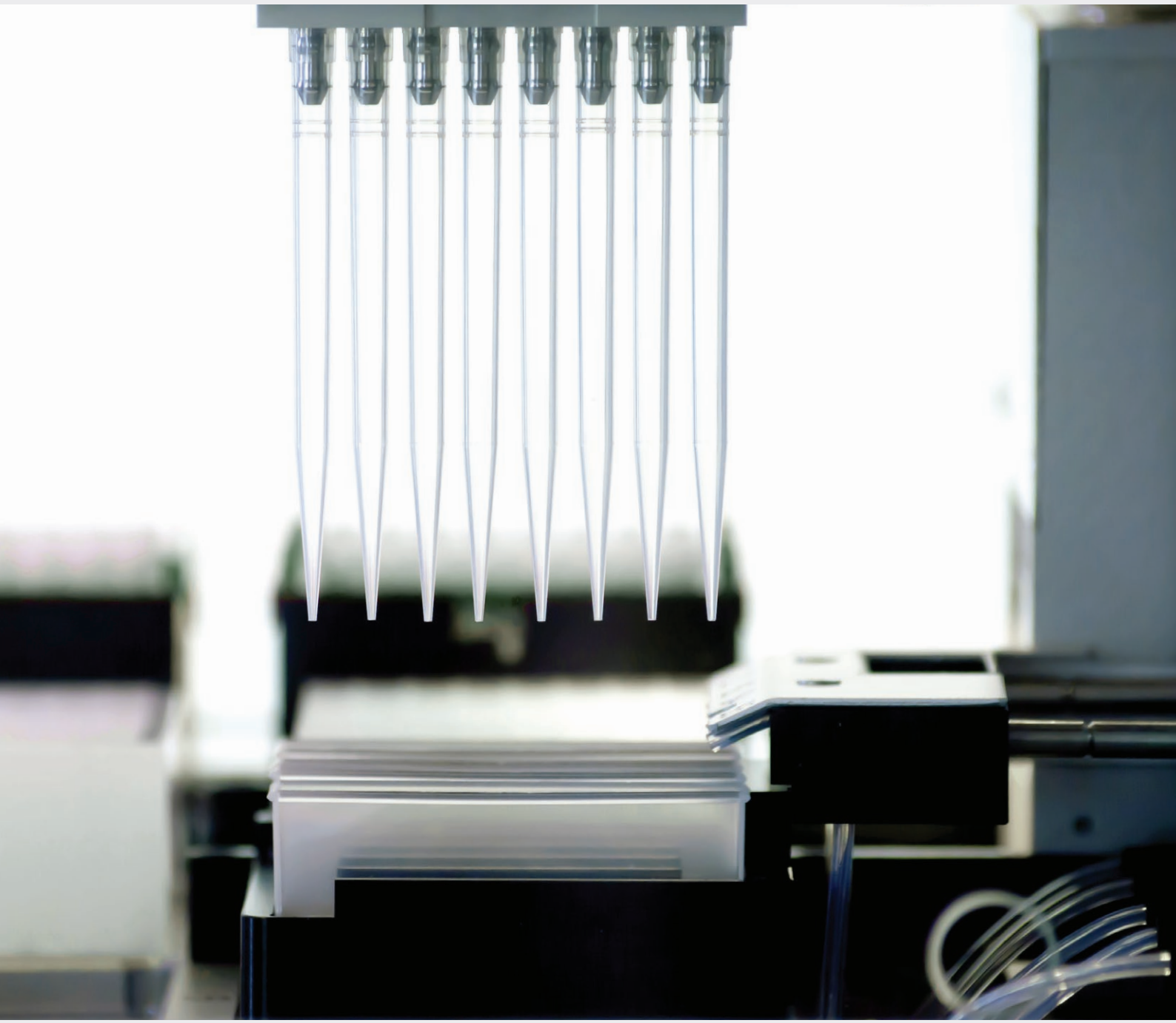
Biotage® Extrahera™

Getting Started Guide



Biotage®

Biotage® Extrahera™

Getting Started Guide

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System Overview

Biotage® Extrahera™ is an automation system for consumable based sample preparation methods. Extrahera can automate Supported Liquid Extraction (SLE), Solid Phase Extraction (SPE), Phospholipid Depletion (PLD), and Protein Precipitation (PPT). The system also offers two filtration methods, Filtration (extraction media already containing sample) and Filtration+ (with sample load and optional pretreatment).

Liquid Handling

Extrahera is equipped with five solvent inlets found on the right hand side of the system (S1-S5). Solvents are pumped into five 25-mL, disposable solvent reservoirs inside the system (in position **5** on the working area) where the robot can aspirate solvent using disposable pipette tips; see Figure 1. A maximum of 4 or 8 tips can be used depending on the system setup.

It is possible to have an extra solvent rack with five 25-mL or 100-mL, solvent reservoirs in position **6** on the working area. These reservoirs have to be filled manually by the user.

To ensure that the system cannot be set to aspirate more liquid into a pipette tip than it can accommodate, the maximum aspiration volume has to be specified for each pipette tip that is configured in the software.

After completion of a liquid operation, the robot can be programmed to move the pipette tips either back to the pipette tip rack (to be reused) or to the pipette tip waste bin. Note that it is only possible to reuse a sample pipette tip when mixing following pretreatment and when loading a sample in aliquots; see "Large Sample Volumes" below.

The system contains corrosion sensitive parts. Best practice is to avoid sustained continuous exposure to acidic and basic vapors by always removing the solvent reservoirs when the system is not in use, and cleaning the system following usage. Usage of acids containing chloride ions, e.g. hydrochloric acid (HCl), in the solvent pumps is not supported at any concentration.

Large Sample Volumes

When a sample load volume exceeds the specified capacity of the selected extraction plate or column rack, the sample is dispensed in aliquots, with application of positive pressure in-between each aliquot.

A user can choose to either reuse the sample pipette tip or use a new sample pipette tip for each aliquot. This is defined when setting up a method in the software's **Manage Methods** view. If the run requires more than the available quantity of sample pipette tips, the system will automatically pause when it runs out of tips and prompt the user to load more.

Working Area

The working area has seven positions (see Figure 2):

- **1**: Solvent pipette tips.
- **2**: Sample pipette tips.
- **3**: Extraction plate or columns.
- **4**: Sample plate or test tubes.
- **5**: Solvent reservoirs, 5 x 25 mL. Filled using the five solvent pumps.
- **6**: Optional solvent reservoirs, 5 x 25 mL or 5 x 100 mL. Manually filled. Note that the 100-mL reservoir has a maximum fill volume of 90 mL.
- **WASTE**: Waste bin for used pipette tips.

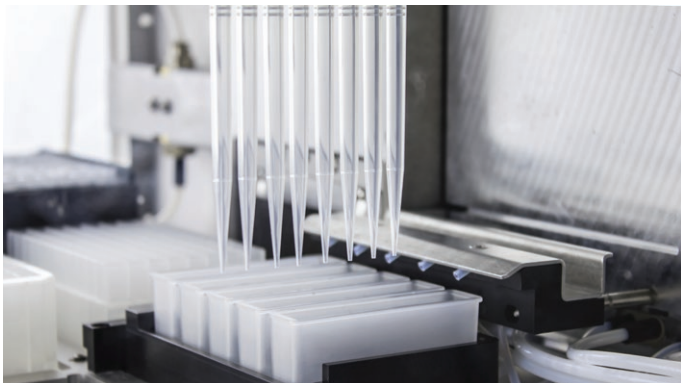


Figure 1. The robot is aspirating solvent from the solvent reservoirs inside the system using disposable pipette tips.

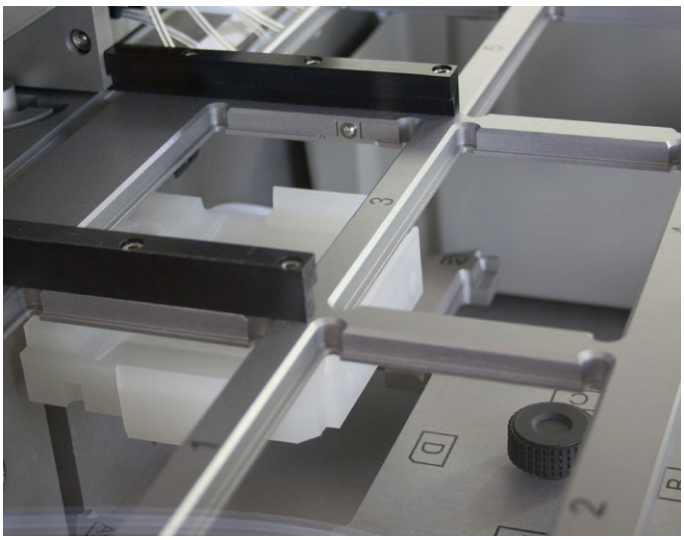


Figure 2. The working area has seven positions (1-6 and WASTE) and the carousel has four positions (A-D).

Carousel and Lifter

The carousel has three positions for collection plates or racks with test tubes (**A**-**C**) and one for a flow-through plate (**D**), which is used for guiding waste into the extraction waste collector eliminating the risk of cross-contamination.

Before each operation in the method, the carousel moves the flow-through plate or the specified collection plate/rack to the inner carousel position. When in position, the plate/rack is moved up by a lifter to a position just underneath the extraction plate or columns (see Figure 3), so that the Luer fittings of the plate or columns cannot splash droplets onto each other or adjacent wells or tubes during the operation.

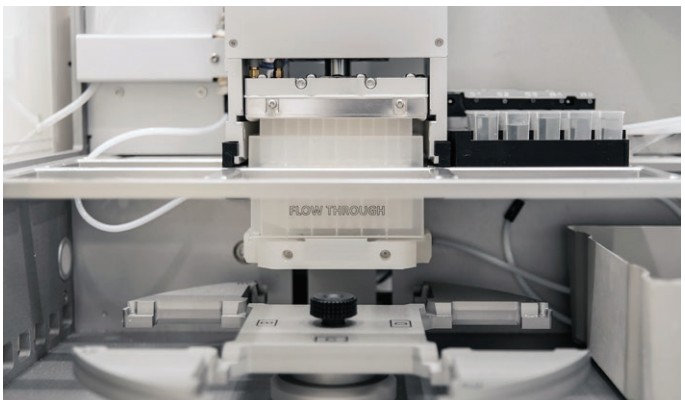


Figure 3. The plate/rack is lifted up from the carousel to just underneath the extraction plate or columns, located in position 3 on the working area.

Columns, Test Tubes, and Plates

The following formats are supported:

Extraction: 96-well extraction plates, 96-array plates for 1- and 2-mL wells, 96-position extraction racks for 1-mL (tabless) columns (A format), 48-well extraction plates, and 24-position extraction racks for 1-, 3-, and 6-mL (tabless) columns (A, B, and C format).

Sample: 1 mL x 96, 1.4 mL x 96, 2 mL x 96, 5 mL x 48, and 10 mL x 24 sample plates, and 12 x 75 mm, 16 x 75 mm, 18 x 75 mm, 13 x 100 mm, and 16 x 100 mm test tubes.

Collection: 1 mL x 96, 2 mL x 96, 5 mL x 48, and 10 mL x 24 collection plates and 24-position collection rack for 12 x 75 mm, 16 x 75 mm, and 18 x 75 mm test tubes.

Flow-through plate: The flow-through plate is used for guiding waste into the extraction waste collector eliminating the risk of cross-contamination. The plate can be cleaned and reused. There are three types of flow-through plates, one for 96 format (P/N 414201SP), one for 48 (P/N 414516SP), and one for 24 (P/N 414203SP).

Pressure Unit

The pressure unit is designed to process extraction plates or columns using pressurized gas. There are two pressure heads available, one for 48 and 96 formats and one for 24 format.

The gas connected to the **AIR** port (at the right side of the system) is used to seal the plate or columns. Note that the pressure has to be adjusted according to how many positions in the plate or column rack that are populated. Use 6 ± 0.2 bar (0.6 ± 0.02 MPa; 87 ± 3 PSI) for a fully populated plate/rack and lower to approximately 4 bar (0.4 MPa; 58 PSI) when populating 50% of the plate/rack and 3 bar (0.3 MPa; 44 PSI) when populating 25%.

The gas connected to the **N_2** port is used when processing samples. The processing pressure is adjustable from 0 to 5 bar in the software, while the pressure into the system has to be set to 6 ± 0.2 bar (0.6 ± 0.02 MPa; 87 ± 3 PSI). The gas flow when processing is between 0 and 10 mL/min during all operations except for plate dry, where it is approximately 600 mL/min. Note that older systems (unless upgraded) do not have dual flow and always process at 0 to 10 mL/min.

Whether compressed air and/or nitrogen is/are used, the gas should be free of moisture-, particulates, and hydrocarbons. This is essential to prevent sample contamination and general fouling of the pressure unit.

Pressure Gradients

When setting up a method, it is possible to create pressure gradients for conditioning, equilibration, sample load, wash, collection, and elution steps. For more information, see page 10.

Waste Kit and Vacuum

A laboratory vacuum source, or a vacuum pump (sold separately, P/N 356330SP), is required for collecting the waste in the 5-liter waste reservoir outside the system.

Always ensure that there is sufficient volume in the waste reservoir before starting a run.

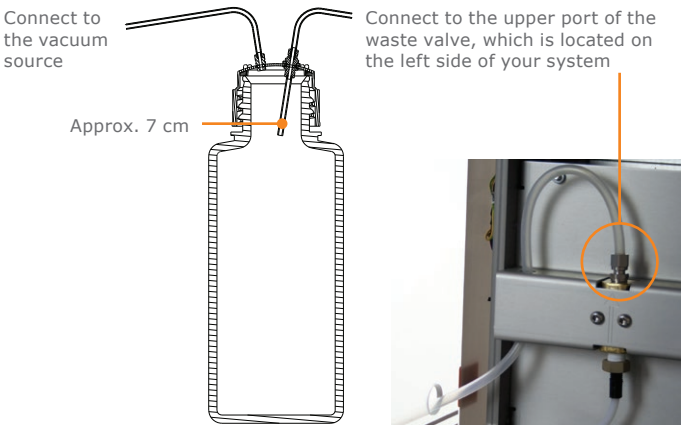


Figure 4. The setup of the waste kit.

