AFFINITY PURIFICATION SYSTEM

Profinia™ Protein Purification System

FAQs

Instrument, Firmware, Parts, and Accessories

1 What are the advantages of the Profinia Protein Purification System?

The system is configured for automated affinity purification with optional integrated desalting. The advantages of the system include the following:

- Ready-made buffer kits
- Preprogrammed methods
- Easily modified templates
- Easy-to-install prepacked cartridges
- Automated purification and cleaning procedures
- Optional desalting cartridge in line with affinity cartridge

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2 What kind of tags can I use for affinity purification on the Profinia System?

The Profinia System can be used to purify any affinity-tagged protein, if appropriate cartridges are available. This will include, but is not limited to, proteins containing polyhistidine tags, GST, MBP, *Strep*-tag, and Profinity eXact™ Purification Tags. The system can also purify monoclonal or polyclonal antibodies with protein A or G cartridges.

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3 Can I perform ion exchange or gel filtration on the Profinia System?

The system is not designed for ion exchange or gel filtration. However, desalting can be done with or without affinity methods. In addition, simple step wash and elution ion exchange methods can also be run.

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4 Can I use the Profinia System in the cold room?

Yes, you can use the Profinia System in the cold room with the operation temperature set at 4°C. Temperature can be set under Data/Utilities then Diagnotic/Maintainance Functions.

The system can also be run at room temperature while keeping the sample and eluted fraction cold by placing them in the cooling units (purchased separately, catalog #6200401).

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5 Where can I find a schematic of the Profinia System lines and valves?

Sections 9.32, 13.2, and 13.2.6 in the Profinia System manual show a schematic diagram of the Profinia System fluidic path.

BIO-RAD

6 If the power is shut off during my purification process, do I lose all the data? Can I continue the run I had started before power-off?

There is no loss of data. The run data file is saved on the Profinia instrument and, if you are running Profinia Software, the run data file will also be saved on the PC.

If the sample was loaded on the system but was not yet loaded onto the cartridge, you will be able to direct the sample from the system to a specified fraction in the manual mode (see section 9.3.2 of the instruction manual).

If the sample was bound on the cartridge, you will be able to program a custom method that will skip the previously executed steps by setting the CV values for those steps to zero. See Program Methods mode, section 7.4.1 of the manual.

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7 What is the power consumption of the Profinia System? What is recommended for choosing a surge suppressor or uninterruptible power supply (UPS)?

During operation, the Profinia System draws a maximum of 40 volt-amps (VA) or 0.4 A at 100 V, 0.33 A at 120 V, or 0.17 A at 240 V.

Select a surge suppressor rated to deliver enough current for the system (40 VA).

Select a UPS with a rating of at least 80 VA (twice the draw of the Profinia System) that supplies power through the longest expected power outage.

More detailed information can be found in the Profinia System hardware manual, section 13.2.8.

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8 I need longer tubing for water, waste, buffer, or sample lines. How do I get it?

See section 13.2.6 of the Profinia System manual for details.

- PTFE FEP tubing (catalog #7500603)
- Fittings (catalog #7500553)

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9 How long will the lamp in the Profinia System last?

The estimated service life for the mercury/phosphor lamp in the Profinia System is 1,200 hours of lamp operation. The system turns the lamp off when it is not required (before the method starts and between runs). The manual operation screen shows the lamp reference voltage. We recommend replacing the lamp when the reference voltage is 0.2 V or less. The shelf life of a spare lamp is about 5 years.

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10 What is the pressure limit for the Profinia System?

The upper pressure limit for the Profinia System is 45 psi.

Kits/Buffers

1 What are the commercially available buffers for affinity purification on the Profinia System?

For native IMAC and GST purification, the following kits are available:

- Native IMAC or GST starter kits containing native IMAC buffers, IMAC or GST cartridges, and *E. coli* control lysate
- Native IMAC or GST purification kits containing native IMAC buffers and IMAC or GST cartridges
- Native IMAC or GST buffer kits containing native IMAC or GST buffers

For desalting purposes, the following kits are available:

- Desalting purification buffer kit containing desalting buffer and desalting cartridges
- Desalting buffer kit containing desalting buffer only

The desalting kits can also be used when desalting steps are included with Profinity eXact Fusion-Tag and custom purification methods. Refer to Bulletins 5701, 5741, 5744, and 5725 for buffer components.

