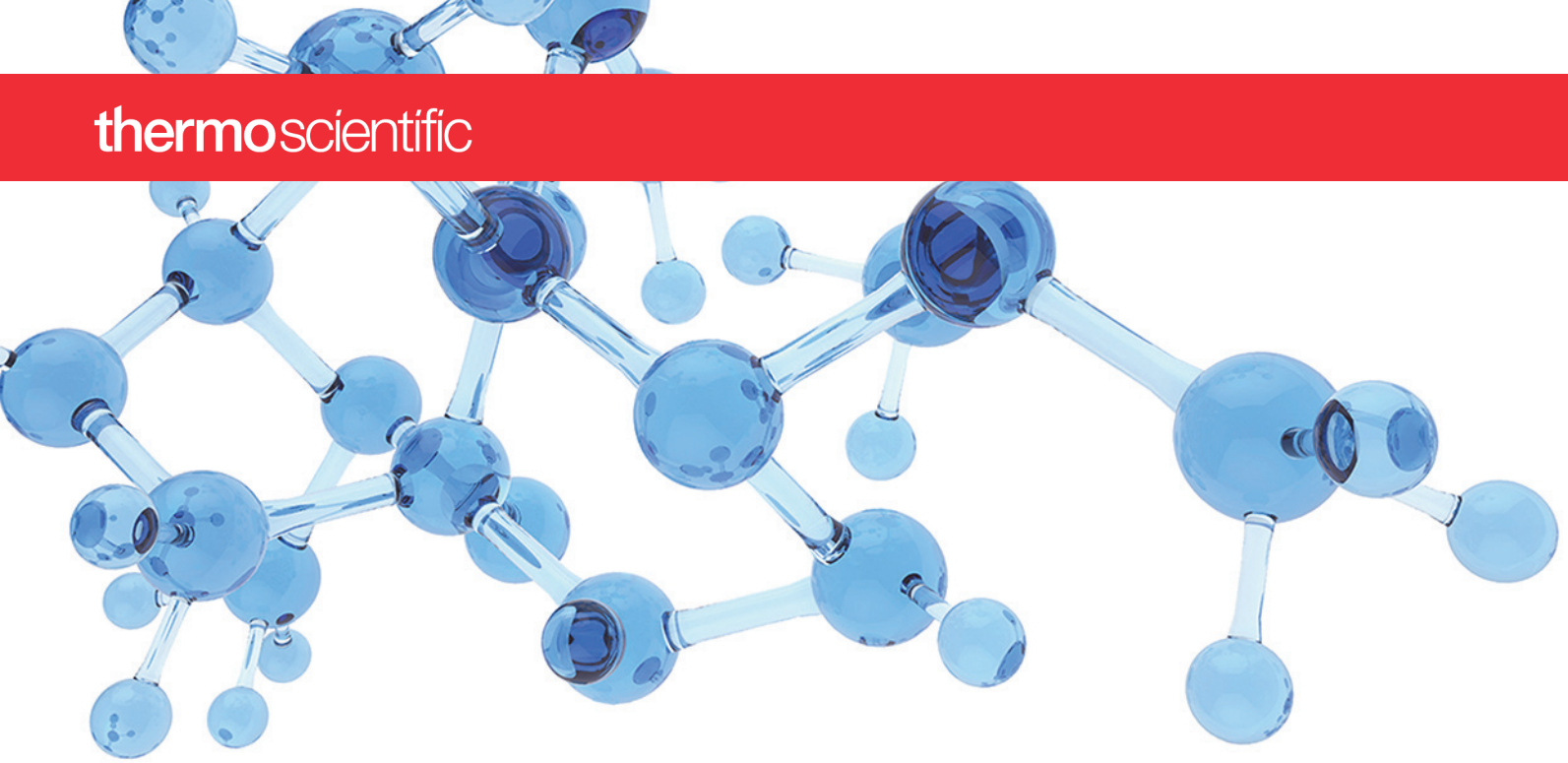
thermoscientific



Prelude LX-4 MD

HPLC for in vitro diagnostic use

Operator Manual

65000-98007 Revision A • July 2020

ThermoFisher SCIENTIFIC

Release history: Revision A, July 2020.

IVD **In vitro diagnostic medical device.**

Regulatory Compliance

Thermo Fisher Scientific performs complete testing and evaluation of its products to ensure full compliance with applicable North American and European regulations. Your system meets the applicable requirements in the electromagnetic compatibility (EMC) and product safety standards described in this section.

Unauthorized changes that you make to your system will void regulatory compliance and may defeat the built-in protections for your instrument. Some examples of unauthorized changes include using replacement parts or adding components, options, or peripherals that Thermo Fisher Scientific has not qualified and authorized. Unauthorized changes can also result in bodily injury and/or damage to your system and laboratory.

Ensure continued compliance with regulatory standards:

- Follow all installation instructions provided in the documentation that comes with your system.
- Order replacement parts (as specified in the instrument manual) and additional components, options, and peripherals directly from Thermo Fisher Scientific or an authorized representative.

EMC

This device has been evaluated by TÜV Rheinland of North America, Inc. and complies with the following EMC standards: IEC 61326-2-6 and subordinate EMC standards, FCC Title 47 CFR 15, Subpart B, Class A: 2015.

FCC Compliance Statement

THIS DEVICE COMPLIES WITH PART 15 OF THE FCC RULES. OPERATION IS SUBJECT TO THE FOLLOWING TWO CONDITIONS: (1) THIS DEVICE MAY NOT CAUSE HARMFUL INTERFERENCE, AND (2) THIS DEVICE MUST ACCEPT ANY INTERFERENCE RECEIVED, INCLUDING INTERFERENCE THAT MAY CAUSE UNDESIRED OPERATION.

CAUTION Read and understand the various precautionary notes, signs, and symbols contained inside this manual pertaining to the safe use and operation of this product before using the device.

Note 1. It is the responsibility of Thermo Fisher to provide equipment electromagnetic compatibility information to the customer or user, if requested by the customer or user.

Note 2. It is the responsibility of the user to ensure that a compatible electromagnetic environment for the equipment can be maintained in order that the device will perform as intended.

Notice on Lifting and Handling of Thermo Scientific Instruments

For your safety, and in compliance with international regulations, the physical handling of this Thermo Fisher Scientific instrument ***requires a team effort*** to lift and/or move the instrument. This instrument is too heavy and/or bulky for one person alone to handle safely.

Notice on the Proper Use of Thermo Scientific Instruments

In compliance with international regulations: This instrument must be used in the manner specified by Thermo Fisher Scientific to ensure protections provided by the instrument are not impaired. Deviations from specified instructions on the proper use of the instrument include changes to the system and part replacement. Accordingly, order replacement parts from Thermo Fisher Scientific or one of its authorized representatives.

Contents

Preface

The Thermo Scientific™ Prelude LX-4 MD™ is a liquid chromatography (LC) instrument that runs high-performance liquid chromatography (HPLC) and ultra-high-performance liquid chromatography (UHPLC). You can run up to four LC channels at the same time by using one detector.

The Prelude LX-4 MD control application controls the Prelude LX-4 MD instrument components, while interfacing with the data processing software. This manual describes how to use the Prelude LX-4 MD control application to manage instrument components and create the LC and autosampler (AS) methods.

Contents

Intended Use

The Prelude LX-4 MD instrument is intended to separate drugs or compounds from a sample solution and introduce these separated drugs or compounds into a detector. It is for in vitro diagnostic use.

Intended Users

To operate the Prelude LX-4 MD instrument for the purpose of running routine samples, you must have experience in general laboratory procedures, knowledge of laboratory safety procedures, and training on the instrument by a qualified instructor. If the instrument might be exposed to biological samples, laboratory safety training must include biohazard safety procedures and precautions. When your work involves chemical hazards, laboratory safety training must include chemical hazard safety training. If you must decontaminate the Prelude LX-4 MD instrument, see "Decontaminating the Instrument" on page 188.

When you use the Prelude LX-4 MD instrument to optimize or create laboratory methods for analyzing sample components, you must have experience developing methods for HPLC instruments, and knowledge of compound interactions with stationary phases and mobile phases.

Related Documentation

The Prelude LX-4 MD instrument includes complete documentation. In addition to this manual, you can access the *Prelude LX-4 MD Preinstallation Requirements Guide* as a PDF file and have two ways to access the Prelude LX-4 MD Help.

❖ To view the product manuals

From the Microsoft™ Windows™ taskbar, choose **Start > All Apps** (Windows 10) **or All Programs** (Windows 7) **> Thermo Instruments > Manuals > *Manual Name***.

❖ To view the Prelude LX-4 MD Help from the Direct Control window

1. From the Microsoft Windows taskbar, choose **Start > All Apps** (Windows 10) **or All Programs** (Windows 7) **> Thermo Prelude LX-4 MD > Direct Control**.

   The Direct Control window opens.

2. Choose **Help > Help**.

❖ To view the Prelude LX-4 MD Help from the instrument method area

1. From the Instrument Method page of the data processing software, click **Prelude LX-4 MD**.

   The LC Method Editor window opens.

2. Choose **Help > Prelude LX-4 MD Help**.

Special Notices, Symbols, and Cautions

Make sure you follow the cautions and special notices presented in this manual. Cautions and special notices appear in boxes; those concerning safety or possible system damage also have corresponding caution symbols. For specific cautionary information and caution label locations, see Chapter 9, "Operating Hazards."

For complete definitions, see Table 1.

Table 1. Notices, symbols, labels, and their meanings (Sheet 1 of 2)

Notice, symbol, or label	Meaning
CAUTION	Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the product.
IMPORTANT	Useful information for efficiently operating the system.
Note	Highlights information of general interest.
Tip	Highlights helpful information that can make a task easier.
	For in vitro diagnostic use.
	Manufacturer
	Serial number
	Consult the instructions for use.
	**Caution:** Observe the operational steps provided to a safely operate the instrument and avoid personal injury.
	**Chemical hazard:** Observe safe laboratory practices and procedures when handling chemicals. Only work with volatile chemicals under a fume or exhaust hood. Wear gloves and other protective equipment, as appropriate, when handling toxic, carcinogenic, mutagenic, corrosive, or irritant chemicals. Use proper procedures to handling wet parts of the instrument.
	**Heavy object:** Never lift or move the instrument by yourself; you can suffer personal injury or damage the instrument. Use suitable lifting equipment as needed.

Table 1. Notices, symbols, labels, and their meanings (Sheet 2 of 2)

Notice, symbol, or label	Meaning
	**Risk of electric shock:** This instrument uses voltages that can cause electric shock and personal injury. Before servicing the instrument, shut it down and disconnect it from line power. While operating the instrument, keep covers on.
	**Hot surface:** Avoid burns. Allow any heated components to cool before touching them.
	**Risk of Puncture:** Sharp component can puncture your skin.
	**Risk of eye injury:** Eye injury can occur from splattered chemicals, airborne particles, or sharp objects. Wear safety glasses when handling chemicals or servicing the instrument.
	**Trip obstacle:** Be aware of cords, hoses, or other objects located on the floor.
	**Biological Hazard:** The instrument might have been exposed to potentially infectious biological hazardous materials. To avoid infection, refer to the biohazard safety precautions described by your company's standard operating procedures. This might include, but is not limited to, wearing protective clothing, and hand, eye, nose, and mouth protection.

Contacting Us

Contact	Email	Telephone	QR Code
U.S. Technical Support	us.techsupport.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752	
U.S. Customer Service and Sales	us.customer-support.analyze@thermofisher.com	(U.S.) 1 (800) 532-4752	
Global support	❖ **To find global contact information or customize your request** 1. Go to thermofisher.com. 2. Click **Contact Us**, select the country, and then select the type of support you need. 3. At the prompt, type the product name. 4. Use the phone number or complete the online form. ❖ **To find product support, knowledge bases, and resources** Go to thermofisher.com/us/en/home/technical-resources. ❖ **To find product information** Go to thermofisher.com/us/en/home/brands/thermo-scientific.		

Note To provide feedback for this document, go to surveymonkey.com/s/PQM6P62 or send an email message to Technical Publications (techpubs-lcms@thermofisher.com).

1

Installation Procedures

A Thermo Fisher Scientific field service engineer installs the Prelude LX-4 MD instrument at your laboratory. Refer to the *Prelude LX-4 MD Preinstallation Requirements Guide*.

During the installation visit, the service engineer performs the following tasks:

- Installs the instrument components that were removed or not installed prior to shipping, including the autosampler injection unit.
- Loads the required applications. If you are using an existing computer, the service engineer loads the applications onto the computer at installation.
- Configures the hardware components by using the Thermo Foundation™ platform.
- Calibrates the hardware component positions as necessary.
- Registers the data processing software and configures it for use with the Prelude LX-4 MD instrument. Without this registration, you can use the application for a limited time.
- Prepares and installs the mobile phases.
- Runs a system verification test.

2

Principles of Operation

This chapter describes the operating principles of the Prelude LX-4 MD instrument.

Contents

The Prelude LX-4 MD instrument incorporates the features described in Table 2.

Table 2. Prelude LX-4 MD instrument features

Feature	Description
HPLC (high-performance liquid chromatography)	Liquid chromatography is a mechanism used to separate the analytes from other components by using interactions between buffered solvents (the mobile phase) and column packing material (the stationary phase). An HPLC instrument uses a pumping device to force the mobile phase through a closed column.
UHPLC (ultra-high-performance liquid chromatography)	The pumps, valves, and connections contain components designed to operate at high pressures up to 1000 bar (15 500 psi). These components allow you to run UHPLC methods, which provide optimal separation and speed.
Cross-sequential optimization	The Prelude LX-4 MD instrument contains four LC channels that can run simultaneously by using one detector, maximizing efficiency.
Method flexibility	Because of the flexibility of the Prelude LX-4 MD instrument, you can develop your own methods to run new, unique tests on the instrument.

HPLC Technology

HPLC is a technology that is used to separate a mixture in solution into its individual components. The separation is based on the samples' components partitioning between the mobile and stationary phases. The mobile phase is made from solvents or water, and the stationary phase is the solid packing material of the analytical column.

Components that favor the conditions of the stationary phase have a stronger interaction with the column packing material, and therefore require a longer time to pass through the analytical column. To elute the analytes and other compounds that bind to the stationary phase, the solvent composition of the mobile phase changes so that the analytes favor the conditions of the mobile phase and flow out of the column.

UHPLC Technology

The Prelude LX-4 MD instrument can use UHPLC columns, which have smaller diameter tubes and particles than columns used in traditional HPLC and provide ultra-high performance. The Prelude LX-4 MD instrument is optimized to withstand the high pressures that are produced when using UHPLC columns.

Cross-Sequential Optimization

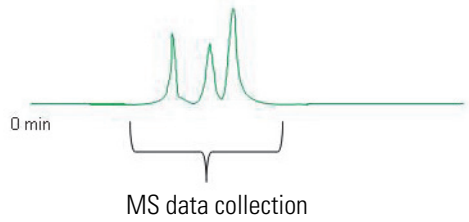
The Prelude LX-4 MD instrument has four LC channels. The channels have separate injectors, tubing, heaters, pumps, columns, and solvents. The channels are synchronized to a single detector. Each channel operates independently, permitting four different assays to run simultaneously.

The cross-sequential optimization on the Prelude LX-4 MD instrument refers to running up to four LC channels simultaneously with staggered sample starts, which brings the productivity of four separate, parallel LC instruments to a single detector. Cross-sequential optimization ensures the maximum performance of your detector with little to no idle time so that you can save money and boost productivity without compromising data quality or sensitivity.

Single-Channel HPLC Instrument

During a method in a single LC instrument, the detector analyzes samples for only a fraction of the total method time. Figure 1 shows an illustration of the data collection time within the method of a single-channel HPLC instrument.

Figure 1. Single LC/MS instrument

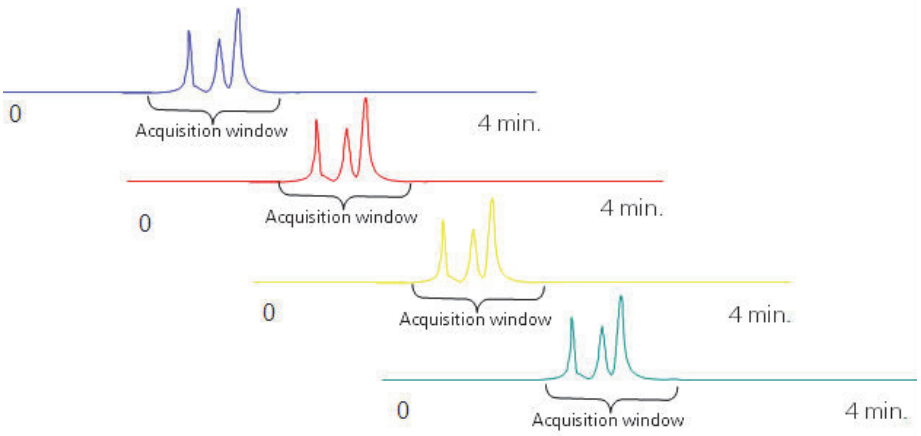


Multiple-Channel HPLC Instrument

With the Prelude LX-4 MD instrument, cross-sequential channels connect to a single detector. Each channel operates independently, permitting four methods to run simultaneously. The Prelude LX-4 MD instrument generates the throughput of four traditional LC/MS systems, while maximizing the productivity of one detector.

Figure 2 shows an illustration of the data collection times when four channels run simultaneously, but with staggered starts so that the data collections do not occur at the same time.

Figure 2. Four LC channels synchronized to a single detector



If one of the channels stops working during a run, samples that are not specifically linked to this channel will continue to run.

3

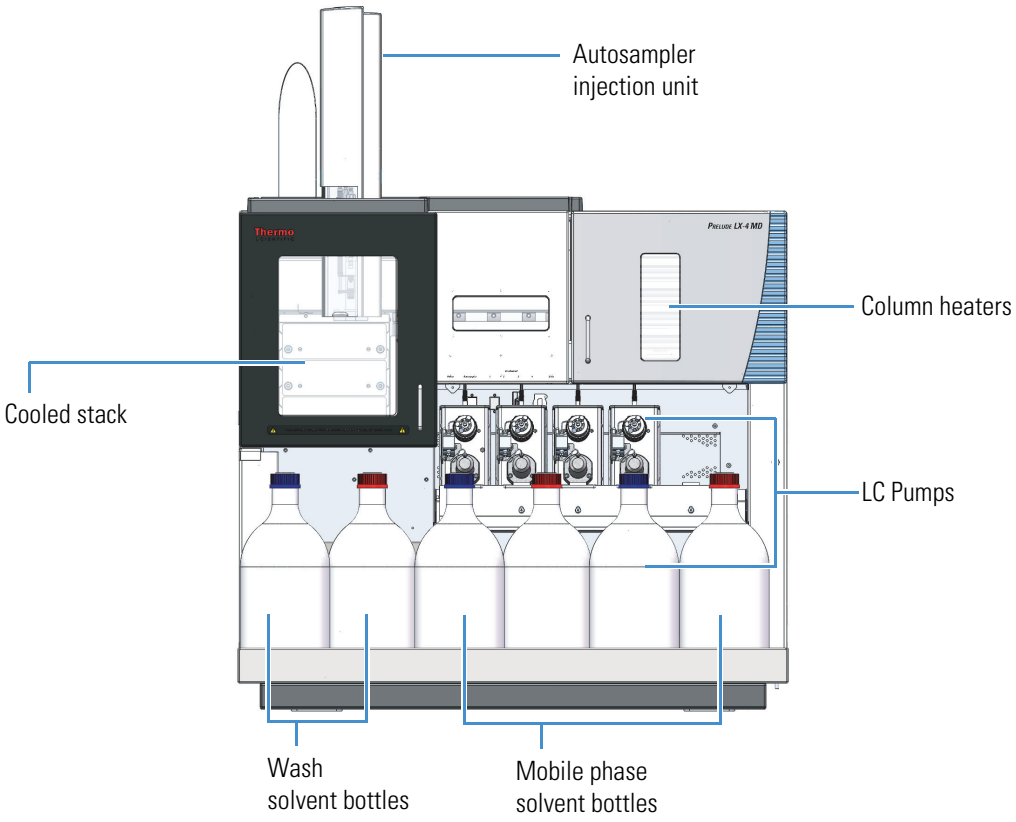
Instrument Components

This chapter describes the instrument components, including the autosampler, solvent bottles, columns, pumps, and control application.

Contents

Figure 3 shows the Prelude LX-4 MD instrument and its components.

Figure 3. Prelude LX-4 MD instrument with components labeled (Model B autosampler shown)



Autosamplers

The autosampler (AS) automates sample handling tasks, such as getting the sample, injecting the sample, and cleaning the syringe and injectors valves.

Note This release of the Prelude LX-4 MD version 1.3 software supports two types of autosamplers, which are referred to as Model A and Model B. For more details, see "Supported Autosampler Models" on page 76.

The following topics describe the components that make up the autosampler:

- Injection Unit
- Cooled Stack
- Wash Stations
- Wash Solvent Bottles
- Injector Valves

Injection Unit

The injection unit contains a 100 µL syringe for withdrawing sample from a sample container and dispensing it into an injection port. The syringe can withdraw 1–100 µL of sample volume. Use the recommended sample volume of 10–80 µL. The withdrawn volume of sample displaces the contents of the loop of tubing, called the sample holding loop, such that sample never enters the syringe barrel.

Cooled Stack

The sample cooled stack contains three drawers. Each drawer can hold up to two sample trays. The cooled stack maintains a temperature between 4 and 40 degrees Celsius. Table 3 lists some sample tray types and their capacities.