Safety

The ventilated system enclosure protects the user against mechanical hazards and potentially harmful solvents and/or vapors. The system cannot be operated when:

- the door is open, and/or
- the integral system ventilation fan is not working.

Figure 5. The outlet of the Extrahera top ventilation.

Adjustable Touch Screen

Extrahera is operated through a 12" touch screen that is adjustable for analyst height, and can be front facing or side facing if the unit is placed at the end of a workbench. There are two ports for connecting USB memory devices below the touch screen.

Audible Alarm and Lighting

An audible warning will sound when a run has been completed or if an error has occurred.

To provide better visibility for the user, there are lamps available inside the system. These can be turned on and off in the software (in the **Maintenance** view). There is one control for the upper lamps and one for the lower.

Power Failure

Warning

- If the system is found with the door closed and the power off, ensure to ventilate the system properly before turning the system back on.

The system has open solvent reservoirs. If the ventilation fails and solvent vapors are not removed, an explosive environment could be generated. If the system is found with the door closed and the power off, you must:

1. Ventilate the system properly by opening the door manually using the T25 Torx screwdriver supplied with the system; see Figure 6. Ensure to take the necessary precautions to avoid exposure to potentially harmful solvents and/or vapors.
2. Remove all solvent reservoirs and remove any spillage before turning the system back on.

We do not recommend that the system is left unattended for an extended period of time when using flammable solvents.

Figure 6. Open the door manually by turning the screw counterclockwise using the T25 Torx screwdriver supplied with the system.

Manage Solvents, Sample Types, Plates, Racks, and Pipette Tips

Note: The robot is a precision instrument, i.e. it is important that you enter the correct values when setting up solvents, sample types, plates, racks, and pipette tips in the **Data Administration** view.

Manage Solvents

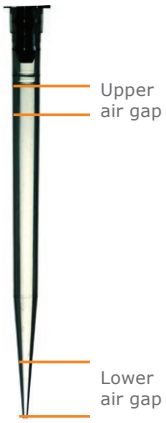
Note: Usage of acids containing chloride ions, e.g. hydrochloric acid (HCl), in the solvent pumps is not supported at any concentration.

The system comes with a number of predefined solvents. If desired, these can be copied and edited to your preferences.

To add, copy, edit, view, and delete solvents, press **Data Administration** in the main menu and then **Manage Solvents**.

The following parameters are available:

- **Solvent name:** The name of the solvent that will be displayed in the software.
- **Solvent description:** The solvent including all additives.
- **Aspiration flow rate (mL/min):** The flow rate that will be used when aspirating the solvent.
- **Dispense flow rate (mL/min):** The flow rate that will be used when dispensing the solvent.
- **Chlorinated?:** Whether the solvent is chlorinated (Yes) or not (No).
- **Serial dispensing allowed?:** Whether serial dispensing is allowed (Yes) or not (No). Note that serial dispensing is faster, while individual dispensing has a higher precision.
- **Lower air gap flow rate (mL/min):** The flow rate that will be used when aspirating and dispensing the lower air gap.
- **Lower air gap volume (µL):** The volume of the lower air gap.
- **Upper air gap flow rate (mL/min):** The flow rate that will be used when aspirating and dispensing the upper air gap.
- **Upper air gap volume (µL):** The volume of the upper air gap.
- **Upper air gap dispense pause (ms):** The amount of time that the system will pause between the solvent dispensation and the upper air gap dispensation. The default value of 300 ms can be adjusted when dealing with viscous, volatile, or low surface tension solvents.



- **Requires tip conditioning?:** Whether the pipette tip requires conditioning (Yes) or not (No).
- **Conditioning, number of times:** The number of conditioning iterations. This field is only enabled when **Requires tip conditioning** is set to **Yes**.
- **Conditioning flow rate (mL/min):** The flow rate that will be used when conditioning the pipette tip. This field is only enabled when **Requires tip conditioning** is set to **Yes**.
- **Conditioning volume (% of tip capacity):** The percentage of the pipette tip that will be filled with solvent when conditioning the tip. This field is only enabled when **Requires tip conditioning** is set to **Yes**.

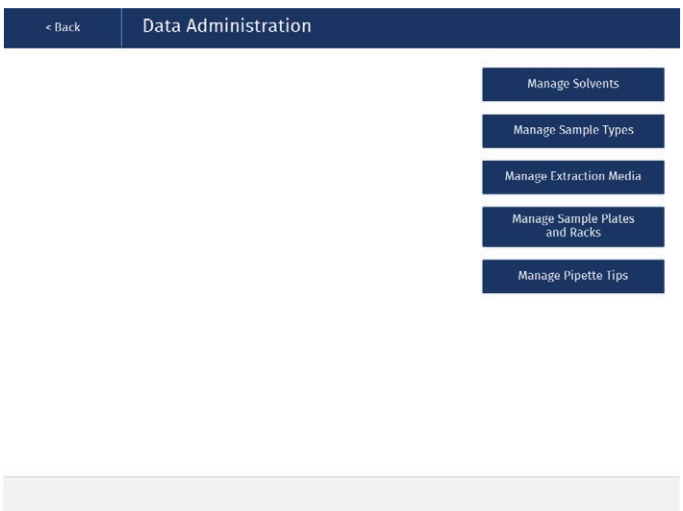


Figure 7. The Data Administration view.

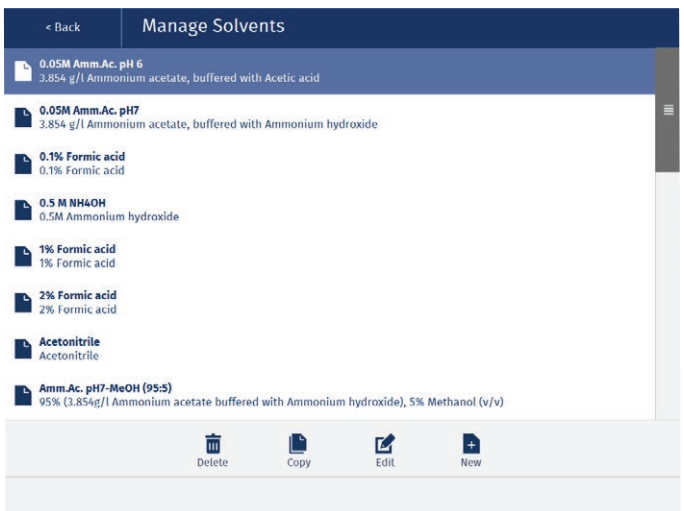


Figure 8. The Manage Solvents view.

- **Aspirate post dispense?:** Whether air is aspirated into the pipette tip after dispensing solvent (Yes) or not (No). The aspiration volume will be the same as for the upper air gap; see **Upper air gap volume (µL)** above.

Manage Sample Types

To add, edit, copy, and delete sample types, press **Data Administration** in the main menu and then **Manage Sample Types.** These settings are important if you work with a wide variety of different sample matrices whose liquid handling properties can vary.

The following parameters are available:

- **Sample name:** The name of the sample that will be displayed in the software.
- **Sample description:** A description of the sample.
- **Aspiration flow rate (mL/min):** The flow rate that will be used when aspirating samples.
- **Dispense flow rate (mL/min):** The flow rate that will be used when dispensing samples.
- **Lower air gap flow rate (mL/min):** The flow rate that will be used when aspirating and dispensing the lower air gap.
- **Lower air gap volume (µL):** The volume of the lower air gap.
- **Upper air gap flow rate (mL/min):** The flow rate that will be used when aspirating and dispensing the upper air gap.
- **Upper air gap volume (µL):** The volume of the upper air gap.
- **Upper air gap dispense pause (ms):** The amount of time that the system will pause between the sample dispensation and the upper air gap dispensation. The default value of 300 ms can be adjusted when dealing with viscous or pretreated samples that behave differently.
- **Aspirate post dispense?:** Whether air is aspirated into the pipette tip after dispensing sample (Yes) or not (No). The aspiration volume will be the same as for the upper air gap; see **Upper air gap volume (µL)** above.

Manage Extraction Media

To add, edit, copy, and delete extraction plates and column racks, press **Data Administration** in the main menu and then **Manage Extraction Media**.

The following parameters are available:

- **Name:** The name of the extraction plate or column rack that will be displayed in the software.
- **Manufacturer:** The manufacturer.
- **Part number:** The manufacturer's part number.
- **Capacity volume (µL):** The amount of liquid that each well or column can accommodate. If the sample load volume (in the SPE method) exceeds this, the sample will be dispensed in aliquots, with application of positive pressure in-between each aliquot. Note that this aliquot feature is disabled when the capacity volume is set to zero, which is the default value.
- **Format:** The number of wells or columns (24, 48, or 96).
- **Comment:** Optional information about the plate or rack.

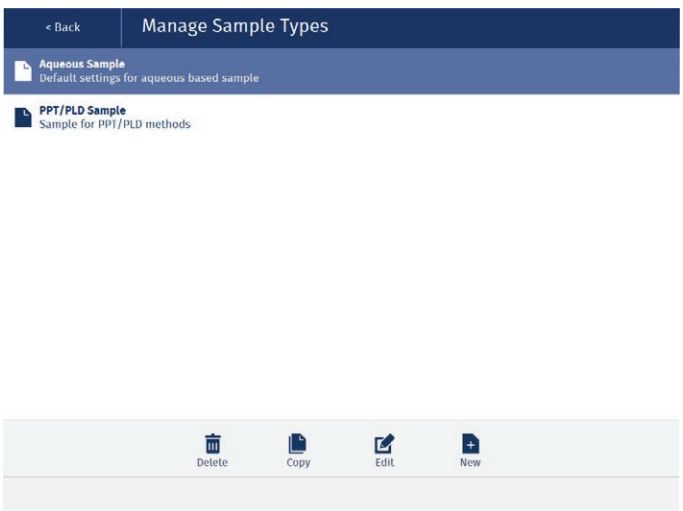


Figure 9. The Manage Sample Types view.

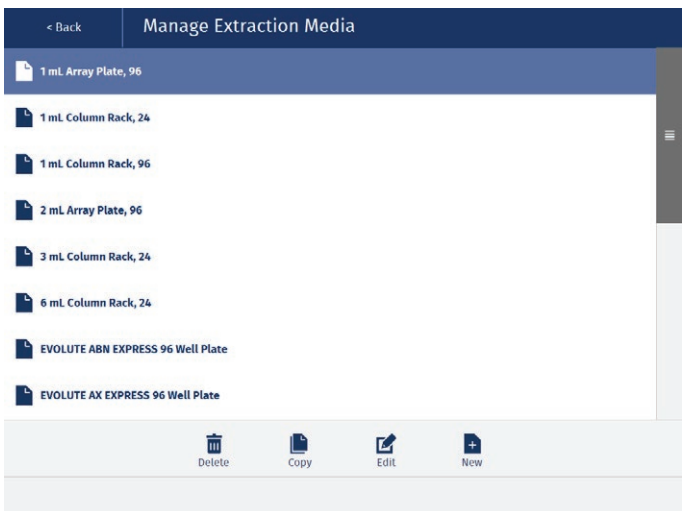


Figure 10. The Manage Extraction Media view.

- **Solvent/Sample dispensation height (mm):** The height where the solvent/sample is dispensed by the pipette tip, measured from the robot's top position.*
- **Aspiration height (mm):** The height where the sample mixture is aspirated by the pipette tip during mixing in a PPT or PLD plate or column rack, measured from the robot's top position.*

Manage Sample Plates and Racks

To add, edit, copy, and delete sample plates and racks, press **Data Administration** in the main menu and then **Manage Sample Plates and Racks**.

The following parameters are available:

- **Name:** The name of the sample plate or rack that will be displayed in the software.
- **Capacity volume (µL):** The maximum amount of liquid that each well or test tube can accommodate.
- **Format:** The number of wells or test tubes (24, 48, or 96).
- **Aspiration height (mm):** The height where the sample is aspirated and premixed by the pipette tip, measured from the robot's top position.*
- **Pretreatment dispensation height (mm):** The height where pretreatment solvent is dispensed by the pipette tip, measured from the robot's top position.*

Manage Pipette Tips

To add, edit, copy, and delete pipette tips, press **Data Administration** in the main menu and then **Manage Pipette Tips**.

The following parameters are available:

- **Name:** The name of the pipette tip that will be displayed in the software.
- **Manufacturer:** The manufacturer.
- **Part number:** The manufacturer's part number.
- **Capacity (µL):** The maximum amount of liquid that can be aspirated. This setting ensures that the system cannot attempt to aspirate more liquid into the pipette tip than it can accommodate.
- **Length (mm):** The length of the pipette tip.

* The pipetting heights can be entered manually or set using a wizard (see Figure 11). To open the software wizard, press **Tune Pipetting Heights...**. Note that only one type of pipette tip is required for the tuning of the pipetting heights.

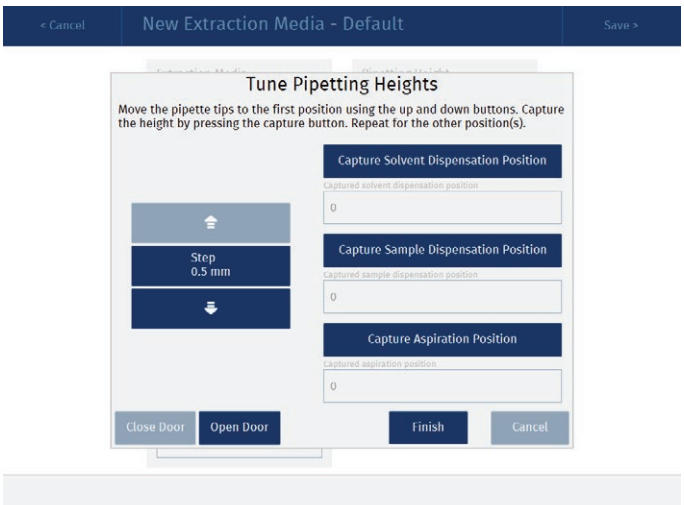


Figure 11. Wizard for setting the pipetting heights for an extraction plate or column rack.