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2 Should I dilute the concentrated buffers to 1x before use?

Do not dilute the buffers provided in the kits. The instrument does the dilution automatically.

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3 Can I use different concentrations of buffers than the buffers in the buffer kits?

Yes. If different concentrations are used, you will need to select the purification method from the Program Methods mode and enter the correct buffer concentrations (up to 5x). The system will dilute the concentrated buffers to 1x when needed.

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4 If I have a choice to use either a protein A or protein G cartridge to purify my antibody, can I use the same buffer for both?

No. We suggest using different binding and elution buffers. Refer to Bulletins 5701 and 5712.

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5 Can I use detergent in my purification buffers?

Yes. SDS (up to 10%) and Triton X-100 (up to 5%) are compatible with the Profinia System. Appendix A of the Profinia System manual lists system specifications and chemical compatibilities.

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6 Can I make the buffers myself?

Yes. Buffer compositions for native IMAC, denaturing IMAC, GST, and desalting are listed in Bulletin 10006044. Buffers specific to Protein A and G Methods, Profinity eXact, MBP, and *Strep*-tag are found in Bulletins 5701, 5725, 5741, and 5744. Buffers need to be filtered through a 0.45 µm or 0.2 µm filter.

Cartridges

1 How do I install my cartridges properly on the Profinia System?

To prevent leaks, it is always recommended to install the cartridge from the bottom end and then the top end according to the instructions on the screen. It is important to remember not to over tighten the cartridge.

2 What are the positions for installing the affinity and desalting cartridges?

The desalting cartridge can only be installed on the right while the affinity cartridge can be installed at both positions.

3 What cartridges are available from Bio-Rad to purify antibodies?

Bio-Rad provides Bio-Scale™ Mini Affi-Prep® Protein A and UNOsphere SUPrA™ Cartridges (1 ml and 5 ml) and Bio-Gel® P-6 Desalting Cartridges (10 ml and 50 ml) for this application. The 1 and 5 ml protein A and G cartridges from other vendors (GE Healthcare and Thermo Scientific) are compatible with the preset Profinia Methods.

4 How many times can I use the cartridges to purify my proteins?

It is recommended that you use cartridges for affinity purification no more than five times.

5 Can all the proteins be washed away after the cartridge cleaning step in the preprogrammed methods?

Cleaning buffers for different cartridges vary and therefore the effect of cleaning could be different. To get minimal contamination, we strongly suggest using a dedicated cartridge for each protein.

6 Do I have to use Bio-Rad cartridges with the Profinia System?

No. You can use cartridges from other vendors with proper adaptors. For GE Healthcare and Thermo Scientific Pierce Cartridges, you can use our Luer to 10-32 Adaptor Fittings Kit (catalog #7320112) as the bottom adaptor.

7 What is the recommended flow rate for the Bio-Scale Mini Cartridges used on the Profinia System?

The flow rate could vary depending on the properties of the protein to be purified, the cartridge, the resin, and the system. The instrument can manually pump 0.2 to 20 ml/min, but programmable steps have limits. Refer to Table 6.1, Table 9.1, and Table 9.2 in the manual, or view steps in different preprogrammed purification methods for the flow rate we used in some of our preprogrammed protocols.

Samples

1 How much sample can I load? Do I need to watch the sample loading process to make sure the sample is fully loaded?

If you use preprogrammed methods (Bio-Rad Methods), you can only load up to 50 ml of sample. Use Program Methods mode if more than 50 ml of sample is needed. You can load up to 999 liters of sample using the Program Methods mode.

You do not need to closely monitor sample loading if the End of Sample fuction is enabled (by default) since the system can detect the end of the sample and stop the loading process to prevent air bubbles from entering the system.

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2 Do I need to filter my lysate before loading my samples? How often do I need to change the inlet filters?

Yes, you should filter your lysate through 0.45 µm or 0.2 µm filters before loading to prevent clogged cartridges, elevated pressures, and reduced cartridge life.

The Profinia System has one inlet filter before the buffer and sample flow into the cartridges. The inlet filter needs to be changed every 3 to 6 months depending on how often you use the system.

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3 Why is my sample only partially loaded (sample skipping)?