Table 3. Sample tray types

Sample tray type	Capacity
2 mL vials	54 vials per tray, two trays per drawer
Well plates	2 plates per drawer (size and number can vary according to your laboratory's requirements)

Wash Stations

The Prelude LX-4 MD instrument has an advanced syringe wash mechanism where the syringe components can be cleaned using minimal plunger movement, reducing the wear on the syringe and on the syringe motor. It uses a wash station at the sample injector, which minimizes time to travel and wear on gears. The wash station uses two wash solutions for maximum efficiency while handling samples.

Wash Solvent Bottles

Two wash bottles store the wash solutions that the instrument uses to wash the autosampler needle and injector ports. One bottle generally holds an aqueous solution and one bottle generally holds an organic solution. A filter is attached to the solvent lines to filter out particulates.

Figure 4 on page 10 and Figure 5 on page 11 show the wash solvent bottle locations on the Prelude LX-4 MD instrument.

Note The wash bottle with a blue cap typically contains the Wash 1 solvent—water or an aqueous solution. The wash bottle with a red cap typically contains the Wash 2 solvent—an organic solvent mixture.

Injector Valves

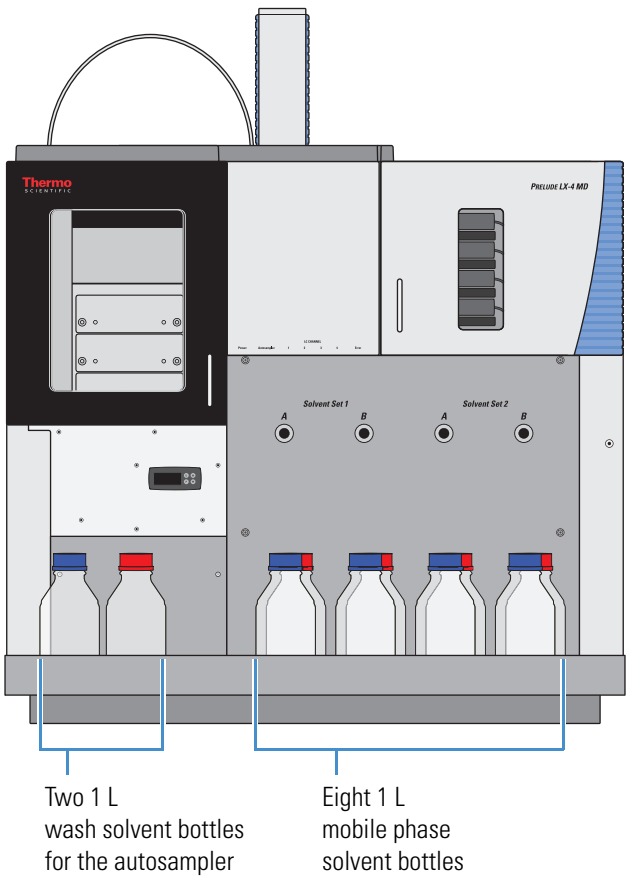
The Prelude LX-4 MD instrument uses four injector valves to inject sample into any one of the four independent LC channels.

The syringe assembly injects the sample into the valve and into a 100 µL sample loop that holds the sample. The valve changes position, which opens the sample loop into the appropriate mobile phase flow where the sample travels to the HPLC column. You can program injector valve wash steps to perform before and after the sample injection.

Mobile Phase Solvent Bottles

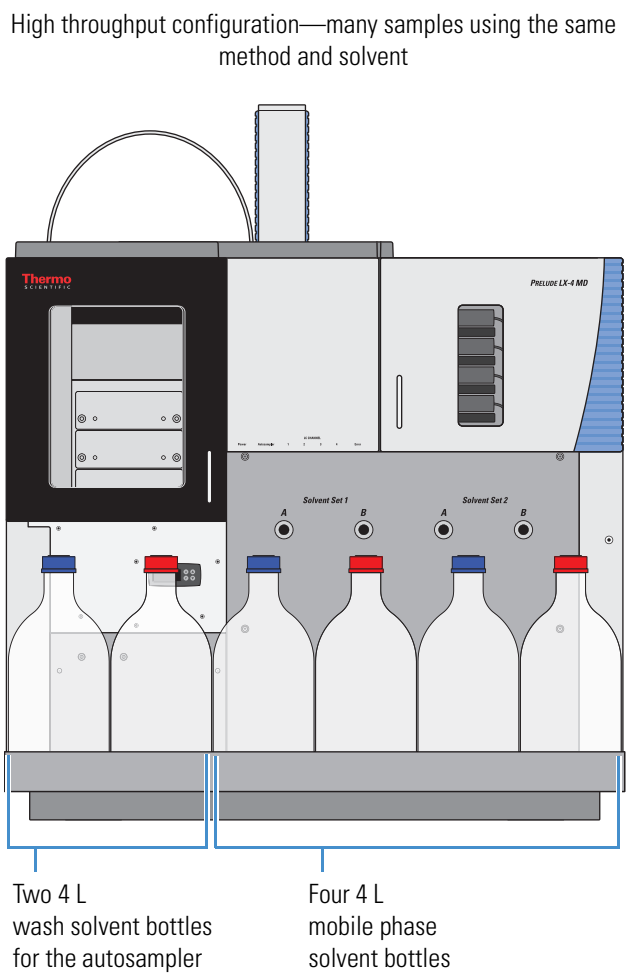
Each channel on the Prelude LX-4 MD instrument can use two mobile phase solutions at any percentage during a method to provide for gradient elutions and flexibility during the method. The instrument comes with eight 1 L bottles for use as mobile phase reservoirs. With this arrangement, each channel can run a different method. See Figure 4.

Figure 4. Prelude LX-4 MD instrument with 1 L bottles (Model A autosampler shown)



Or, the solvent tray can accommodate four 4 L bottles, where channels share a set of solvents A and B. With this arrangement, the system can run with longer run times, but all channels must use the same mobile phases. See Figure 5.

Figure 5. Prelude LX-4 MD instrument with 4 L bottles (Model A autosampler shown)



Note The mobile phase bottle with a blue cap typically contains the mobile phase A solvent—an aqueous solution. The mobile phase bottle with a red cap typically contains the mobile phase B solvent—an organic solvent mixture.

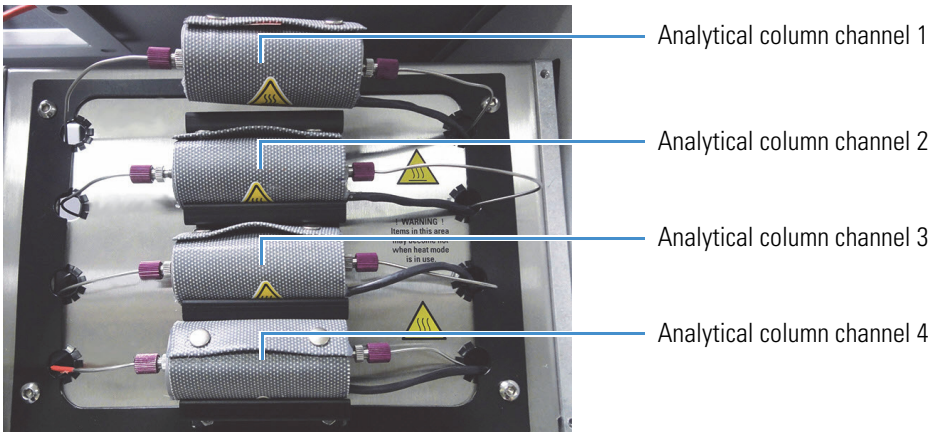
The Prelude LX-4 MD instrument's shipment includes only the 1 L bottles, not the 4 L bottles.

Columns

The instrument uses one HPLC column per channel for separating components in a liquid by using HPLC or UHPLC technology. The columns are available in a variety of chemistries and geometries to better accommodate your methods.

You can use a column heater sleeve, one for each column, to warm the columns to meet your method requirements and to stabilize the column temperature during the run. Figure 6 shows the column compartment with columns and column heater sleeves installed.

Figure 6. Column compartment showing columns with sleeves

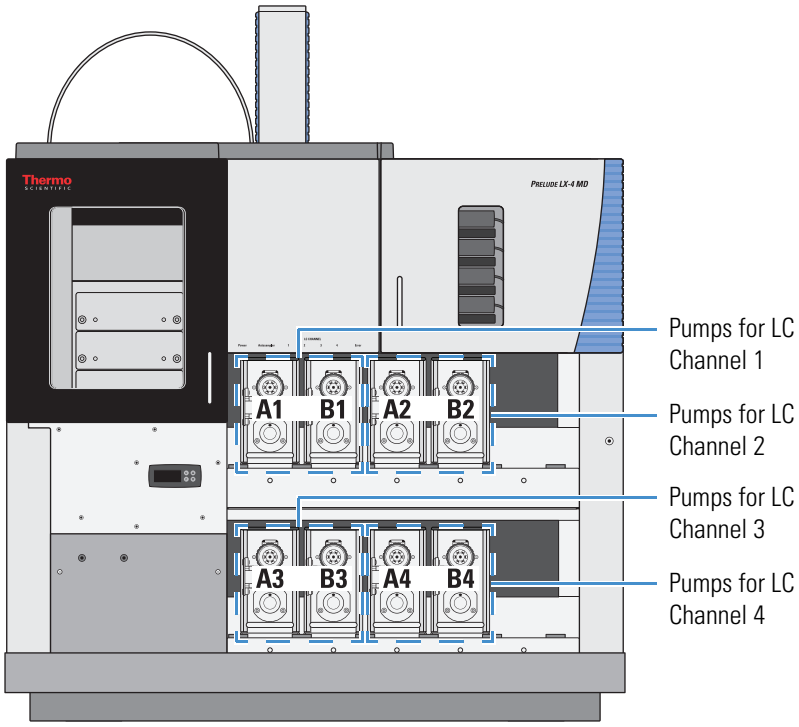


Pumps

The Prelude LX-4 MD instrument contains eight pumps that force the solvents through the HPLC columns, and then to the detector. The pumps can reach pressures up to 1000 bar (15 500 psi). This high pressure, combined with the instrument and column design, provides the instrument environment in which UHPLC is achieved. Each pump operates by using a single piston syringe, which draws 3 mL of fluid (approximately 2.8 mL after compression) and dispenses it according to the method instructions.

The Prelude LX-4 MD instrument contains four LC channels that operate independently. Each LC channel uses two pumps so that each channel can dispense two solvents (A and B). This allows for gradient solvent changes during the method. See Figure 7.

Figure 7. Prelude LX-4 MD instrument showing pump locations behind the solvent bottles



Instrument Control Software

The Prelude LX-4 MD instrument control software controls the LC pumps, valves, and autosampler. You use the Prelude LX-4 MD instrument control software to view the status of these components. You can access the instrument control software from the data processing software.

If you develop a method, you also use the Prelude LX-4 MD instrument control software to create the autosampler and LC method steps. These become part of the instrument method that you create for the LC/MS analysis.

4

Performance Characteristics

The Prelude LX-4 MD instrument is an LC instrument that performs HPLC and UHPLC. It separates sample components prior to analysis on a detector. Figure 8 shows the front and side views of the instrument, and Figure 9 on page 17 shows the instrument's placement on a worktable.

Specifications for the Prelude LX-4 MD instrument follow:

- For dimensions of the instrument and table, see Table 4 on page 19.
- For environmental specifications, see Table 5 on page 19.
- For pump operating specifications, see Table 6 on page 19.
- For electronic specifications, see Table 7 on page 20.

Figure 8. Prelude LX-4 MD instrument dimensions (Model A autosampler chassis)

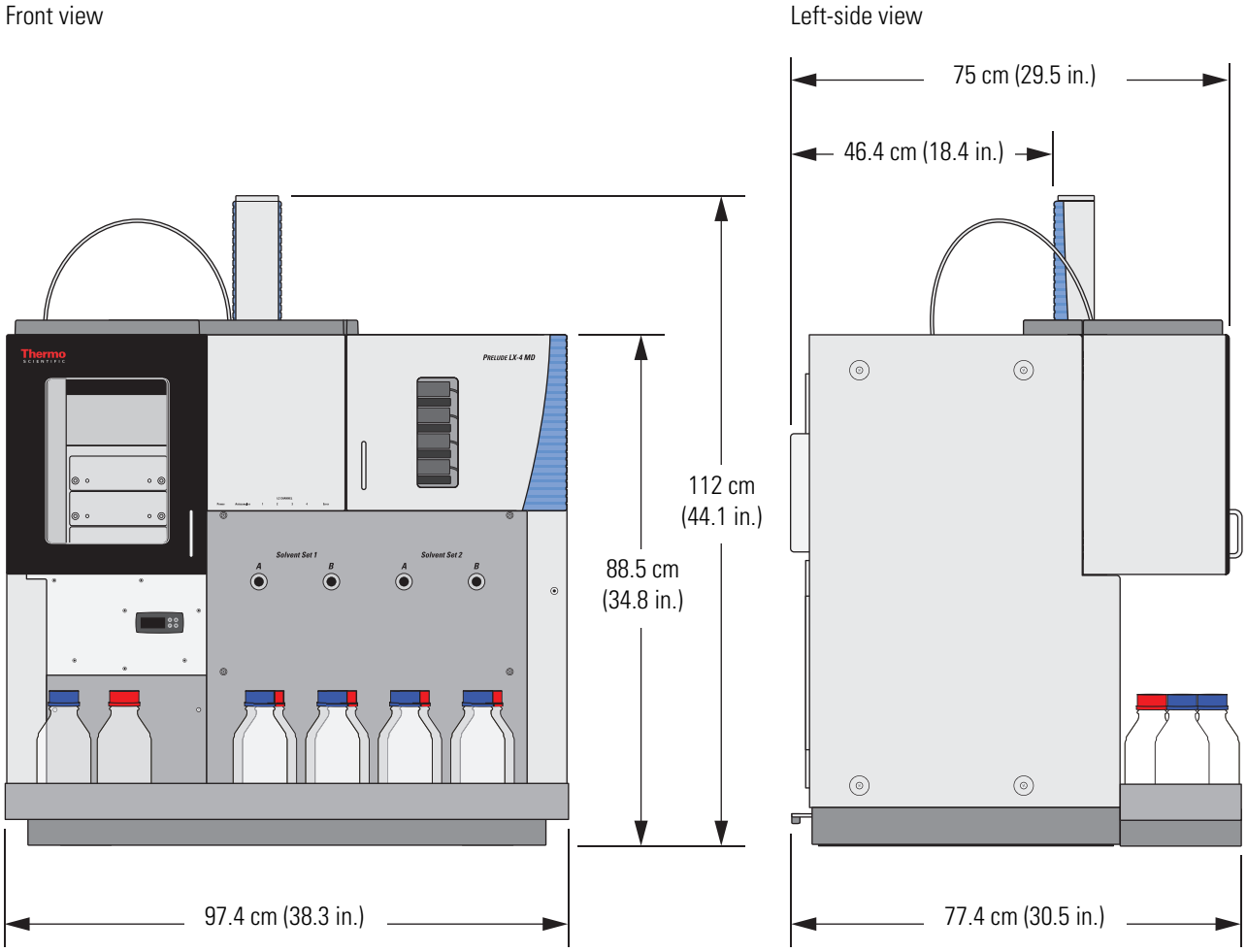
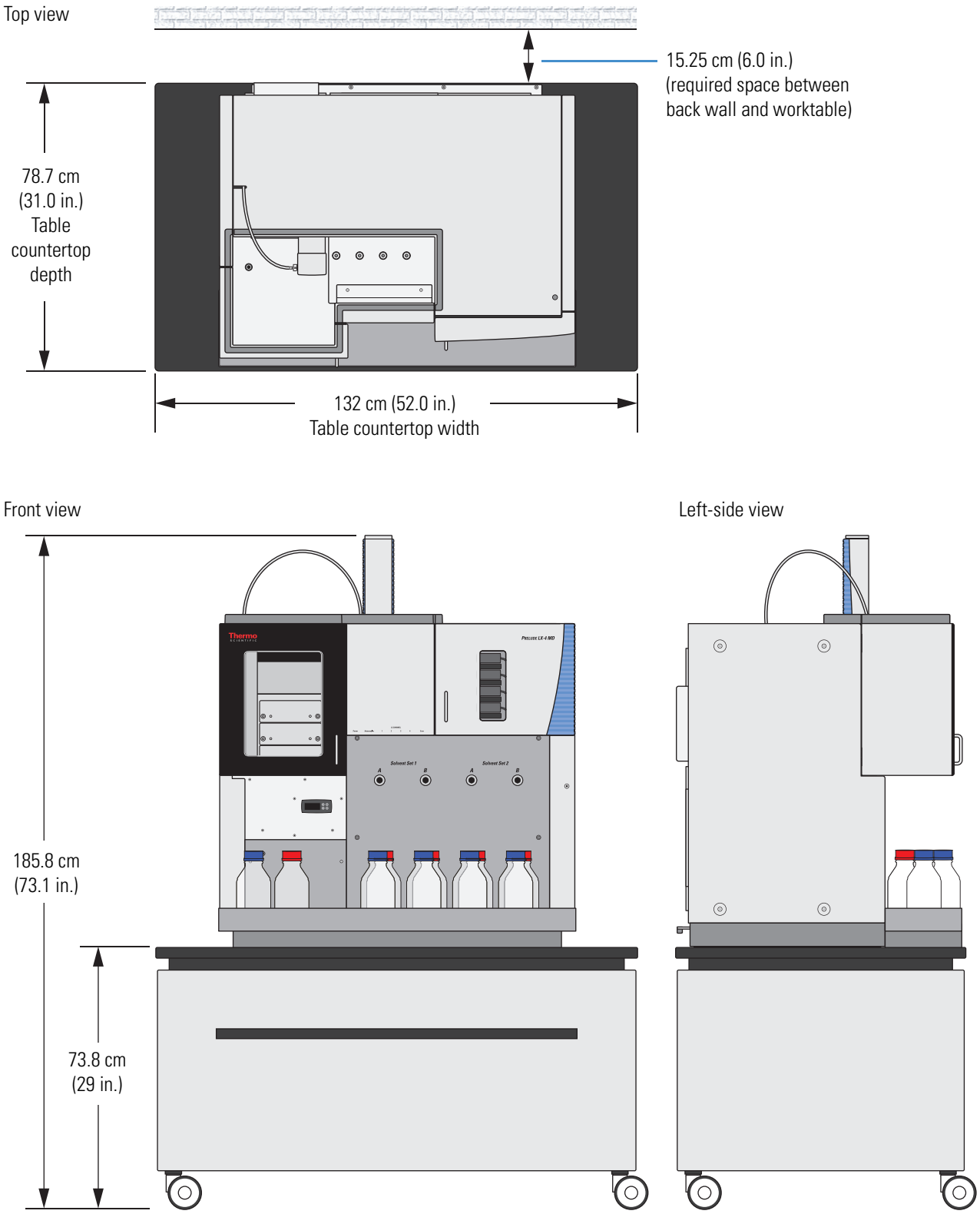
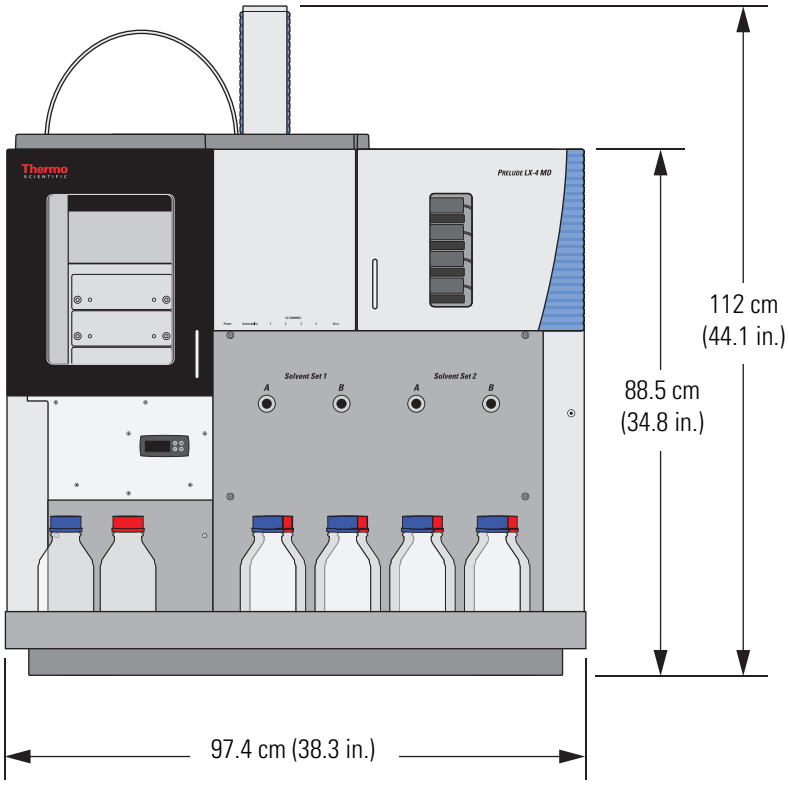


Figure 9. Front, side, and top views with table



Front view



Left-side view

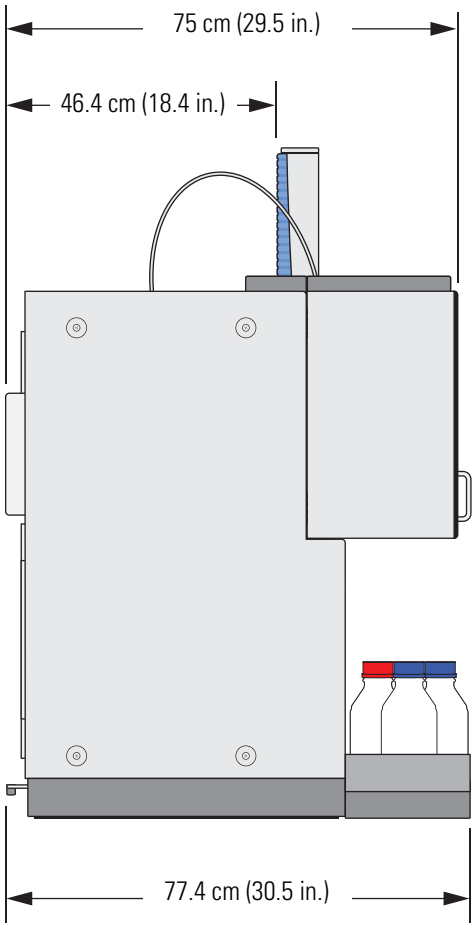


Table 4. Instrument and table dimensions

Component	Dimensions
Instrument (without table) See Figure 8 on page 16.	Model A chassis: 97.4 × 112 × 77.4 cm (*w* × *h* × *d*) (38.3 × 44.1 × 30.5 in.) (*w* × *h* × *d*)
	Model B chassis: 97.4 × 124.3 × 78.8 cm (*w* × *h* × *d*) (38.3 × 48.9 × 31.0 in.) (*w* × *h* × *d*)
Table See Figure 9 on page 17.	132.0 × 73.8 × 78.7 cm (*w* × *h* × *d*) (52.0 × 29.0 × 31.0 in.) (*w* × *h* × *d*) Required space between back wall and table = 15.25 cm (6.0 in.).
Instrument and table See Figure 9 on page 17.	132.0 × 185.8 × 78.7 cm (*w* × *h* × *d*) (52.0 × 73.1 × 31.0 in.) (*w* × *h* × *d*)

Table 5. Environmental specifications or recommendations

Environmental condition	Specification or recommendation
Location	Indoor use only
Relative humidity range	40–80% noncondensing
Temperature	18–27 °C (64.4–80.6 °F)
Altitude (operating)	Sea level to 2000 meters (6560 feet)
Pollution Degree	2

Table 6. Pump operating specifications

Specification	Description
Maximum operating pressure	1000 bar (15 500 psi)
Minimum programmable flow rate	10 µL/min
Flow rate range	10–2000 µL/min
Pressure signal accuracy	0.25% of full scale
Residual pulsation	< 1% for flow > 10 µL/min
Flow rate accuracy	0.5% of set point (optimal range)
Gradient composition accuracy	±0.5% of set point (5–95%)
Gradient composition range	0–100%

Table 7. Electronic specifications

Specification	Description
Communications	LAN and USB
Power	120/230 Vac ±10%, 50/60 Hz, 6.7 A Maximum Voltage fluctuation not to exceed ±10% of the nominal voltage

5

Operating Procedures to Start a Run

This chapter provides a workflow and procedures for running samples on the Prelude LX-4 MD instrument.