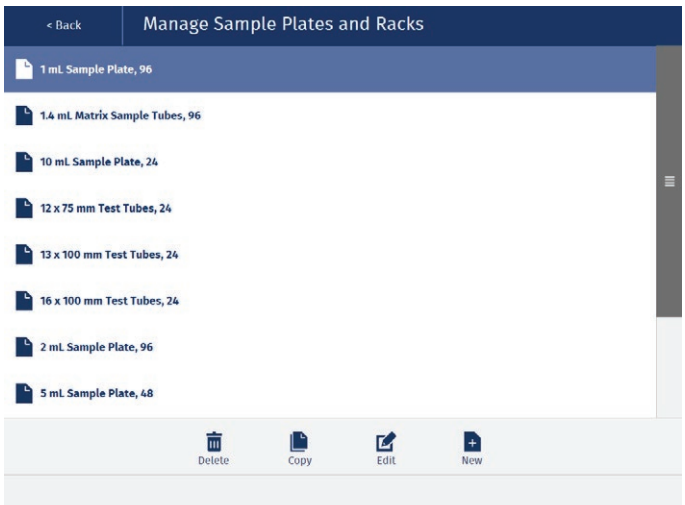


Figure 12. The Manage Sample Plates and Racks view.

Set Up a Method

Methods can be created, copied, edited, viewed, locked, imported, exported, and deleted in the **Manage Methods** view. Enter the view by pressing **Manage Methods** in the main menu.

New, Copy, Edit, Lock, and Delete

To create a new method, press **New** and then select the type of method, i.e. press:

- **SLE** (Supported Liquid Extraction)
- **SPE** (Solid Phase Extraction)
- **PPT/PLD** (Protein Precipitation/Phospholipid Depletion)
- **Filtration** (collects filtrate from an extraction media that is preloaded with sample)
- **Filtration+** (sample load and filtration preceded by an optional pretreatment)

The method will be saved in the **User methods** folder.

To copy or edit a method, select the folder containing the method, select the method, and then press **Copy** or **Edit**.

To save changes in a displayed method, press **Save** in the top pane. To return to the **Manage Methods** view without saving, press **Cancel** in the top pane. Methods that are highlighted with an exclamation mark (❗) in the **Manage Methods** view (see Figure 13) do not have all the necessary information to be run and are not displayed in the **Run** view, and cannot be locked.

To lock a user-defined method, set the check box (☑) in front of the method and press **Lock** and then **Yes** to confirm. The method is moved to the **Locked methods** folder (🔒) and cannot be unlocked, modified, or deleted.

To delete methods, set the check boxes (☑) in front of the methods that you want to delete and press **Delete** and then **Yes** to confirm. Note that all methods will be deleted if you set the check box in the header.

Export and Import

Methods can be exported to a USB memory device for backup or to be used on other Extrahera systems.

To export methods, connect a USB memory device to one of the two USB ports underneath the touch screen, set the check boxes (☑) in front of the methods that you want to export, and press **Export.** To export the selected methods as PDF files instead, press **Export PDF**. Note that you can select all the methods in the folder by setting the check box in the header.

To import methods from a USB memory device, connect the device and press **Import**, select the file that contains the methods you want to import in the appearing dialog and press **Import**. If you import a method with a solvent, plate, or rack that is not available on the system, it will also be imported. The decision is based on the name of the solvent, plate and rack, and will not consider their settings.

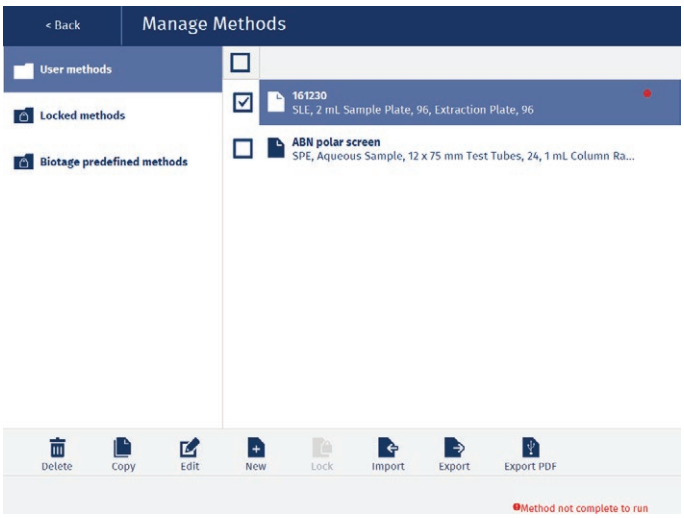


Figure 13. User methods highlighted with ❗ do not have all the necessary information to be run and are not displayed in the Run view.

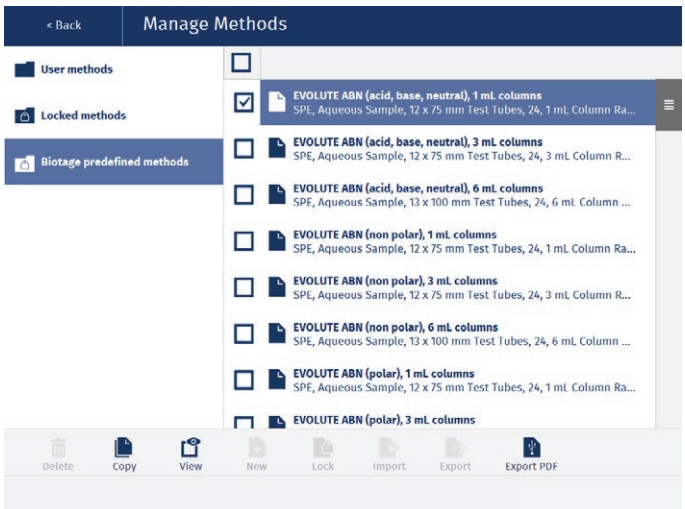


Figure 14. The system comes with a number of predefined methods. They can be copied and edited to your preferences.

Predefined Methods

The system comes with a number of predefined Biotage methods. If desired, these can be copied and then edited to your preferences. The copy will be saved in the **User methods** folder. Note that the predefined methods are locked and cannot be modified or deleted. To view a predefined method, select it and press **View**.

Operations

The following operations can be enabled or disabled when setting up a method:

Operation	SLE	SPE	PPT/PLD	Filtration	Filtration+
Pretreatment	✓	✓[3]			✓
Conditioning		✓			
Equilibration		✓			
Load/Solvent load	✓[1]	✓[1]	✓[2]		✓[4]
Wash		✓			
Elution	✓	✓			
Collection			✓	✓	✓

Table 1. Method operations that can be enabled or disabled.

[1] Sample load.

[2] The sample is either added before or after the solvent depending on a system setting. To change this setting, press **Maintenance** in the main menu and then disable or enable the **Add solvent first** option in the **PPT/PLD Mode** field. This has to be done before you set up the method.

[3] The pretreatment step can be moved to take place immediately before load.

[4] A load operation is incorporated into the collection operation.

Parameters

Available method parameters are listed in alphabetical order:

- **Advanced pressure settings:** Press **Edit...** to set up a pressure gradient. For more information, see page 10.
- **Air push after last elution?:** Whether to apply positive pressure with a gas flow of approximately 10 mL/min after the last elution (Yes) or not (No). SLE only.
- **Air push time (s):** The amount of time, in seconds, that positive pressure with a gas flow of approximately 10 mL/min will be applied to the extraction plate or columns to trigger gravity loading or elution. This field is only enabled for elution when **Air push after last elution?** is set to **Yes**. SLE only.
- **Collect in position:** In which position the collection plate/rack or the flow-through plate (waste) is to be loaded onto the carousel.
- **Collection plate height (%):** How high, in percentage of the maximum height, that the collection plate/rack is moved up by the lifter. See Table 2 on page 10 for guidelines. Filtration only.
- **Conditioning solvent:** The solvent to be used for pipette tip conditioning. SPE only.
- **Dispose solvent tips?:** Whether the pipette tips will be disposed of after the solvent load (Yes) or reused (No). PPT/PLD (solvent first) only.
- **Dispose solvent tips after each step?:** Whether the pipette tips will be disposed of after each step (Yes) or reused (No). Note that it is not possible to reuse a pipette tip after sample dispensation. SPE, SLE, and Filtration+ only.
- **Extraction media:** The type of extraction plate or columns.
- **Method comment:** Optional information about the method.
- **Method name:** The name of the method.

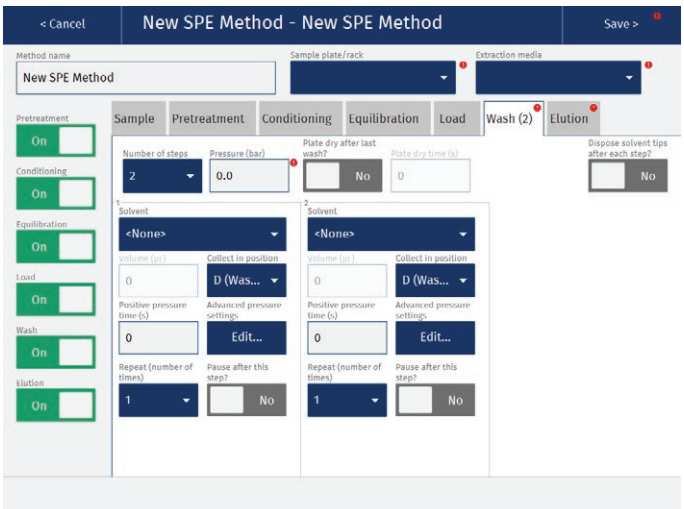


Figure 15. Setting up an SPE method. The exclamation mark (❗) shows you which information is still required to run the method.

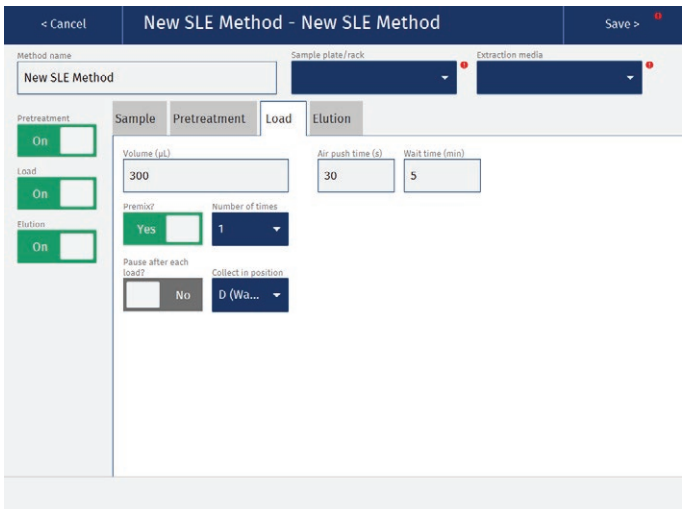


Figure 16. Setting up an SLE method.

- **Mix number of times:** The number of mixing iterations when mixing the sample with a solvent following pretreatment (except for PPT/PLD). Not available for Filtration.
- **Mix volume (µL):** The aspiration volume when mixing the sample with a solvent following pretreatment (except for PPT/PLD). Not available for Filtration.
- **Move pretreatment step:** Use the arrow buttons to move the pretreatment operation to the desired position, before conditioning (default) or before load. SPE only.
- **Number of steps:** The number of steps the operation contains. SPE, SLE, and Filtration+ only.
- **Number of times:** The number of mixing iterations in the sample plate. This field is only enabled when **Premix?** is set to **Yes**. Not available for Filtration.
- **Pause after this step/last step/each load?** Whether to pause after a certain step or operation (Yes) or not (No). When the system is paused, it is possible to open the door and check for clogged wells or columns. SPE, SLE, and Filtration+ only.
- **Plate dry after last elution?:** Whether to apply positive pressure with a gas flow of approximately 600 mL/min after the last elution (Yes) or not (No). SPE only.
- **Plate dry after last wash?:** Whether to remove the last residual solvent from the sorbent bed using positive pressure with a gas flow of approximately 600 mL/min (Yes) or not (No). SPE only.
- **Plate dry time (s):** The amount of time, in seconds, that positive pressure with a gas flow of approximately 600 mL/min will be applied to remove the last residual solvent from the sorbent bed. This field is only enabled when **Plate dry after last wash/elution?** is set to **Yes**. SPE only.
- **Positive pressure time (s):** The amount of time, in seconds, that positive pressure will be applied to the extraction plate or columns. Not available for SLE. Note that this field is disabled when a pressure gradient is enabled; see "Pressure Gradients" on page 10.
- **Premix?:** Whether the sample will be premixed (Yes) or not (No) before it is loaded into the extraction plate or column. Not available for Filtration.
- **Pressure (bar):** The pressure, in bar, that will be applied to the extraction plate or columns. Not available for SLE. Note that this setting will not be used when a pressure gradient is enabled; see "Pressure Gradients" on page 10.
- **Repeat (number of times):** The number of times this step will be repeated. SPE and SLE only.
- **Reuse sample tips:** Whether to reuse a sample pipette tip when mixing following pretreatment or when loading a sample in aliquots (Yes) or not (No). Not available for Filtration.
- **Rinsing?:** Whether to remove the last residual sample from the sample plate/rack by rinsing the wells or test tubes with solvent, and then transferring the liquid to the extraction media (Yes) or not (No). SPE only.
- **Rinse solvent:** The solvent to be used to rinse the wells or test tubes in the sample plate/rack. SPE only.
- **Rinse volume (µL):** The amount of rinsing solvent to be used for each well or test tube in the sample plate/rack. SPE only.
- **Sample type:** The type of sample. This setting is important if you work with a wide variety of different sample matrices whose liquid handling properties can vary.
- **Starting sample volume in plate/rack (µL):** The amount of available liquid in each well or test tube in the sample plate/rack, prior to starting the method.
- **Sample plate/rack:** The type of sample plate or test tubes.