Partial loading of the sample is caused by premature detection of air in sample air detectors. This can happen for a few reasons:

1. Air in sample — minimize presence of air bubbles; if needed, degas sample.
2. Air in system from previous run — make sure that the cleaning steps from the previous run completed the sample line-cleaning process. If the sample line water wash is overlooked the system will have air in the sample air detectors and cause the partial loading of sample in the subsequent run.
3. Air sensor out of calibration — recalibrate the air sensors. To calibrate go to Data/Utilities, then Calibrate Functions.

If you suspect there is air in the system, do the following:

Prior to starting a run, position water in all sample and buffer inlet lines and perform the Clean All Inlet and Outlet lines function until all air is removed from the system. This function is available under Data/Utilities then Diagnostic/Maintainance Functions.

Methods

1 When do I use Bio-Rad Methods, Program Methods, or Saved Methods?

If you want to use preprogrammed methods, choose Bio-Rad Methods. If you want to modify a purification protocol, choose Program Methods and alter the preprogrammed templates. If you have saved the program methods to your own filename, you can go directly to Saved Methods to find it.

2 Can I add or delete method steps when programming a method on the Profinia System?

Method steps that display the Edit key on the screen are editable. (See section 7. Program Methods in the system manual.) A step can be deleted by programming a CV value of zero. Steps cannot be added to the method template.

3 What type of antibodies can I purify using the Protein A and G Methods?

In general, protein A and G are for isolation of IgG antibodies. Table 1 in Bulletin 5726 lists their binding specificity for different subclasses of IgG from different species.

4 Can we use resins of protein A or G other than the ones from Bio-Rad on the Profinia System?

Yes, you can. We have tested GE Healthcare, Thermo Fisher Scientific, and Millipore resins of protein A and protein G and they all work well with our Profinia Protein A and G Methods.

5 What is the purity and yield for antibody purification on the Profinia System with Protein A and G Methods?

The yield and purity for antibody purification with Protein A and G Methods vary based on the capacity and the relative affinity of the antibody to the selected ligand. Typical results give a minimum of 70% purity. Yield varies by manufacturer, antibody species, and testing criteria.

6 Can I use other resins like protein D, L, or P on Profinia Protein A and G Methods?

Yes. You will need to use the buffers and chromatography conditions recommended by the manufacturer and then alter the Protein A and G Method in Program Mode to match accordingly.

7 When do I use the Profinity eXact purification methods on the Profinia System?

When you want to purify a protein by affinity-tag purification methods, but want a tag-free protein at the end, you can use this combination. You would normally use an affinity-tag system and then cleave the tag with a protease following purification. A repurification step is then needed to remove the tag and the protease. The Profinia System in combination with the Profinity eXact Fusion-Tag System simplifies this workflow with its on-column purification and cleavage of the tag.

8 What do I need to use the Profinity eXact purification methods on the Profinia System?

You will need Bio-Scale Mini Profinity eXact Cartridges (1 ml or 5 ml) and desalting cartridges if you want to desalt proteins on the Profinia System. For the formulations of all the buffers recommended for Profinity eXact purification, refer to Bulletin 5725.

9 What is the purity and yield for protein purification with the Profinity eXact Methods on the Profinia System?

The yield and purity for Profinity eXact purifications are dependent on the protein, fusion linker, and time. Typical purity is >90%. See the Profinity eXact System instruction manual (Bulletin 10011260) for more details on expression and purification.

10 In the denaturing IMAC method, has my protein been renatured after purification?

No. After purification the eluent still contains 6 M urea. Gradual dilution or dialysis into native buffer can aid refolding of some proteins. If renatured protein is desired after IMAC purification, Native IMAC Method can be run with integrated desalting, provided the denatured protein can be refolded on column when no urea or less concentrated urea (<6 M) is used in wash buffer 1. However, it does not work for all proteins. If it works, it will save a lot of time and effort. Refer to Bulletin 5870 for details.

11 Is it possible to do a run using both IMAC and GST cartridges together?

Running different cartridge types (IMAC and GST) together in a single automated method is theoretically possible, but not recommended. This would require complicated programming and manual pausing of the system at the correct times to change the reagents. The system will not prime the buffer lines and some of the IMAC buffers would be pumped onto the GST cartridge. For optimum performance it is best to run the IMAC and GST as separate methods.

