at too high a concentration.</td>
</tr>
<tr>
<td>Low Purity of His-Tagged Target Protein</td>
<td>Increase the concentration of imidazole in the sample and wash buffers to minimize binding by host proteins. Higher imidazole in the sample may decrease binding of His-tagged proteins, resulting in lower yield. Optimization of imidazole concentration is necessary to balance higher purity and yield.</td>
</tr>
<tr>
<td>Purification and Buffer Kits</td>
<td>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 kits that have labile reagents (glutathione in GST kits), 4°C is the recommended long-term storage temperature.</td>
</tr>
<tr>
<td>Proper Storage of Solutions and Kits</td>
<td>Once opened and used with the instrument, all reagents should be stored at 4°C for up to 3 months. After insertion into the instrument, the solutions are no longer sterile and require storage at 4°C.</td>
</tr>
<tr>
<td></td>
<td>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 be discarded.</td>
</tr>
<tr>
<td>Addition of Additives or Reagents to the Solutions</td>
<td>The solutions are provided as concentrates, and are diluted by the Profinia instrument. If another component need to be added to one of the solutions (for example, a protease inhibitor), it should be added as a concentrate and brought to the final concentration of the solution (for example, 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.</td>
</tr>
<tr>
<td>Addition of Urea and Glutathione</td>
<td>Urea and glutathione are supplied in convenient, premeasured aliquots for each buffer bottle. These reagents 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.</td>
</tr>
<tr>
<td>Topic</td>
<td>Tips and Recommendations</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>For longer-term storage, any unused buffer can be stored at –20°C and used within 6 months. 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.</td>
<td></td>
</tr>
</tbody>
</table>
## Appendix G
### Profinia™ System Annual Maintenance

The list of maintenance procedures in Table G should be performed regularly to ensure optimum instrument performance and longevity.

<table>
<thead>
<tr>
<th>Maintenance Procedure</th>
<th>Date Performed</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examine the instrument to note any obvious damage or spills.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Determine whether the instrument has been malfunctioning, and note any problems (if desired, use a Profinia starter kit to verify operation).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Examine all external tubes (buffer inlet tubes, sample inlet tubes, cartridge inlet and outlet fittings) and replace any that are worn or damaged.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Examine the diluent and waste bottles and tubing; clean, repair, or replace as necessary.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Examine the cartridge connectors; replace if worn or damaged.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Run the system ethanol wash and the procedure to clean all lines and valves (section 13.1.4).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replace the inline filter disk.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check UV monitor sample and reference voltage (section 13.2.3). Replace UV lamp if Vref is &lt;0.2 V. Clean or replace UV flow cells if Vsample is &lt;65% of Vref.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calibrate the pH probe (if installed), at pH 4 and 10 (section 9.6.2). Measure the pH of a pH 7 standard; replace the probe if the pH reading is &lt;6.9 or &gt;7.1.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check the pump flow rate (section 9.2.2) and note the results.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Check the conductivity monitor calibration with a standard solution, recalibrate if necessary (section 9.2.4).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With instrument power off, remove the fluidics compartment cover (section 13.2) to:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Correct any leaks and repair any damaged tubing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Replace the pump seals and the seals in the piston guides (section 13.2.2).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Clean or replace the pump check valves (two inlet, two outlet; section 13.2.1).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maintenance Procedure</td>
<td>Date Performed</td>
<td>Comments</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------</td>
<td>----------</td>
</tr>
<tr>
<td>4. Replace the UV lamp, if necessary (section 13.2.3).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Check UV flow cell assembly (section 13.2.4).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After replacing any system parts or components, run the system ethanol wash, then the procedure to flush all inlet and outlet lines (section 13.1.4).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recheck the pump flow rate and note the results.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Note that the pressure reads zero with the system stopped; re-zero the sensor if necessary. Install a calibrated gauge and 20 psi backpressure regulator at the pressure sensor outlet. Compare the readings of the gauge and the sensor, recalibrate the sensor if not within ±4 psi. Replace the pressure sensor if calibration is not successful.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Replace the back panel and reconnect all tubing and cables.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If desired, run a starter kit separation to validate system operation.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## Appendix H
### Profinia™ Instrument Cleaning and Maintenance Log

<table>
<thead>
<tr>
<th>User Information</th>
<th>Cleaning</th>
<th>Maintenance</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Short Term</td>
<td>Long Term</td>
<td>Inline Filter Replacement</td>
</tr>
<tr>
<td>Operator</td>
<td>Lamp Replacement</td>
<td>Notes</td>
<td>Notes</td>
</tr>
</tbody>
</table>


Appendix I
Profinia Protein A and G Method

1.1 Background Information
The Profinia protein purification system is an automated, user-friendly instrument for the purification of affinity-tagged proteins and antibodies. The system has been designed to use standard chromatography buffers, resins, and reagents with easy-to-use pre-programmed purification methods stored on the instrument. There are five main types of applications currently used in the Profinia system: native IMAC, denaturing IMAC, GST, desalting, and protein A and G. The Protein A and G method will be referred to as the Protein A method throughout this section. This section provides information on the use of protein A methods in the Bio-Rad Methods and Program Methods modes of the Profinia instrument.

1.2 Description of Method
The Profinia protein A method is designed for the affinity purification of antibodies (typically IgG) from serum, ascites, or hybridoma cell culture supernatant using protein A (or in some cases, protein G) chromatography media. The instrument has two main types of protein A methods that can be used for the purification of IgG: protein A affinity, and protein A plus integrated desalting. Both methods are designed to use either 1 ml or 5 ml pre-packed protein A cartridges and 10 ml or 50 ml P6 desalting cartridges (respectively), when using the protein A plus integrated desalting methods. Cartridges for protein A applications are available from Bio-Rad as 1 ml and 5 ml Bio-Scale Mini Affi-Prep protein A cartridges that are designed for direct connection to the Profinia instrument. Each ml of Bio-Rad Affi-Prep protein A resin typically yields 5–15 mg of purified IgG, depending on the species, sample type, and antibody subclass. The Profinia protein A methods allow the selection of one or two samples per run, a choice of standard or low flow-rates for sample loading (1 ml cartridges, 1 or 0.5 ml/min; 5 ml cartridges, 5 or 2.5 ml/min) or extended cartridge washing for each sample, similar to the options available for the IMAC and GST pre-programmed methods. Following system priming and cartridge equilibration, the protein A method is programmed to load sample from the S1 and/or S2 ports onto the cartridge. The available pre-programmed Bio-Rad protein A methods are designed for loading volumes between 2–50 ml per sample. For larger sample volumes, the protein A methods can be customized to load volumes (in ml or L) ranging between 0–999. Refer to Section 11 for system requirements when using large sample or buffer volumes on the Profinia instrument. Table 1 summarizes the method steps for a typical Bio-Rad Profinia 1 ml protein A plus 10 ml desalting method with 1 sample. The steps outlined in Table 1 are similar for the 5 ml protein A methods; however, flow-rates are increased proportionally to accommodate large volumes of the 5 ml protein A and 50 ml desalting cartridges. In the case of the protein A affinity only methods (without integrated desalting), the steps associated with the desalting cartridge are absent, and elution of the protein A cartridge is collected directly into 1D (or 2D) fraction without desalting.
1.3 Required Equipment and Materials

Bio-Rad Affi-Prep protein A 1 ml and 5 ml Bio-Scale Mini cartridges are designed to be used with the pre-programmed protein A methods on the Profinia instrument. In addition, a number of other pre-packed protein A cartridges available in 1 ml and 5 ml sizes from other chromatography suppliers have been tested and can be used with the pre-programmed protein A methods. When using cartridges from other suppliers, it may be necessary to obtain the correct fittings to adapt the cartridges to the luer-style fittings of the Profinia instrument cartridge connection ports. Protein A methods that include integrated desalting will also require the use of Bio-Rad Bio-Gel P6 desalting cartridges, which are available in 10 ml and 50 ml sizes, to match the corresponding cartridge sizes required in the pre-programmed protein A methods. Complete Profinia buffer and purification kits specific for protein A methods are not available; however, Table 2 lists the most common recommended buffers and reagents used for protein A purifications. All buffers and solutions can be made or purchased as concentrates as described in Table 2. Prepared buffers should use high quality, chromatography grade reagents and should be pre-filtered through a 0.2 or 0.45 µm filter to remove particulates prior to use with the system. Although the buffer concentrations are preset in the protein A methods, buffer concentrations of 1x, 2x, 3x, 4x, and 5x can be selected and saved as an alternative protein A method in the Program Method mode on the Profinia instrument.