Contents

Note The Prelude instrument control software (version 1.3) supports two Prelude LX-4 MD models that use different autosamplers (AS), referred to in the manual as Model A and Model B respectively. For more information, see "Supported Autosampler Models" on page 76.

Getting Started

The procedures in this chapter assume the following:

- You have read and are familiar with the operating hazards for this instrument. See Chapter 9, "Operating Hazards."
- You have calibrated and prepared the detector. For information on maintaining, preparing, and running the detector, refer to the documentation that comes with the detector and data processing software.
- The data processing software contains an instrument method that includes steps for running the Prelude LX-4 MD instrument. To create a new method, create the detector method steps by using the data processing software. Then add autosampler and LC instrument method steps by using the Prelude LX-4 MD control application. See Chapter 7, "Creating an Autosampler Method," and Chapter 8, "Creating an LC Method."

- The data processing software contains templates for running a preview batch, a calibration batch, and an unknown samples batch using your instrument method. For information on creating batch templates, refer to the documentation that comes with the data processing software.

Preparing the Instrument Before Each Run

This topic describes the procedures that you must perform before each run.

Installing Columns

If your instrument does not contain a HPLC column, or a column has expired, see "Replace the Analytical Column" on page 155.

Preparing the Samples

Check that all samples are free of particulates. For best results, centrifuge the sample to remove particulates prior to loading them onto the instrument.

Some laboratories choose to add an internal standard (IS) to each sample. This is a compound that is chemically similar to the analyte, but the detector can distinguished it from the analyte. You can use the IS to monitor or account for sample loss and variations during the HPLC separations.

Sample preparation procedures are often specific to the tests that you want to run. Your laboratory will determine the sample preparation procedures that are required for the tests that you want to run. Refer to your laboratory's standard operating procedure for sample handling tips and procedures.

Preparing the Mobile Phases

Prepare fresh aqueous mobile phases daily, and visually examine all mobile phases for particulates. See "Change the Solvent Bottles" on page 153 and "Prepare the Mobile Phase Solvents" on page 142.

Prime the instrument lines if you installed new mobile phases or the instrument has been idle for more than 24 hours. See "Priming the LC Pumps" on page 182.

Preparing the Channels

Note You can have the instrument automatically trigger channel preparation in situations where the instrument has been idle for a period of time. In the Prelude Configuration dialog box, select Require prep if idle more than *x* hours check box, and then set the number of hours. See "Editing Instrument Settings" on page 63. If you enable this option, simply turning on the instrument through the application layer causes the instrument to prime the pumps.

Use the following procedure to perform the channel preparation procedure manually for each channel that you want to run before each sample run. The instrument performs the appropriate number of primes and the pump seal check.

❖ To prepare the channels manually

1. From the Microsoft Windows taskbar, choose **Start > All Apps** (Windows 10) **or All Programs** (Windows 7) **> Thermo Prelude LX-4 MD > Direct Control**.

   The Direct Control window opens and displays the features that apply to your model of Prelude LX-4 MD (see Figure 10 for Model A and Figure 11 for Model B).

Figure 10. Direct Control window (Model A autosampler)

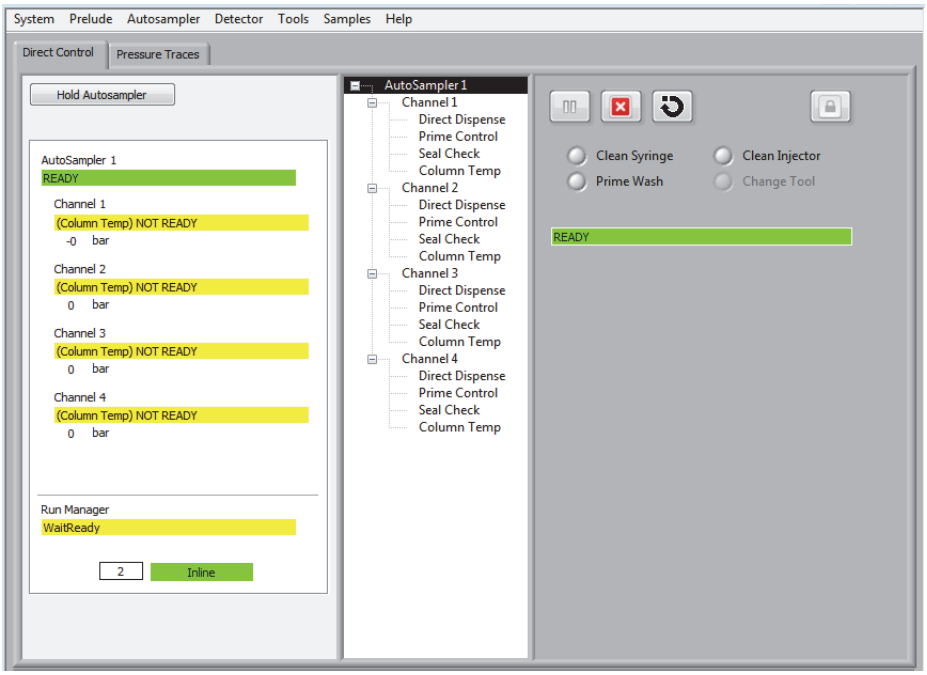
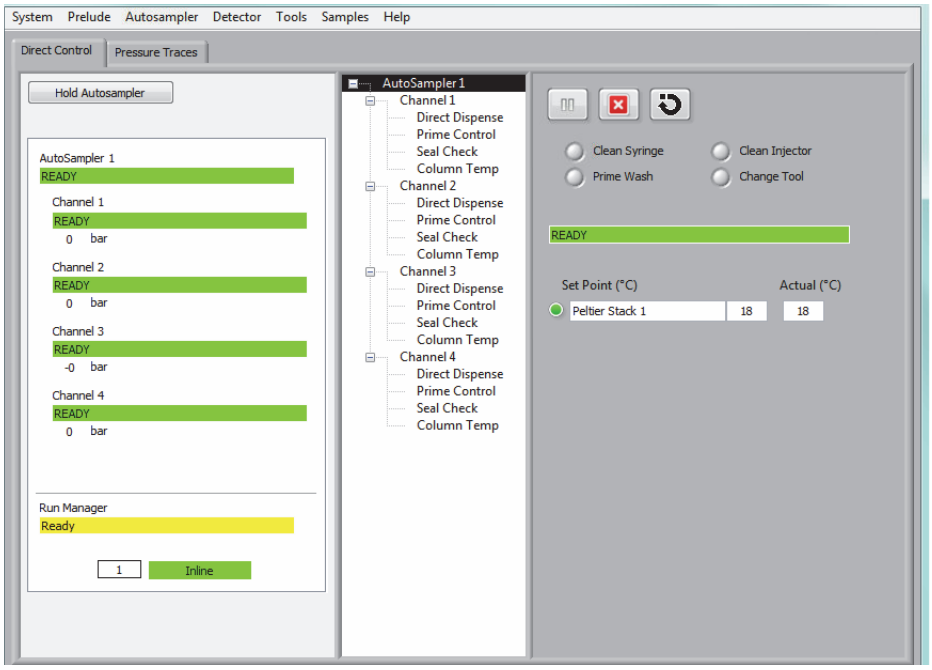
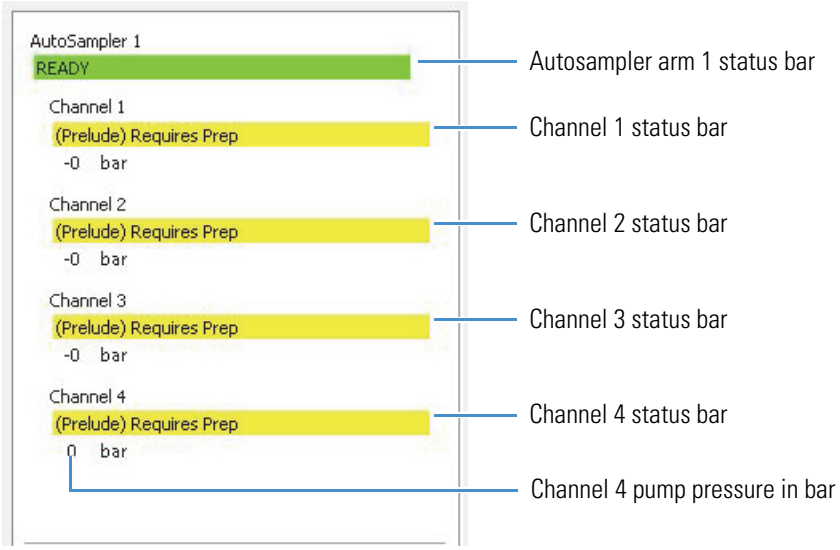


Figure 11. Direct Control window (Model B autosampler)



2. The left Status pane shows the status bars for each channel (see Figure 12).

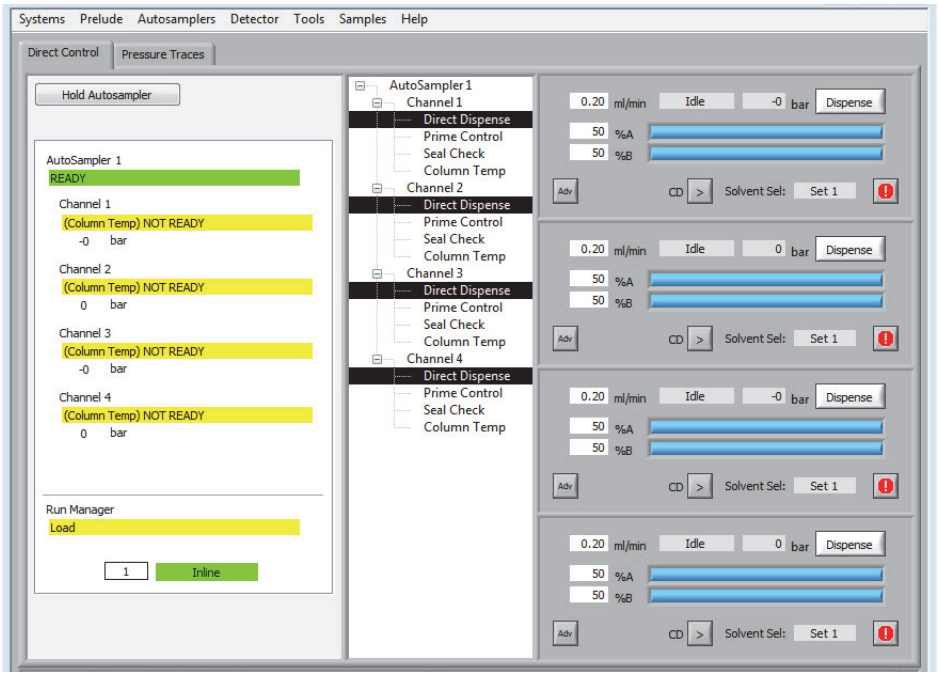
Figure 12. Status pane



3. If the Direct Control window displays the autosampler options as seen in Figure 10 on page 23, click **Direct Dispense** under Channel 1, 2, 3, or 4.

   The Channel status bars appear on the right (see Figure 13).

Figure 13. Direct Control window showing Direct Dispense selected for all channels



4. Verify that the solvent set that is assigned to the channel is the solvent set that you want to use to prepare the channel. See "Assigning Bottle Sets" on page 46.

5. Do any of the following:

   - To prepare all four channels, choose **Prelude > Prepare All**.
   - To prepare one channel, choose **Prelude > Channel *n* > Prepare**.

   The preparation procedure starts.

6. When the procedure is complete, close the window.

Preconditioning the LC Channels

You can precondition all of the LC channels in the system at one time by using the Precondition command.

Precondition the pumps at the start of each new run and when instructed to do so in a maintenance or troubleshooting procedure.

Note This feature preconditions the LC pumps to the initial conditions of a single method for all selected channels.

The Precondition command has two options:

- To Pending—Set the pumps and heaters to the starting conditions of the LC method for the pending sequence queue.

- To Method—Navigate to the instrument method that you want to use. The software updates all of the pump flow rates, compositions, and heater temperatures.

CAUTION Always preheat any column heaters that are in use to prevent column overpressure once flow is started.

Note To avoid the possibility of shutting off any column heaters that might be used in your method prior to running a sequence, set the LC Timeout value to 60 minutes.

❖ **To precondition all of the system LC channels**

1. Open the Direct Control window.
2. Choose **Systems > Precondition >** (either) **To Pending** (or) **To Method**.

   Note The To Pending option automatically loads the starting parameters for the next pending method on each channel if samples are in the sample queue.

3. Turn on the column heaters and wait for the temperature to reach the set point.
4. Start the analysis by doing one of the following:
   - If you are using the To Pending option, start the analysis in the LCMS control application.
   - If you are using the To Method option, submit the sequence that you want to run.

Monitoring the Run

During the run, you can do any of the following:

- View the instrument status. See "Viewing the Status Pane from the Direct Control Window" on page 36.
- Pause the autosampler. See "Controlling the Model A Autosampler" on page 49, or "Controlling the Model B Autosampler" on page 52.
- View the pressure trace. See "Monitoring the Pump Pressure" on page 161.

6

Operating Procedures to Control System Components

Use the procedures in this chapter for direct control of the pumps, valves, column heater, and autosampler through the Prelude LX-4 MD control application rather than through a method.

Note When you run a method, method settings override most direct control settings.

Contents

Accessing the Direct Control Window

See these procedures for using the Direct Control window:

- To access the Direct Control window
- To view the same information for all channels simultaneously
- To view information for individual channels

❖ To access the Direct Control window

1. From the Microsoft Windows taskbar, choose **Start > All Apps** (Windows 10) or **All Programs** (Windows 7) **> Thermo Prelude LX-4 MD > Direct Control**.

   The Direct Control window opens (see Figure 14).

Figure 14. Direct Control window (Model B interface shown)

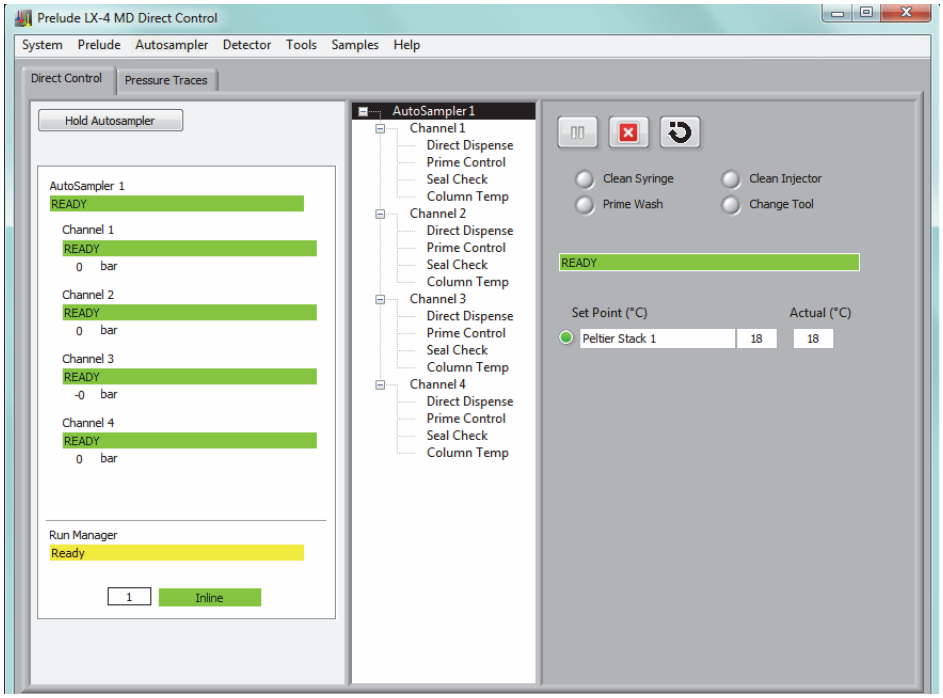


Table 8 describes the features of the Direct Control window.

Table 8. Direct Control window parameters

Parameter	Description
Tabs	
Direct Control	The Direct Control tab contains three panes: to the left, middle, and right. • Left pane—Displays the Prelude LX-4 MD status pane information. See Table 10 on page 36. • Middle pane—Lists these autosampler and channel control options: – Autosampler: Shows the controls for performing autosampler options. See "Controlling the Model A Autosampler" on page 49. – Direct Dispense: Shows the controls for dispensing mobiles phases throughout the system. See "To dispense solvent through the system" on page 40. – Prime Control: Shows the controls for priming the pumps. See "Priming the LC Pumps" on page 182. – Seal Check: Shows the controls and results for the seal check test. See "Performing the Pump Seal Check Test" on page 181. – Column Temp: Shows the column heater controls. See "To change a column heater temperature" on page 61. • Right pane—Shows the controls for the option you select in the middle pane.
Pressure Traces	Displays the pump pressure traces. See "To view the pressure trace in the Direct Control window" on page 163.

Table 9 describes the menu items of the Direct Control window.

Table 9. Direct Control window menu items (Sheet 1 of 4)

Item	Description
Menu bar	
Provides access to more channel and system commands, and system information. The main menu items are Systems, Prelude, Autosamplers, Detector, Tools, Samples, and Help.	
Systems	These commands turn all channels on or off: • All On (Ctrl + O): Turns on the column heaters for all four channels. Turning on the channels also causes the syringes to refill if they are not already full, and might also run the channel preparation if the channels have been idle and you select the Require prep if idle more than *x* hours check box in the Prelude Configuration dialog box. See "Editing Instrument Settings" on page 63. • All Off (Ctrl + X): Turns off all the column heaters for all four channels. See "To turn on or off all LC channels" on page 38.

Table 9. Direct Control window menu items (Sheet 2 of 4)

Item	Description
Prelude	These commands access the options that control all four channels at once or individual channels: • Prepare All: Primes the pump three times and performs a seal check test (by default). See "To dispense solvent through the system" on page 40. You can define the number of primes and the seal check option in the Prelude Configuration dialog box. See "Editing Instrument Settings" on page 63. • Change All Bottles: Initiates the procedure for changing all the solvent and wash bottles. See "To replace the solvent bottles" on page 153. • Prime All: Flows fluid from the solvent bottles to the pumps and then to waste. The fluids do not reach the columns. See "Priming the LC Pumps" on page 182. • Seal Check All: Performs a seal check for all eight pumps. See "Performing the Pump Seal Check Test" on page 181. • Abort All: Stops the pumps for all channels. Stops any AS or LC method that is in progress. See "To turn on or off all LC channels" on page 38 and "To stop the autosampler" on page 51. • Continuous Flow Mode: Use to flow the mobile phase to the detector during the manual loop injection or compound optimization. See "To flow mobile phase to the detector for manual loop injection or compound optimization (Continuous Flow Mode)" on page 43. • All to Bottle Set 1: Changes the active bottle set to Bottle Set 1 for all channels, and dispenses fluids from Bottle Set 1 throughout the systems. See "Assigning Bottle Sets" on page 46. • All to Bottle Set 2: Changes the active bottle set to Bottle Set 2 for all channels, and dispenses fluids from Bottle Set 2 throughout the systems. See "Assigning Bottle Sets" on page 46. • Channel 1, Channel 2, Channel 3, and Channel 4: Use to access options for individual channels. Options are Prepare, Change Bottles, Prime, Seal Check, Bottle Set 1, and Bottle Set 2.