12 In the method steps of native and denaturing IMAC and GST runs, Elute-1 and Elute-2 are listed. In desalting runs, Elute-1, Elute-2, and Elute-3 are listed. What do they mean?

Affinity Elute-1 and Elute-2

Elute-1: The elution step starts and system looks for the start of the protein peak.

Elute-2: The protein peak was detected and the protein peak is collected or diverted to the desalting cartridge.

Desalting Elute-1, Elute-2, and Elute-3

Elute-1: The desalting step starts and system looks for the start of the protein peak.

Elute-2: The peak was detected and the protein peak is collected.

Elute-3: Salt is removed from the desalting cartridge and is diverted to waste.

13 Why is there no desalting step after the denaturing IMAC method?

The denaturing IMAC method is used for purification of insoluble proteins. The protein would very likely precipitate during a desalting step, so that step is not included in the method.

14 How do I find out the value of the extinction coefficient (A_{280} of 1 mg/ml)?

If you know the protein sequence, copy and paste it to any software available on a web search to calculate A_{280} value.

Purification

1 How can I load a large volume (>200 ml) of protein sample?

If the sample size is over 200 ml, we suggest using external tubing (see Profinia System accessories in our catalog) for sample loading, with the container placed on the bench. Loading volume for the Profinia System is up to 999 liters.

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2 How do I purify MBP-tagged and *Strep*-tagged proteins on the Profinia System?

We have validated the purification of MBP- and *Strep*-tagged proteins on the Profinia System. Please refer to Bulletins 5741 and 5744 for details.

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3 Can I use 20% glycerol in my protein purifications?

We do not recommend using a 20% glycerol solution at the flow rates used in the preprogrammed GST or IMAC plus desalting method templates because the column equilibration flow rate will cause an unacceptable increase in backpressure from the desalting cartridge with this solution.

However, reducing the column equilibration flow rate to 2 ml/min in the Program Methods mode will eliminate the backpressure problem. In addition, the concentration of the desalting buffer containing 20% glycerol will need to be changed to 1x (change proportioning from 5x to 1x), which can be done using the following steps:

1. Create a custom affinity-desalting method by selecting Program Methods from the startup screen.
2. Select desired affinity-desalting method.
3. Select Edit Method at the bottom of the Enter Run & Sample Information screen.
4. Scroll to the Equilibrate C2 step and change the flow rate to 2 ml/min and the concentration of B4 to 1x.
5. Save the method and proceed with the separation.

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4 How do I know that my purification is completed?

Although the purified protein can be used right after collection, the whole purification is not completed until the Home screen appears. See the Maintenance section of the system manual, question 1.

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5 I don't see the protein peak in the elution fraction. Why?

The reasons for the absence of the protein peak could be:

- Protein is not expressed or is present at a very low concentration
- Protein does not bind to the cartridge resin (due to either the protein or the column). In this case, the protein would go to the flowthrough fraction and not to the elution fraction
- Protein does not have UV absorbance at 280 nm, even if it is present in the elution fraction (analyses of the fractions would detect the protein, if present)

6 I do see UV absorbance around 0.05 in the elution fraction of my IMAC purification. But SDS-PAGE analysis of my elution fraction does not detect any protein. Why?

The absorbance of imidazole at the concentration used in the elution buffer is approximately 0.05. The low absorbance peak in the elution step of IMAC purification is likely due to the presence of imidazole. The peak you observed is the imidazole peak, not the protein peak.

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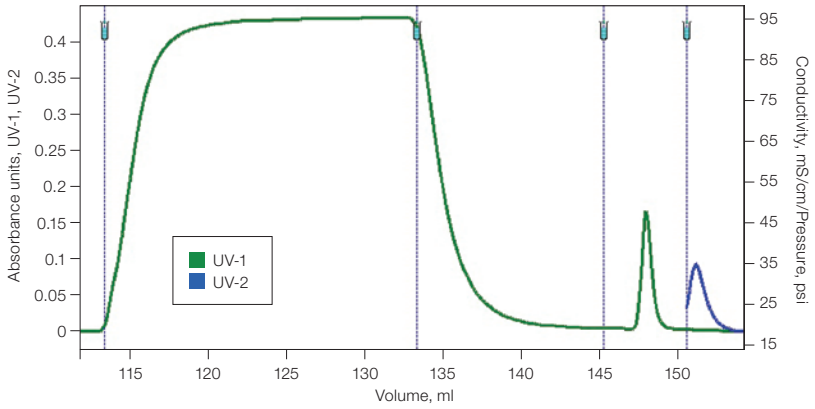
7 During my purification using affinity with desalting methods, the leading edge of the desalting peak is not collected and some of my protein is lost. How can I improve the collection of the desalting peak?

