AFFINITY PURIFICATION

Profinia™ Protein Purification System

Operation Guide for Purification of Proteins

New Instrument Startup Procedure

1 Remove Plugs and Caps Before Powering On

- Remove the shipping plugs (black) from the DI water port and waste port located at the back of the instrument and replace with the connectors to the DI water bottle and waste bottle, respectively (label bottles to avoid cross-contamination)
- Remove the plastic caps from the ends of the buffer, sample, and fraction dispensing lines (the system is stored in 20% ethanol during shipping)

2 Prepare System for First Run

- Connect power cord and turn power switch on (power switch is located at the right side of the instrument)
- Remove all of the air and 20% ethanol from the system lines by running the Clean All Inlet and Outlet Lines utility from the Diag/Maint Functions menu

  On the home screen, from the **Data/Utilities** dropdown menu, select **Diag/Maint Functions** and then **Clean All Inlet and Outlet Lines**. Follow the onscreen directions.
- Repeat the cleaning procedure above until no air bubbles are observed entering the waste bottle

Instrument Settings

- **Temperature setting** (4°C or room temperature) — Be sure to set the temperature at 4°C if the system is operating in a coldroom

  On the home screen, from the **Data/Utilities** dropdown menu, select **Diag/Maint Functions**. Scroll down and highlight **Select Method Temperature**. Press **Select** and set the correct temperature. Press **OK.**

  Note: The flow rates for the desalting cartridge are reduced at the 4°C setting to prevent overpressure in the system. All other flow rates remain the same. When the 4°C setting is selected, the letter C is displayed in the upper right corner of the screen if the purification method includes the desalting step.

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BIO-RAD

2 Prepare System for First Run (continued)

- **End of sample and end of reagent detection** — The Profinia instrument has three separate air detection sensors, one air sensor for each of the two sample lines and one for system eluent air detection. As a default, they are all turned on

  Sample air detectors — These sensors detect air bubbles when the entire sample is loaded and allow automatic advance to the wash step

  Reagent air detector — This sensor will detect air bubbles that result from reagent depletion. When air is detected, the system will pause and alert the user to make sure a specific reagent bottle has no bubble or has enough buffer. With pressing of the START button, the system will reprime the lines and continue the method at the step where it paused

  To access sensor options, select **Diag/Maint Functions** from the **Data/Utilities** dropdown menu on the home screen, and highlight **Select End of Sample/Reagent Detection.** Press **Select.**

Notes and recommendations: End of Reagent/Sample detection should always be enabled. For really large volumes of sample, End of Reagent/Sample detection can be disabled to prevent partial loading of the whole sample due to occasional bubble appearance.

3 Start Your Run

Profinia Methods

- **Bio-Rad methods** — This menu contains preprogrammed methods for commonly used affinity-tagged protein purifications. Select the method type and available method-specific options you wish to run, and follow the onscreen instructions

  Programmable options for Bio-Rad methods: 1) 1–50 ml sample volumes; **2)** A_{280} of 1 mg protein/ml: enter the value if known (default is 1.0); value will be used to calculate total protein and protein concentration.

- **Program methods** — This menu contains all the preprogrammed method templates, but the templates can be customized using the programming options shown below:

  Programmable options: 1) 1–999 ml or 1–999 L sample volumes; **2)** A_{280} of 1 mg protein/ml: enter the value, if known (default is 1.0); value will be used to calculate total protein and protein concentration; **3)** flow rates and column volumes (CVs) for method steps; **4)** peak detection settings: Peak Detection Delay, Max Peak Volume, Peak Detection Sensitivity (see section 6.8.2 of the Profinia system manual for details); **5)** protein collection volumes.

Run Data Collection

- **Real-time data transfer to Profinia software** — Make sure that the USB cable is connected to the USB port at the back of Profinia instrument and to the USB port of the PC so that communication has been established PRIOR to starting the run
- **Profinia instrument data collection** — When the run is started, the run data is stored into the Run Data file memory (available for transfer to a USB memory stick immediately after the run; data for the last three runs can be accessed from the home screen (Data/Utilities menu ↳ Data menu))

4 Clean Lines and Export Data When Run Is Completed

- **End-of-run cleaning** — When the run is completed, the display will show instructions for cleaning of the sample and fraction lines. Follow the onscreen instructions to wash BOTH the sample lines and ALL the fraction lines. When the cleaning procedure is completed, the run results are displayed and can be downloaded

  Caution: If air is allowed to enter one or both of the sample lines during the end-of-run cleaning procedure, there could be premature end-of-sample triggers for your next run. Sample lines should always be in water during automated system wash.