Table 9. Direct Control window menu items (Sheet 3 of 4)

Item	Description
Autosampler	These commands control important operational aspects of the autosampler. The menu options include: • (Model A autosampler) AS Object Viewer: View a list of autosampler objects. See "To change the tray type configuration for a sample tray" on page 52. • (Model A and Model B autosamplers) Configure Trays: Configure tray types for the cooled stack sample cooled stack. See "Configuring the Peltier Stack Drawer Tray Types" on page 57. • (Model A and Model B autosamplers) Prime All Wash Lines: Perform standard daily autosampler maintenance. See "Prime the Autosampler Wash" on page 149. • (Model A and Model B autosamplers) Tool Wash evaluation: Check the autosampler wash pump system. See "Autosampler Wash Pump Evaluation Tool" on page 154. • (Model B autosampler) Update Configuration Data: Updates the Model B autosampler configuration data.
Detector	These commands direct the mobile phase flow through a selector valve or bypass valve. To control the flow through a selector valve, select a channel that is inline with the detector with this sub-menu command: Source. Then choose the system channel that you want to flow to the detector: Channel 1, Channel 2, Channel 3, or Channel 4. The bypass valve directs the flow exiting the selector valve to the detector or to waste. To control the flow through the bypass valve, choose one of these commands: • Inline: Mobile phase flows to the detector. • Bypass: Mobile phase flows to waste. See "To control the bypass valve" on page 49 and "To control the selector valves" on page 48.

Table 9. Direct Control window menu items (Sheet 4 of 4)

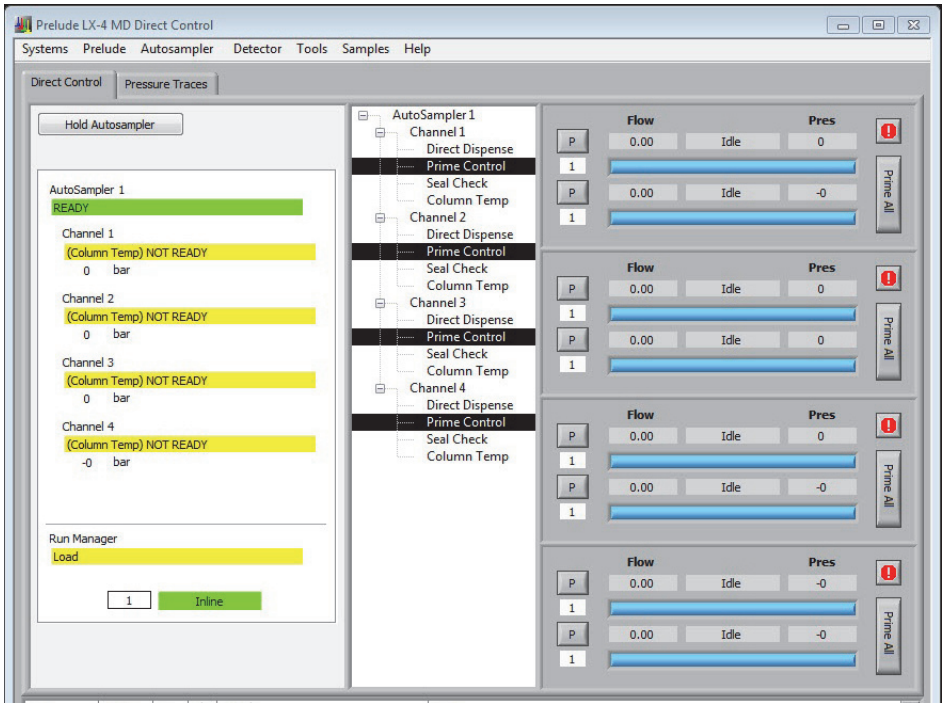
Item	Description
Tools	These commands access the Sequence Log Viewer, Event Log Viewer, autosampler object information, Maintenance dialog box, Options dialog box, and Tree Selection: • Sequence Log Viewer: Navigate to the stored sequences to view the sample list, instrument method, sample event information, and pressure traces. See "Accessing the Sequence Log Viewer" on page 174. • Event Log Viewer: Navigate to stored event logs. See "To view events by the Event Log Viewer" on page 172. • Maintenance: View the total number of injections for each channel and component, and the number of injections for each component since the previous reset. See "Track the Number of Injections—System Counts Tab" on page 143. • Options: Assign the LC Timeout value. See "Changing the LC Timeout Value" on page 62. • Preferences: Customize the displayed pump pressure units. See "Changing the Displayed Pump Pressure Units" on page 42 • Tree Selection: Select whether you want the Direct Control window to display channel information individually or display all channel information simultaneously. Options are Simultaneous and Individual.
Samples	This command accesses Recover Samples, which assigns samples as pending that have been previously skipped due to an autosampler error. This command applies when the On AS Error: Skip Sample and Continue check box is selected. See "Editing Logic Settings" on page 66.
Help	Opens Help for the Prelude LX-4 MD control application.

❖ To view the same information for all channels simultaneously

Choose **Tools > Tree Selection > Simultaneous**.

The Direct Control window shows the same information type for all channels (see Figure 15).

Figure 15. Direct Control showing channel information simultaneously

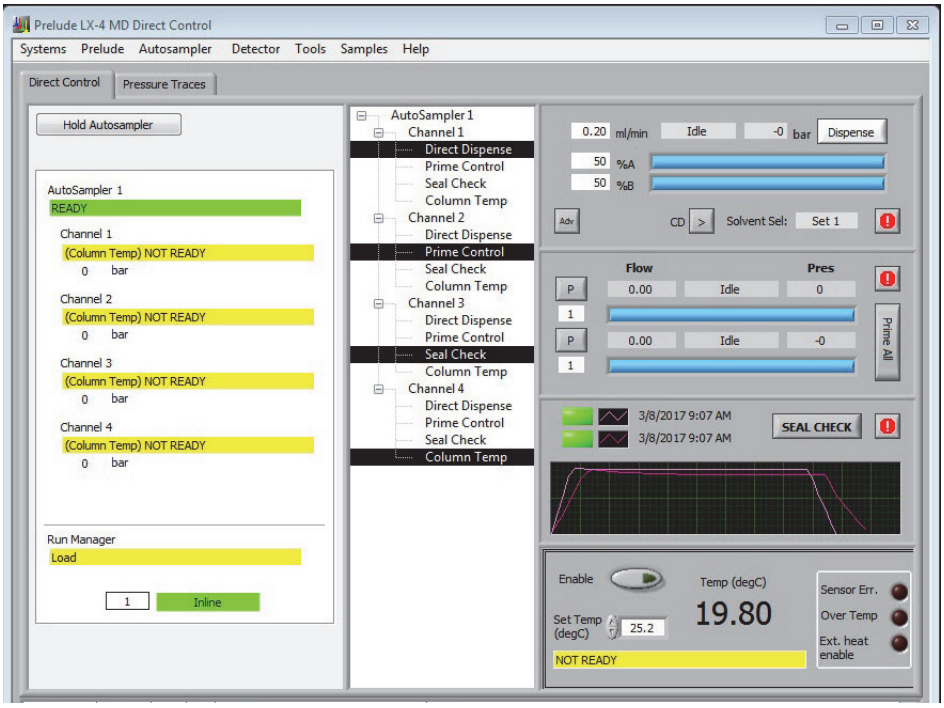


❖ To view information for individual channels

Choose **Tools > Tree Selection > Individual.**

You can select the information that you want to display for each channel. The type of information shown can be different for each channel (see Figure 16).

Figure 16. Direct Control showing different information for each channel



Viewing the Status Pane from the Direct Control Window

❖ To view the Prelude LX-4 MD Status Pane

Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.

The Prelude LX-4 MD Status pane appears at the left side of the window (see Figure 17).

Figure 17. Prelude LX-4 MD Status pane

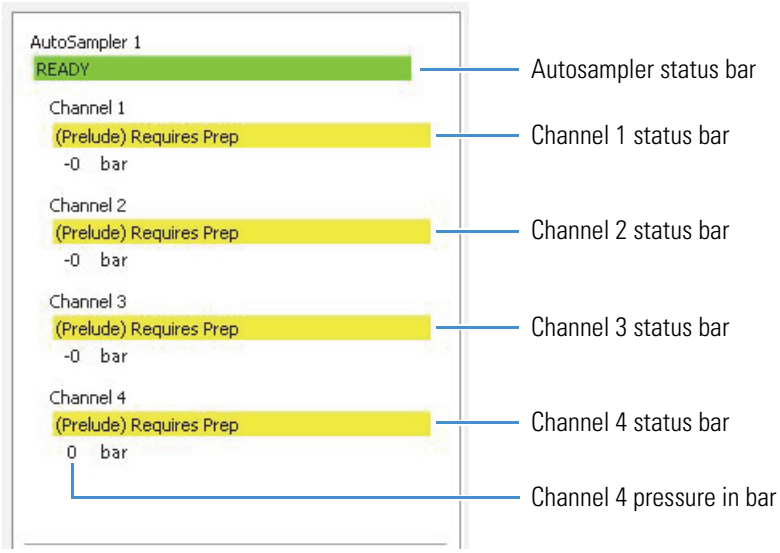


Table 10 describes the Prelude LX-4 MD Status pane.

Table 10. Status pane information and commands (Sheet 1 of 2)

Item	Description
Status bars for autosamplers and channels	The bar color indicates the pump or probe status. A status message appears in the bar to provide specific information on the pump or probe condition. For a definition of the status colors, see Table 11.

Table 10. Status pane information and commands (Sheet 2 of 2)

Item	Description
Channel pressure	The channel pressure appears below the status bar for each channel. It displays the current pump pressure in bar. The channel pressure is measured after the solvent mixing.
Right-click menu	Accessed by right-clicking any of the channel status bars. The menu commands apply to the selected channel and are as follows: • On: Turns on the column heater and changes the column status to Ready. This command also causes the syringes to refill if they are not already full, and might also run the channel preparation if the channels have been idle and you select the **Require prep if idle more than *x* hours** check box, where *x* is a value in hours, in the Prelude configuration dialog box. • Off: Turns off the column heater and changes the channel status to Idle. • Standby: Turns on heaters. • Enable/Disable: Turns off the column heater. The status bar is grayed out. The channel will not run during a method and cannot be selected to perform any task in the Direct Dispense window.

Table 11 describes the channel's status by bar color.

Table 11. Channel status bars

Status	Color	Description
Off line	Gray	The Prelude LX-4 MD control application cannot establish a communication link with one or more channel components—heaters or pumps.
Not Ready	Yellow	Communication between the application and components has been established, but the instrument is not ready to begin. The heaters might not have reached the set temperature. The pumps might be busy or not at the top of stroke.
Ready	Green	The channel is ready to run methods.
Running	Blue	The channel is currently running a method.
Error	Red	A channel component is in an error state.

Controlling the Pumps

Control the system pumps according to the following procedures and sections:

- To turn a channel on or off
- To turn on or off all LC channels
- To turn on or off one or more LC channels
- To access the Direct Dispense area
- To dispense solvent through the system
- To prime the pumps
- Changing the Displayed Pump Pressure Units
- Placing the LC Pumps into Continuous Flow Mode
- Setting a Continuous Flow Mode Timeout
- Setting a High-Pressure Limit for the System LC Pumps

❖ To turn a channel on or off

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. In the left status pane, right-click the LC Channel that you want to turn on or off.

   A list of shortcut menu commands appears.
3. Do one of the following:
   - To turn off the selected LC channel, choose **Disable**.
   - To turn on the selected LC channel, choose **Enable**.

> **Note** The system does not use a channel that is off during a sequence.

❖ To turn on or off all LC channels

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Do one of the following:
   - To turn on all LC channels, choose **System > All On**.

     All column heaters turn on, and the LC status for all channels changes to Ready. The pumps fill the syringe if they are not already full.
   - To turn off all LC channels, choose **System > All Off**.

All column heaters turn off, and the LC status for all channels changes to Standby.

❖ To turn on or off one or more LC channels

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. In the left status pane, do one of the following:
   - To turn on a channel, right-click the status bar of the channel you want to turn on, and choose **On**.
   - To turn off a channel, right-click the channel you want to turn off, and choose **Off**.

     The heater on the selected channel turns off.

❖ To access the Direct Dispense area

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. In the middle pane, under the appropriate channel, click **Direct Dispense**.

   The Direct Dispense information appears (see Figure 18).

Figure 18. Direct Control window showing Direct Dispense information

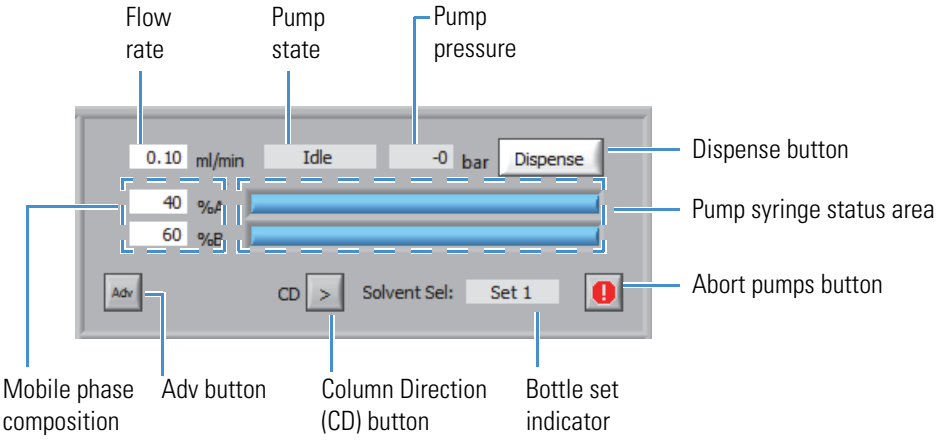


Table 12 describes the parameters in the Direct Dispense area.

Table 12. Direct Dispense area parameters (Sheet 1 of 2)

Parameter	Description
Adv button	Specifies the LC Pump high-pressure limit.
Column-direction valve state (CD button)	Indicates the position of the column-direction valve. Click the CD button to change the direction the fluid flows through the column.
Flow rate	Indicates the flow rate for dispensing the fluid through the channel.

Table 12. Direct Dispense area parameters (Sheet 2 of 2)

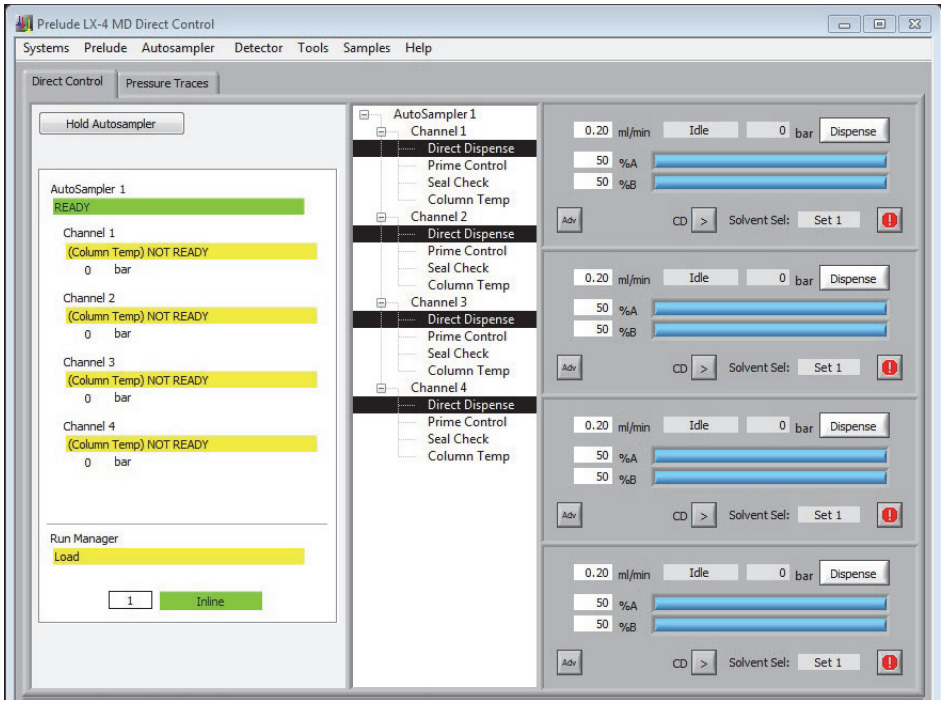
Parameter	Description
Pump state	Indicates the status of the pumps for the channel.
Pump pressure	Shows the pump pressure reading in bar after solvents A and B are mixed.
Dispense (Stop/Refill) button	Click to have the pump start dispensing. The Dispense button changes to a Stop/Refill button. Click **Stop/Refill** to stop dispensing and refill.
Pump syringe status	Indicates the syringe status as it fills and empties during the dispensing procedure.
Abort pumps	Click to cancel the current operation and halt the pump.
Bottle set indicator	Indicates the bottle set that the channel uses to dispense solvent.
Mobile phase composition	Displays the percentage of solvents A and B used for dispensing.

❖ To dispense solvent through the system

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. In the middle pane, under the appropriate channel, click **Direct Dispense**.

   The dispensing information appears (see Figure 19).

Figure 19. Direct Control window showing dispensing information



3. (Optional) To change the column-direction valve, click the **CD** icon,  .

   The valve changes position, which changes the direction of the flow through the column. The Valve icon turns yellow and shows the arrow pointing in the opposite direction,  .

4. Type the appropriate solvent percentage for each solvent in the %A and %B boxes.

5. (Optional) To change the flow rate from the default value, type the flow rate in the mL/min box.

   IMPORTANT Do not exceed the recommended flow rate for the column. Refer to the documentation included with your analytical column.

6. Verify that the solvent set selected is the solvent set you want the system to dispense. See "To determine which bottle set is assigned to a channel" on page 46.

7. (Optional) To change the solvent set that the instrument will dispense through the system, do one of the following:

   - To change the solvent set to solvent 1 for all four channels, choose **Prelude > All to Bottle Set 1**.
   - To change the solvent set to solvent 2 for all four channels, choose **Prelude > All to Bottle Set 2**.

- To change the solvent set to solvent 1 for one channel, choose **Prelude > Channel *n* > Bottle Set 1**.
- To change the solvent set to solvent 2 for one channel, choose **Prelude > Channel *n* > Bottle Set 2**.

8. Click **Dispense**.

   The pump status bar shows the pump syringe dispensing and filling, and the Dispense button changes to a Stop/Refill button.

9. To stop the dispensing, click **Stop/Refill**.

   The pump syringes stop dispensing and begin refilling.

> **Note** If you do not click Stop/Refill, the pumps stop dispensing when the LC timeout limit has elapsed or when you click the Abort button, .

❖ **To prime the pumps**

See "Priming the LC Pumps" on page 182.

Changing the Displayed Pump Pressure Units

You can set the pump pressure units that are displayed according to your laboratory's requirements. Changes in units take effect after you close and then reopen application windows that display pressure values.

> **Note** Pump pressure unit display settings are saved and persist for each Windows user account.

You can configure the following units of pressure displayed in the Direct Control window, Sequence Log Viewer, and all pressure plot windows:

- bar (default setting)
- psi (pounds/square inch)
- kPa (kilopascal)
- MPa (Megapascal)

❖ **To change the displayed pump pressure units of measure**

> **Note** Changes to the pressure units take effect after closing and then reopening application windows that display pressure readings.

1. Open the Direct Control window.
2. Choose **Tools > Preferences**.

   The Preferences dialog box appears.

3. From the Display Pressure Units list, select a unit of measure that you want to display, and then click **OK**.
4. For your changes to take effect, close and restart all open Thermo Scientific applications that display pressure readouts.

Placing the LC Pumps into Continuous Flow Mode

With Continuous Control Mode, the LC pumps run without interruption, moving mobile phase solvents to the detector until they are turned off manually, or until a preset timeout time expires (see "Setting a Continuous Flow Mode Timeout" on page 44).

This feature is useful for mass spectrometer method development tasks and for bakeout operations, such as bringing a system online for the first time, or restarting a system that has been turned off for an extended period.

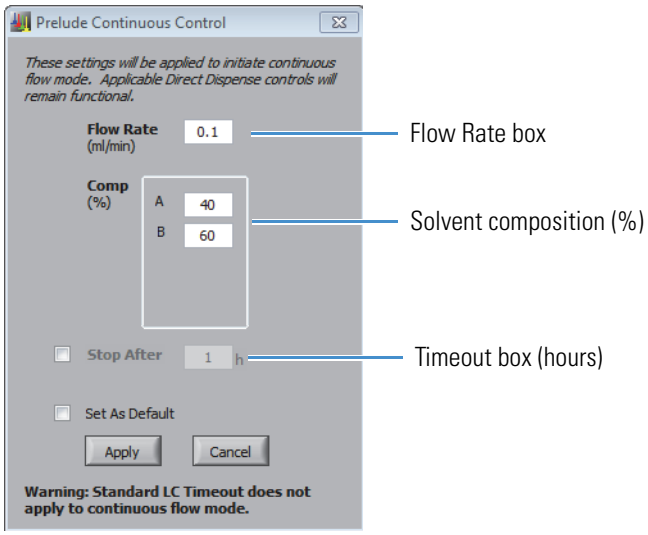
Note Channels 1 and 2 are used as the solvent sources for continuous flow, alternating between the two.

❖ **To flow mobile phase to the detector for manual loop injection or compound optimization (Continuous Flow Mode)**

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Confirm that the bottle set is filled with the appropriate solvent or fill the bottles as needed. Confirm that both channel 1 and channel 2 are using the same bottle set.
3. Choose **Prelude > Continuous Flow Mode.**

   The Prelude Continuous Control dialog box opens (see Figure 20).