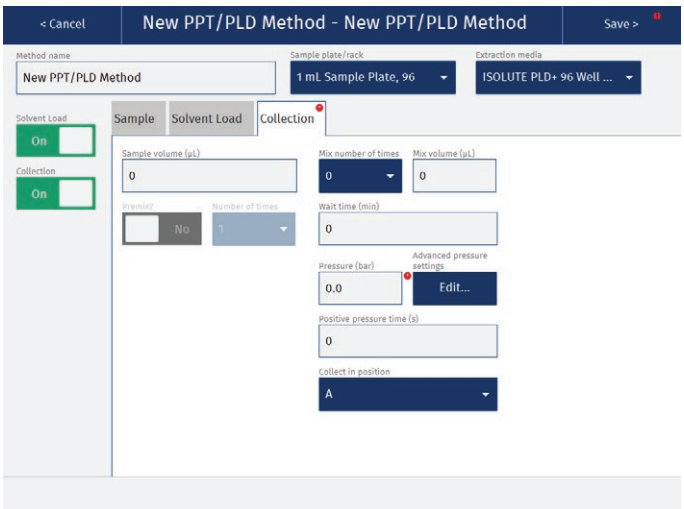


Figure 17. Setting up a PPT/PLD method with the sample added after the solvent.

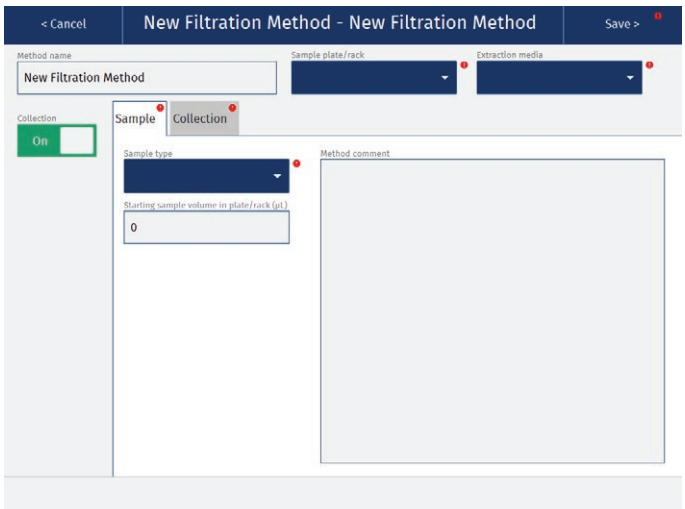


Figure 18. Setting up a Filtration method, to collect filtrate from an extraction media that is preloaded with sample.

- **Solvent:** The solvent to be used for this step. Not available for Filtration.
- **Tip conditioning?:** Whether to condition the pipette tip with a solvent (Yes) or not (No). SPE only.
- **Volume (µL):** For a sample load operation, it specifies the amount of sample that will be loaded into the extraction plate or column. Otherwise, it specifies the amount of solvent that will be used. Not available for Filtration. Note that if the sample load volume exceeds the specified capacity of the selected extraction plate or columns, the sample will be dispensed in aliquots, with application of positive pressure in-between each aliquot. This large volume feature is disabled when the capacity of the selected extraction plate or columns is set to zero, which is the default value.

  Note: When loading a sample in aliquots, the sample pipette tip can be reused. See **Reuse sample tips** above.
- **Wait time (min):** The amount of time to wait to complete the process step. Not available for Filtration.

Pressure Gradient

When setting up an SPE method, it is possible to create a pressure gradient for any conditioning, equilibration, load, wash, or elution step. Pressure gradients are also available for elution steps in SLE methods, and for the collection step in PPT/PLD, Filtration, and Filtration+ methods. To set up a pressure gradient, press the **Edit...** button for the desired step (see Figure 15).

The following parameters are available:

- **Use advanced pressure settings?** Whether to use a pressure gradient (Yes) or not (No).
- **Number of steps:** The number of steps the pressure gradient contains.
- **Pressure (bar):** The pressure, in bar, that will be applied to the extraction plate or columns.
- **Positive pressure time (s):** The amount of time, in seconds, that positive pressure will be applied to the extraction plate or columns.
- **Air push/Plate dry:** Whether to apply positive pressure with a gas flow of approximately 10 mL/min (air push) or 600 mL/min (plate dry) after the pressure gradient has been completed (Yes) or not (No).
- **Air push time/Plate dry time (s):** The amount of time, in seconds, that positive pressure will be applied to remove the last residual solvent from the sorbent bed. This field is only enabled when **Air push/Plate dry?** is set to **Yes**.

Collection Plate/Rack Height (Filtration Only)

When setting up a Filtration method, you need to specify how high the collection plate/rack is to be moved up by the lifter toward the extraction media. The height is specified in percentage of the maximum height. The table below lists the approximate settings for the **Collection plate height (%)** parameter for some combinations of collection plate/rack and extraction media.

Collection Plate/Rack	Extraction Media	Height (%)
1/2 mL Collection Plate, 96	Extraction Plate, 96	80
1/2 mL Collection Plate, 96	1 mL Array Plate, 96	69
1/2 mL Collection Plate, 96	1 mL Column Rack, 96	69
5 mL Collection Plate, 48	Extraction Plate, 48	80
10 mL Collection Plate, 24	1 mL Column Rack, 24	69
10 mL Collection Plate, 24	3 mL Column Rack, 24	58
10 mL Collection Plate, 24	6 mL Column Rack, 24	55
12/16/18 x 75 mm Test Tubes	1 mL Column Rack, 24	33
12/16/18 x 75 mm Test Tubes	3 mL Column Rack, 24	22
12/16/18 x 75 mm Test Tubes	6 mL Column Rack, 24	19

Table 2. Examples of settings for the Collection plate height (%) parameter that is used in the Filtration method.

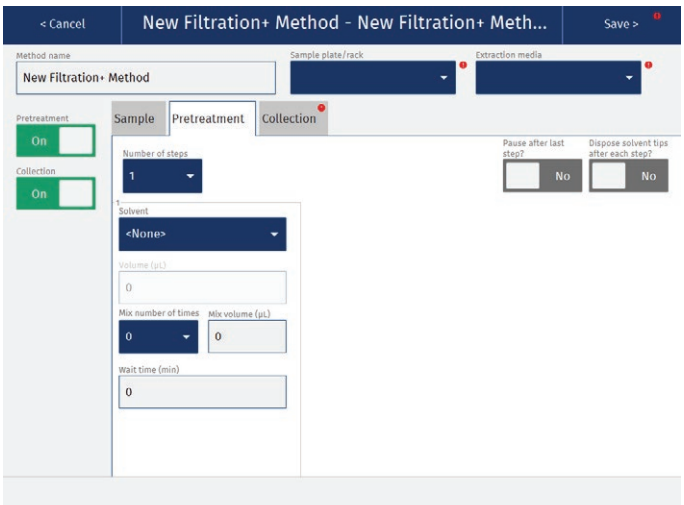


Figure 19. Setting up a Filtration+ method, which offers the options of pretreatment and collection.

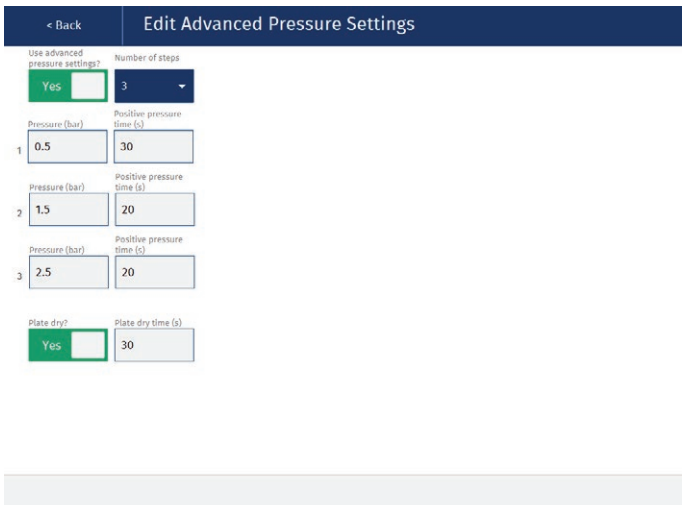


Figure 20. In the Edit Advanced Pressure Settings view, you can set up a pressure gradient for a conditioning, equilibration, sample load, wash, collection, or elution step.

Run Single or Multiple Methods

Warning

» All samples and waste should be treated as potentially biohazardous.

Verify System Setup

Verify the following before operating the system:

1. Ensure that all connections are properly connected and tightened; see the "Connections" section in the "Biotage® Extrahera™ Installation and Safety" document, P/N 414157.
2. Ensure that the extraction waste collector is in place.
3. Ensure that the vacuum is turned on and that there is sufficient volume in the waste reservoir.
4. If using a vacuum pump, ensure that the vacuum pump fumes are directed into a proper ventilation system.
5. Ensure that the pipette tip waste bin (below the **WASTE** position on the working area) is empty.
6. Ensure that there is a solvent rack, with reservoirs in all five rack positions, in position **5** on the working area and that the solvent feeder is in position (see Figure 38 on page 17).

Prepare and Run

Select Method(s)

7. In the main menu, press either **Run Single Method** to run one method or **Run Multiple Methods** to run two or more methods on the same sample plate/rack.

Note: Do not perform a run with multiple Filtration methods.

8. Select the method(s) you want to run and press **Prepare Run**. Only methods relevant to the current system configuration (24, 48, or 96 format) are displayed.

   When selecting multiple methods, only methods using the same sample plate/rack as the first selected method will be displayed; see Figure 21. You can select up to 12 (96 format) or 6 (48/24 format) methods, using a maximum of 10 solvents.

9. If desired, enter the **Sample Plate/Rack ID**, **Extraction Media Lot No**, and **Collection Plate/Rack ID**.

Load Extraction Plate or Column Rack and Adjust the Pressure

10. Load the correct type of extraction plate or column rack (see the method summary in the **Prepare Run** view) into position **3** on the working area.

    If using an array plate or column rack with empty positions, load the wells or columns symmetrically from the outside in so that the pressure head can be positioned horizontally over the plate/rack. Note that you have to use all positions in a plate/rack column. In other words, the minimum amount of wells or columns that can be loaded is 8 for the 24 format and 16 for the 96 format (see Figure 23).

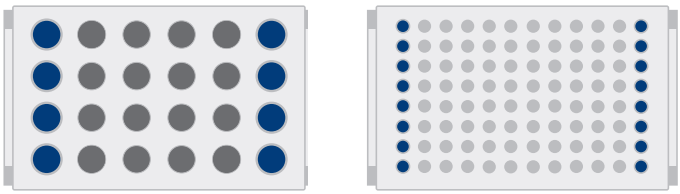


Figure 23. Load the columns symmetrically from the outside in and use all positions in a plate/rack column.

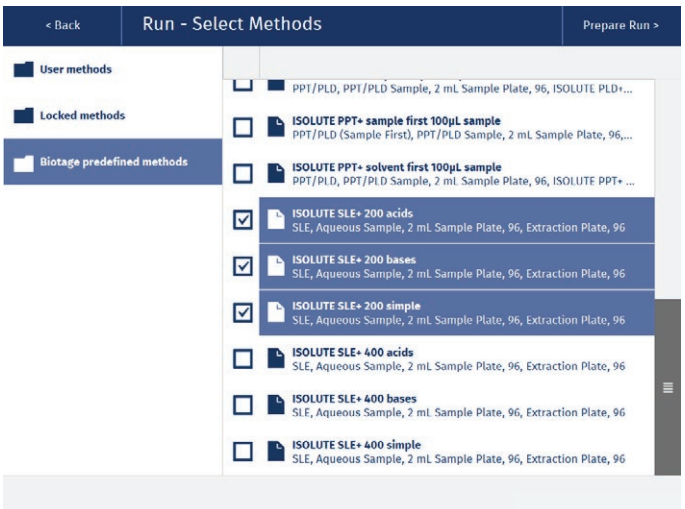


Figure 21. When using a 96-format system, you can select up to 12 methods using the same sample plate/rack and a maximum of 10 solvents.

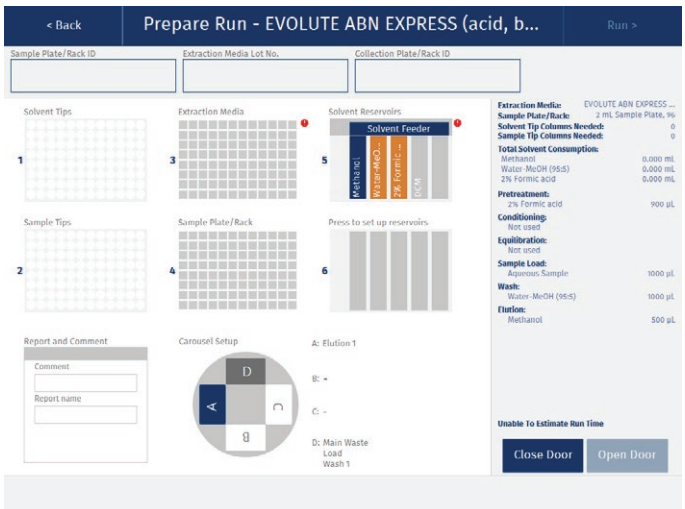


Figure 22. In the Prepare Run view, you set up the tips, assign solvents, and select the columns to be used in the plates and racks.

11. Adjust the pressure of the gas connected to the **AIR** port using the external pressure regulator.

    Use 6 ± 0.2 bar (0.6 ± 0.02 MPa; 87 ± 3 PSI) for a fully populated plate/rack and lower to approximately 4 bar (0.4 MPa; 58 PSI) when populating 50% of the plate/rack and 3 bar (0.3 MPa; 44 PSI) when populating 25% of the plate/rack.