When the affinity and desalting cartridges are in series, the leading edge of the affinity peak will start to elute from the desalting cartridges at approximately 3.0 ml from the point where the affinity was detected. To ensure that the entire desalting peak is collected, the affinity peak elution volume can be lowered (for example to 2.5 ml) in the Program Methods.

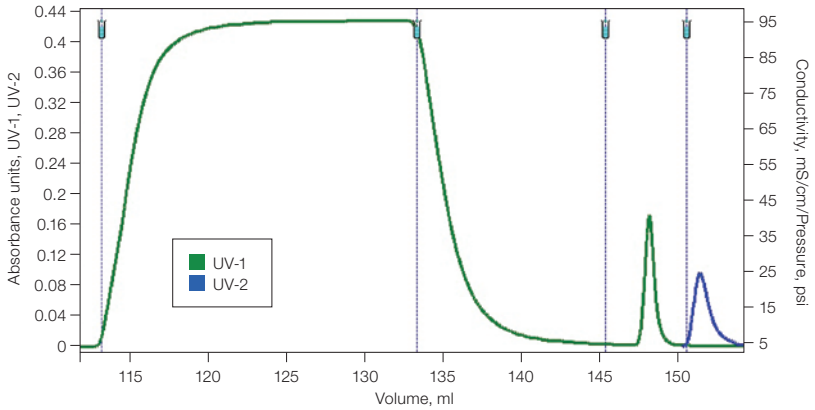
Procedure:

- In Home screen select Program Method
- Select the method and proceed to Edit Method
- Select the step “Elute-2 C1”
- Select the CV parameter and enter 2.7 ml or less (check your chromatogram to determine the optimum amount)
- Click OK and save the method and start the run

Chromatogram Missing Part of the Desalting Peak



Chromatogram With Full Desalting Peak After Settings Were Corrected



Software

1 What are the newest software and firmware versions available for the Profinia System?

The newest version of the Profinia System has UI firmware 1.40 (since June 10, 2008), PV firmware 2.01, and software version 2.0. You can upgrade the firmware from the website free of charge. Contact technical support for new software.

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2 What is new in version 2.0 of Profinia Software?

The new version of Profinia Software has improved presentation of Protein A and G Methods and Profinity eXact Methods data, and it opens run data files faster. It is also compatible with Windows XP and Vista operating systems. The software has also been specifically validated for the following languages: English, Chinese, Japanese, and German.

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3 What are the error codes displayed by the software in the Profinia System Log tab?

The Profina instruction manual Appendix C lists system error codes that can appear under the Log tab. It also lists possible causes and solutions along with the meaning of error codes. Also refer to Appendix D for more information.

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4 What are the advantages of using Profinia Software? How can it help with recording, reporting, and presenting my data?

Profinia Software is not necessary to run the Profinia System, but it broadens the utility of the system by allowing you to view, manage, save, and report the data in both chromatogram and table formats. You can collect real-time data during the run, and after the run you can generate standard or custom reports for data analysis and presentation. The software allows you to view and print a record of the method steps used for the chromatography run.

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5 Can I control the Profinia System with Profinia Software?

No. The Profinia instrument is controlled through the touch screen on the instrument. The software is used to record, display, and report run and method information. The software does not control the instrument.

Maintenance

1 How do I maintain the instrument for regular use?

After each run, the instrument prompts the user to allow an automatic line rinse with water and then with 20% ethanol, before the Home screen appears. It is important to complete the whole process by selecting the proper cleaning methods on the screen. If the system is used weekly, it is sufficient to do a short-term cleaning while less frequent use of the system requires long-term storage cleaning at the end of each run.

Before a run, select Data/Utilities, Diagnostic/Maintainance Functions, then Clean All Inlet/Outlet Lines to rinse all of the system lines and make sure there are no bubbles in the system.

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2 Are there any other maintenance tasks?

Please check the Maintenance section of the Profinia System manual for details.

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