When using the Profinia protein A plus integrated desalting methods, the Profinia desalting purification kit or individually packaged Profinia buffers can be used to supply most of the reagents recommended for the protocol. If the Profinia desalting purification kit is used, a protein A elution/regeneration buffer, such as those recommended in Table 2, will need to be prepared separately (see Table 2 for buffer compositions). Protocols for preparation of recommended protein A elution/regeneration buffers are included in Section 5 of this appendix.
In order to preserve the activity of labile antibodies when using acidic elution conditions, it is recommended that 1 M Tris-HCl, pH 8 be added to the elution fractions (1D and 2D) containing purified antibodies when not using integrated desalting so that the final pH of the collected fractions is adjusted to pH 7–8.

Accessories to the Profinia instrument that can be used for protein A methods include Profinia cooling blocks, cartridge connection fittings, and external sample/buffer tubing. Complete catalog and ordering information can be found in Section 4 of this appendix.

Table 2. Recommended formulations for buffers and solutions for 1 ml protein A plus 10 ml desalting method

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Conc. [l]</th>
<th>Concentrated Formulation</th>
<th>Working 1x Formulation</th>
<th>Volume [ml]</th>
<th>Port #</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein A Bind/Equilibration/Wash Buffer (Recommended: same as Profinia Desalting Buffer)*</td>
<td>5x</td>
<td>685 mM NaCl, 13.5 mM KCl, 21.5 mM NaHPO₄, 40.5 mM Na₂HPO₄, pH 7.0 (pH 7.4 upon dilution)</td>
<td>137 mM NaCl, 2.7 mM KCl, 4.3 mM NaHPO₄, 8.1 mM Na₂HPO₄, pH 7.4</td>
<td>35 ml</td>
<td>B1</td>
<td>620-0216</td>
</tr>
<tr>
<td>Protein A Bind/Equilibration/Wash Buffer (Optional)</td>
<td>5x</td>
<td>100 mM NaHPO₄, pH 7.0</td>
<td>20 mM Na₂HPO₄, pH 7.0</td>
<td>35 ml</td>
<td>B1</td>
<td>-</td>
</tr>
<tr>
<td>Protein A Bind/Equilibration/Wash Buffer (Optional)</td>
<td>5x</td>
<td>100 mM Tris, pH 8.0</td>
<td>20 mM Tris, pH 8.0</td>
<td>35 ml</td>
<td>B1</td>
<td>161-0718</td>
</tr>
<tr>
<td>Protein A Elution/Regeneration Buffer (Recommended) &amp;</td>
<td>5x</td>
<td>500 mM Sodium Citrate, pH 9.0</td>
<td>100 mM Sodium Citrate, pH 9.0</td>
<td>40 ml</td>
<td>B3</td>
<td>-</td>
</tr>
<tr>
<td>Protein A Elution/Regeneration Buffer (Optional) &amp;</td>
<td>5x</td>
<td>1750-2000 mM Glycine, pH 2.6</td>
<td>350-400 mM Glycine, pH 2.6</td>
<td>40 ml</td>
<td>B3</td>
<td>161-0717</td>
</tr>
<tr>
<td>Profinia Desalting Buffer (PBS)*</td>
<td>5x</td>
<td>685 mM NaCl, 13.5 mM KCl, 21.5 mM NaHPO₄, 40.5 mM Na₂HPO₄, pH 7.0 (pH 7.4 upon dilution)</td>
<td>137 mM NaCl, 2.7 mM KCl, 4.3 mM NaHPO₄, 8.1 mM Na₂HPO₄, pH 7.4</td>
<td>200 ml</td>
<td>B4</td>
<td>620-0216</td>
</tr>
<tr>
<td>Cleaning Solution 1*</td>
<td>2x</td>
<td>1000 mM NaCl, 100 mM Tris, pH 8.0</td>
<td>100 mM NaCl, 50 mM Tris, pH 8.0</td>
<td>126 ml</td>
<td>B5</td>
<td>620-0217</td>
</tr>
<tr>
<td>Cleaning Solution 2*</td>
<td>4x</td>
<td>2000 mM NaCl, 400 mM NaOAc, pH 4.5</td>
<td>500 mM NaCl, 100 mM NaOAc, pH 4.5</td>
<td>126 ml</td>
<td>B6</td>
<td>620-0218</td>
</tr>
<tr>
<td>Storage Solution*(Desalting cartridge)</td>
<td>2x</td>
<td>4% C₂H₅OH.70 (benzyl alcohol)</td>
<td>2% C₂H₅OH.70 (benzyl alcohol)</td>
<td>200 ml</td>
<td>B7</td>
<td>620-0219</td>
</tr>
<tr>
<td>20% Ethanol (system and Protein A cartridge)</td>
<td>1x</td>
<td>20% Ethanol (v/v)</td>
<td>20% Ethanol (v/v)</td>
<td>200 ml</td>
<td>B8</td>
<td>-</td>
</tr>
<tr>
<td>Optional Buffer for Manual Neutralization (used for Protein A affinity only methods)</td>
<td>1x</td>
<td>-</td>
<td>1M Tris, pH 8</td>
<td>Variable</td>
<td>N/A</td>
<td>161-0718</td>
</tr>
</tbody>
</table>

* These buffers and solutions are available as part of the Profinia desalting kit (catalog #620-0228)
† Volumes needed for the 5 ml protein A methods will be corresponding larger to accommodate the increase in cartridge capacity and size
‡ These catalog numbers are for dry ingredients that can be used to prepare working buffer solutions
△ See Section 5 for suggested recipes for these buffers

1.4 Recommended Storage Conditions

Buffers in the Profinia purification kits should be stored between 4 and 22°C. Once they are used with the instrument, all reagents should be stored at 4°C for up to 3 months. After insertion into the instrument, the solutions are no longer sterile. It is important to check solutions for particulates and clarity before reusing. If there is any indication of particulates, contamination or microbial growth, the solutions should be discarded. Affi-Prep protein A and P6 desalting Bio-Scale Mini cartridges should be stored at 4°C in the appropriate storage solutions listed in Table 2. Refer to the manufacturer’s recommendations for storage of other protein A cartridges that are compatible with the Profinia system.
Section 2
Guidelines When Using Protein A Methods

2.1 Sample Preparation Information
Affi-Prep protein A cartridges are convenient, ready-to-use devices for the isolation and purification of monoclonal and polyclonal antibodies. Protein A binds specifically and with high affinity to the Fc portion of immunoglobulins, in particular IgG. Bio-Rad protein A cartridges are designed for routine affinity purification of most IgGs from serum, ascites, and cell culture supernatant. The binding capacity of protein A depends on the species, subtype, and source of the particular IgG. In addition, the total capacity of protein A cartridges also depends on several other factors, such as flow rate during sample application, sample concentration, the temperature at which the purification is carried out, and the composition of the buffers used for the separation. Profinia protein A methods are compatible with most commercially available protein A cartridges and are optimized to provide fast, easy, and reproducible chromatographic separations including integrated desalting and cartridge regeneration.

Protein A generally binds to IgG most effectively between pH 7–9, although the optimal binding pH varies by species. Elution is generally achieved by decreasing the pH (2.5–4). When possible, the sample should be adjusted to the composition of the binding/equilibration buffer (see Table 2 for recommendations). If a concentrated source of IgG is used, such as serum, then this is best accomplished by diluting the sample with binding buffer to a minimum of a 1:5 ratio (or greater). Increased recoveries of concentrated samples can be achieved by using sample load volumes of 5 ml or greater. In cases where the concentration of the sample is low and the sample volume is large (for example, > 25 ml for a 1 ml cartridge, such as with cell culture supernatants) it may not be practical to dilute the sample further with binding buffer. When working with dilute samples, the pH of the sample should be checked and adjusted to an optimum binding pH (~7–9) with an appropriate buffer, such as 1 M Tris pH 8 or similar, in order to maximize binding to the protein A cartridge. Immediately prior to loading on the instrument, samples should be filtered through a 0.2 or 0.45 µm filter to prevent clogging of the system filter and cartridge. This is especially important when loading large volumes of serum or plasma.