    Note that the gas connected to the **N_2** port always have to be 6 ± 0.2 bar (0.6 ± 0.02 MPa; 87 ± 3 PSI).

12. Press the illustration of the extraction plate or column rack, and then set up each plate/rack in the **Select Columns** view (see Figure 24):
    a. If running a single method, select the plate/rack columns to be used. To use all columns, enable the **Use all columns** option. To disable or enable a column, press it or its check box.
    b. If running multiple methods, select a method for each plate/rack column to be used.
    c. When done, press **Save** in the top pane.

Load Pipette Tips

13. Load the necessary amount of solvent pipette tips (see the method summary on the right in the **Prepare Run** view) into a pipette tip rack holder and place it in position **1** on the working area.
14. Press the illustration of the solvent tips in the software, and set up the tips according to what you have loaded onto the system; see Figure 25. When done, press **Save** in the top pane.

    If you are using the same type of pipette tip in all rack columns, enable the **Use same tips in all columns** option. Note that the pipette tip selected for column 1 will be selected for all columns.

    If the pipette tip has been used in a previous run, the used solvent is displayed in the **Used with solvent** field. To empty a column, disable the **Use same tips in all columns** option, press **Empty** for that column, and unload the pipette tips.
15. Load the necessary amount of sample pipette tips (see the method summary on the right in the **Prepare Run** view) into a pipette tip rack holder and place it in position **2** on the working area.

    Note: If the run requires more than the available quantity of sample pipette tips, the system will automatically pause when it runs out of tips and prompt the user to load more.
16. Press the illustration of the sample tips in the software and set up the tips according to what you have loaded onto the system. When done, press **Save** in the top pane.

    If you are using the same type of pipette tip in all rack columns, enable the **Use same tips in all columns** option. Note that the pipette tip selected for column 1 will be selected for all columns.

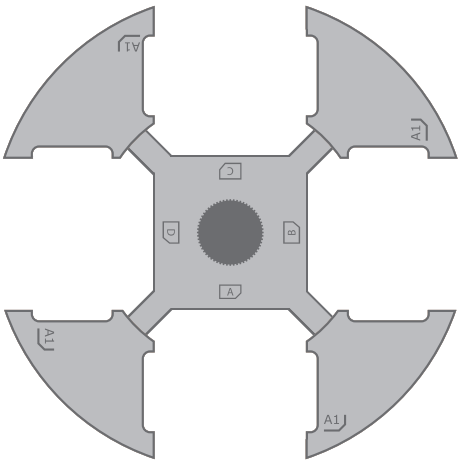


Figure 26. Ensure that the collection plates/racks are loaded correctly into the carousel. Note the location of the A1 position of the plate/rack.

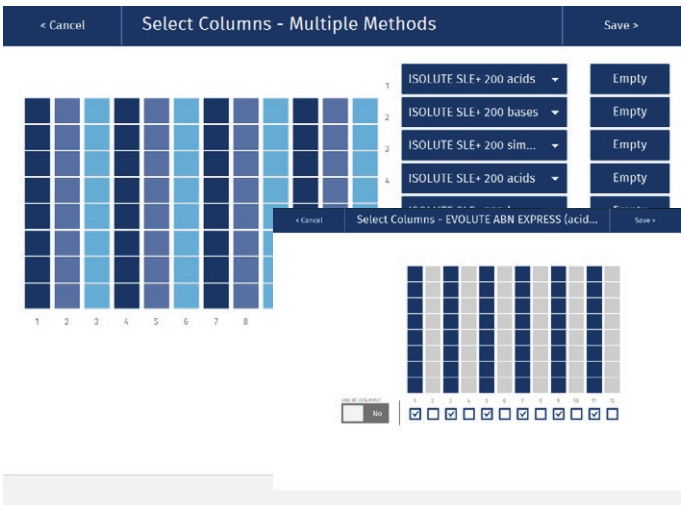


Figure 24. The Select Columns view when running multiple methods (seen in the back) and when running a single method (seen in the front).

Figure 25. If you are using the same pipette tip in all rack columns, enable the Use same tips in all columns option.

Load Collection Plate(s) or Rack(s)

17. Load 24-, 48-, or 96-well collection plate(s) or rack(s) onto the carousel as specified in the method; see the **Carousel Setup** in the **Prepare Run** view. Ensure to load them in the correct direction; see the location of the A1 position of the plate/rack in Figure 26.
18. Load a 24-, 48-, or 96-well flow-through plate, which is used for guiding waste into the extraction waste collector eliminating the risk of cross-contamination, into position **D** on the carousel.

Load and Assign Solvents and Prime

19. If more than five solvents are required to run the method(s), setup a solvent rack in position **6** on the working area. Note that position **6** can be used even when less than six solvents are required to run the method(s).

    To set up a solvent rack in position 6 on the working area:

    a. Press the illustration of the optional solvent reservoirs in the software (position 6).

    b. In the **Select Position 6 Accessory** view, select the desired reservoir size, 25 mL or 100 mL, and press **Save**. Note that the 100-mL reservoir has a maximum fill volume of 90 mL and that the reservoirs in position **6** have to be filled manually and cannot be refilled during the run.

    c. Press the illustration of the optional solvent reservoirs (position **6**) and assign a solvent to each reservoir to be used; see Figure 27. If a reservoir is orange, the total volume of that solvent is less than required. Add another reservoir with the same solvent or consider having the solvent in one of the five solvent bottles (see step 20).

20. Ensure that the solvent bottles (S1-S5) contain the necessary amount of the solvents needed for the run; see **Total Solvent Consumption** in the method summary on the right in the **Prepare Run** view.

    When any of the solvents used on the system has to be exchanged, the miscibility of the solvent presently connected and the new solvent to be connected are important and have to be considered. The use of a miscible co-solvent is advised which will minimize the time taken for successful priming and therefore the volume of solvent wasted during multiple prime occurrences.

    Ensure that no foreign matter (e.g. molecular sieve) is present in the bottles. If necessary, filter the liquids. Use appropriate caps on the bottles to prevent harmful solvent vapors from escaping and the contents from being spilled. Always place the bottles on the side of the system.

21. Press the illustration of the solvent reservoirs in the software (position **5**) and ensure that the solvent bottles (S1-S5) are assigned correctly in the **Assign Solvents** view (Figure 28). If necessary, change using the drop-down lists.
22. If one or more solvent reservoirs are orange in the **Assign Solvents** view (see Figure 28), prime the solvent inlet line(s):

    a. Ensure that the solvent reservoir is empty. If not, replace it with a new one.

    b. Press **Prime...** and enter the prime volume. 15 mL is required to fill the solvent inlet line and pump with the new solvent. Note that the solvent reservoir can contain a maximum of 25 mL.

    c. Press **Prime**.

    d. Ensure that no air bubbles are visible in the tubing. If necessary, empty the solvent reservoir and prime again.

    e. When you have finished priming, press **OK** in the top pane and repeat the procedure for any other inlet that needs to be primed.

    f. When you have finished priming all solvent inlets that have an orange solvent reservoir, press **Save** in the top pane.

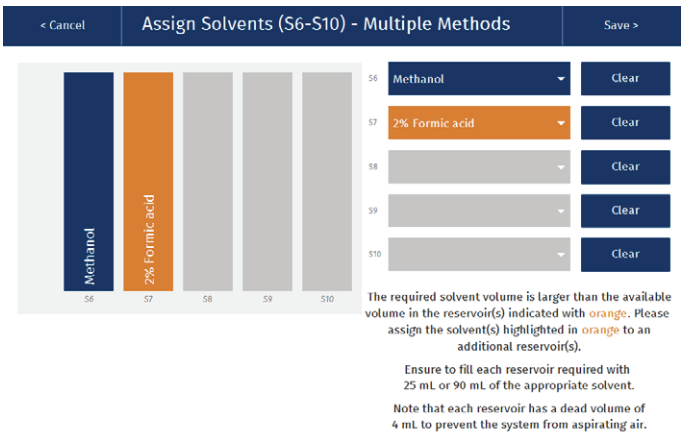


Figure 27. The reservoirs in position 6 have to be filled manually and cannot be refilled during the run. If a reservoir is orange, the total volume of that solvent is less than required.

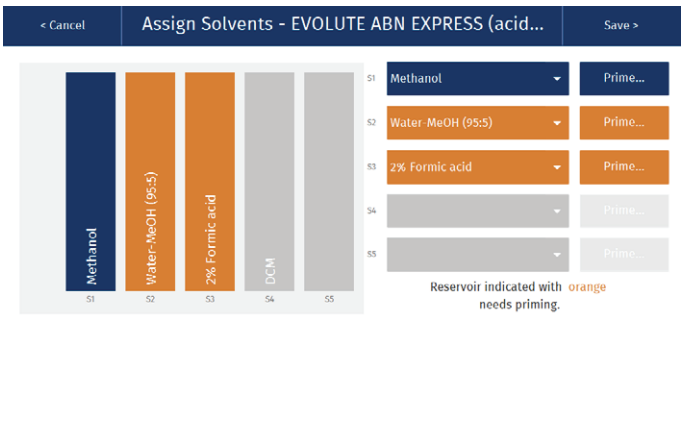


Figure 28. An orange solvent reservoir needs to be primed. A grey reservoir is either empty or not used in this run.

23. Ensure that the solvent feeder is in position, i.e. that it is blue in the **Prepare Run** view. The solvent feeder is orange when pushed back.
24. If using an extra solvent rack, fill it with solvent reservoirs containing the solvents defined in step 19 c and load the rack into position **6** on the working area.

    Ensure to fill each reservoir required with 25 mL or 90 mL (the maximum fill volume for the 100-mL reservoir) of the appropriate solvent.

    Note that each reservoir has a dead volume of 4 mL to prevent the system from aspirating air.

Set Up the Report (Optional)

25. Press the **Report and Comment** field in the **Prepare Run** view.
26. Enable or disable sections in the report in the **Select Report Sections to Include** field; see Figure 29.
27. Add a comment in the report by pressing the **Comment** text box.
28. Enter the name of the report by pressing the **Report name** text box. The name will be used in the **Report** view and in the file name when exporting the report to a USB memory device. If no name is entered, the method name will be used.

Load the Sample Plate or Rack and Start the Run

29. Load your sample plate or rack into position **4** on the working area. Ensure that the correct type of plate or test tubes is used; see the method summary on the right in the **Prepare Run** view.
30. To run, press **Run** in the top pane and then **Run** to confirm.

Note: A method has to be run at least once before an estimated time can be calculated and displayed in the **Running** view; see Figure 30.

Pause or Abort a Run

If you need to pause or abort a run that is in progress, press **Pause** or **Abort** in the **Running** view (see Figure 30). Note that the system will finish the task in progress before it pauses or ends the run. To undo the pause, press **Cancel Pause**.

When the **Pause** dialog opens (see Figure 31), you will be able to open the door and check for clogged wells or columns, and enter a pause comment that will be displayed in the report. The report will also show when and for how long the door was opened. If you wish to resume the run, press **Close Door** and then **Resume**. If you wish abort the run, press **Abort Run**.

When the **Abort** dialog opens, enter an abort comment for the report (if desired) and then press **Restore** to dispose of the pipette tips in use (if any) and restore the system, i.e. return the pipette head, pressure unit, etc., to their home positions.

When the run has been cancelled, please ensure that the correct number of pipette tips are available in the pipette tip rack holders (positions **1** and **2** on the working area) according to the setup in the **Prepare Run** view before starting a new run.

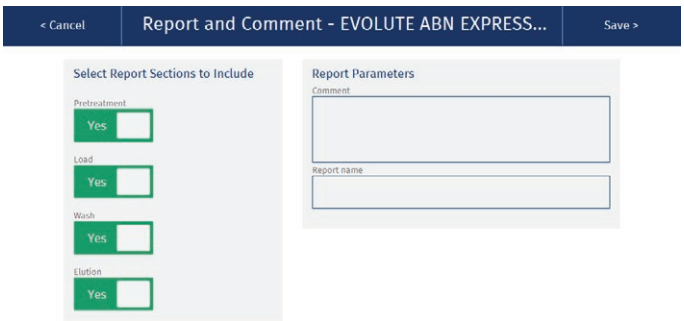


Figure 29. In the Report and Comment view, it is possible to enter the report name, enable or disable sections and add a comment in the report.

Figure 30. The progress of the run is displayed in the Running view. When the run has been completed, it is possible to go directly to the report by pressing View Report.

Unload the Run and Empty Waste

Warning

- All samples and waste should be treated as potentially biohazardous.
- Handle chemical and liquid waste according to the Safety Data Sheets and to local/national guidelines on laboratory safety procedures.

When a run is completed:

1. Remove the used sample plate or rack, extraction plate or column rack, and collection plates or racks.
2. Empty the pipette tip waste bin.
3. If the waste reservoir is full, turn off the vacuum and then empty the waste reservoir.
4. If you are leaving the system, you should also:
   a. Empty the solvent reservoirs and replace them with new, empty ones.
   b. Clean the extraction waste collector and the flow-through plate; see "Clean the Accessories" on page 16.

View and Export Reports

Reports can be viewed, exported, and deleted in the **Reports** view; see Figure 32. Enter the view by pressing **Reports** in the main menu. The reports are organized in folders by month of creation. The reports can be sorted in chronological or alphabetical order within a folder by pressing the **Run Date** or **Report Name** header. To display a report, select it and press **View**.

To save reports as PDF files on a USB memory device, connect the device to one of the two USB ports underneath the touch screen, select the desired reports by setting the check boxes (☑) in front of the reports, and press **Export Selected to USB**. Note that you can select all reports by setting the check box in the header. The file name will be the report name + run date + run time (when started), e.g. EVOLUTE_CX_EXPRESS_20150216_1610.pdf.

To delete reports, set the check boxes (☑) in front of the reports that you want to delete and press **Delete**. Note that all reports will be deleted if you set the check box in the header.