Figure 20. Prelude Continuous Control dialog box



4. In the Flow Rate box, enter the target flow rate in mL/min.

   Note Make sure you enter a flow rate that is compatible with the analytical column.

5. In the Comp A box, type the appropriate mobile phase percentage of solvent A.

   When you click outside the box, solvent B automatically adjusts its value to maintain a total value (A + B) of 100 percent.

6. To assign these values as the default values for this window, select the **Set As Default** check box.

7. Ensure that the LC flow is inline to the detector (the bypass valve is not in the Bypass position). See "To control the bypass valve" on page 49.

8. Click **Apply** to start the flow to the detector.

   The Direct Dispense area shows the channel 1 pump syringes dispensing the mobile phase. While the pump from channel 1 refills its syringe, channel 2 dispenses the mobile phase, and when the pump from channel 2 refills its syringe, channel 1 dispenses again, and the selector valve switches, accordingly.

9. To stop the flow, click **End** in the Direct Dispense area of the Direct Control window or clear **Continuous Flow Mode** from the Prelude menu.

IMPORTANT After you initiate Continuous Flow Mode, do not walk away from the system because the LC timeout setting does not stop the solvent flow. Instead, you can set a timeout time to shut off the LC pumps using the Continuous Flow Mode Timeout feature (see Setting a Continuous Flow Mode Timeout).

Setting a Continuous Flow Mode Timeout

In Continuous Flow mode, you can set a timer (timeout value) to shut off the LC pumps. Use this feature, for example, if there is a chance that the system will be unattended for a long period of time, or when solvent overconsumption might occur.

❖ To set a timeout value for the Continuous Flow mode

1. Open the Direct Control window.
2. Choose **Prelude > Continuous Flow Mode**.

   The Prelude Continuous Control dialog box appears (see Figure 20 on page 43).

3. Select the **Stop After** check box.
4. Type the value in hours when you want the LC pumps that are running in Continuous Flow Mode to shut off, and then click **OK**.

Tip Add a decimal fraction to the hour value to fine-tune the timeout time. For example, typing **1.75** in the Stop After (timeout) box specifies that the pumps shut off after 1 hour and 45 minutes.

The timeout value is set and the pumps will shut off when the time expires.

Related Topics

- Controlling the Pumps
- Placing the LC Pumps into Continuous Flow Mode

Setting a High-Pressure Limit for the System LC Pumps

You can set a high-pressure limit for the Prelude LC pumps using the Adv button.

CAUTION HPLC or UHPLC columns can have different maximum pressure ratings. Columns might be damaged if the system backpressure is higher than the rating for the columns that are in use. To prevent damage to your system's LC columns, Thermo Fisher Scientific recommends that you set a pump high-pressure limit that is no higher than the column's maximum pressure rating.

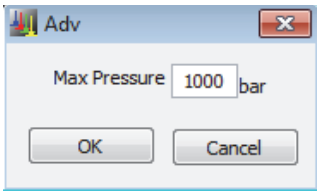
❖ To set a high-pressure limit on the system pumps

1. Open the Direct Control window.
2. From the middle pane, under the appropriate channel, select **Direct Dispense**.

   The system Channel information appears in the right pane.
3. Click **Adv** for the pump that you want to set.

   The Adv dialog box appears (Figure 21).

   Figure 21. Adv dialog box, maximum LC pump pressure setting

4. Type the high pressure limit value in the box for the selected pump, and then click **OK**.

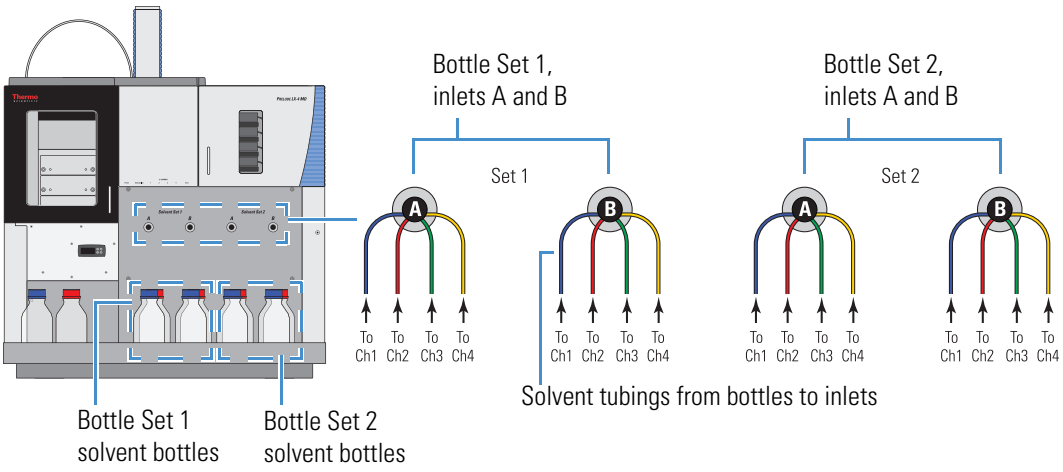
   The LC pump high-pressure limit is set.
5. Repeat step 3 through step 4 for each channel and pump, as needed.

Assigning Bottle Sets

For flexibility with bottle configurations, the instrument has two bottle sets. Each channel can use Bottle Set 1 or Bottle Set 2. Each bottle set has entry ports A and B on the instrument.

Each entry port has a line to supply mobile phase for each channel, which provides flexible solvent arrangements. The solvent lines on the instrument appear as shown in Figure 22.

Figure 22. Diagram of Bottle Sets 1 and 2 showing inlets and tubing



A method can use Bottle Set 1 or Bottle Set 2, but it must use entry ports from the same bottle set. Use the LC Method Editor to assign the bottle set selection to a method. See "Assigning Bottle Sets to a Method" on page 117.

Tip If you are working in a high-throughput environment and running more than one channel under the same method, Thermo Fisher Scientific recommends that the channels share mobile phase bottles to ensure that there is no variation in the dispensed solvents.

To assign the bottle set for priming or dispensing fluids, or to perform other procedures outside a method, do the following:

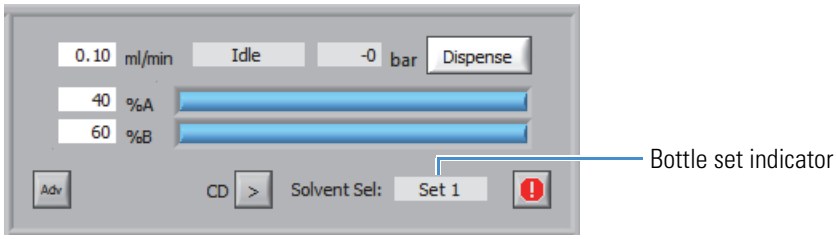
- To determine which bottle set is assigned to a channel
- To assign the bottle set for one channel
- To assign the bottle set for all channels

❖ To determine which bottle set is assigned to a channel

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. In the middle pane, click **Direct Dispense** for the channel that you want to view.

   In the Direct Dispense area in the right pane, the bottle set indicator shows which bottle set the channel uses (Set 1 or Set 2, see Figure 23).

Figure 23. Direct Dispense area

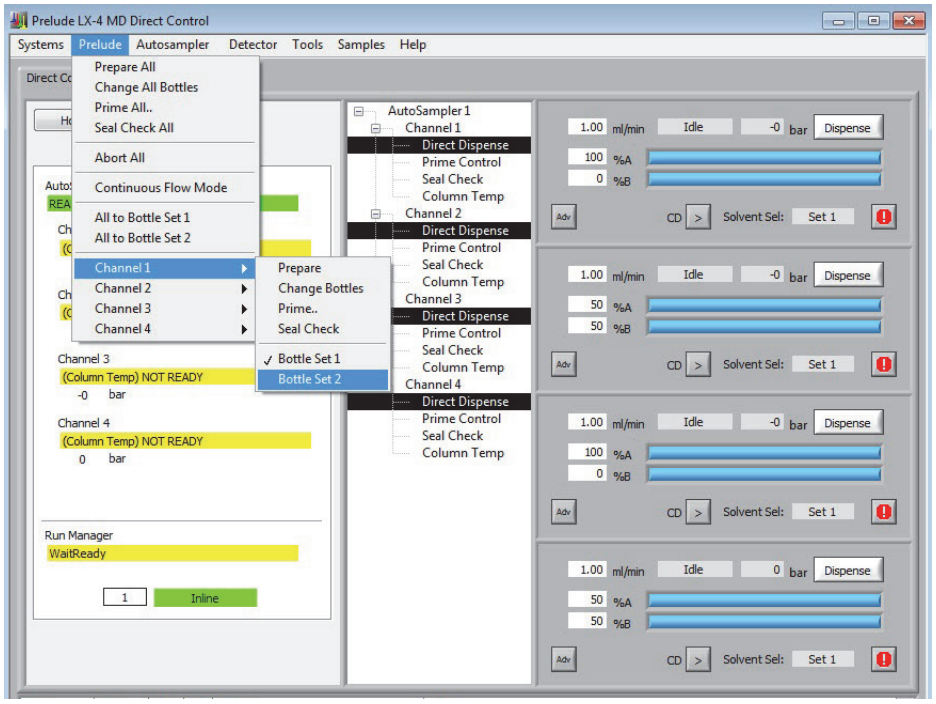


❖ To assign the bottle set for one channel

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Prelude > Channel *n***, where *n* is the channel you want to change.
3. Do one of the following:
   - To use the solvents in Bottle Set 1, choose **Bottle Set 1**.
   - To use the solvents in Bottle Set 2, choose **Bottle Set 2**.

   See Figure 24.

Figure 24. Channel bottle set assignment



❖ To assign the bottle set for all channels

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Do one of the following:
   - To change the active bottle set to Bottle Set 1 for all channels, choose **Prelude > All to Bottle Set 1**.
   - To change the active bottle set to Bottle Set 2 for all channels, choose **Prelude > All to Bottle Set 2**.

Controlling the Valves

Follow these procedures:

- To control the column-direction valve
- To control the selector valves
- To control the bypass valve

❖ To control the column-direction valve

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Click **Direct Dispense** for the channel you want to view.
3. Click the **CD** (column-direction valve) icon,  .

   The valve changes position, which changes the direction of the flow through the column. The Valve icon turns yellow and shows the arrow pointing in the opposite direction,  .

❖ To control the selector valves

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Detector > Source**, and select the system channel that you want to flow to the detector.

❖ To control the bypass valve

Note The bypass valve directs the flow exiting the selector valve to the detector or to waste.

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Detector > Bypass** to switch the position of the bypass valve.

   The Bypass indicator in the Direct Control window and Status pane indicates the position of the bypass valve.

   - When the valve directs the mobile phases to bypass the detector and flow to waste, "Bypass" appears in a red status bar.
   - When the valve directs the mobile phases to flow to the detector, "In line" appears in a green status bar.

Controlling the Model A Autosampler

Follow the procedures provided to perform common Model A autosampler tasks.

- To access the Model A autosampler features
- To change the cooled stack temperature
- To pause the autosampler
- To stop the autosampler
- To reset the autosampler
- To clean the autosampler syringe
- To clean an autosampler injector
- To change the tray type configuration for a sample tray

❖ To access the Model A autosampler features

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Select the autosampler in the middle pane.

   The autosampler options appear (see Figure 25).

❖ To change the cooled stack temperature

Note The cooled stack temperature is preset at +10 °C.

1. At the front of the instrument, locate the digital temperature display below the cooled stack drawer.
2. Quickly press P (less than 2 seconds) until the display shows “SP1”.
3. Use the increment/decrement keys to select the desired temperature value.
4. Press P again.

Tip Pressing P for 2 seconds or longer enables a programming function and blocks the temperature controls. Leave the unit untouched for approximately 2 minutes to return the display to the actual temperature reading.

Figure 25. Autosampler options (Model A autosampler)

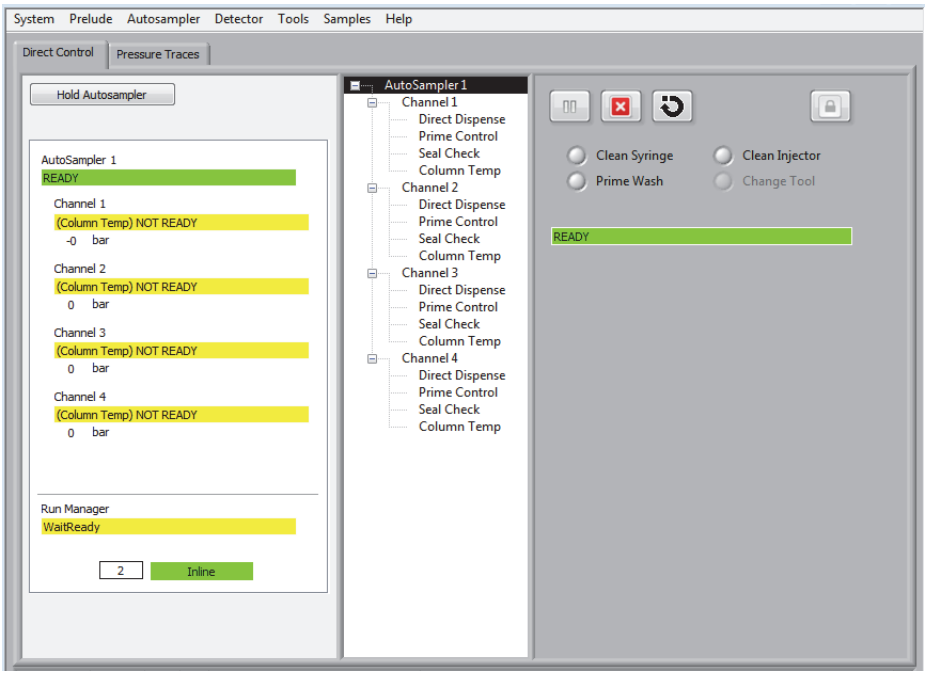


Table 13 describes the autosampler control parameters.

Table 13. Autosampler control parameters

Parameter	Description
Pause 	The autosampler pauses at the earliest possible moment. It can pause between the steps of a method without canceling the current action. It does not stop the queue. The Pause icon becomes the Continue icon (). When you want to continue sampling, click the **Continue** icon.
Stop 	The autosampler cancels the current action, the sample method, or both.
Reset (XYZ) 	The autosampler arm returns to the origin (0,0,0) and resets the XYZ coordinates based on that position.
Unlock Terminal 	(Model A autosampler) Unlocks the AS terminal. Typically used during service calls. Do not use this button unless you have been instructed to do so by a service representative or Technical Support.
Change Tool	(Model B autosampler) Initiates movement of the AS arm for changing the syringe or needle. See "Change a Needle or a Syringe (Model A Autosampler)" on page 151.
Clean Syringe	Opens the dialog box for cleaning the syringe. See "Clean the Syringe" on page 150.
Clean Injector	Opens the dialog box for cleaning the injector. See "Cleaning the Injector" on page 186.
Prime Wash	Opens the dialog box for priming the AS wash. See "Prime the Autosampler Wash" on page 149.
Status bar	Indicates the status of the autosampler.

❖ To pause the autosampler

Click the **Pause** icon,  . See Table 13.

❖ To stop the autosampler

Click the **Stop** icon,  . See Table 13.

❖ To reset the autosampler

Click the **Reset** icon,  . See Table 13.

❖ To clean the autosampler syringe

Select the **Clean Syringe** option. See Table 13.

❖ To clean an autosampler injector

Select the **Clean Injector** option. See Table 13.

❖ To prime the autosampler wash

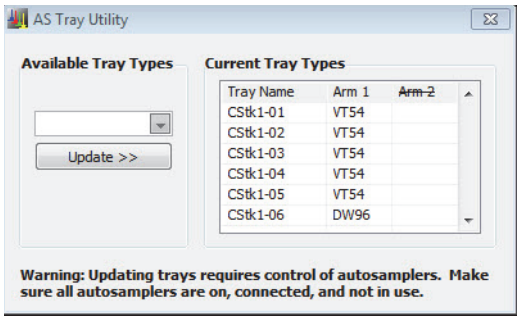
Select the **Prime Wash** option. See Table 13.

❖ To change the tray type configuration for a sample tray

1. In the Direct Control window, choose **Autosampler > Configure Trays**.

   The AS Tray Utility dialog box opens (see Figure 26).

   Figure 26. AS Tray Utility dialog box



2. In the Current Tray Types list, select the tray that you want to configure.
3. In the Available Tray Types list, select the tray type for your tray configuration.
4. Click **Update** to save your changes.

Controlling the Model B Autosampler

The following topics provide information on various Model B autosampler tasks, including information on configuring the Peltier stack tray types and temperature control.

Follow the procedures provided to perform common Model B autosampler tasks.

- To access the Model B autosampler features
- To pause the autosampler
- To stop the autosampler
- To clean an autosampler syringe
- To clean an autosampler injector
- To prime the AS wash pumps
- Configuring the Peltier Stack Drawer Tray Types
- Changing the Peltier Stack Drawer Tray Types

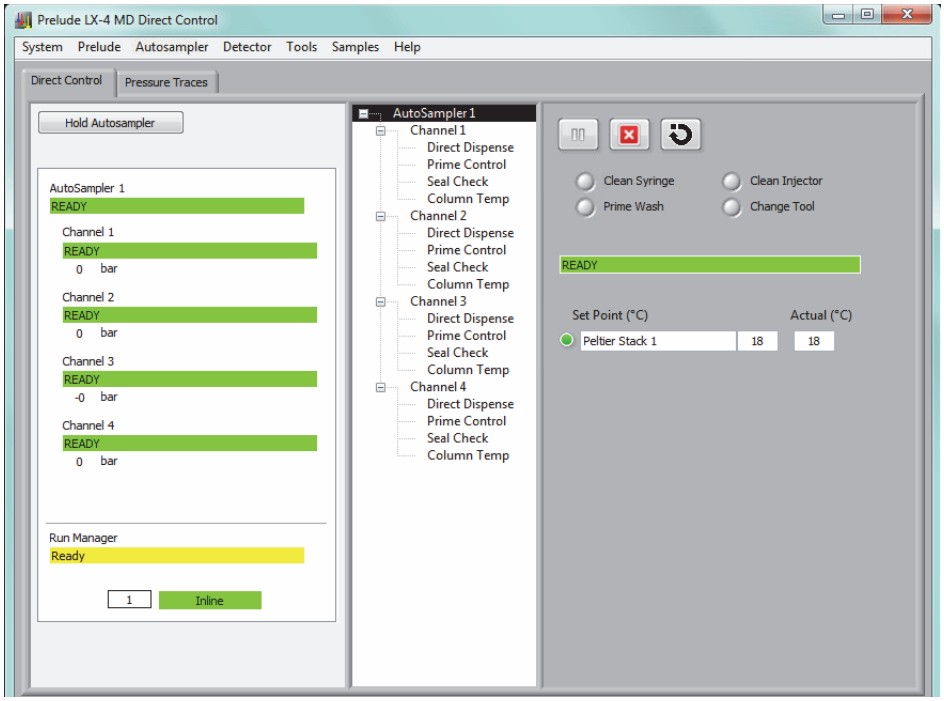
- Controlling the Peltier Stack Temperature
- Viewing the Peltier Stack Temperature in a Sequence Log
- Controlling the Column Heater Temperature

❖ To access the Model B autosampler features

1. Open the Direct Control window.

   The Direct Control window opens (Figure 27)

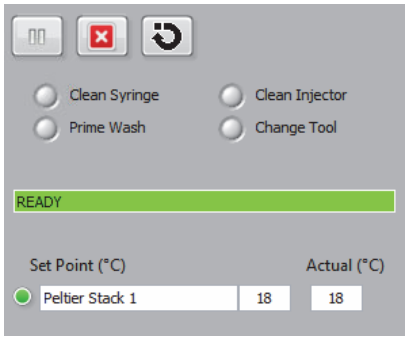
Figure 27. Autosampler options (Model B autosampler)



2. Select the autosampler in the middle pane.

   The autosampler options appear (Figure 28).

Figure 28. Direct Control autosampler options



❖ To pause the autosampler

1. Click the **Pause** icon,  .

   The autosampler completes the method for the current sample but does not draw additional samples. The Pause icon changes to the Continue icon.

2. When you want to continue sampling, click the **Continue** icon.

❖ To stop the autosampler

Click the **Abort** icon,  .

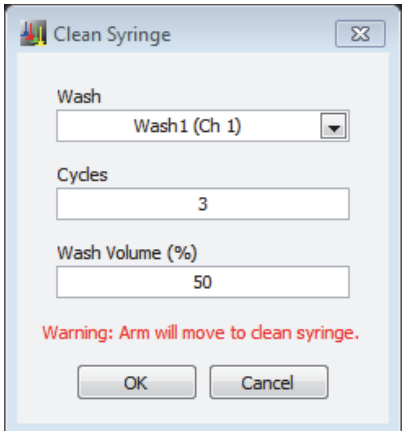
The autosampler cancels the current operation.

❖ To clean an autosampler syringe

1. Select the **Clean Syringe** option.

   The Clean Syringe dialog box appears.

   Figure 29. Clean Syringe dialog box

2. In the Wash list, select the wash solution and position of the Channel Injector wash cup where you want to clean the syringe.
3. In the Cycles box, type the number of rinse cycles that you want.
4. In the Wash Volume box, type the syringe volume (%) that you want, and then click **OK**.

Note

- A lower syringe volume decreases travel and wear on the syringe plunger and barrel.
- A higher syringe volume can assist in clearing syringe needle blockages.
- The wash system pumps solution during a syringe draw and stops pumping when syringe set volume (%) is reached. The Wash Volume (%) is then dispensed from the syringe. This occurs in one cycle.