2.2 Protein A Cartridge Selection
Profinia protein A methods are designed to use 1 ml or 5 ml protein A cartridges. Since the total IgG content of serum differs between species, use a sample size such that the amount of IgG in the sample does not exceed the maximum cartridge binding capacity. The concentration of antibody in cell culture supernatant can vary considerably between hybridoma clones (5–50 µg/ml). The approximate cartridge binding capacity for a 1 ml Affi-Prep protein A cartridge is approximately 15–20 mg of human IgG. If the anticipated amount of IgG in the starting sample exceeds the capacity of a 1 ml cartridge, then it is recommended to either select the 5 ml cartridge purification option or decrease the sample volume applied to a 1 ml cartridge. To minimize the possibility of cross contamination, it is suggested that individual protein A and desalting cartridges are dedicated to the purification of a unique sample or IgG species, as in the case of hybridoma cell culture supernatants. Profinia protein A methods include regeneration steps for the protein A cartridge to strip any remaining bound immunoglobulins following elution. For methods that include integrated desalting, using the recommended buffers in Table 2 will ensure that the Bio-Rad P6 Desalting cartridges are cleaned, stored, and ready for re-use at the completion of the method.
For additional cleaning-in-place options for protein A cartridges, refer to the Utilities section of the manual for information on the affinity cartridge cleaning-in-place method and the information provided from the manufacturer of the protein A cartridge used.

2.3 Protein A Method Parameters and Instruction for Use

The default protein A method in the Profinia protein A purification system requires the use of buffers as concentrates which are then proportioned to a 1x formulation by the instrument using the preprogrammed methods. The formulations provided in Table 2 list the most commonly recommended buffers for protein A purifications, as well as cleaning and storage solutions designed for optimal regeneration and cartridge re-use.

Optimal binding and elution conditions for IgG are both sample type and antibody dependent. In general, binding/equilibration/wash buffers for protein A purifications should be between pH 7–9. The recommended buffer for binding/equilibration/washing is the Profinia desalting buffer (pH 7.4). Two commonly recommended elution buffers for Protein A separations are solutions of sodium citrate (pH 2.7–4) or glycine (pH 2.5–3). For methods that include integrated desalting, elution with a sodium citrate buffer at pH 3 is recommended (see Section 5 of the appendix). In addition, the width of the elution peak is influenced by the concentration of the elution buffer; higher buffer concentrations produce narrower elution peaks. In cases where milder elution conditions are required, use of protein A affinity only methods is recommended to ensure that the entire IgG elution peak is collected.

When preparing for a protein A purification, bottles should be clearly labeled with the buffer name and the buffer position number (1–8) for insertion in the correct location on the instrument. The position number on the bottle must match the position number on the instrument to properly run the preprogrammed methods. Follow the on-screen guide on the instrument for the correct set-up of a protein A method and refer to the Quick Start Guide in Section 4 of the instrument manual for detailed instructions. The sample load should be in the range of 2–50 ml; however, a minimum load volume of 5–10 ml is recommended to avoid sample loss due to small volumes. The recommended $A_{280}$ for 1 mg/ml solution of IgG is 1.4. A typical run profile for 1 ml of human serum diluted 1:5 in 1x PBS (Profinia desalting buffer) and using the recommended buffer formulations in Table 2 is shown in Figure 1.

Note: For Protein G purifications, it may be necessary to reduce the affinity peak diversion volume from 3 ml (for a 1 ml protein A and G plus desalting method) to 2.5–2.7 ml in Program Method Mode in order to optimize recovery of the antibody.
**Section 3**

**Frequently Asked Questions and Troubleshooting**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Possible Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody detected in 1A (or 2A) flow-through fractions</td>
<td>Cartridge binding capacity exceeded</td>
<td>Load a smaller volume of starting material or increase cartridge size so that the binding capacity is not exceeded</td>
</tr>
<tr>
<td></td>
<td>Sample or equilibration buffer not at neutral pH or correct ionic strength for optimum binding</td>
<td>Dilute sample with additional binding buffer or adjust pH to ensure neutral sample pH</td>
</tr>
<tr>
<td></td>
<td>Sample loading is too fast for efficient binding</td>
<td>Choose the “Low” flow rate option in the Protein A methods and, if necessary, dilute sample to reduced concentration of IgG</td>
</tr>
<tr>
<td>No antibody detected in 1D (or 2D) elution fraction</td>
<td>Sample does not contain immunoglobulin or species/subclass of IgG does not have affinity for Protein A</td>
<td>Check starting sample on SDS-PAGE to ensure that antibody is present and refer to cartridge manual to confirm specificity of species/subclasses for Protein A resin</td>
</tr>
<tr>
<td>Antibody lacks function in downstream assay or appears degraded on SDS-PAGE</td>
<td>Antibody is acid labile</td>
<td>Increase pH of elution buffer and use Protein A Custom method to collect larger volume from elution fraction</td>
</tr>
<tr>
<td></td>
<td>Antibody is temperature sensitive</td>
<td>Carry out purification at 4°C and set Profinia instrument to cold-room operation setting</td>
</tr>
<tr>
<td>Low purity of antibody on SDS-PAGE or residual contamination from starting material</td>
<td>Sample load and wash steps do not return to baseline with standard Protein A method wash option</td>
<td>Select “Extended” wash option for Protein A method during setup to increase wash time</td>
</tr>
</tbody>
</table>

**Fig. 1.** Typical chromatogram and SDS-PAGE for Bio-Rad 1 ml Protein A plus 10 ml desalting method.
Section 4
Ordering Information

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>732-0111</td>
<td>Luer to M6 Adaptor Fittings Kit</td>
</tr>
<tr>
<td>732-0112</td>
<td>Luer to 10-32 Adaptor Fitting Kit</td>
</tr>
<tr>
<td>732-0113</td>
<td>Luer to BioLogic System Fittings Kit</td>
</tr>
<tr>
<td>750-0603</td>
<td>PTFE FEP Tubing, 1/8&quot;, for pre-pump connections, 15’</td>
</tr>
<tr>
<td>731-8211</td>
<td>Silicone Tubing, 1.6 mm ID, 0.8 mm wall</td>
</tr>
<tr>
<td>620-0401</td>
<td>Profinia Cooling Accessory</td>
</tr>
<tr>
<td>620-0404</td>
<td>Profinia Inline Filter Assembly Replacement</td>
</tr>
<tr>
<td>620-0228</td>
<td>Profinia Desalting Kit</td>
</tr>
<tr>
<td>620-0224</td>
<td>Profinia Desalting Buffer Kit, 10 applications</td>
</tr>
<tr>
<td>732-4600</td>
<td>Bio-Scale Mini Affi-Prep Protein A Cartridges, 5 x 1 ml</td>
</tr>
<tr>
<td>732-4602</td>
<td>Bio-Scale Mini Affi-Prep Protein A Cartridges, 1 x 5 ml</td>
</tr>
<tr>
<td>732-5302</td>
<td>Bio-Scale Mini Profinity Desalting Cartridges, 1 x 10 ml</td>
</tr>
<tr>
<td>732-5312</td>
<td>Bio-Scale Mini Profinity Desalting Cartridges, 1 x 50 ml</td>
</tr>
<tr>
<td>620-0216</td>
<td>5x Profinia Desalting Buffer, 200 ml</td>
</tr>
<tr>
<td>620-0217</td>
<td>2x Profinia Cleaning Solution 1, 125 ml</td>
</tr>
<tr>
<td>620-0218</td>
<td>4x Profinia Cleaning Solution 2, 125 ml</td>
</tr>
<tr>
<td>620-0219</td>
<td>2x Profinia Storage Solution, 200 ml</td>
</tr>
</tbody>
</table>

Section 5
Protocol for Protein A Elution/Regeneration Buffer Preparation

500 mM Sodium Citrate, pH 3:
For 250 ml, add the following to 200 ml of high quality water:
19.75 g Anhydrous citric acid
6.53 g Sodium citrate dihydrate
Stir solution until thoroughly dissolved. Adjust buffer volume to 0.25 L, filter sterilize (0.2 micron filter), and transfer to sterilized bottles compatible with the Profinia buffer ports.