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4 Clean Lines and Export Data When Run Is Completed (continued)

- **Exporting the Run Data file to a portable memory device** — The End of Run screen displays the Export Data button. Insert a USB memory device into the USB port directly below the screen. After the instrument recognizes the device (up to 60 seconds), the Export Data button will be active (the cross is removed). Press **Export Data** to transfer the data file to the memory device

  Note: If the system does not recognize a USB drive, try a different one.

- **Completing the purification cycle** — At the end of the cleaning cycles, the system asks for either short- or long-term storage. If the instrument will be used within a week, use short-term storage. Otherwise, choose long term. The purification cycle is not complete until the home screen appears

Profinia Instrument Operation Guide

Operation	Description
Number of samples per run	Two samples per run using the same cartridge (affinity or affinity plus desalting method) or one sample per cartridge (affinity only methods).
Sample volume	1–50 ml in preprogrammed Bio-Rad methods mode. 1–999 ml and 1–999 L in Program methods mode.
Sample loading options	**End of sample detection disabled** — The sample volume entered for the run must be 0.5 ml less than the volume in the sample tube (0.5 ml is needed for priming). **End of sample detection enabled** — The sample volume entered can be equal to or greater than the volume in the sample tube. When the system detects air in the sample line, the method stops loading sample and proceeds automatically to the wash step.
Buffer concentrations	The Profinia instrument can dilute buffers to 1x from concentrations up to 5x. **Bio-Rad methods** — Buffers at the indicated concentrations must be used for correct dilutions. Do not dilute the designated concentrated buffers, as the instrument automatically dilutes concentrated buffers to 1x working solution. **Program methods** — Buffers at concentrations from 1x to 5x (integer only) can be used. Make sure that the correct concentration is programmed for each buffer position. Once programmed, the instrument will automatically calculate and dilute concentrated buffers to 1x working solution.
Add or delete method steps	**Bio-Rad methods** — No steps can be changed; they are preprogrammed. **Program methods** — Steps can be altered, but new steps cannot be added to a method. Steps can be skipped by programming 0 CV for that step.
Program parameters	Sample wash volume, elution volume, flow rate, and diverting volume (from purification column to desalting column) can be programmed.
Peak detection parameters	There are several parameters for peak detection: **Peak detection sensitivity** — This is a global setting available in the Calibration Functions menu. From the Home screen, select **Data/Utilities,** then **Calibration Functions**. Peak detection sensitivity settings range between 0.1 (most sensitive) to 10 (least sensitive); the default setting is 1.0 (good for >0.15 mg purified protein).

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Profinia Instrument Operation Guide (continued)

Operation	Description
Peak detection parameters (continued)	The Peak Detect Delay and the Max Peak Volume settings are available in the Elute-1 step of the method for both the affinity and desalting peaks. These settings can be edited in Program method only. In the Elute-1 step, the system starts to look for the protein peak. The program advances to the next step once the protein peak is detected. **Peak Detect Delay** — This value indicates the amount of time the Elute-1 step will take before peak detection is activated. While the automatic peak detection is active, the light bulb icon displays on the screen. Peak delay represents the time required for the elution buffer to reach the cartridge inlet line and is automatically calculated based on the flow rate in Bio-Rad methods. **Max Peak Volume** — This represents the maximal reasonable volume of elution buffer that is needed to start protein elution from the cartridge. This volume is used in cases where the protein peak is not automatically detected. When this volume of elution buffer is reached, the protein should have eluted from the cartridge. Now the Elute-1 step will stop and the Elute -2 step will start. During the Elute-2 step, the system will collect the protein in the fraction tube or divert a set volume to the desalting cartridge. **Note for IMAC methods:** If no protein is detected, the rise in slope that occurs when the imidazole elutes will trigger the peak detection. The A_{280} of eluted imidazole is ~0.05. Possible reasons that a protein peak is not detected: 1. The protein has not bound to the cartridge resin and is in the flowthrough (incorrect cartridge or buffer is used or cartridge is damaged). 2. The protein is not expressed or is at very low levels (check the total, soluble, and pellet fractions of the sample on SDS-PAGE). 3. The protein does not absorb at 280 nm.
Run data files (extension .ofi)	**Profinia instrument** — The Profinia instrument stores up to three run data files in memory. Run data files that were transferred to a memory stick are indicated by an asterisk (*). If none of the three data files have been transferred, the letter M displays in the upper right corner of the screen. When a new run is started, the oldest run data file that was transferred will be overwritten. In the case that none of the run data files were transferred, the oldest run data file will be overwritten. This run data file can be transferred to the Profinia software or it can be viewed in compatible spreadsheet software, for example, Microsoft Excel. **Data transfer to Profinia software** — Data are transferred in real time via a USB cable connection between the PC and the Profinia instrument. Communication must be established prior to starting a run. Profinia software automatically creates a “Profinia Data” folder or the user can create a new folder for storing the data file.

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