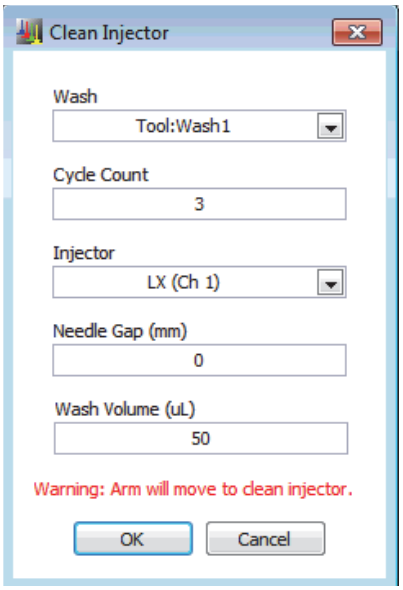
The dialog box closes. The autosampler arm moves to the configured wash station and starts the cleaning operation. The autosampler arm moves back to its standby position when the operation is complete. For more information on setting the parameters and their use in an autosampler method, see Chapter 7: "Creating an Autosampler Method."

❖ To clean an autosampler injector

1. Select the **Clean Injector** option.

   The Clean Injector dialog box opens.

   Figure 30. Clean Injector dialog box

2. In the Wash list, select the wash that will clean the injector.
3. In the Cycles Count box, type the number of cleaning cycles that you want.
4. Set the Injector, Needle Gap, and Wash Volume parameters as needed, and then click **OK**.
5. In the Injector list, select the injector that you want to clean.
6. In the Needle Gap box, type the needle gap (mm) that you want, from 0 to 10 mm.
   - To clean the injector port only, set the gap to 0 mm.
   - To clean the exterior of the needle that is exposed to sample, set the gap to a value greater than 0, which backs the needle out of the injector port.

7. In the Wash Volume (µL) box, type the volume that you want the syringe to draw, and then click **OK**.

Note

- A lower syringe volume decreases travel and wear on the syringe plunger and barrel.
- The wash system pumps solution during a syringe draw and stops pumping when wash set volume (µL) is reached. The Wash Volume (µL) is then dispensed from the syringe. This occurs in one cycle.

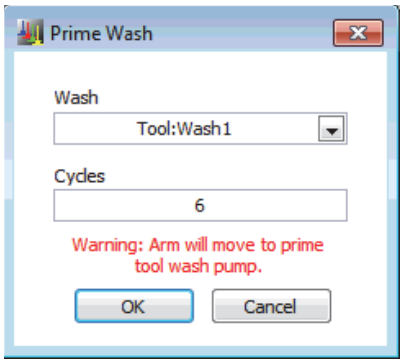
The dialog box closes. The autosampler arm moves to the injector port to start the cleaning operation. The autosampler arm moves back to its standby position when the operation is complete. For more information on setting the parameters and their use in an autosampler method, see Chapter 7: "Creating an Autosampler Method."

❖ To prime the AS wash pumps

1. Select the **Prime Wash** option.

The Prime Wash Pump dialog box appears.

Figure 31. Prime Wash pump dialog box



2. In the Wash list, select the wash pump solution (1 or 2) that you want to prime.
3. In the Cycles box, type the number of priming cycles that you want, and then click **OK**.

Note Thermo Fisher Scientific recommends setting the prime cycle count to 15 to 20 cycles if the system has been inactive for a long period of time. For short periods of inactivity—overnight, for example—3 to 5 prime cycles are sufficient.

The dialog box closes. The selected wash pump starts the priming operation. For more information on setting the parameters and their use in an autosampler method, see Chapter 7: "Creating an Autosampler Method."

Configuring the Peltier Stack Drawer Tray Types

Configure the tray type for the Model B autosampler by running the Configure Trays command from the Direct Control window.

❖ **To configure the Peltier Stack tray type for the Model B autosampler**

1. Open the Direct Control window.
2. Choose **Autosamplers > Configure Trays**.

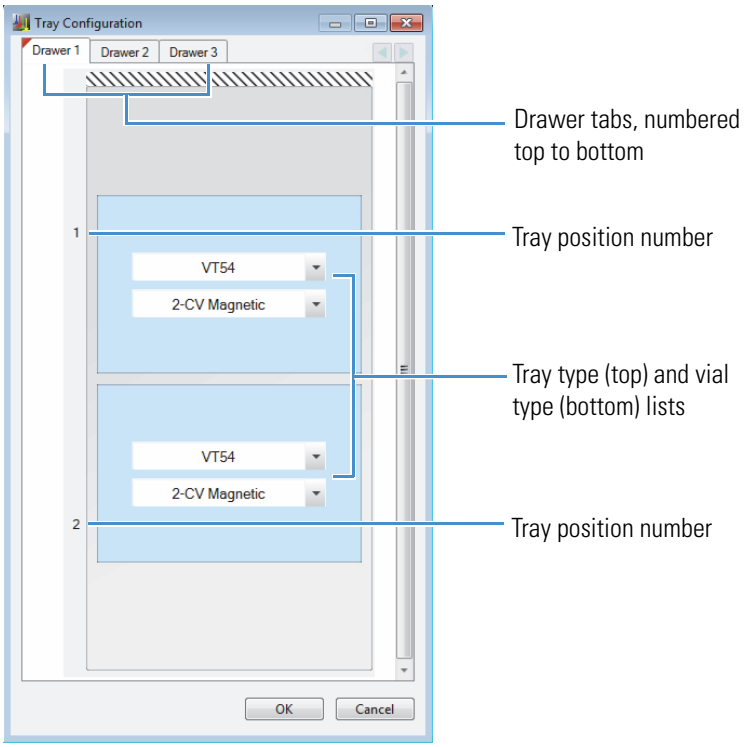
   The Tray Configuration dialog box appears (see Figure 32).
3. Click the tab for the tray drawer that you want to configure, and then choose the tray and vial type that you want to configure from the tray and vial lists.
4. Click **OK**, or click **Cancel** to exit without saving the changes.

Changing the Peltier Stack Drawer Tray Types

With the Configure Tray command, you can make changes to the autosampler tray and vial types that hold samples in the Peltier stack drawers for analysis. When you choose this command, the Tray Configuration dialog box appears, showing the available drawers as clickable tabs. Click the tab for the drawer you want to configure according to your lab's sample requirements (see Figure 32).

Note The stack drawer hardware for the Model B Peltier stack module is numbered from *front to back*, which is the opposite from the Model A Peltier stack module.

Figure 32. Tray Configuration dialog box example, Model B autosampler



You can make changes to the following tray and vial options for each Peltier stack drawer by using the Tray Configuration dialog box:

- Each drawer can be configured for one or two trays.
- Each tray position can be configured to use a different tray type. Supported tray types appear in the tray type list (top).
- Each supported vial type appears in the vial type list (bottom) for the tray type selected.

Controlling the Peltier Stack Temperature

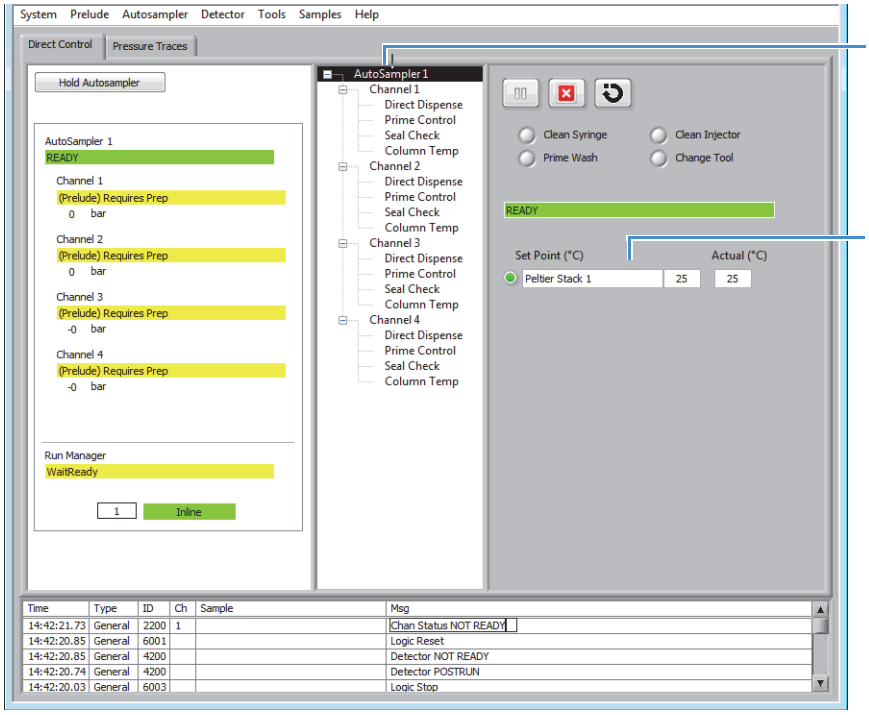
You can set the target temperature for the installed Peltier (cool) stack module from the Direct Control window. Temperatures can be set to an accuracy of one-tenth of a degree Celsius.

❖ To set the Peltier stack temperature

1. Open the Direct Control window.
2. In the middle pane, click **Autosampler1**.

   The stack (reference) number, the set temperature value, and the current (actual) temperature readout appear in the right pane (see Figure 33).

Figure 33. Direct Control window, Peltier Stack temperature settings



3. In the Set Point box (°C) for the selected Peltier stack, type the temperature in degrees Celsius that you want, and then press ENTER.

   The temperature for the Peltier stack is set. The module cools—or warms—until it reaches the configured temperature (Figure 33).

Viewing the Peltier Stack Temperature in a Sequence Log

The Model B autosampler Peltier Stack temperature control status and set points are recorded at the start of each sample acquisition. You can view the sequence record of these items by reviewing the sequence log file (*.tslx file extension).

❖ **To view the recorded Peltier stack temperature for a sample (Model B autosampler)**

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Tools > Sequence Log Viewer**.

   The Sequence Log Viewer opens.
3. Click **File > Open**, then navigate to and select a sequence log file (*.tslx) that you want to review.

Tip You can quickly open recent sequence log files by clicking **File > Recently Accessed** or **Recently Submitted**

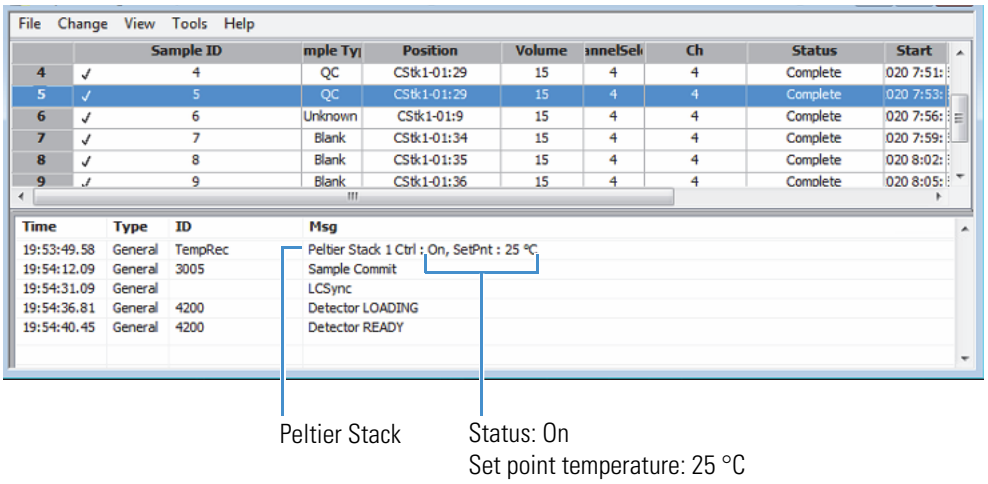
The sequence log file opens to the last view (either Events or Pressure) used in the Sequence Log Viewer.

4. If the current view is set to Pressure, click **View > Events**, and then click a sample ID of interest.

   The event view for that sample opens in the bottom pane of the Sequence Log Viewer (see Figure 34).

In general, the first line of the log message displays the temperature control status and its Set Point temperature value. See Figure 34 for a sequence record example of a Peltier Stack.

Figure 34. Sequence Log Viewer, Peltier Stack On/Off status and set point values



Related Topics

- Accessing the Sequence Log Viewer
- Controlling the Peltier Stack Temperature
- Configuring the Peltier Stack Drawer Tray Types
- Changing the Peltier Stack Drawer Tray Types

Controlling the Column Heater Temperature

During a run, the LC method controls the column temperature. To control the column temperature outside a method, follow these procedures:

- To change a column heater temperature
- To turn the heater on or off
- To use the automatic shutoff

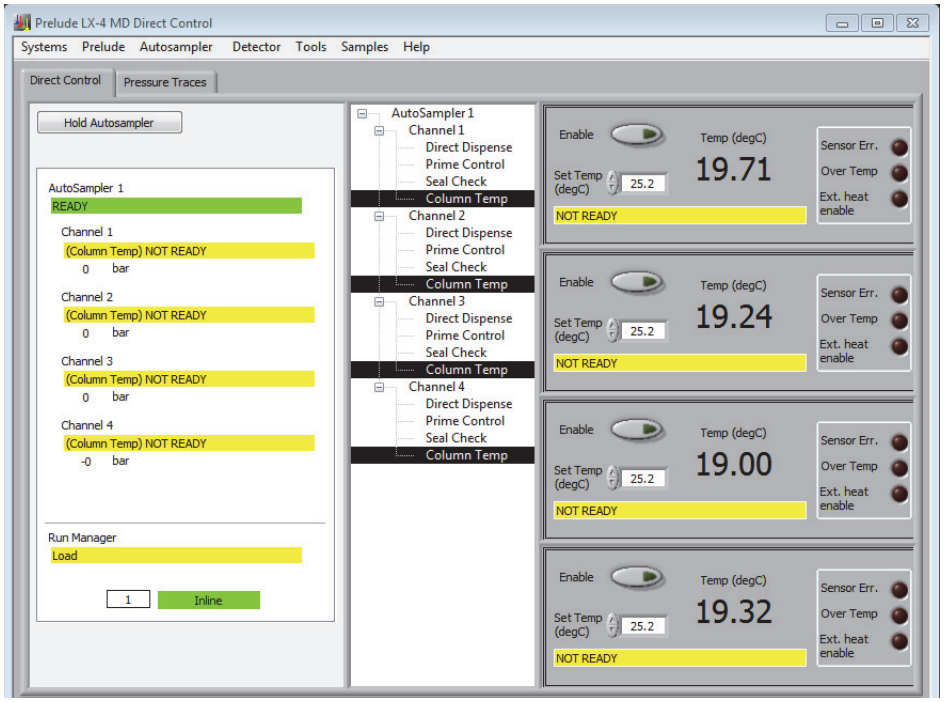
❖ To change a column heater temperature

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. In the middle pane, select the heater that you want to control.

   The heater options open to the right (see Figure 35).

> **Note** The names of your heaters might be different from the names of the heaters in Figure 35 because you can change the names during the heater configuration.

Figure 35. Direct Control window showing heater options for all four channels



3. In the Set Temp box, select or type the temperature in Celsius that you want the heater to reach.
4. If the heater is off, start it. See "To turn the heater on or off."

❖ To turn the heater on or off

1. To turn on the heater, click the **Enable** button.

   The heater temperature adjusts to the temperature assigned in the Set Temp (degC) box.

   When the heater is on, the Enable button appears light green (see Figure 36).
2. To turn off the heater, click the **Enable** button again.

   When the heater is off, the Enable button appears dark green (see Figure 36).

Figure 36. Enable button showing on and off states

Heater on Heater off

❖ **To use the automatic shutoff**

See "Changing the LC Timeout Value."

Changing the LC Timeout Value

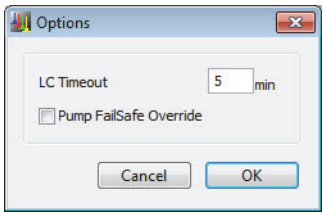
Enter the amount of elapsed time after which you want the column heaters or pumps (if in dispensing state) to turn off, when the rest of the system has been idle.

❖ **To change the LC Timeout value**

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Tools > Options**.

   The Options dialog box opens (see Figure 37).

   Figure 37. Options dialog box

3. In the LC Timeout box, type the amount of time that you want to elapse without a sample request or other command before the column heaters or pumps turn off.
4. Select the **Pump Failsafe Override** check box if you want the heaters to stay on when samples are pending but no samples or channels are running for more than an hour.

   The Failsafe feature turns the devices off when there are potential samples pending but there is no activity for more than an hour. This circumstance is rare.

Editing Instrument Settings

The service engineer configures the Prelude LX-4 MD control application at the time of the installation visit. However, you can change the instrument settings to better meet your laboratory's needs.

❖ To change the Prelude LX-4 MD instrument settings

1. Close all Thermo Scientific applications that are open.
2. From the Start menu, choose **Start > All Apps** (Windows 10) or **All Programs** (Windows 7) **> Thermo Foundation > Instrument Configuration**.

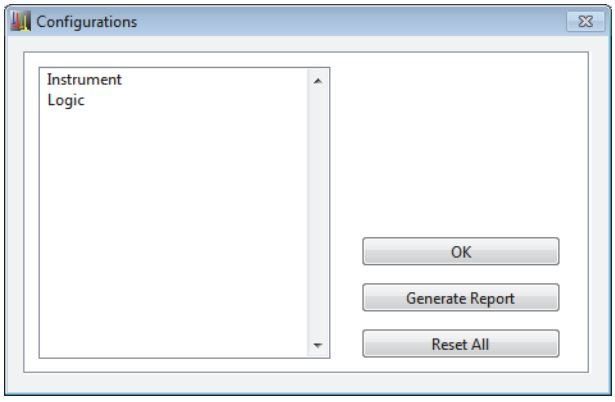
   The Thermo Foundation Instrument Configuration window opens.
3. On the right side of the window, select the Prelude LX-4 MD icon (see Figure 38) and click **Configure**.

   Figure 38. Prelude LX-4 MD icon

   The Configurations dialog box opens (see Figure 39).

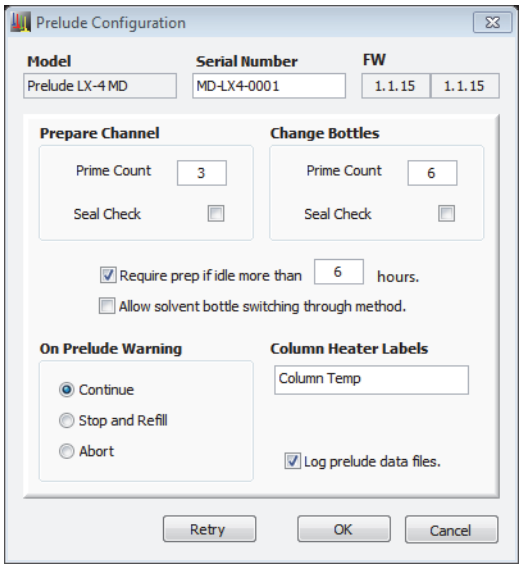
   Figure 39. Configurations dialog box

4. In the left box, click **Instrument**.

   The Prelude Configuration dialog box opens (see Figure 40).

Figure 40. Prelude Configuration dialog box



5. Edit any of the options described in Table 14.
6. Do one of the following:
   - To save your changes, click **OK**.
   - To discard your changes and return the settings to the previous selections, click **Cancel**.
   - If communication with the Prelude LX-4 MD is not established, click **Retry**.

Table 14. Prelude Configuration dialog box options (Sheet 1 of 3)

Option	Description
Serial Number	Type the serial number of the Prelude LX-4 MD instrument.
Prepare Channel	
Prime Count	Type the number of times to prime when preparing the channel.
Seal Check	Select this check box to perform a seal check as part of the channel preparation.
Change Bottles	
Prime Count	Type the number of times to prime when changing the bottles.
Seal Check	Select this check box to perform a seal check as part of changing the bottles.

Table 14. Prelude Configuration dialog box options (Sheet 2 of 3)

Option	Description
Require prep if idle more than *x* hours.	Select this check box to enable the automatic trigger for channel preparation, and then type how many hours of idle time initiates the trigger.
Allow solvent bottle switching through method.	Select this check box to allow the method to switch the currently used bottles to another bottle set and run the Change Bottles protocol. Clear this check box to prevent a sequence submission if it does not use the bottles currently requested by the instrument. For example, you manually prepare your instrument and prime the system by using Bottle Set 1 on all four channels. You then submit a sequence by using a method that requests Bottle Set 2. • If you select this check box, the system switches to Bottle Set 2 and runs the Change Bottles protocol. • If you clear this check box, the system fails validation and does not run the submitted sequence.
On Prelude Warning These are options for the system to perform when receiving a pump warning (*not* an error). For example, you set the method to use 2.95 mL of organic solvent and to typically run at 100 bar (1450 psi). Over time and use, the backpressure on the system rises to 300 bar (4351 psi). At the higher pressure, the total volume in the pump head might decrease to 2.93 mL. The Prelude LX-4 MD application posts a warning that the pump has reached the end of the travel before the end of the method.	
Continue	Posts a warning message, continues the current sample as best as possible, refills the pumps, and then proceeds to the next sample. The system performs these tasks until an error occurs or an operator intervenes.

Table 14. Prelude Configuration dialog box options (Sheet 3 of 3)

Option	Description
Stop and Refill	Stops the channel, posts a warning message, refills the pumps, and then proceeds to the next sample. The system performs these tasks until an error occurs or an operator intervenes. Each sample that triggers the warning generates a separate warning message. The system sees this warning as a premature stop and stops the detector if the warning occurs prior to the end of the data window.
Abort	(Default) Stops the channel, posts a warning message, and does not refill the pump or become ready again.
Column Heater Labels	
Label	Type the label for the column heater.
Log prelude data files	Select this check box if you want to log all of the pressure data and create .pdat log files. Service personnel might request these files for troubleshooting and diagnostic purposes.