1750 mM Glycine, pH 2.5:
For 250 ml, add the following to 160 ml of high quality water:
32.84 g Glycine
Stir solution until thoroughly dissolved then adjust to pH 2.5 by titration with 6 N HCl (~29.5 ml). Use appropriate precautions when working with HCl (wear gloves and goggles and work in a well ventilated area or hood). Adjust the buffer volume to 0.25 L, filter sterilize (0.2 micron filter), and transfer to sterilized bottles compatible with the Profinia buffer ports.
Appendix J
Profinia Profinity eXact Method Manual

Section 1

1.1 Background Information
The Profinia protein purification system is an automated, user-friendly instrument for the purification of affinity tagged proteins and antibodies. The system uses standard chromatography buffers, resins, and reagents along with the easy to use pre-programmed purification methods stored on the instrument. There are six main types of applications currently available in the Profinia system: native IMAC, denaturing IMAC, GST, desalting, Protein A and G, and eXact. This section provides information on the use of the eXact methods in the Bio-Rad Methods and Program Methods modes of the Profinia instrument.

1.2 Description of Method
The Profinia Profinity eXact method is designed for the affinity purification of tag-free target protein from lysates prepared using the Profinity eXact E. coli-based expression system and purification cartridges. The instrument has two main types of eXact methods that can be used for the purification of Profinity eXact affinity-tagged proteins: eXact affinity and eXact plus integrated desalting. Both method types are designed for either 1 ml or 5 ml pre-packed eXact cartridges and 10 ml or 50 ml P6 desalting cartridges (respectively) when using the eXact plus desalting methods. Cartridges for eXact applications are available from Bio-Rad as 1 ml and 5 ml eXact Bio-scale mini cartridges that are designed for direct connection to the Profinia instrument. Each ml of Bio-Rad eXact resin typically yields 2–3 mg of purified, tag-free target protein, depending on the concentration of the target protein in the starting lysate.

The Profinia eXact methods allow selection of one or two samples per run, a choice of single wash or two wash steps, and three preset incubation times for tag cleavage (0.5 hrs, 2 hrs, and 15 hrs). In the Programed Methods mode, the incubation step can be set to any interval up to 999 hrs. The eXact method also allows manual completion of the incubation step, at the users discretion, advancing the program to the protein elution step. Following system priming and cartridge equilibration, the eXact method is programmed to load sample from the S1 and/or S2 ports onto the cartridge. The available pre-programmed Bio-Rad eXact methods are designed for loading volumes between 2–50 ml per sample; however, the recommended minimum load volume is 10 ml. For larger sample volumes, the Program eXact methods can be customized to load in (ml) or (L) volumes ranging between 0–999. Refer to Section 11 for system requirements when using large sample or buffer volumes on the Profinia instrument. Table 1 summarizes the method steps for a typical Bio-Rad Profinia 1 ml eXact + 10 ml Desalting Method with 1 sample. The steps outlined in Table 1 are similar for the 5 ml eXact methods; however, flow-rates are increased to accommodate the larger volumes of the 5 ml eXact and 50 ml desalting cartridges. For eXact affinity only methods (without integrated desalting), the steps associated with the desalting cartridge are absent, and elution of the eXact cartridge is collected directly into 1D (or 2D) fractions without desalting.
### 1.3 Required Equipment and Materials

Bio-Rad Profinia eXact 1 ml and 5 ml Bio-Scale Mini cartridges are designed to be used with the pre-programmed eXact methods on the instrument. Profinia eXact methods that include integrated desalting require the use of Bio-Gel P6 Desalting cartridges, available in 10 ml and 50 ml sizes, to match the corresponding 1 ml and 5 ml affinity cartridge sizes required in the pre-programmed eXact methods. Complete Profinia buffer and purification kits specific for eXact methods are not available; however, Table 2 lists the most common recommended buffers and reagents used for eXact purifications. All buffers and solutions can be made or purchased as concentrates as described in Table 2. Triggering anions should be avoided in buffers used to prepare crude lysates and for the binding and wash steps buffers. A list of triggering anions and buffer and chemical compatibilities are listed in Tables 3 and 4.

Prepared buffers should use high quality, chromatography grade reagents and should be pre-filtered through a 0.2 or 0.45 µm filter to remove particulates prior to use with the system. Although the buffer concentrations are preset to 1x in the Bio-Rad eXact methods, buffer concentrations of 1x, 2x, 3x, 4x, and 5x can be selected and saved as an alternate eXact method. Although the buffer concentrations are preset to 1x in the Bio-Rad eXact methods, buffer concentrations of 1x, 2x, 3x, 4x, and 5x can be selected and saved as an alternate eXact method.

**Note:** Due to the larger volumes required (>250 ml) for some configurations of the 5 ml eXact methods, external containers may be required for some buffers when the eXact methods with the 2 sample options are selected. In such cases, external buffer containers may be used to accommodate the larger buffer volumes required, or the eXact method may be customized in the Program Method mode to select buffer concentrates (automatic dilution) for any buffer position required for the method.

Accessories to the Profinia instrument that can be used for eXact methods include Profinia cooling blocks and external sample/buffer tubing. Complete catalog and ordering information can be found in Section 4 of this appendix.
Table 2. Recommended Formulations for Buffers and Solutions for 1 ml eXact + 10 ml Desalting Method

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Conc [x]</th>
<th>Formulation</th>
<th>Volume†</th>
<th>Port #</th>
<th>Catalog #</th>
</tr>
</thead>
<tbody>
<tr>
<td>eXact Bind/Equilibration/Wash Buffer</td>
<td>1x</td>
<td>100 mM Sodium Phosphate, pH 7.2</td>
<td>65 ml</td>
<td>B1</td>
<td>-</td>
</tr>
<tr>
<td>eXact Wash Buffer 2 (Optional)</td>
<td>1x</td>
<td>100 mM Sodium Phosphate, 300 mM Sodium Acetate, pH 7.2</td>
<td>35 ml</td>
<td>B2</td>
<td>-</td>
</tr>
<tr>
<td>eXact Elution Buffer (Recommended)</td>
<td>1x</td>
<td>100 mM Sodium Phosphate, 100 mM Sodium Fluoride, pH 7.2</td>
<td>40 ml</td>
<td>B3</td>
<td>-</td>
</tr>
<tr>
<td>Profinia Desalting Buffer (PBS)*</td>
<td>1x</td>
<td>137 mM NaCl, 2.7 mM KCl, 8.3 mM Na2HPO4, 8.1 mM KH2PO4, pH 4.4</td>
<td>75 ml</td>
<td>B4</td>
<td>620-0216</td>
</tr>
<tr>
<td>eXact Elution (Optional)</td>
<td>1x</td>
<td>100 mM Sodium Phosphate, 20 mM Sodium Acetate, pH 7.2</td>
<td>40 ml</td>
<td>B5</td>
<td>620-0217</td>
</tr>
<tr>
<td>eXact Regeneration Solution</td>
<td>1x</td>
<td>100 mM Phosphoric Acid</td>
<td>55 ml</td>
<td>B6</td>
<td>-</td>
</tr>
<tr>
<td>eXact Storage Solution</td>
<td>1x</td>
<td>100 mM Sodium Phosphate</td>
<td>60 ml</td>
<td>B7</td>
<td>-</td>
</tr>
<tr>
<td>20% EtOH (system storage solution)</td>
<td>1x</td>
<td>20% EtOH (v/v)</td>
<td>35 ml</td>
<td>B8</td>
<td>-</td>
</tr>
</tbody>
</table>

*These buffers and solutions are available as part of the Profinia Desalting Kit (catalog # 620-0228) or individually as concentrates. The buffers will need to be manually diluted to 1x for use with the default eXact method, or a custom method can be created in the Program Methods mode to accommodate the concentrates, if desired.

†Volumes used for the 5 ml eXact methods will be correspondingly larger to accommodate the increase in cartridge capacity and size.