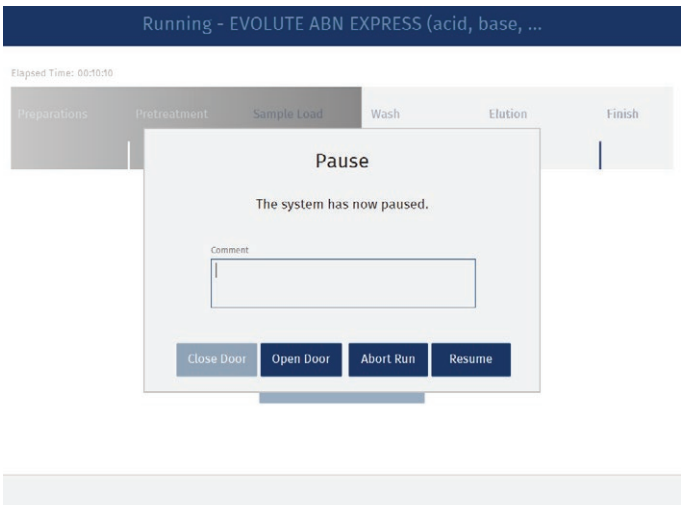


Figure 31. The Pause dialog.

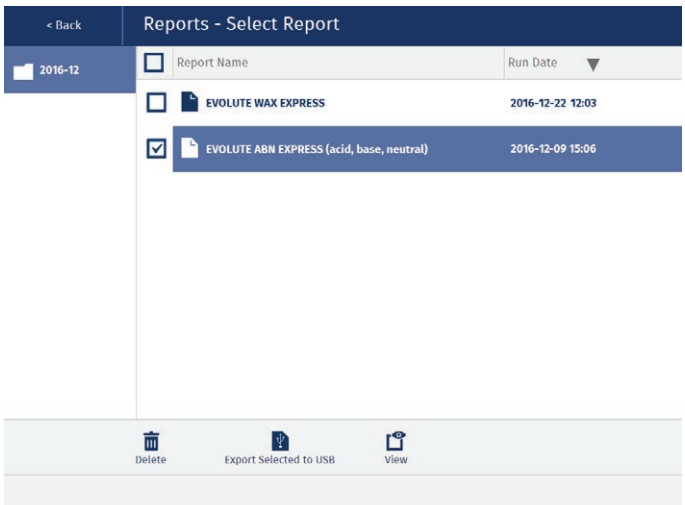


Figure 32. The Reports view. The reports can be sorted in chronological or alphabetical order.

Maintenance

Clean the Exterior of the System

Warning

- Ensure that the system is turned off and the power cord is disconnected before cleaning.
- Never leave solvents, samples, or waste inside the system when the ventilation is turned off.

If the touch screen has been contaminated by chemicals, it must be cleaned immediately.

1. Shut down the system by pressing **Shut Down** in the main menu and then **Yes** to confirm.
2. When the message saying that it is safe to turn off the system appears on the screen, turn off the system and disconnect the power cord.
3. If the solvent rack contains liquid, empty the rack or place it in a fume hood until the system has been switched back on.
4. Clean the touch screen and the exterior of the system, using a soft and clean cloth. The cloth can be dry or lightly dampened with a neutral detergent or alcohol.
5. When the system has been cleaned, connect the power cord and turn on the system.

Clean the Interior of the System

Warning

- Ensure that the system is turned off and the power cord is disconnected before cleaning.
- All samples and waste should be treated as potentially biohazardous.
- Never leave solvents, samples, or waste inside the system when the ventilation is turned off.
- Handle chemical and liquid waste according to the Safety Data Sheets and to local/national guidelines on laboratory safety procedures.

1. Shut down the system by pressing **Shut Down** in the main menu and then **Yes** to confirm.
2. When the message saying that it is safe to turn off the system appears on the screen, turn off the system and disconnect the power cord.
3. If the solvent rack contains liquid, empty the rack or place it in a fume hood until the system has been switched back on.
4. Clean the interior of the system, using a soft and clean cloth. The cloth can be dry or lightly dampened with a cleaning solution that is suitable for the residues. If desired, the carousel can be removed for better access by unscrewing the center knob (see Figure 33). **Note:** Only use water, IPA, or ethanol when cleaning the door and the side walls.
5. Allow the system to dry completely before reconnecting the power cord and turning on the system.

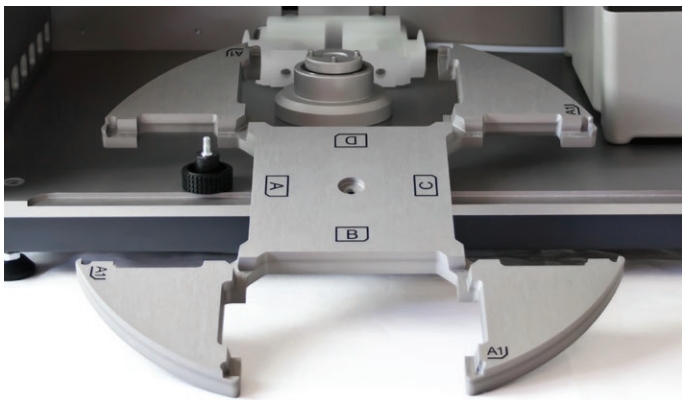


Figure 33. The carousel can be removed for better access when cleaning the interior of the system.

Clean the Accessories

When necessary, clean the column rack (if used), sample rack (if used), solvent rack, pipette tip rack holders, pipette tip waste bin, extraction waste collector, and flow-through plate using a dishwasher program for plastic with a maximum cleaning temperature of 95°C.

To clean without using a laboratory dishwasher, use soap, water and/or ethanol.

Note that the solvent reservoirs used inside the system are disposable.

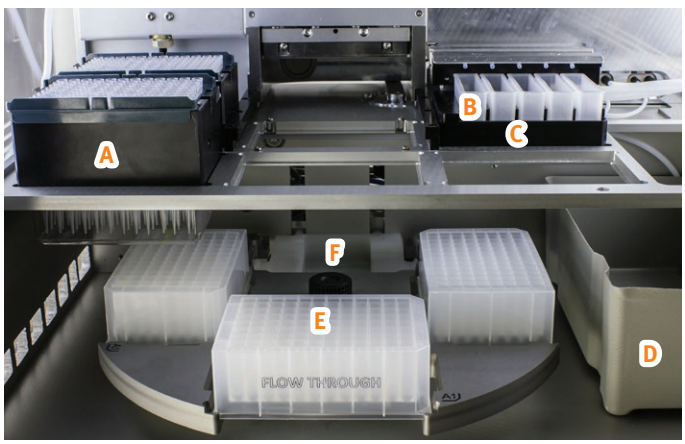


Figure 34. A = pipette tip rack holder, B = solvent reservoir, C = solvent rack, D = pipette tip waste bin, E = flow-through plate, and F = extraction waste collector.

Remove the Extraction Waste Collector

1. Press **Maintenance** in the main menu.
2. If there is liquid in the extraction waste collector or in the waste tubing, ensure that the vacuum is on and then press **Open** in the **Waste Valve** field to remove the liquid. When done, press **Close** and turn off the vacuum.
3. If applicable, close the door by pressing **Close** in the **Door** field.
4. Press **Raise** in the **Extraction Waste Collector** field.
5. Open the door by pressing **Open** in the **Door field**.
6. Remove the extraction waste collector by pulling it straight out.
7. Unscrew the waste tube connected on the right side of the extraction waste collector; see Figure 35.

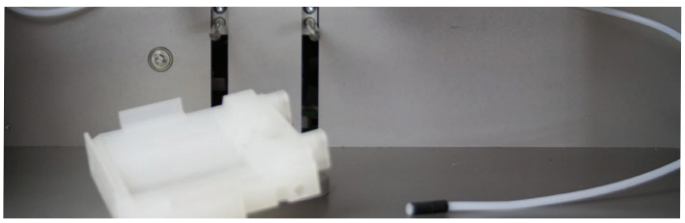


Figure 35. Removing the extraction waste collector. In this image, the carousel has been removed, which is not required.

Replace the Solvent Tubing

Warning

- Ensure that the system is turned off and the power cord is disconnected before replacing the tubing.
- Never leave solvents, samples, or waste inside the system when the ventilation is turned off.
- Use only tubing, nuts, and ferrules supplied by Biotage.
- Use caution when finger-tightening fittings to prevent stripped threads or crushed ferrules.
- Handle chemical and liquid waste according to the Safety Data Sheets and to local/national guidelines on laboratory safety procedures.

1. Empty the solvent inlet lines of liquid by flushing with air:
   a. Remove the solvent inlet lines from their bottles and place them in an empty, clean bottle.
   b. Ensure that you have five empty solvent reservoirs in the solvent rack.
   c. Press **Maintenance** in the main menu and then **Flush Solvent Inlets...**.
   d. Enter the flush volume for S1. 25 mL is required to empty the solvent inlet line and pump of liquid.
   e. Press **Flush** for S1.
   f. When you have finished flushing the inlet line with air, repeat steps d to e for the other solvent inlet lines (S2-S5).
2. Shut down the system by pressing **Shut Down** in the main menu and then **Yes** to confirm.
3. When the message saying that it is safe to turn off the system appears on the screen, turn off the system and disconnect the power cord.
4. Remove the solvent rack and empty it of solvent.
5. Remove the solvent feeder by pulling it straight out.
6. To replace the five solvent inlet lines (see Figure 36), unscrew them from the back wall and mount new ones. Ensure to tighten the screw connectors properly.

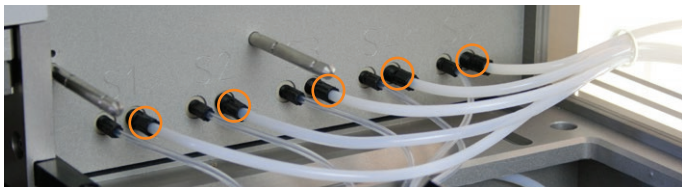


Figure 36. The solvent inlet connections at the back wall.

7. To replace the five solvent tubes between the back wall and the solvent feeder:
   a. Unscrew the tubes from the back wall.
   b. Open the solvent feeder by unscrewing the two screws and remove the old tubes.
   c. Reassemble the solvent feeder with new tubes.
   d. Connect the new tubes to the back wall. Ensure to tighten the screw connectors properly.
8. Put the solvent feeder back in place. Ensure that the tubes are positioned correctly; see Figure 37.

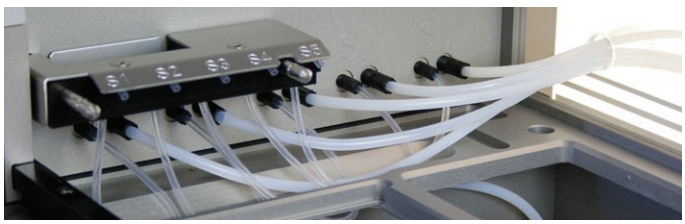


Figure 37. All the solvent tubes in position.

9. Put the solvent rack back in place and pull out the solvent feeder; see Figure 38.

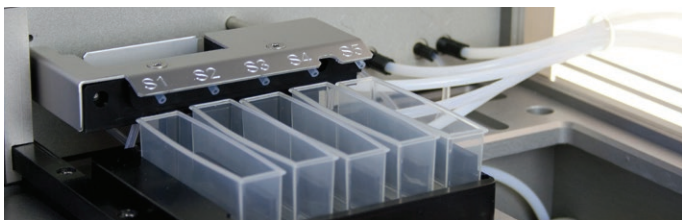


Figure 38. The solvent feeder in position.

10. When done, connect the power cord and turn on the system.

11. Press **Maintenance** in the main menu and check all tubes and connections for leaks using the Flush Solvent Inlets function. If a leak is detected, tighten the solvent's both screw connectors on the back wall. If air is visible in one of the tubes between the back wall and the solvent feeder, tighten the inlet line connector for that solvent.

Note: A solvent that is not cleared from its inlet line (S1-S5) in the **Flush Solvent Inlets** view will appear in the same position in the **Prepare Run** view.

Clean or Replace the Waste Tubing

Warning

- Clean the waste tubing regularly to avoid leakage caused by the tubing getting clogged.
- Use only tubing, nuts, and ferrules supplied by Biotage.
- Use caution when finger-tightening fittings to prevent stripped threads or crushed ferrules.

Clean the Waste Tubing

1. Press **Maintenance** in the main menu.
2. If applicable, press **Open** in the **Waste Valve** field to remove any liquid in the extraction waste collector. When done, press **Close**.
3. Press **Close** in the **Door** field (if applicable), and then **Raise** in the **Extraction Waste Collector** field.
4. Press **Open** in the **Door** field.
5. Remove the extraction waste collector by pulling it straight out.
6. Unscrew the waste tube and put it in a container with a cleaning solution that is suitable for the residues.
7. Press **Open** in the **Waste Valve** field to clean the waste tubing. When done, press **Close**.
8. Put the waste tube and extraction waste collector back in place and remove the container with the cleaning solution.

Replace the Waste Tubing

1. If there is liquid in the extraction waste collector or in the waste tubing, ensure that the vacuum is on, press **Maintenance** in the main menu and then **Open** in the **Waste Valve** field to remove the liquid. When done, press **Close**.
2. To replace the waste tube connected between the extraction waste collector and the waste valve (see A in Figure 39):
   a. Press **Maintenance** in the main menu, press **Close** in the **Door** field (if applicable), and then **Raise** in the **Extraction Waste Collector** field.
   b. Press **Open** in the **Door** field.
   c. Remove the extraction waste collector by pulling it straight out.
   d. Unscrew the waste tube and replace it. Ensure to secure the tube in the correct position using the holders/clips above the extraction waste collector; see Figure 39.
   e. Put the extraction waste collector back in place.
3. To replace the waste outlet tube connected between the waste valve and the waste reservoir (see B in Figure 39):
   a. Turn off the vacuum.
   b. Release the pressure in the tube by pressing **Maintenance** in the main menu and then **Open** in the **Waste Valve** field.
   c. If applicable, press **Open** in the **Door** field.
   d. Unscrew the tube and replace it.
   e. Press **Close** in the **Waste Valve** field.
4. When done, put the solvent rack back into the system, connect the power cord, and turn on the system.