Editing Logic Settings

The service engineer configures the Prelude LX-4 MD control application at the time of the installation visit. However, you can change the logic settings to better meet your laboratory's needs.

❖ To change the Prelude LX-4 MD logic settings

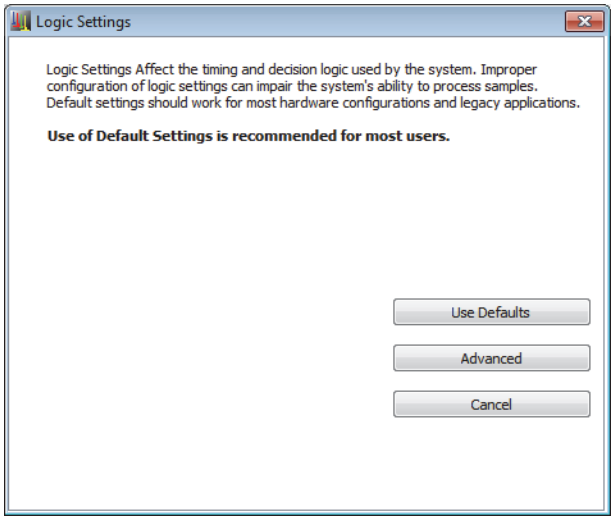
1. Close all Thermo Scientific applications that are open.
2. From the Start menu, choose **Start > All Apps** (Windows 10) or **All Programs** (Windows 7) **> Thermo Scientific Foundation *x.x* > Instrument Configuration**.

   The Thermo Foundation Instrument Configuration window opens.
3. On the right side of the window, select the Prelude LX-4 MD icon (see Figure 38 on page 63) and click **Configure**.

   The Configurations dialog box opens. See Figure 39 on page 63.
4. In the left box, click **Logic**.

   The Logic Settings dialog box opens (see Figure 41).

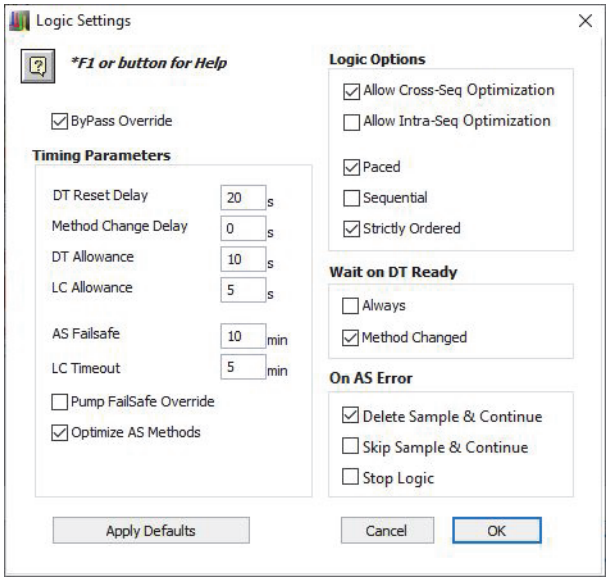
Figure 41. Logic Settings dialog box



5. Click **Advanced**.

   The Advanced Logic Settings dialog box opens (see Figure 42).

Figure 42. Advanced Logic Settings dialog box



6. Edit any of the options described in Table 15.
7. Do one of the following:
   - To save your changes, click **OK**.
   - To discard your changes and return the settings to the previous selections, click **Cancel**.
   - To apply the factory-set settings, click **Apply Defaults**. Then, click **OK** to save the default values.

Table 15. Advanced logic settings dialog box options (Sheet 1 of 4)

Option	Description
Bypass Override	Do one of the following: • Select this check box if you want fluid exiting the column to flow to the detector only during the data collection time specified in the LC method. At other times, the fluid flows to waste. • Clear this check box if you want the fluid to flow to the detector as directed by the selector valve.
Timing Parameters	
DT Reset Delay	Type the value in seconds to indicate how long the detector takes to return to a ready state after it has completed the data collection. The Prelude LX-4 MD control application uses this information to forecast when a detector is ready for the next acquisition and to determine when a detector's ready or not-ready state might indicate an error. This time value acts as a buffer between detector acquisitions to allow time for the detector to prepare for acquisition.
Method Change Delay	Type the amount of additional time that you want the system to wait before sampling whenever a new instrument method runs. This parameter equilibrates the column and detector conditions before the system runs the next method.
DT Allowance	Type the amount of allowed time for the detector to respond to a trigger. Following a trigger signal, this is the period of time that the system allows before generating an error when it detects no state change. This accounts for software lag.
LC Allowance	Type the amount of allowed time for the LC pumps to respond to a trigger, similar to DT Allowance for the detector.
AS Failsafe	Type the amount of allowed elapse time (idle time) before the autosampler cancels the sample. This option applies when the autosampler is waiting to complete an injection. An injection requires certain conditions before it can proceed (Channel Ready, sometimes Detector Ready). If these conditions are not met within the specified time, the autosampler cancels the sample.

Table 15. Advanced logic settings dialog box options (Sheet 2 of 4)

Option	Description
LC Timeout	Type the amount of allowed elapse time without a command before the channel turns off.
Pump Failsafe Override	If you select the Pump Failsafe Override and the Allow Cross-Seq Optimization check boxes, and samples are waiting or pending, the column heaters do not shut off when the LC Timeout time has elapsed.
Optimize AS Methods	Select this check box if you want the Prelude LX-4 MD control application to calculate the optimal system starts based on stored AS method timing values from previous runs of the autosampler method. If you clear this check box, the control application uses the timing values you entered into the Prior to Sample and Pre-inject Total boxes in the AS Method Editor window.
Logic Options	
Allow Cross-Seq Optimization	**Note** • Thermo Fisher Scientific recommends choosing this option (selected by default) for most applications. • When turned off, the system runs the sequence queue in order and stops if a specific sample cannot be processed. Specifies the software to run multiple sequences concurrently, or to skip sequences, which maximizes sample throughput. This option also allows the system to process higher priority sequences first, regardless of their location in the queue. When selected, this check box activates the Allow Intra-Seq Optimization and the Sequential check boxes. Default: On (selected)

Table 15. Advanced logic settings dialog box options (Sheet 3 of 4)

Option	Description
Allow Intra-Seq Optimization	Instructs the system to process samples out-of-order within an individual sequence. The order of samples within a sequence is maintained by default. Sequence processing stops when a sample in the sequence cannot be run. When using Intra-Seq optimization with the "Channel Select" feature on a per-sample basis, the ordering of sampling can move within a sequence according to channel availability. For more information, see "Assigning Channels to the Method" on page 115 Default: Off (not checked) **Note** The first sample in a sequence must always be the first sample to be processed.
Paced	Instructs the system to delay starting a sample to achieve normalized spacing between data windows. This option helps to avoid irregular timing between samples that can result when methods have not been optimized to run across the number of available channels. Default: Off (not checked) **Tip** This option works best when the same method—or methods—with identical timing characteristics are run across all of the available channels.
Sequential (Not recommended for most applications)	This check box becomes active when Allow Cross-Seq Optimization is selected. This option prioritizes the sample order over sample processing speed, even when another channel might be available to start sooner. The queue runs in order, but the system can skip samples that cannot be run due to the operational state of the LC channels to which they are assigned. See Allow Cross-Seq Optimization. Default: Off (not checked)

Table 15. Advanced logic settings dialog box options (Sheet 4 of 4)

Option	Description
Strictly Ordered	This option gives precedence to the next available channel in the channel number order even if another channel can start running its samples sooner. This option prioritizes load balancing over speed. Default: Off (not checked)
Wait on DT Ready	
Always (Not recommended for most applications)	Select this check box if you want the control application to receive the detector-ready signal before injecting the next sample under all conditions.
Method Changed (Recommended for most applications)	Select this check box if you want the control application to receive the detector-ready signal before injecting the next sample if the next sample uses a different instrument method.
On AS Error	
Delete Sample and Continue	Select this check box if you want the control application to delete any sample with an autosampler error and to continue with the next sample. The sample remains in the batch, but the control application deletes it from the data processing software queue.
Skip Sample and Continue	Select this check box if you want the control application to skip any sample with an autosampler error and to continue with the next sample. The sample remains in the batch and in the data processing software queue.
Stop Logic	Select this check box if you want the control application to stop sampling whenever a sample has an autosampler error.

Assigning Values by Using the Sample List

This section describes procedures for assigning certain values in the sample list for each sample. In some sample runs, you might want to change a component setting for specific samples by using the same method and batch. All of the values described in this section, except the method variables, are usually assigned in the method rather than the sample list. These values, including method variables, are assigned in the sample list as a method development procedure. Perform these procedures only if instructed to do so by your standard operating procedure or as an experimental procedure if you have advanced knowledge of the system.

Follow these procedures:

- To assign the column temperature in the sample list
- To assign values to a method variable in the sample list

❖ To assign the column temperature in the sample list

CAUTION Be aware that the heater operates at different temperatures during the run and can, at times, be too hot to handle, depending on the settings you enter. To prevent burns, Thermo Fisher Scientific recommends waiting 15–20 minutes after the heater is turned off before handling.

IMPORTANT When you assign the temperature in the sample list, consider the following:

- The system injects the sample after the temperature has reached the entered value. For best results, allow more time for the temperature to equilibrate. You can do this by adding a wait time to the autosampler method before the sample injection and by scheduling multiple injections of the same sample.
- Enter samples into the sample list with lower heater temperatures first; then enter samples in order of increasing temperatures.

1. In the sample list, create a custom column for each column heater. Name the sample list column the same as the column heater name. For information on creating custom columns, refer to the documentation that comes with the data processing software. View the Direct Control window on your system for the name of your column heaters.
2. In the new column, type the temperature that you want to set for each sample.

   Figure 43 shows an example of a sample list with column temperature values set for each sample.

   Figure 43. Sample list showing temperature settings for each sample

	SampleName	Sample Type	File Name	Sample ID	Path	Inst Meth	Proc Meth	Position	Inj Vol	Column Temp
1	Sample 1	Unknown	B01	CStk1-01:1	C:\Xcalibur\Data	C:\Xcalibur\methods\Two		CStk1-01:1	10.00	25
2	Sample 2	Unknown	B02	CStk1-01:1	C:\Xcalibur\Data	C:\Xcalibur\methods\Two		CStk1-01:1	10.00	30
3	Sample 3	Unknown	B03	CStk1-01:1	C:\Xcalibur\Data	C:\Xcalibur\methods\Two		CStk1-01:1	10.00	35
4	Sample 4	Unknown	B04	CStk1-01:1	C:\Xcalibur\Data	C:\Xcalibur\methods\Two		CStk1-01:1	10.00	40
5	Sample 5	Unknown	B05	CStk1-01:1	C:\Xcalibur\Data	C:\Xcalibur\methods\Two		CStk1-01:1	10.00	45
*									0.00	

You might want to assign column temperatures in a sample list if you want to evaluate different temperatures in a method. With this feature, you do not have to create a new method for each temperature that you evaluate.

❖ To assign values to a method variable in the sample list

1. Create a custom column and name it the same name as the method variable. For information on method variables, see "Allowing Method Variables During a Run" on page 123. For information on creating custom columns, refer to the documentation that comes with the data processing software.

2. Enter the variable value for each sample in the new column.

You might want to assign values to a method variable in a sample list if you are evaluating different conditions during method development. With this feature, you do not have to create a new method for each set of conditions you evaluate.

Extracting the Method from the Raw Data File

Extracting a method from a raw data file that shows optimal results after modifying a method step can be useful, particularly if the step was modified by using method variables. By extracting the method using this procedure, you save the method with the same conditions as those used to obtain the raw data file.

❖ To extract the method from the raw data file

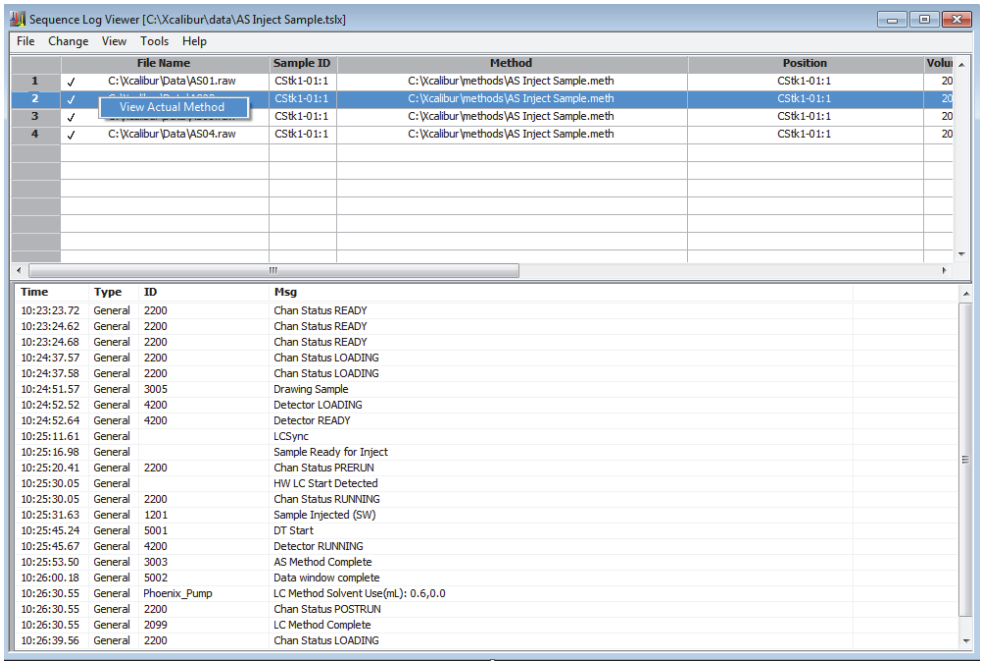
1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Tools > Sequence Log Viewer**. The Sequence Log Viewer opens.

> **Note** The Sequence Log Viewer is a separate application. You can also open it by choosing **Start > All Apps** (Windows 10) **or All Programs** (Windows 7) **> Thermo Prelude LX-4 MD > Sequence Log Viewer**.

3. Do one of the following:

- Right-click the appropriate sample line in the sequence, and then choose **View Actual Method** (see Figure 44).

Figure 44. Extracting the LC method from the raw data file



The Sequence Log Viewer extracts the method from that sample's raw data file and the method window opens. The name of the extracted method file appears in the method window as *raw data file name_original method name*.meth.

–or–

a. Choose **Sequence Log Viewer > Tools > Extract Method from Raw File**.

b. Navigate to the raw data file that has the method you want to extract, select the file, and click **OK**.

c. Navigate to the folder where you want to save the extracted method, select the folder, and click **OK**.

The saved method opens showing the LC method steps.

7

Creating an Autosampler Method

The autosampler executes all tasks associated with drawing the sample from the sample vial, injecting it into the LC instrument, and washing the syringe and injectors. This chapter describes how to create or edit an autosampler method, which controls the autosampler functions.

When you create an autosampler method, you can maximize the sample delivery and minimize carryover by determining the best wash, sample aspiration, and sample dispensing steps for the autosampler.

Contents

Supported Autosampler Models

The Prelude instrument control software (version 1.3) supports two Prelude LX-4 MD models that use different autosamplers (AS), which are referred to as follows in this document:

- "Model A"—Prelude LX-4 MD systems that use a DLW (Dynamic Load-Wash) wash system and CStack temperature controlled sample drawer system running on instrument control software version 1.2 or earlier.
- "Model B"—Prelude LX-4 MD systems that use the LCMS-P wash system and Peltier Stack temperature controlled sample drawer system running on instrument control software version 1.3 or later.

Note Consult with your Thermo Fisher Scientific sales representative to confirm the specific model that you purchased.

Related Topics

- Access the Autosampler Method Editor
- Import and Convert AS Methods

Access the Autosampler Method Editor

❖ To open the AS Method Editor window

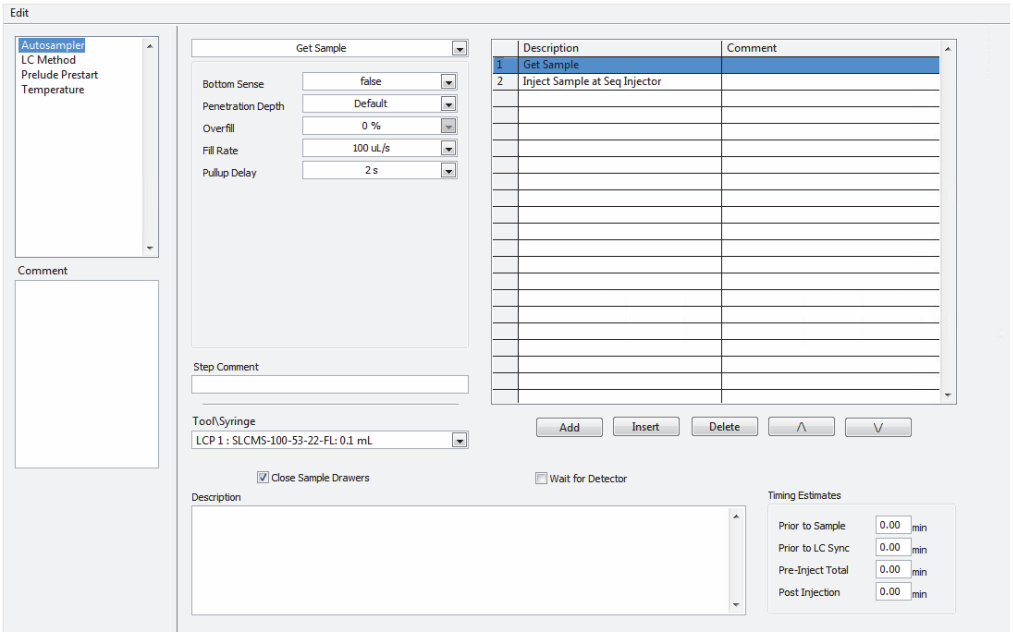
1. Open the instrument method that you want to view.

   For information on accessing the instrument method, refer to the documentation provided with the data processing software.

2. From the left pane, click the Prelude LX-4 MD icon.

   The AS Method Editor window opens (see Figure 45).

Figure 45. AS Method Editor window, Model B autosampler (example)



Create an Autosampler Method

The Prelude LX-4 MD control application saves the autosampler method you create as part of an instrument method in the data processing software. Instrument methods have a .meth file name extension.

During the autosampler method, the autosampler draws sample from the vial and injects it into the LC systems. To program this action, you use a minimum of two method step types: Get Sample and Inject Sample.

To clean the injector and syringe, add clean steps to the AS method. If you have biological samples, insert the Get Sample and Inject Sample step types into the AS method between two or more aqueous washes to avoid precipitating the sample with an organic wash.

Tip Always include clean steps in your method to ensure effective cleaning of the needle and syringe.

Use the autosampler method editor to create and edit methods.

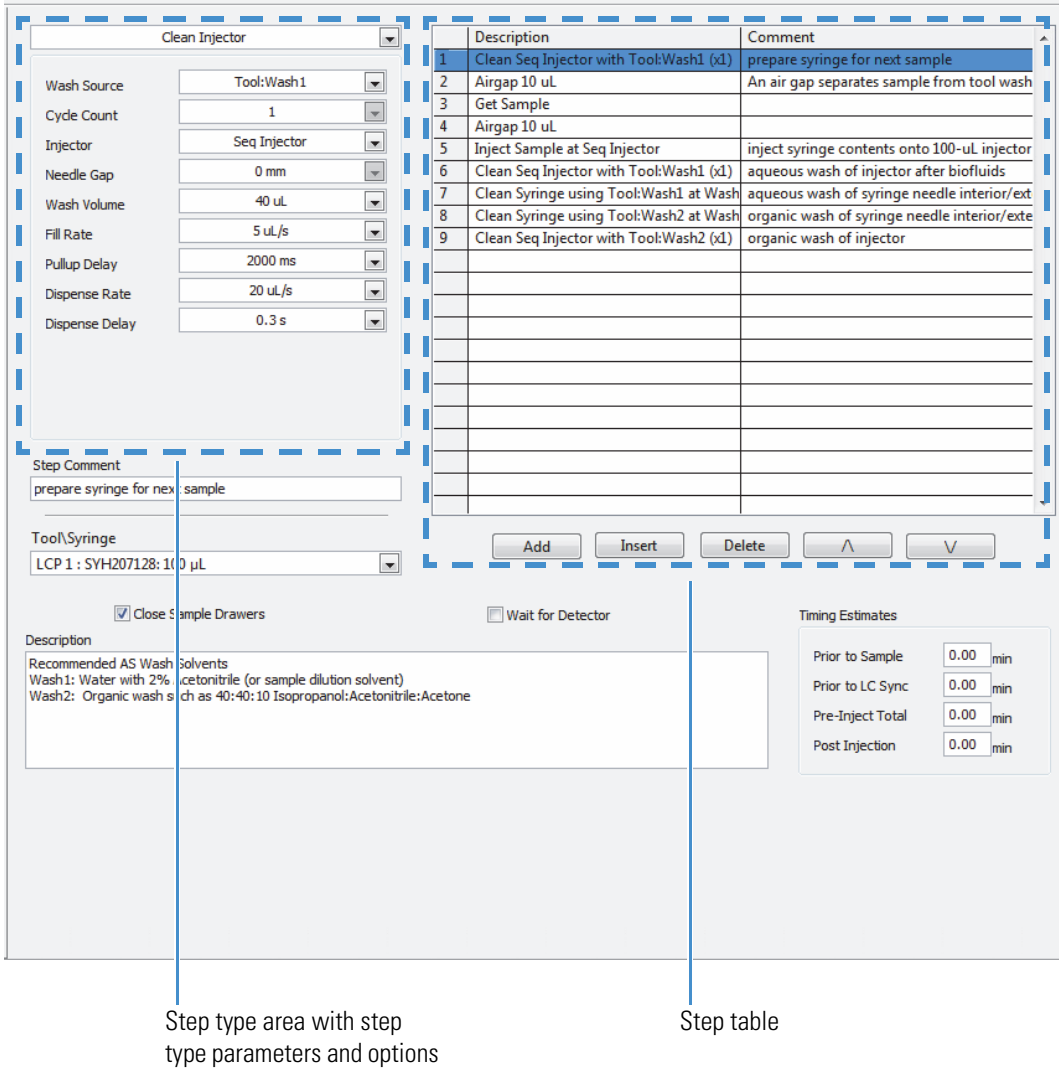
❖ To open the autosampler method editor window

1. Navigate to and then double-click the instrument method (.meth) file that you want to view.

   The AS Method Editor opens to the Step Control page and displays the AS method steps in the step table (Figure 46).