Table 3. Triggering Anions for the Profinity eXact Tag System

<table>
<thead>
<tr>
<th>Anion</th>
<th>Compound</th>
<th>Fast Cleavage</th>
<th>Moderate Cleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>F⁻</td>
<td>NaF, KF</td>
<td>100 mM</td>
<td>5 mM</td>
</tr>
<tr>
<td>N₃⁻</td>
<td>NaN₃</td>
<td>10 mM</td>
<td>1 mM</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>NaNO₂</td>
<td>5 mM</td>
<td>1 mM</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>NaHCO₃</td>
<td>1000 mM</td>
<td>25 mM</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>NaCl, KCl</td>
<td>&gt;1000 mM</td>
<td>75 mM</td>
</tr>
</tbody>
</table>
Table 4. Buffer and Chemical Compatibilities for the Profinity eXact Tag System

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Description</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salts</td>
<td>Sodium Acetate</td>
<td>≤ 3M</td>
</tr>
<tr>
<td></td>
<td>NaCl or KCl</td>
<td>Avoid use in lysis and wash buffers for highest yields; use sodium acetate instead</td>
</tr>
<tr>
<td>Buffers</td>
<td>Tris-HCl</td>
<td>Substitute Tris-acetate or Tris-phosphate</td>
</tr>
<tr>
<td>Acids</td>
<td>HCl</td>
<td>Substitute acetic or phosphoric acid</td>
</tr>
<tr>
<td>Detergents</td>
<td>Non-ionic</td>
<td>≤ 5% (w/v)</td>
</tr>
<tr>
<td></td>
<td>Zwitterionic</td>
<td>≤ 5% (w/v)</td>
</tr>
<tr>
<td></td>
<td>Ionic</td>
<td>Do not use</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>PMSF, Calbiochem cocktail, Roche Protease inhibitor, Complete mini tablet</td>
<td>1x</td>
</tr>
<tr>
<td></td>
<td>BD Binscience cocktail</td>
<td>2x</td>
</tr>
<tr>
<td>Lysis Solutions</td>
<td>Lysis &amp; Extraction Reagent (Bio-Rad), B-PER phosphate (Pierce), BugBuster (EMD), FastBreak Cell Lysis (Promega)</td>
<td>1x</td>
</tr>
<tr>
<td></td>
<td>ReadyPrep Lysis (Epitector), CellLyte Express (Sigma)</td>
<td>Do not use</td>
</tr>
<tr>
<td>Denaturants</td>
<td>Guanidine-HCl</td>
<td>Do not use</td>
</tr>
<tr>
<td></td>
<td>Urea</td>
<td>2M binding capacity unchanged; 4M possible capacity loss; 9M reduced capacity and purity</td>
</tr>
<tr>
<td>Other additives</td>
<td>CaCl₂</td>
<td>≤ 5 mM when used with MES, MOPS, or PIPES buffers; do not use with phosphate buffers</td>
</tr>
<tr>
<td></td>
<td>MgCl₂</td>
<td>≤ 5 mM; Do not use with fluoride containing buffers; use Na₂SO₄ as an alternative triggering ion</td>
</tr>
</tbody>
</table>

1.4 Recommended Storage Conditions

Purchased buffers obtained from Profinia purification kits should be stored between 4–22°C. Once used with the instrument, all reagents should be stored at 4°C for up to 3 months. After insertion into the instrument, the solutions are no longer sterile. Prior to use, it is important to check solutions for particulates and clarity before reuse. If there is any indication of particulates, contamination, or microbial growth, the solutions should be discarded. Bio-Rad Profinity eXact and P6 Desalting Bio-Scale Mini cartridges should be stored at 4°C in the appropriate storage solutions listed in Table 2.

Section 2

eXact Method Guidelines

2.1 Sample Preparation Information

Bio-Rad Profinity eXact cartridges are convenient, ready-to-use devices for the capture, cleavage, and purification of proteins expressed from the eXact system expression vector. The Profinity eXact resin utilizes an immobilized, engineered protease that both recognizes
and binds to the N-terminal co-expressed affinity tag in the fusion protein. Subsequent to the column binding and washing steps, the eXact protease performs a specific, controlled cleavage and removal of the tag from the fusion directly on the cartridge, resulting in the release of highly purified recombinant protein with a native N-terminus. This cleavage reaction is triggered with a sodium fluoride-containing buffer and can take between 30 min to 15 hrs (overnight). During elution, the cleaved Profinity eXact tag remains tightly associated with the resin’s immobilized protease, eliminating the need for additional steps to remove the protease and the tag. The yield of Profinity eXact cartridges is approximately 3 mg/ml of purified maltose binding protein (from Profinity eXact Control Protein Lysate). The total capacity of Profinity eXact cartridges will also depend on several other factors, such as flow rate during sample application, sample concentration, the temperature at which the purification is carried out, and the composition of the buffers used for the separation. The Profinia eXact methods are designed for the recommended buffer formulations provided in Table 2 (see the eXact System Manual for additional information) and are optimized to provide fast, easy, and reproducible chromatographic separations including integrated desalting and cartridge regeneration.

The protease used in the Profinity eXact system generally binds most effectively at neutral pH using a sodium phosphate or tris-based buffer that does not contain Cl- ions or other triggering anions (see Table 3). Elution is generally achieved by triggering anions such as fluoride or azide. When possible, the sample should be prepared in the composition of the binding/equilibration buffer. Refer to Section 5.3 of the Profinity eXact System Manual for detailed sample preparation guidelines.

Note: increased recoveries of target protein can be achieved during purification by using sample load volumes of 10 ml or greater. This is best accomplished by diluting the sample to a minimum of 10 ml into binding buffer. In cases where the concentration of the sample is low and the sample volume is large (i.e. > 25 ml for a 1 ml cartridge) it may not be practical to dilute the sample further with binding buffer. When working with dilute samples, the flow-rate of the sample loading step may be adjusted in the Profinia Programmed Method mode in order to minimize the cleavage and loss of the sample that may occur under prolonged sample loading conditions. Immediately prior to loading on the instrument, samples should be centrifuged to remove cellular debris and filtered through a 0.2 or 0.45 µm filter to prevent clogging of the Profinia system filter and cartridge. This is especially important when loading large volumes of cell lysate. Samples should be kept on ice or cooled using the Profinia Cooling Accessory until ready to load on the system.

2.2 eXact Method Cartridge Selection

The Profinia eXact methods are designed to use 1 ml or 5 ml Profinity eXact cartridges. Since the target protein content of cell lysates varies between preparations and different proteins, use a sample size such that the amount of target protein in the sample does not grossly exceed the maximum cartridge binding capacity. The cartridge binding capacity for a 1 ml Bio-Rad eXact cartridge is approximately 3–4 mg target protein. If the anticipated amount of target protein in the starting sample exceeds the capacity of a 1 ml cartridge, then it is recommended to either select the 5 ml cartridge purification option or to decrease the sample volume applied to a 1 ml cartridge. To minimize the possibility of cross contamination, it is recommended that individual Profinity eXact and desalting cartridges are dedicated to the purification of a unique samples or target protein. The Profinia eXact methods include regeneration steps for the Profinity eXact cartridge to strip the bound affinity tag following elution. In the methods that include integrated desalting, use of the recommended buffers in Table 2 will ensure that the Bio-Rad P6 Desalting cartridges are cleaned, stored, and ready for re-use at the completion of the method.
For additional cleaning-in-place options for eXact affinity and desalting cartridges, refer to the Utilities section of the manual for information on the Affinity and Desalting Cartridge Cleaning-In-Place method.

2.3 eXact Method Parameters and Instruction for Use

The default Bio-Rad eXact method requires the use of buffers at 1x concentrations which are used by the preprogrammed methods stored on the instrument. The formulations provided in Table 2 list the recommended buffers for eXact purifications, as well as cleaning and storage solutions designed for optimal regeneration and cartridge re-use. The concentration of any buffer position used in the Profinia eXact method can be customized in the Program Methods mode to 1x, 2x, 3x, 4x, and 5x. This option is particularly useful when selecting 5 ml eXact purification methods with the 2 sample option, as the volume of buffer required for some steps may exceed the capacity of standard bottles provided with the instrument (~250 ml). In such cases, a custom eXact method may be programmed with buffer concentration values between 2x–5x (the default is 1x) in order to minimized the volume of buffer required by the method.