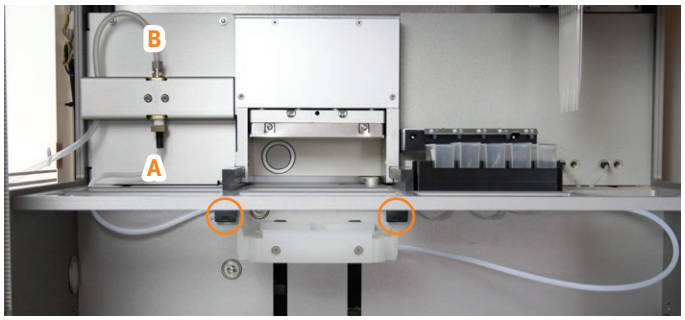


Figure 39. The two waste tubes (A and B). Note the two tube holders/clips above the extraction waste collector.

Clean or Replace the Pressure Head Seal

1. If applicable, open the door by pressing **Maintenance** in the main menu and then **Open** in the **Door** field.
2. Remove any plates/racks located in position **3** and **4** on the working area.
3. Remove the two screws holding the pressure head to the pressure unit; see Figure 40.

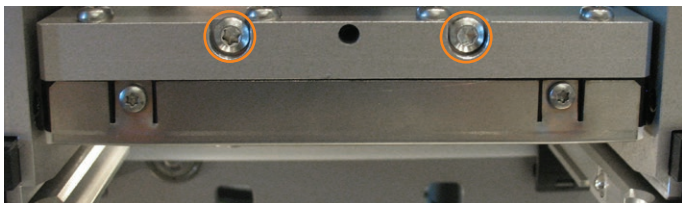


Figure 40. The two screws attaching the pressure head to the pressure unit.

4. Remove the pressure head by pulling it down and then pulling it out.

   Note: If you have a system with only one gas tube connected to the pressure head, you have to tilt the pressure head horizontally to be able to pull it out.
5. Disconnect the gas tubes from the pressure head by pushing in the collar of each connector against the fitting and pulling the tubing out.
6. Put the pressure head on a clean and lint-free surface.
7. Pull off the seal.

8. Either clean the seal using ethanol or similar and put it back in place, or replace it.
9. Reconnect the gas tubes. Ensure that the tubes are properly fastened by pulling on them. Ensure that the tubes are on the left side of the bracket in Figure 41.
10. Put the pressure head back in place using the two screws.

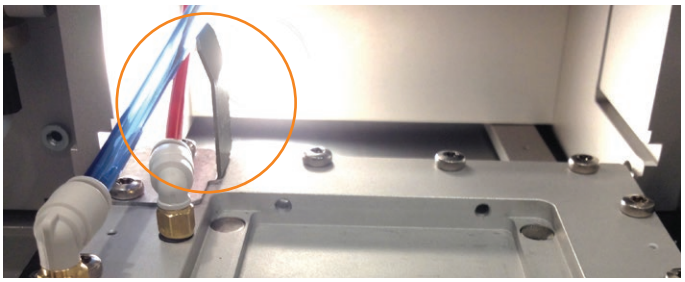


Figure 41. Ensure that the gas tubes are on the left side of the highlighted bracket.

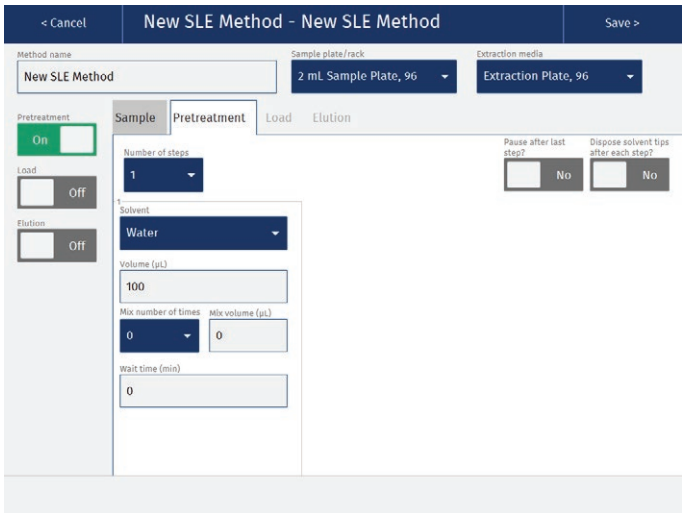


Figure 42. A calibration method for 100 µL when using a 96-format system.

Pipette Pump Calibration and Adjustment

You need a 24-position sample rack or a 96-well sample plate, the appropriate number of tubes for individual weighing (see "Calibrate"), a scale with 0.1 mg readability, and deionized water.

Set Up Calibration Methods

Set up a calibration method for each calibration volume as described below. Use calibration volumes that are appropriate for your applications, e.g. 100 and 1000 µL.

1. Press **Data Administration** in the main menu.
2. Set up a 24-position sample rack or 96-well sample plate with tubes for individual weighing with the appropriate values for the aspiration and dispensation heights. For more information, see "Manage Sample Plates and Racks" on page 6.
3. Press **Manage Methods** in the main menu.
4. Set up an SLE method for each calibration volume containing only a pretreatment operation using the default water and the sample plate or rack created in the previous steps; see Figure 42. To be able to run the method, you will also have to select a sample type in the **Sample** tab and an extraction plate or column rack of the appropriate format in the **Extraction media** drop-down list.

Calibrate

Calibrate an aspiration volume as described below. If the accuracy is not within specification, adjust as described in "Adjustment" on page 20 and then calibrate again. If the precision is outside the specification, see "Pipette Pump" on page 22.

1. Weigh empty tubes and place them in the sample plate or rack. We recommend that you use 12 tubes for each pipette nozzle, i.e. in total 48 or 96 tubes.
2. Press **Run Single Method** in the main menu.
3. Select the method for the volume to be calibrated and press **Prepare Run** in the top pane.
4. Press the illustration of the sample plate or rack, enable the **Use all columns** option or select e.g. every second column and run the plate/rack in several runs (see note below), and then press **Save** in the top pane.

   Note: To minimize evaporation, the plate/rack can be divided into several runs.
5. Prime the solvent pump until water comes out of the solvent feeder:
   a. Press the illustration of the solvent reservoirs.
   b. Press **Prime...** for the solvent inlet line that contains the water.
   c. Ensure that the solvent reservoir is empty. If not, replace it with a new, empty one.
   d. Enter the prime volume. 15 mL is required to fill the solvent inlet line and pump with water. Note that the solvent reservoir can contain a maximum of 25 mL.
   e. Press **Prime**.
   f. If necessary, empty the solvent reservoir and prime again. Ensure that there are no air bubbles visible in the tubes. If you have problems with air bubbles, check the screw connectors.
   g. When you have finished priming, press **OK** and then **Save** in the top pane.

   Note that you only need to prime before the first calibration.
6. If desired, enter the **Sample Plate/Rack ID**.
7. Press **Run**. If using a 24-format system and 48 tubes, you need to run the method at least twice.
8. When finished, weigh the individual tubes again and calculate:
   a. Individual volumes. Take temperature into account when choosing the water density value.
   b. Average, accuracy, and coefficient of variation (CV) for the individual channels and the entire set.

9. If the accuracy is outside the specification, adjust the system as described below. The technical specification is ±2.0% at 50 µL and 100 µL, ±1.5% at 500 µL, and ±1.0% at 1000 µL, but it is your responsibility to ensure that the result is within tolerance for your applications.

Adjustment

If the accuracy is outside the specification, adjust the system as described below and repeat the calibration.

1. Press **Maintenance** in the main menu.
2. Press **Calibrate Pipette Pump...**.
3. Set **Desired volume one** to the first calibration volume.
4. Set **Obtained volume one** to the average for the entire set at the first volume calculated in the calibration step above.
5. Set **Desired volume two** to the second calibration volume.
6. Set **Obtained volume two** to the average for the entire set at the second volume calculated in the calibration step above.
7. Press **Calculate**. The calculated calibration coefficient and offset is displayed.
8. If you want to use the calculated values, press **Apply** and then **Save** in the top pane.
9. Verify that the accuracy is within specification by repeating the calibration.

It is possible to restore an old set of calibration coefficient and offset values by selecting the values, pressing **Restore Selected Values**, and then pressing **Save** in the top pane. Note that the coefficient and offset values that are used when performing a calibration are taken into account when calculating new values.

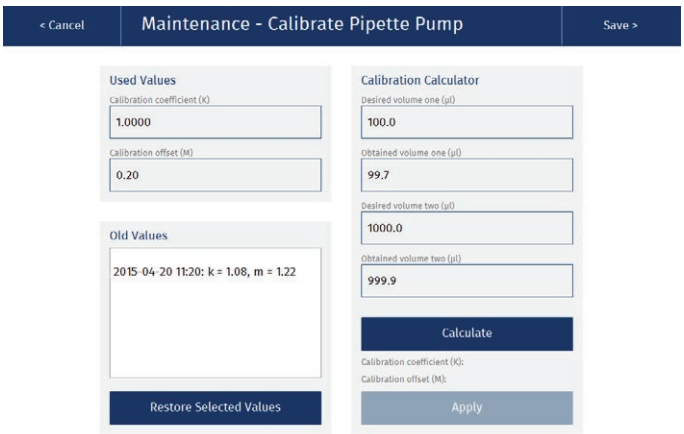


Figure 43. The Calibrate Pipette Pump view.

Replace the Fuses

Warning

- Ensure that the system is turned off and the power cord is disconnected before replacing the fuses.
- Use only exact replacement fuses supplied by Biotage. Incorrect fuses create a potential fire hazard.
- Never leave solvents, samples, or waste inside the system when the ventilation is turned off.

1. Shut down the system by pressing **Shut Down** in the main menu and then **Yes** to confirm.
2. When the message saying that it is safe to turn off the system appears on the screen, turn off the system and disconnect the power cord.
3. If the solvent rack contains liquid, empty the rack or place it in a fume hood until the system has been switched back on.
4. Unscrew the two fuse holders at the power inlet on the right side of the system; see Figure 44.
5. Clean the new fuses using a cloth lightly dampened with ethanol and wipe them dry with a dry cloth.

   Note: Do not touch the metal surfaces with your hands after the fuses have been cleaned.
6. Replace both of the old fuses.
7. Put the two fuse holders back in place.

Note: If the fuses blow shortly after replacing them, please contact Biotage® 1-Point Support™.

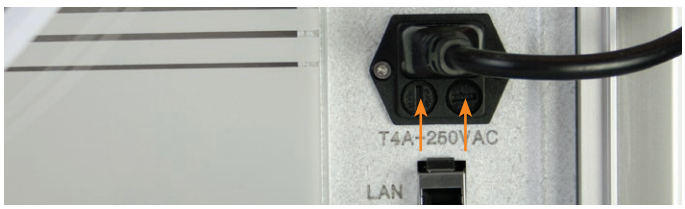


Figure 44. Fuse holders at the power inlet.

Manual Control

If you need to control the system manually e.g. to perform method development, press **Maintenance** in the main menu, press **Close** in the **Door** field (if applicable), and then **Manual Control...**.

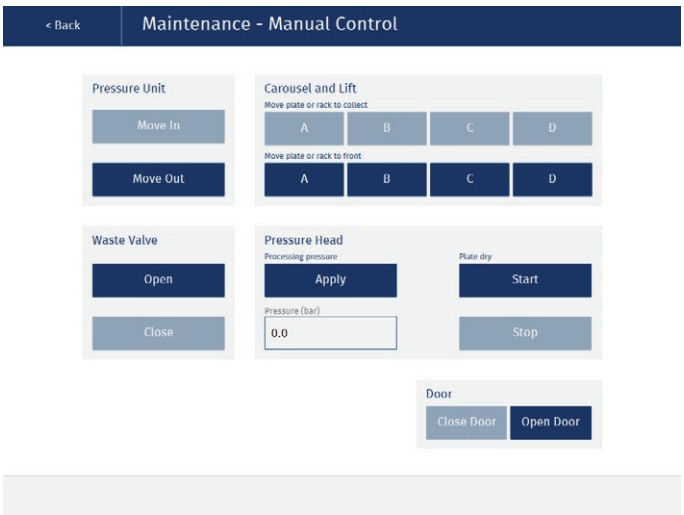


Figure 45. The Manual Control view.

Pressure Unit

To process an extraction plate or column rack, first move the pressure head in position by pressing **Move Out** in the **Pressure Unit** field.

To unload an extraction plate or column rack, press **Move In** in the **Pressure Unit** field. To open the door, press **Open Door** in the **Door** field.

Carousel and Lift

To lift a flow-through plate or a collection plate/rack to the position just underneath the extraction plate or columns, press its position (**A-D**) on the carousel in the **Move the plate or rack to collect** field. Note that the pressure unit has to be in its outer position.

To unload or load a flow-through plate or a collection plate/rack, press its position (**A-D**) on the carousel in the **Move the plate or rack to front** field. This lowers the lift and moves the selected position to the outer position. To open the door, press **Open Door** in the **Door** field.

Waste Valve

When using a flow-through plate, ensure that the vacuum is on and then press **Open** in the **Waste Valve** field before applying pressure.

Pressure Head

To apply a processing pressure with a gas flow of between 0 and 10 mL/min, enter the desired pressure (up to 5 bar) in the **Pressure (bar)** text box in the **Pressure Head** field and then press the **Apply** button. When done, press **Stop**.

To apply a plate dry with a gas flow of approximately 600 mL/min, press **Start** in the **Pressure Head** field. When done, press **Stop**.

Note that older systems (unless upgraded) do not have dual flow and always process at 0 to 10 mL/min.

Troubleshooting

If you need to restore the system to its initial state after startup, press **Maintenance** in the main menu, press **Re-Initialize System**, and then **Yes** to confirm.