Optimal binding and elution settings for the eXact system are preset in the eXact method. There are single wash and two wash method options. The two wash method option allows the use of a second, optional buffer for increased wash stringency prior to elution. The default Bio-Rad eXact methods allow three choices of incubation times, 0.5 hrs, 2 hrs, and 15 hrs. The optimal incubation time should be empirically determined and will be target protein dependant; however, the cleavage reaction for most eXact fusion proteins occurs within 30 min at room temperature. The incubation time can also be customized in the Program Methods mode for durations outside the three standard options in the default method.

Note: For cleavage reactions performed overnight, it is generally recommended to perform the incubation at 4°C to minimize target protein degradation or denaturation. If the Profinia instrument is stored and operated in a cold room, the cold room setting should be enabled in the Utilities menu.

In general, binding/equilibration/wash buffers for eXact purifications should follow the recommendations found in Table 2 and the eXact System Manual. The recommended buffer for desalting is the Bio-Rad Profinia desalting buffer (pH 7.4). When preparing for an eXact purification, bottles should be clearly labeled with the buffer name and the buffer position number (1–8) for insertion in the correct location on the instrument. The position number on the bottle must match the position number on the instrument to properly run the preprogrammed methods. Follow the on-screen guide on the instrument for the correct set-up of an eXact method and refer to the Quick Start Guide in Section 4 of the instrument manual for detailed instructions. The sample load should be in the range of 2-50 ml; however, a minimum load volume of 10 ml is recommended to avoid sample loss due to line priming and tubing dead volumes. The recommended A280 for 1 mg/ml solution will be target protein dependant.

A typical run profile for 10 ml of Profinity eXact-tagged Maltose Binding Protein lyophilized lysate (diluted 1:5 in 1x eXact Binding/Wash buffer) and using the recommended buffer formulations in Table 2 is shown in Figure 1.
Profinity eXact affinity-tagged maltose binding protein (MBP) was purified from bacterial lysate by Profinia chromatography using the preset affinity plus desalting method and recommended buffers. A Chromatogram of a Profinity eXact purification using a 1 ml Bio-Scale Mini™ Profinity eXact cartridge and a 10 ml Bio-Scale™ Mini Bio-Gel® P6 desalting cartridge. B Samples were run on a 4–20% Criterion™ Tris-HCl gel. Lanes 1, Precision Plus Protein™ standards (PPstd); lane 2, column load (Load); lane 3, column flow-through fraction (1A); lane 4, column wash (1B); and lane 5, eluted and desalted tag-free MBP (1D).

### Section 3

#### Frequently Asked Questions and Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Possible Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein detected in 1A (or 2A) flow-through fractions</td>
<td>Cartridge binding capacity exceeded</td>
<td>Load a smaller volume of starting material or increase cartridge size so as to not exceed binding capacity</td>
</tr>
<tr>
<td></td>
<td>Sample or equilibration buffer not at optimum pH or contains triggering anions such as Cl⁻</td>
<td>Dilute sample with additional binding buffer or adjust pH to &lt;7 to ensure optimum sample pH; remove triggering anions from buffers used to prepare cell lysate and wash buffer</td>
</tr>
<tr>
<td></td>
<td>Protein is sensitive to temperature</td>
<td>Maintain lysates at 4°C prior to loading and run purification in cold room or pre-chill buffers</td>
</tr>
<tr>
<td>No protein detected in 1D (or 2D) elution fraction</td>
<td>Sample does not contain target protein or target protein does not have affinity tag needed for eXact system</td>
<td>Check starting sample on SDS-PAGE to ensure that target protein is present and target protein (with affinity tag) is intact</td>
</tr>
<tr>
<td>Protein lacks function in downstream assay or appears degraded on SDS-PAGE</td>
<td>Protein is denatured or insoluble or is unstable in elution buffer for long periods of time</td>
<td>Modify buffer conditions and use integrated desalting method to ensure quick buffer exchange into a stabilizing buffer,</td>
</tr>
<tr>
<td></td>
<td>Protein is sensitive to temperature</td>
<td>Carry out purification at 4°C and set Profinia instrument to cold-room operation setting</td>
</tr>
</tbody>
</table>
Low purity of target protein on SDS-PAGE or residual contamination from starting material. Sample load and wash steps do not return to baseline with standard eXact method wash option. Select two wash option for eXact method during setup to add secondary wash step and/or decrease load volume to prevent over saturation of resin; increase ionic strength of buffers (see eXact System Manual for details).

Section 4
Ordering Information

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>156-3004</td>
<td>Profinity eXact Antibody Reagent</td>
</tr>
<tr>
<td>750-0603</td>
<td>1/8” OD PTFE Tubing</td>
</tr>
<tr>
<td>731-8211</td>
<td>1.6 mm ID, 0.8 mm Wall Silicone Tubing</td>
</tr>
<tr>
<td>620-0401</td>
<td>Profinia Cooling Accessory</td>
</tr>
<tr>
<td>620-0404</td>
<td>Profinia Inline Filter Assembly Replacement</td>
</tr>
<tr>
<td>620-0228</td>
<td>Profinia Desalting Kit</td>
</tr>
<tr>
<td>620-0224</td>
<td>Profinia Desalting Buffer Kit, 10 applications</td>
</tr>
<tr>
<td>732-4646</td>
<td>BioScale Mini eXact Cartridges, 2 X 1 ml</td>
</tr>
<tr>
<td>732-4648</td>
<td>BioScale Mini eXact Cartridges, 1 X 5 ml</td>
</tr>
<tr>
<td>732-5312</td>
<td>BioScale Mini Profinity Desalting Cartridges, 1 X 50 ml</td>
</tr>
<tr>
<td>620-0216</td>
<td>5x Profinia Desalting Buffer, 200 ml</td>
</tr>
<tr>
<td>620-0217</td>
<td>2x Profinia Cleaning Solution, 1, 125 ml</td>
</tr>
</tbody>
</table>
Appendix K
Profinia Program Methods Planning Worksheets

The worksheets included in this section are designed to help plan desired changes to step parameters when customizing a program method. The worksheets provide an overview of all main method steps and parameters associated with the steps; parameters that are not editable are indicated by an “X” in the corresponding position. Worksheets are included for the following method types:

- Native IMAC only
- Native IMAC plus desalting
- Denaturing IMAC
- GST only
- GST plus desalting
- Protein A and G only
- Protein A and G plus desalting
- Profinity eXact
- Profinity eXact plus desalting
- Affinity only
- Affinity plus desalting
- Desalting only
# Native IMAC Only Program Method Planning Worksheet

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

**Method Type:** Native IMAC Only

<table>
<thead>
<tr>
<th>Method Name:</th>
<th>Sample 1 Settings</th>
<th>Sample 1 Settings</th>
<th>Sample 2 Settings</th>
<th>Sample 2 Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method Step</strong></td>
<td><strong>Step Description</strong></td>
<td><strong>Position / Reagent</strong></td>
<td><strong>Buffer Conc.</strong></td>
<td><strong>Frac.</strong></td>
</tr>
<tr>
<td><strong>PRIMING STEPS</strong></td>
<td>Set equipment</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td><strong>EQUILIBRATING C1/C2</strong></td>
<td>Equilibrate the IMAC cartridge C1</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>Equilibrate C1/C2</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
<tr>
<td><strong>LOADING SAMPLE</strong></td>
<td>Load sample to C1</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>Load sample to C2</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
<tr>
<td><strong>WASHING</strong></td>
<td>Wash 1 C1/C2</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>Wash 2 C1/C2</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
<tr>
<td><strong>ELUTING</strong></td>
<td>Elute affinity 1-C1/C2</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>Elute affinity 2-C1/C2</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
<tr>
<td><strong>CARTRIDGE CLEANING</strong></td>
<td>Cartridge 1-C1/C2</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
<tr>
<td></td>
<td>Cartridge 2-C1/C2</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
<tr>
<td><strong>STORING</strong></td>
<td>Store cartridge</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
<tr>
<td><strong>END OF RUN CLEANING STEPS</strong></td>
<td>Clean equipment</td>
<td>x</td>
<td>1.5x</td>
<td>x</td>
</tr>
</tbody>
</table>

*Sample Notes:*
Native IMAC Plus Desalting Program Method Planning Worksheet

Use this worksheet to plan a native IMAC plus desalting method. General method step and reagent descriptions are in the right portion of the worksheet; specific programming parameters in the left. An "X" in any parameter indicates it is not an editable setting for the corresponding method step.

<table>
<thead>
<tr>
<th>Method Type: Native IMAC + Desalting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affinity Cartridge Size:</td>
</tr>
<tr>
<td>Desalting Cartridge Size:</td>
</tr>
<tr>
<td># of Samples:</td>
</tr>
<tr>
<td>Sample 1 Name &amp; Volume:</td>
</tr>
<tr>
<td>Sample 2 Name &amp; Volume:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method Name:</th>
<th>Sample 1 &amp; 2 Settings</th>
<th>Sample 1 Settings</th>
<th>Sample 2 Settings</th>
<th>Peak Detection Parameters</th>
<th>Peak Detection Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method Step</td>
<td>Buffer Conc., 1–5x</td>
<td>Frac. # (S1/S2)</td>
<td>Flow Rate, m/min</td>
<td>CV</td>
<td>Max Peak Detect Vol.</td>
</tr>
<tr>
<td>PRIMING STEPS</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>EQUILIBRATING C2 (equilibrating the desalting cartridge)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Equilibrate D1-D2</td>
<td>Wash cartridge with water</td>
<td>D2: distilled water</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Equilibrate C2</td>
<td>Equilibrate with desalting buffer</td>
<td>B4: desalting buffer</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>EQUILIBRATING C1 (equilibrating the IMAC cartridge)</td>
<td></td>
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<tr>
<td>Equilibrate D1-C1</td>
<td>Wash cartridge with water</td>
<td>D1: distilled water</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Equilibrate C1</td>
<td>Equilibrate with equilibration wash 1 buffer</td>
<td>E1: equilibration wash 1 buffer</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>LOADING SAMPLE</td>
<td>Load sample to C1</td>
<td>Sample loaded to IMAC cartridge</td>
<td>X</td>
<td>1A/2A</td>
<td>X</td>
</tr>
<tr>
<td>WASHING</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Wash 1 C1</td>
<td>Wash IMAC cartridge with equilibration wash 1 buffer</td>
<td>E1: equilibration wash 1 buffer</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Wash 2 C1</td>
<td>Wash IMAC cartridge with wash 2 buffer</td>
<td>E2: wash 2 buffer</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>ELUTING</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elute affinity 1-C1</td>
<td>Start station until purified protein peak is detected</td>
<td>B4: station buffer</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Elute affinity 2-C1</td>
<td>Stop after purified protein peak in desalting cartridge</td>
<td>B5: station buffer</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Elute desalting 1-C2</td>
<td>Start station until desalting protein is detected</td>
<td>B4: desalting buffer</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Elute desalting 2-C2</td>
<td>Collect desalted protein peak in fraction tube</td>
<td>B4: desalting buffer</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Elute desalting 3-C2</td>
<td>Remove solub from desalting cartridge</td>
<td>B4: desalting buffer</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CARTRIDGE CLEANING</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clean 1-C2</td>
<td>Clean desalting cartridge</td>
<td>B4: cleaning solution 1</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Clean 2-C2</td>
<td>Clean IMAC cartridge</td>
<td>B5: cleaning solution 2</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Clean 2-C1</td>
<td>Clean IMAC cartridge</td>
<td>B5: cleaning solution 2</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Clean 3-C2</td>
<td>Clean desalting cartridge</td>
<td>B4: cleaning solution 1</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Clean 3-C1</td>
<td>Clean IMAC cartridge</td>
<td>B5: cleaning solution 2</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>STORING</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Store C</td>
<td>Storage buffer IMAC cartridge</td>
<td>B7: storage solution</td>
<td>W/W</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

204
### Native Denaturing IMAC Program Method Planning Worksheet

Use this worksheet to plan a native denaturing IMAC method. General method steps and reagent descriptions are in the right portion of the worksheet; specific programming parameters in the left. An "X" in any parameter indicates it is not an editable setting for the corresponding method step.

**Method Type:** Denaturing IMAC

<table>
<thead>
<tr>
<th># of Cartridges:</th>
<th>Affinity Cartridge Size:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th># of Samples:</th>
<th>Sample 1 Name &amp; Volume</th>
<th>Sample 2 Name &amp; Volume:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Method Name:

<table>
<thead>
<tr>
<th>Method Step</th>
<th>Step Description</th>
<th>Position / Reagent</th>
<th>Sample 1 &amp; 2 Settings</th>
<th>Sample 1 Settings</th>
<th>Sample 2 Settings</th>
<th>Sample 1 Settings</th>
<th>Sample 2 Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Buffer Conc., 1-5x (SI/S2)</td>
<td>Frac.</td>
<td>Flow Rate, ml/min</td>
<td>CV</td>
<td>Peak Detec Delay</td>
</tr>
<tr>
<td>PRIMING STEPS</td>
<td>Not edible</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>EQUILIBRATING C1/C2 (equilibrating the IMAC cartridge)</td>
<td>Wash cartridge with water</td>
<td>B1/denitrozed water</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Equilibrate C1/C2</td>
<td>Equilibrate with equilibration wash-1 buffer</td>
<td>B1/eqilibration wash-1 buffer</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>LOADING SAMPLE</td>
<td>Load sample to C1</td>
<td>Sample load to IMAC cartridge C1</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Load sample to C2</td>
<td>Sample load to IMAC cartridge C2</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>WASHING</td>
<td>Wash-1 C1/C2</td>
<td>Wash IMAC cartridge with equilibration wash-1 buffer</td>
<td>B1/eqilibration wash-1 buffer</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Wash-2 C1/C2</td>
<td>Wash IMAC cartridge with wash-2 buffer</td>
<td>B2/wash-2 buffer</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>ELUTING</td>
<td>Elute 1-1/C1/C2</td>
<td>Start elution until purified protein peak is detected</td>
<td>B3/elution buffer</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Elute 2-1/C1/C2</td>
<td>Collect purified protein peak in fraction tube</td>
<td>B3/elution buffer</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>CARTRIDGE CLEANING</td>
<td>Clean 1-1/C1/C2</td>
<td>Clean IMAC cartridge</td>
<td>B5/cleaning solution 1</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Clean 2-1/C1/C2</td>
<td>Clean IMAC cartridge</td>
<td>B6/cleaning solution 2</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Clean 3-1/C1/C2</td>
<td>Clean IMAC cartridge</td>
<td>B7/steinized water</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>STORING</td>
<td>Store C1/C2</td>
<td>Storeage buffer IMAC cartridge</td>
<td>B7/storeage solution</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>END OF RUN CLEANING STEPS</td>
<td>Not edible</td>
<td></td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

Sample Notes

205
### GST Program Method Planning Worksheet

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

**Method Type:** GST

<table>
<thead>
<tr>
<th>Method Step</th>
<th>Step Description</th>
<th>Position / Reagent</th>
<th>Buffer Conc., 1–5x</th>
<th>Frac. # (S1/S2)</th>
<th>Flow Rate, ml/min</th>
<th>Peak Detect Delay</th>
<th>Max Detect Vol.</th>
<th>Sample 1 Settings</th>
<th>Sample 2 Settings</th>
<th>Sample Notes</th>
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</thead>
<tbody>
<tr>
<td><strong>PRIMING STEPS</strong></td>
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<tr>
<td>EQUILIBRATING C1/C2</td>
<td>(equilibrating the GST cartridge)</td>
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<td></td>
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</tr>
<tr>
<td>Equilibrate C1/C2</td>
<td>Wash cartridge with water</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>B5/denatured water</td>
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</tr>
<tr>
<td>Equilibrate C1/C2</td>
<td>Equilibrate with equilibration/wash buffer</td>
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<td>Bi-equilibration wash buffer</td>
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<tr>
<td><strong>LOADING SAMPLE</strong></td>
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<tr>
<td>Load sample to C1</td>
<td>Sample load to GST cartridge C1</td>
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<tr>
<td>Load sample to C2</td>
<td>Sample load to GST cartridge C2</td>
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<td><strong>WASHING</strong></td>
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<tr>
<td>Wash-1 C1/C2</td>
<td>Wash GST cartridge with equilibration/wash buffer</td>
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<tr>
<td><strong>ELUTING</strong></td>
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</tr>
<tr>
<td>Elute affinity 1/C1/C2</td>
<td>Start elution until purified protein peak is detected</td>
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<td></td>
<td>B5/elution buffer</td>
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</tr>
<tr>
<td>Elute affinity 2/C1/C2</td>
<td>Collect purified protein peak in fraction tube</td>
<td></td>
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<tr>
<td><strong>CARTRIDGE CLEANING</strong></td>
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<tr>
<td>Clean 1/C1/C2</td>
<td>Clean GST cartridge</td>
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<tr>
<td>Clean 2/C1/C2</td>
<td>Clean GST cartridge</td>
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<tr>
<td>Clean 3/C1/C2</td>
<td>Clean GST cartridge</td>
<td></td>
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<tr>
<td>Store C1/C2</td>
<td>Storage buffer GST cartridge</td>
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<tr>
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<td>B7/Storage solution</td>
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<td>END OF RUN</td>
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</tr>
</tbody>
</table>

Sample Notes

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206
# GST Plus Desalting Program Method Planning Worksheet

Use this worksheet to plan a GST plus desalting program method. General method step and reagent descriptions are in the right portion of the worksheet; specific programming parameters in the left. An *X* in any parameter indicates it is not an editable setting for the corresponding method.