Pipette Pump

If you suspect that the pipetting precision or accuracy is incorrect, calibrate and adjust the system as described in "Pipette Pump Calibration and Adjustment" on page 19.

If the precision of the system is outside the specification when measured as described on page 19:

- Check that the correct pipette tip type is used.
- Check that new pipette tips are used.
- Check that the nozzles on the pipette head are securely tightened.
- If the precision is still outside the specification, contact Biotage 1-Point Support.

Pipetting Tips

Check that the pipette tip waste bin has sufficient space for pipette tips being disposed during the run and that the waste bin is correctly positioned with no collision risk for the carousel or the door.

If you see big differences in the volumes being aspirated into the pipette tips, fluid may have entered the pump:

- Contact Biotage 1-Point Support.
- Check that the capacity parameter for the pipette tip is set to the correct value; see "Manage Pipette Tips" on page 6.

If the pipette tips collide with system components or consumables during the process:

- Check that the correct pipette tips are placed in the pipette tip rack holder.
- Check that the sample plate or rack is correctly tuned; see "Manage Sample Plates and Racks" on page 6.
- Check that the extraction plate or column rack is correctly tuned; see "Manage Extraction Media" on page 5.
- Check that the length parameter for the pipette tip is set to the correct value; see "Manage Pipette Tips" on page 6.
- Check that the correct number of nozzles are mounted properly on the pipette head for the current system configuration; eight nozzles are used for the 48/96 format and four for the 24 format.

Power Failure

The system has open solvent reservoirs. If the ventilation fails and solvent vapors are not removed, an explosive environment can be generated. If the system is found with the door closed and the power off, you must ventilate the system properly before turning it back on. Follow the instructions in "Power Failure" on page 3.

Pressure Head

If the pressure head collides with the extraction plate or columns:

- Check that the extraction plate or columns are supported and correctly configured.
- Check that the extraction plate or columns are correctly positioned and level.

Pressurized Air and Nitrogen

If there is a leakage when pressurized air or nitrogen is applied:

- Check that the gas tubing is securely attached.
- Check that the correct gas tubing is used; the outer diameter should be 6 mm and the inner diameter 4 mm. Always use tubing supplied by Biotage.

If the pressure head does not move down or up:

- Check that the **AIR** inlet at the right side of the system is connected to pressurized air or nitrogen and that the pressure is suitable for the method. Use 6 ± 0.2 bar (0.6 ± 0.02 MPa; 87 ± 3 PSI) for a fully populated plate/rack and approx. 4 bar (0.4 MPa; 58 PSI) when populating 50% of a plate/rack and 3 bar (0.3 MPa; 44 PSI) when 25% of a plate/rack.
- Check that all external valves for the incoming air are open.

If the extraction plate or columns do not empty:

- Check that the **N_2** inlet at the right side of the system is connected to nitrogen or pressurized air at 6 ± 0.2 bar (0.6 ± 0.02 MPa; 87 ± 3 PSI).
- Check that the **AIR** inlet at the right side of the system is connected to pressurized air or nitrogen and that the pressure is suitable for the method. Use 6 ± 0.2 bar (0.6 ± 0.02 MPa; 87 ± 3 PSI) for a fully populated plate/rack and approx. 4 bar (0.4 MPa; 58 PSI) when populating 50% of a plate/rack and 3 bar (0.3 MPa; 44 PSI) when 25% of a plate/rack.
- Check that all external valves for the incoming pressurized air and/or nitrogen are open.
- Check that the extraction plate or columns are not damaged.

- Check that the gas tubes are connected properly to the pressure head and that the pressure head seal and tubing is not damaged. See instructions on how to remove the pressure head in "Clean or Replace the Pressure Head Seal" on page 18.
- Check that the correct pressure head is used for the current system configuration, i.e. 24 or 48/96 format.

Robot

If the robot fails to pick up pipette tips or they fall off:

- Check that the correct pipette tip type is used.
- Check that the pipette tip rack holder is in the correct position.
- Check that the pipette tip rack holder and pipette tip rack (delivered with the pipette tips) are not damaged.
- Check that new pipette tips are used.
- Check that the correct number of nozzles are mounted properly on the pipette head for the current system configuration; eight nozzles are used for the 48/96 format and four for the 24 format.

Sample Cross-Contamination

To eliminate the risk of sample cross-contamination, always use a flow-through plate in the waste position (**D**) on the carousel and ensure that the pressure head seal is clean and not damaged (see "Clean or Replace the Pressure Head Seal" on page 18).

Solvent Contamination

If you suspect contamination after changing a solvent:

- Clean the solvent pump and tubing thoroughly using the **Flush Solvent Inlets** function at the **Maintenance** view. Repeat the flush cycle until the pump and tubing are clean.
- If necessary, change the solvent tubing as described in "Replace the Solvent Tubing" on page 17.

Solvent Pumps

If the pumped volume is too low:

- Check the solvent level in the solvent bottle.
- Check that the solvent bottle is placed in level with the system.
- Flush the solvent pump and tubing repeatedly using the Flush Solvent Inlets function at the **Maintenance** view. If this does not help, check that both the screw connectors are securely tightened and flush again.

If solvent leaks from the solvent tubing in the back wall:

- Check that the screw connectors are securely tightened.
- Check that the correct tubing is used. Always use tubing supplied by Biotage.
- Remove spillage; see "Clean the Interior of the System" on page 16.

If solvent leaks from behind the back wall, shut down the system, remove any solvents, samples, and waste inside the system, and contact Biotage 1-Point Support.

Ventilation Fan

If the integral system ventilation fan stops working, shut down the system, remove any solvents, samples, and waste inside the system, and contact Biotage 1-Point Support.

Waste and Lifter

If the system does not evacuate waste:

- Check that the system is connected to a vacuum pump or another vacuum source.
- Check that the vacuum pump or source is operational.
- Check that the vacuum tubing is not blocked.
- Check that the extraction waste collector is not clogged. If clogged, clean as described in "Clean the Accessories" on page 16.
- Check that the tube between the extraction waste collector and the waste reservoir is not clogged or blocked. If necessary, clean or replace the tube as described in "Clean or Replace the Waste Tubing" on page 18.
- Remove any spillage; see "Clean the Interior of the System" on page 16.

If the extraction waste collector leaks, check that the tube between the extraction waste collector and the waste valve is securely tightened.

If the extraction waste collector does not reach its lower position, ensure that nothing is stuck underneath the extraction waste collector.

Switch Between 24, 48, and 96 Format

The system can be configured to process up to 24, 48, or 96 samples during a run.

Hardware Configuration

There are two configuration kits available, one for 24 format and one for 48 and 96 formats. See "Consumables and Accessories" on page 25.

1. Remove any plates/racks located in position **3** and **4** on the working area.
2. Press **Maintenance** in the main menu.
3. If applicable, close the door by pressing **Close** in the **Door** field.
4. Press **Left** in the **Move Pipette Head** field. The pipette head is moved into a position where it can be easily accessed.
5. Open the door by pressing **Open** in the **Door** field.
6. To go from 48/96 to 24 format, remove nozzle A, C, E, and G on the pipette head using the T9 Torx screwdriver supplied with the system; see Figure 46. Place the nozzles in a clean and dust-free container with a lid.

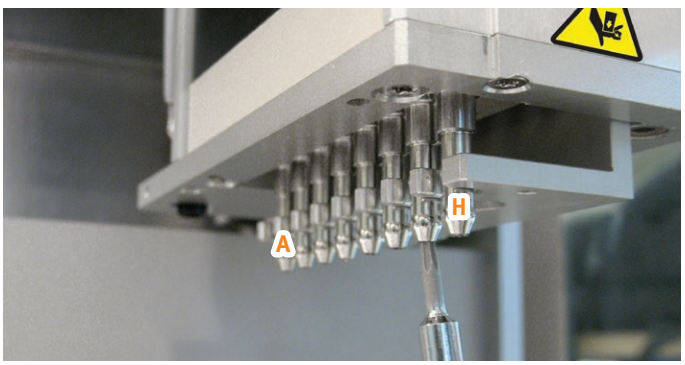


Figure 46. Eight nozzles are used for the 48/96 format and four for the 24 format. Remove or mount nozzels A, C, E, and G using the T9 Torx screwdriver supplied with the system.

7. To go from 24 to 48/96 format, mount four nozzles on the pipette head so that you have a total of eight nozzles.
8. Remove the two screws holding the pressure head to the pressure unit; see Figure 47.

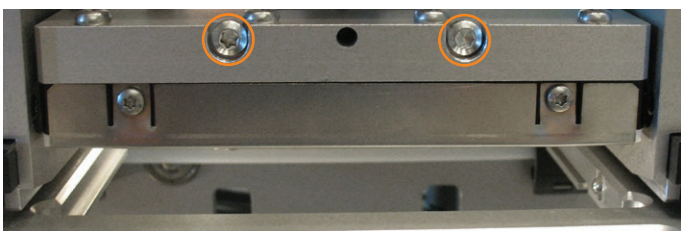


Figure 47. The two screws attaching the pressure head to the pressure unit.

9. Remove the pressure head by pulling it down and then pulling it out.

   Note: If you have a system with only one gas tube connected to the pressure head, you have to tilt the pressure head horizontally to be able to pull it out.
10. Disconnect the gas tubes from the pressure head by pushing in the collar of each connector against the fitting and pulling the tubing out.
11. Put the pressure head in a clean and dust-free container with a lid.
12. Connect the gas tubes to the pressure head of the desired format. Ensure that the tubes are properly fastened by pulling on them. Ensure that the tubes are on the left side of the bracket in Figure 48.

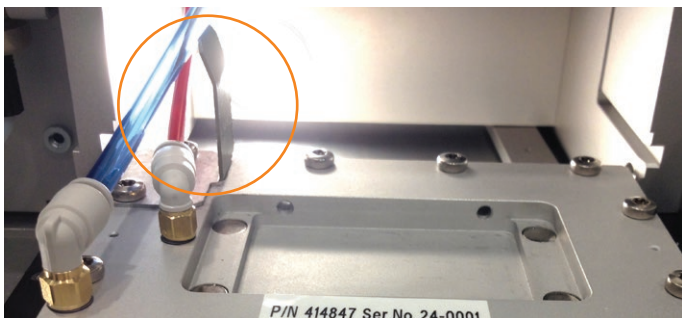


Figure 48. Ensure that the gas tubes are on the left side of the highlighted bracket.

13. Put the pressure head back in place using the two screws.

Software Configuration

1. Press **Maintenance** in the main menu.
2. Select the current configuration of the system (24, 48, or 96 format) from the **Instrument Type** drop-down list.
3. Press **Set** and then **Yes** to confirm.

General Information

Consumables and Accessories

Only genuine Biotage accessories must be used in the system. To order consumables and accessories, see contact information on the back of this document or visit our website www.biotage.com.

Part No.	Description	Qty
414007	Configuration Kit 96 Positions	1
415040	Configuration Kit 96 Positions Dual Flow	1
414008	Configuration Kit 24 Positions	1
415041	Configuration Kit 24 Positions Dual Flow	1
413629SP	Pressure Head Seal 96 Positions	5
414148SP	Pressure Head Seal 24 Positions	5
414253SP	Column Rack 96 x 1 mL (tabless)	1
414169SP	Column Rack 24 x 1 mL	1
414174SP	Column Rack 24 x 3 mL	1
413640SP	Column Rack 24 x 6 mL (tabless)	1
414256SP	Sample Rack 12 x 75 mm 24 Positions	1
414255SP	Sample Rack 13 x 100 mm 24 Positions	1
414254SP	Sample Rack 16 x 100 mm 24 Positions	1
415491	Sample/Collection Rack 12 x 75 mm 24 Positions	1
415585	Sample/Collection Rack 16 x 75 mm 24 Positions	1
415492	Sample/Collection Rack 18 x 75 mm 24 Positions	1
C44651	12 x 75 mm Test Tube	1000
413282	16 x 75 mm Test Tube	1000
414574	18 x 75 mm Test Tube	304
C40707	13 x 100 mm Test Tube	1000
C40708	16 x 100 mm Test Tube	1000
121-5202	96-Well Collection Plate, 1 mL, Square	50
121-5203	96-Well Collection Plate, 2 mL, Square	50
121-5213	96-Well Collection Plate, 2 mL, Round	50
121-5210	48-Well Collection Plate, 5 mL	20
121-5208	24-Well Collection Plate, 10 mL	50
414511SP	Collection Rack 12 x 75 mm 24 Positions	1
414512SP	Collection Rack 18 x 75 mm 24 Positions	1
414578SP	Inserts for 12 x 32 mm vials for Collection Rack 12 x 75 mm 24 Positions	24
413991SP	Solvent Rack for 25 mL Reservoirs	1
414045SP	Solvent Reservoir 25 mL	25
415560SP	Solvent Rack for 100 mL Reservoirs	1
414214SP	Solvent Reservoir 100 mL	5
414330SP	Kit, Solvent Inlet Lines (S1-S5)	5
414579	Solvent Safety Kit (inc. GL45 Caps, Filters and Bottles, Qty 5)	1
413686SP	Tip Rack Holder	1
414141	Biotage Disposable Tips 1000 µL Clear	10x96
414201SP	Flow-Through Plate 96	1
414516SP	Flow-Through Plate 48	1
414203SP	Flow-Through Plate 24	1
414703SP	Spacer for µElution and SPEC Fixed Well Plates	1
414702SP	Matrix Tube retaining Plate	1
414272SP	Waste Kit incl. Waste Reservoir 5 L and Tubing	1
414218SP	Pipette Tip Waste Bin	1
414565SP	Extraction Waste Collector for Lifter	1
414137SP	Waste Tubing	1
411916SP	Fuse, 4A/250VAC, 5x20mm	5
C67361	Mains Cord-Set (EU)	1
C65902	Mains Cord-Set (US/CA)	1
C128195	Mains Cord-Set (UK)	1
356330SP	Vacuum Pump ME1C, 100 to 230VAC 50/60Hz	1

For a complete list, please visit our website www.biotage.com.

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