**Method Type:** GST + Desalting

<table>
<thead>
<tr>
<th>Affinity Cartridge Size:</th>
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</thead>
<tbody>
<tr>
<td>Desalting Cartridge Size:</td>
</tr>
<tr>
<td># of Samples:</td>
</tr>
<tr>
<td>Sample 1 Name &amp; Volume:</td>
</tr>
<tr>
<td>Sample 2 Name &amp; Volume:</td>
</tr>
</tbody>
</table>

**Method Name:**

<table>
<thead>
<tr>
<th>Method Step</th>
<th>Step Description</th>
<th>Position / Reagent</th>
<th>Buffer Conc. 1-5x</th>
<th>Frac. # (S1/S2)</th>
<th>Flow Rate, ml/min</th>
<th>CV</th>
<th>Peak Detection Parameters</th>
<th>Max Peak Detect Vol.</th>
<th>Peak Detect Delay</th>
<th>Flow Rate, ml/min</th>
<th>CV</th>
<th>Peak Detection Parameters</th>
<th>Max Peak Detect Vol.</th>
<th>Peak Detect Delay</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRIMING STEPS</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equilibrating C1 (equilibrating the desalting cartridge)</td>
<td>Equilibrate C1</td>
<td>Wash cartridge with water</td>
<td>DI-deionized water</td>
<td></td>
<td></td>
<td>WW</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equilibrate C1</td>
<td>Equilibrate with desalting buffer</td>
<td>DI-deionizing buffer</td>
<td></td>
<td></td>
<td></td>
<td>WW</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>EQUILIBRATING C1 (equilibrating the GST cartridge)</td>
<td>Equilibrate C1</td>
<td>Wash cartridge with water</td>
<td>DI-deionized water</td>
<td></td>
<td></td>
<td>WW</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Equilibrate C1</td>
<td>Equilibrate with equilibration wash buffer</td>
<td>DI-deionization wash buffer</td>
<td></td>
<td></td>
<td></td>
<td>WW</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOADING SAMPLE</td>
<td>Load sample to C1</td>
<td>Sample load to GST cartridge</td>
<td></td>
<td></td>
<td></td>
<td>WW</td>
<td></td>
<td></td>
<td></td>
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Sample Notes
Protein A Program Method Planning Worksheet

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

**Method Type:** Protein A

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<th>Frac. # (S1/S2)</th>
<th>Flow Rate, ml/min</th>
<th>CV</th>
<th>Peak Detect Parameters</th>
<th>Peak Detect Delay</th>
<th>Max Peak Detect Vol.</th>
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END OF RUN CLEANING STEPS Not editable

Sample Notes
Protein A Plus Desalting Program Method Planning Worksheet

Use this worksheet to plan a GST plus desalting program method. General method step and reagent descriptions are in the right portion of the worksheet, specific programming parameters in the left. An * indicates an optional setting for the corresponding method step.

**Method Type:** Protein A + Desalting

**Affinity Cartridge Size:**

**Desalting Cartridge Size:**

**# of Samples:**

**Sample 1 Name & Volume:**

**Sample 2 Name & Volume:**

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Sample Notes
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<td>Pre-Elute C1 Fill eXact Cartridge with Elution buffer</td>
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<td>Clean-2 C1 Clean eXact Cartridge</td>
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<td>Store C2 Store Desalt Cartridge</td>
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**Method Type:**

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**Sample 1 Name & Volume:**

**Sample 2 Name & Volume:**

### SAMPLE 1 SETTINGS

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<th>Flow Rate ml/min</th>
<th>CV</th>
<th>Peak Detect Delay</th>
<th>Max Peak Detect Vol</th>
<th>Flow Rate ml/min</th>
<th>Peak Detect Delay</th>
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### SAMPLE 2 SETTINGS

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<th>Flow Rate ml/min</th>
<th>CV</th>
<th>Peak Detect Delay</th>
<th>Max Peak Detect Vol</th>
<th>Flow Rate ml/min</th>
<th>Peak Detect Delay</th>
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# Affinity-Only Prinseq Method Planning Worksheet

The following worksheet is an affinity-only program method. Detailed method steps and parameter descriptions are in the right portion of the worksheet. Specific programming parameters in the left portion ("X") may require adjustment to meet the specific requirements for the corresponding method step.

## Method Type:
- **Affinity**

### Method Specifications:
- **No. of Cartridges:**
- **Sample 1 Name & Volume:**
- **Sample 2 Name & Volume:**

## Method Name:

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<td>x</td>
<td>WW</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
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<td>Equilibration C1-C3</td>
<td>Wash column with water</td>
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<tr>
<td></td>
<td>Equilibration C1-C3</td>
<td>Wash column with wash buffer</td>
<td>x</td>
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<tr>
<td>Load sample to C1</td>
<td>Sample load to affinity cartridge (A)</td>
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<td>16/34</td>
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<td>Sample load to affinity cartridge (A)</td>
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<td>16/34</td>
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<td>Wash 1 C1</td>
<td>Wash affinity cartridge with wash buffer</td>
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<td>Wash 2 C1</td>
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<td>Clean 1 C1</td>
<td>Clean affinity cartridge</td>
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<td>Clean affinity cartridge</td>
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## Sample Notes:
# Affinity Plus Desalting Program Method Planning Worksheet

The Affinity Plus Desalting Program is designed to desalt proteins and remove contaminants at the appropriate stage in the method. It is important to plan the method carefully to avoid contamination and ensure efficient purification.

## Method Type:
- Affinity Plus Desalting

## Sample Name & Volume:

### Sample 1 Name & Volume:

### Sample 2 Name & Volume:

## Method Name:

## Method Name:

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<th>Step Description</th>
<th>Position</th>
<th>Buffer Conc. 1 mL (mM)</th>
<th>Flow Rate (μL/ min)</th>
<th>Peak Detection Parameters</th>
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<th>Max. Retent. Vol.</th>
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## Notes:
- Adjust flow rate and buffer concentration according to the specific requirements of your experiment.
- Ensure that all steps are properly washed to avoid contamination.
- Use the correct buffer for each stage to ensure efficient desalting.

## References:
- [Affinity Plus Desalting Protocol](#)
- [Protein Purification Methodology](#)
# Desalting-Only Program Method Planning Worksheet

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

**Method Type:** Desalting Only

**# of Cartridges:**

**Desalting Cartridge Size:**

**Sample Name & Volume:**

**Method Name:**

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<tr>
<th>Method Step</th>
<th>Step Descriptions</th>
<th>Position / Reagent</th>
<th>Buffer Conc., 1–5x</th>
<th>Frac. #</th>
<th>Flow Rate, ml/min</th>
<th>CV</th>
<th>Peak Detect Delay</th>
<th>Max Peak Detect Vol.</th>
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<td>W</td>
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<td>Collect desalted protein peak in fraction</td>
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<td>Remove salts from desalting cartridge</td>
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<td><strong>END OF RUN CLEANING STEPS</strong></td>
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Sample Notes
Trademarks

Pentium is a trademark of Intel Corporation. Windows and Windows XP are trademarks of Microsoft Corporation.