   Note For more information on accessing the instrument method, refer to the documentation provided with the MS control application.

Figure 46. Autosampler method editor example, Model B autosampler shown



- Adding and Deleting Autosampler Method Steps
- Tips for Creating an Autosampler Method
- Converting Model A Autosampler Methods

Validate and Run a New AS Method

You can validate a new AS method by using the Validate & Run command from the autosampler method editor window.

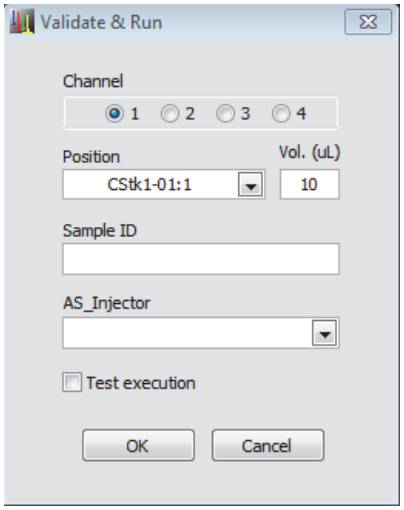
This process is done in two phases; once complete, you can return to the method editor to correct any method errors found, add/insert a new step type, or make any adjustments to a method step parameter.

❖ To validate an autosampler method

1. Open the method in the Autosampler method editor.
2. Choose **Edit > Validate & Run**.

   The Validate & Run dialog box appears (see Figure 47).

   Figure 47. Validate & Run dialog box



   Note Thermo Fisher Scientific recommends that you validate the converted method before selecting the Test Execution option.

3. Select an appropriate test vial position—a blank is recommended—and volume based on the method details or use the default, and then click **OK**.

   One of the following messages appears:

   - If the method validates, the Method is Valid window appears. Click **OK**.
   - If the method does not validate, an error message appears listing which step numbers need additional changes. Click **OK**.

     Make the necessary changes to the step numbers indicated. Repeat the validation from step 2.

4. After confirming that the converted method validates, choose **Edit > Validate & Run** again, and then select the **Test Execution** check box (see Figure 47).
5. Enter an appropriate Channel, (sample vial) Position, (sample volume) Vol. (µL), leave AS_injector blank, and then click **OK**.

   The autosampler runs the new method without submitting a sequence or engaging the LC pumps, which can be in dispense mode.

Note Thermo Fisher Scientific recommends using an innocuous solvent that will not contaminate the LC channel—such as water—when testing the autosampler operation with the Test Execution option selected.

Adding and Deleting Autosampler Method Steps

An AS method contains several steps. Program each step that the autosampler performs during the method as a method step.

❖ To add or delete steps

1. Open the AS method. See "Access the Autosampler Method Editor" on page 77.
2. Do one of the following:
   - To add a step at the end of the method, click **Add**. A blank step box and step number appears at the end of the method.
   - To add a step in the middle of the method, select the step above where you want the step, and click **Insert**. A blank step box and step number appear above the selected step.
3. Edit the step type as necessary. See "Editing Autosampler Step Types" on page 82.

❖ To delete a step

Select the step and click **Delete**.

Tips for Creating an Autosampler Method

Follow these tips while you create an autosampler method.

- For common wash solutions, see "Prepare the Wash 1 and Wash 2 Autosampler Wash Solution Bottles" on page 148.
- Validate new methods or method steps before you run a sequence. See"Validate and Run a New AS Method" on page 79.
- Once you have a satisfactory autosampler method, you can edit it to improve the system's throughput. See "Editing the AS Method for Maximum Throughput" on page 100.
- In your AS method, to separate the sample and solvent, include an Airgap step type before and after the Get Sample step type. See "Airgap" on page 89.
- If you use the default autosampler method that Thermo Fisher Scientific provides with the control application, then Thermo Fisher Scientific also recommends that you also
  - Use the default method for the DLW for Prelude systems equipped Model A autosamplers

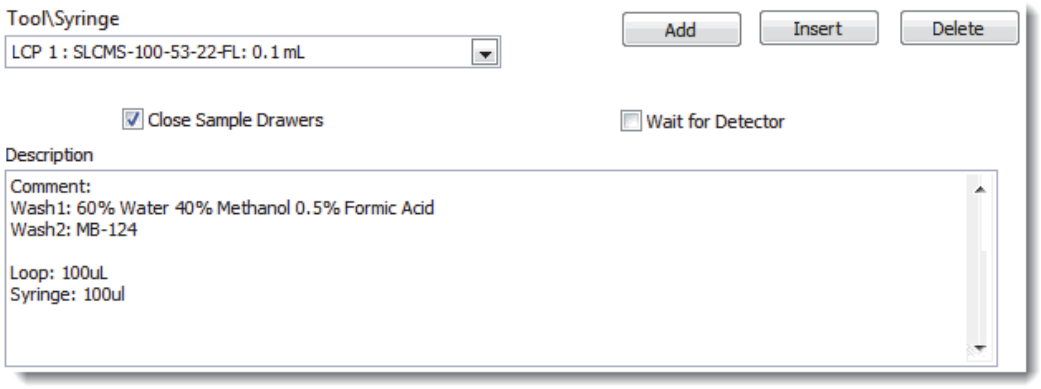
–or–

– Use the LCP default method for Prelude systems equipped Model B autosamplers.

Entering Information on the Method Info Page

Use the Description box at the bottom of the method editor to record information about the method. See Figure 48 for an example of information you might record.

Figure 48. Autosampler method Description box with additional method information, AS Method Editor (Model B autosampler example)



❖ To enter information on the AS method description box

1. Open an AS method. See "Access the Autosampler Method Editor" on page 77.
2. In the Description box, type a description of the autosampler method.

   Suggested items to enter in this box might include wash solution information, syringe volume, loop volume, and so forth.

Editing Autosampler Step Types

❖ To edit an autosampler step type

1. Open the AS Method Editor. See "Access the Autosampler Method Editor" on page 77.
2. Select the method step that you want to edit.
3. Open the Step Type list, and select the step type that corresponds to the task that you want the autosampler to perform during this method step.

   The step types appears in the Method table, and step type options appear below the Step Type box. For a list of the step types, see "Autosampler Step Types."
4. Enter the appropriate parameters for the step type options or use the default values. For a list of the step types and their options, see "Autosampler Step Types."

5. Save the method. See "Saving the Instrument Method" on page 106.

Autosampler Step Types

This section provides specific information about the step types available when you create or edit an AS method. For a summary of these steps and their functions, see Table 16 and Table 17.

- Table 16 lists the most common, basic step types used in AS methods, and are separated from the additional step types by a gray line in the AS method editor.
- Table 17 lists the AS method step types that require advanced knowledge of the system and technology.

Figure 49. Basic AS step types list (AS method editor)

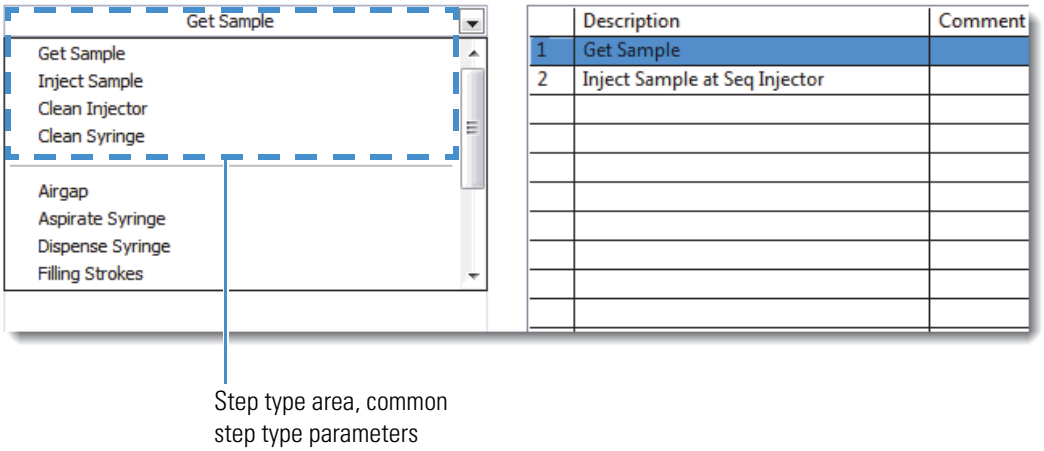


Table 16. Common AS method step types

Step type	Function
Get Sample	The probe moves to the vial position and draws the sample.
Inject Sample	The probe injects the sample into the specified autosampler valve.
Clean Injector	The specified wash solution flushes though the syringe loop and needle interior, and then into the injector port; or using a needle gap setting cleans the syringe needle exterior.
Clean Syringe	The specified wash solution rinses the syringe loop and needle interior.

Table 17. Advanced AS method step types (Sheet 1 of 2)

Step type	Function
Airgap	The syringe moves away from the sample container or injector and draws in air.
Aspirate Syringe	The syringe draws up at its current location. Precede this step type with the Move to Object step type to move the syringe to the appropriate location.
Dispense Syringe	The syringe dispenses a specified volume at its current location. Precede this step type with the Move to Object step type to move the syringe to the appropriate location.
Eject Syringe (Model A only)	The syringe dispenses its entire volume at its current location. Precede this step type with the Move to Object step type to move the syringe to the appropriate location.
Filling Strokes (Model B only)	Moves to sample, fills, and then empties the syringe, effectively mixing the sample. Thermo Fisher Scientific recommends that you precede this step type with the Move to Object step type, which moves the syringe to the appropriate vial location for filling. **Note** Use this step type with care when running biological samples as sample particulate matter can settle and become aspirated by the AS.
Infuse Sample	Moves the autosampler arm to the current injector, switches the injector valve into the fluid path, activates the LC pumps to start their methods, and then injects the sample. Use this step to infuse the sample into the stream, rather than introduce it into the system as a single injection.
Move to Object	Instructs the autosampler arm to move to a specified position.
Move Valve (Model B only)	Instructs the specified injector valve to a standby or an active position.
Rinse Injector	The specified wash solution flushes the injector port. This step type differs from the Clean Injector step in that the Rinse Injector step type does not use the syringe to fill the injector with wash solution.
Rinse Needle (Model A only)	The syringe needle enters a specified wash port. The specified wash solution rinses the inside and outside of the needle by setting the Needle Gap parameter to a value greater than 0 (the default Needle Gap value is 3).
Set Out Signal (Model A only)	Controls the output signal of a non-injector autosampler valve.

Table 17. Advanced AS method step types (Sheet 2 of 2)

Step type	Function
Switch Injector (Model A only)	Instructs the injector valve to change position (Standby, Active, or Toggle)
Wait	Instructs the autosampler to wait a specified amount of time.
Wait for LC Ready	Instructs the autosampler to wait for the LC system to signal that it is ready to accept another sample.
Wait for Signal (Model A only)	Instructs the autosampler to wait for the LC pumps, or other hardware, to be in the ready state.

Get Sample

The Get Sample step type (see Figure 50) instructs the probe to move to the sample vial and draw the sample. For a description of the Get Sample options, see Table 18.

Figure 50. Get Sample step type (Model B autosampler defaults shown)

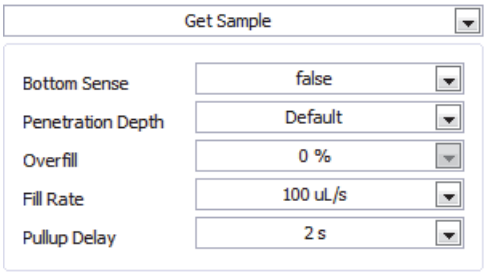


Table 18. Get Sample options, Model B autosampler (Sheet 1 of 2)

Option	Description
Bottom Sense	The sensor function that detects if a needle strikes the bottom of a sample vial. To turn on the bottom sense function, select **true**.
	Note Use this step type with care when running biological samples as sample particulate matter can settle and become aspirated by the AS.
Penetration Depth	The penetration depth of the needle into the sample vial.
Overfill	Additional percentage to aspirate and return to the sample vial.
Fill Rate	Specifies the speed of the plunger as the syringe fills.
	Tip When you use viscous samples, enter a slower fill speed than the default value.

Table 18. Get Sample options, Model B autosampler (Sheet 2 of 2)

Option	Description
Pullup Delay	The delay time between pulling up the plunger and the next action, such as ejecting sample from the syringe or moving the syringe to waste.
	Tip When you use viscous samples, enter a longer pull-up delay than the default value.

Inject Sample

The Inject Sample step type (see Figure 51) instructs the autosampler to inject the sample into the specified autosampler injector. For a description of the Inject Sample options, see Table 19.

Figure 51. Inject Sample step type (Model B autosampler defaults shown)

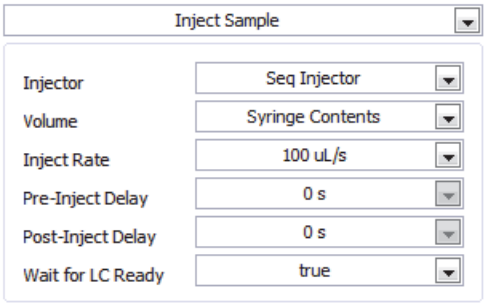


Table 19. Inject Sample options, Model B autosampler (Sheet 1 of 2)

Option	Description
Injector	Specifies the injector that you want to use to inject the sample into the LC system flow path. There are two options: • Seq Injector • LX
Volume	Specifies the sample volume to inject. There are two options: • Syringe Contents • Seq Volume
	Note If Syringe Contents appears, the syringe injects the entire syringe contents. If Seq Volume appears, the syringe injects the volume specified in the sample list.
Inject Rate	Specifies the plunger speed for sample injections.
Pre-Inject Delay	Specifies the delay time prior to sample injection.

Table 19. Inject Sample options, Model B autosampler (Sheet 2 of 2)

Option	Description
Post-Inject Delay	Specifies the delay time after the sample injection.
Wait for LC Ready	Instructs the autosampler to wait until it receives a ready signal from the LC system before making this injection. The default setting is "true" (on).

Clean Injector

The Clean Injector step type (see Figure 52) instructs the probe to wash the injector during the AS method. For a description of the Rinse Injector options, see Table 20.

Figure 52. Clean Injector step type

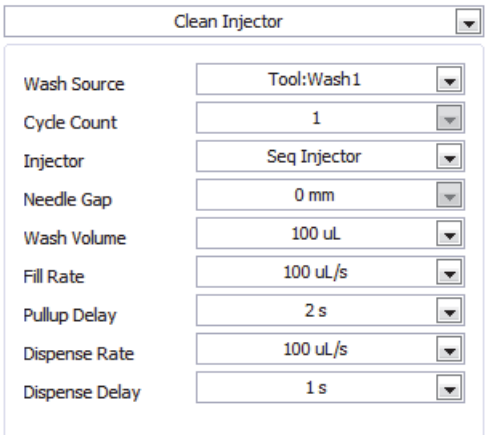


Table 20. Clean Injector options (Sheet 1 of 2)

Option	Description
Wash Source	Specifies the solvent that the autosampler uses to wash the injector. Select **Tool:Wash 1** or **Tool:Wash 2**.
Cycle Count	Specifies the number of Clean Injector step wash cycles. **Tip** Use a cycle count higher or lower based on Wash Volume and Fill Rate settings.
Injector	Specifies the injector that you want to use to rinse.

Table 20. Clean Injector options (Sheet 2 of 2)

Option	Description
Needle Gap	Specifies the height above the normal penetration depth for the injector. Adding a needle gap to the Clean Injector step type is strictly designed to allow for additionally washing the outside of the needle. Needle gap range: 0–10 mm Default: 0 mm
	Tip Thermo Fisher Scientific recommends setting the needle gap to 3 mm to effectively clean the outside of the needle.
Wash Volume	Specifies the syringe volume to aspirate (fill).
	Tip Use lower wash volumes to reduce syringe wear.
Fill Rate	Specifies the speed of the plunger as the syringe fills.
	Tip Decrease the fill rates to increase wash time and reduce syringe wear.
Pullup Delay	Specifies the delay time between pulling up the plunger and starting the rinse cycle.
Dispense Rate	Specifies the rate that wash volume is dispensed from the syringe.
Dispense Delay	Specifies the delay time prior to dispensing wash volume.

Clean Syringe

The Clean Syringe step type (see Figure 53) instructs the probe to move to the waste position at the active wash cup (injector) and flow wash solvent through the needle to waste during the AS method. For a description of the Clean Syringe options, see Table 21.

Tip Use the Clean Syringe step type immediately *after* the Inject Sample step to effectively remove residual sample from the syringe *before* cleaning the injector.

Figure 53. Clean Syringe step type

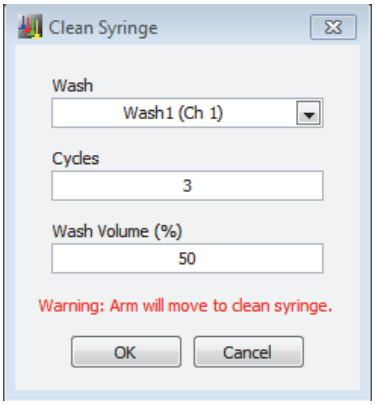


Table 21. Clean syringe options

Option	Description
Wash	Do one of the following: • To use the Wash 1 solution for the wash, select **Wash 1**. • To use the Wash 2 solution for the wash, select **Wash 2**.
Clean Cycles	Specifies the number of cycles to run for the cleaning operation. **Tip** Use a cycle count higher or lower based on your Wash Volume and Fill Rate settings.
Wash Volume	Specifies the amount of wash solvent—as a percent of total syringe volume—to use for the cleaning operation.
Fill Rate	Specifies the speed of the plunger as the syringe fills.
Pullup Delay	Specifies the delay time between pulling up the plunger and starting the cleaning cycle.
Dispense Rate	Specifies the rate that wash solvent is dispensed from the syringe.

Airgap

The Airgap step type (see Figure 54) removes the syringe from the injector or sample container and draws in air. You might want to use this step type before and after the Get Sample step type to avoid mixing solvent and sample. For a description of the Airgap options for both the Model A and Model B autosamplers, see Table 22.

Figure 54. Airgap step type (Model B autosampler defaults shown)

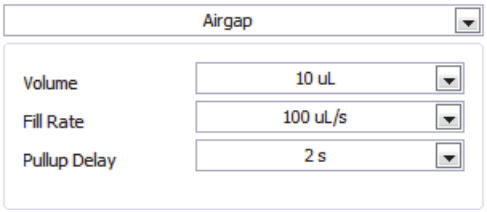


Table 22. Airgap options

Option	Description
Volume	The volume of air that you want the syringe to draw in.
Fill Rate	The speed of the plunger movement as the syringe fills with air.
Pullup Delay	The amount of delay time after the syringe has fully aspirated.

Aspirate Syringe

The Aspirate Syringe step type (see Figure 55) instructs the autosampler syringe to draw a specified volume of fluid from its current location. For a description of the Aspirate Syringe options, see Table 23.

Figure 55. Aspirate Syringe step type

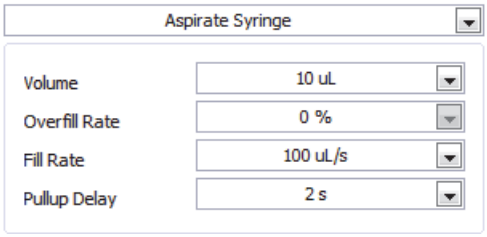


Table 23. Aspirate Syringe options

Option	Description
Volume	Amount of volume to aspirate.
Overfill Rate	Additional percentage to aspirate and return to the sample vial.
Fill Rate	The speed of the plunger movement as the syringe fills.
	Tip When you use viscous samples, enter a slower fill speed than the default value.
Pullup Delay	The delay time between pull-up and ejection or movement of the syringe.
	Tip When you use viscous samples, enter a longer delay time than the default value.

Dispense Syringe

The Dispense Syringe step type (see Figure 56) delivers a specified volume at the needle's current location. For a description of the Dispense Syringe options, see Table 24.

Figure 56. Dispense Syringe step type

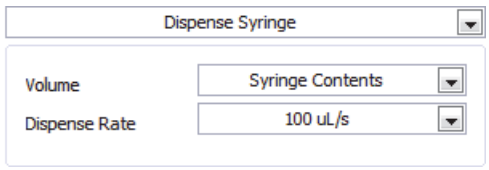


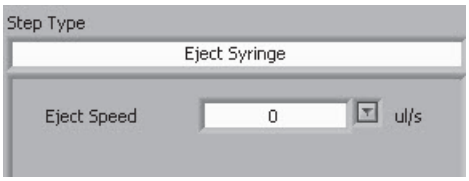
Table 24. Dispense Syringe options

Option	Description
Volume	The volume in the syringe that you want to dispense.
Dispense Rate	The plunger movement speed for the dispensing movement.

Eject Syringe

(Model A autosampler only) The Eject Syringe step type (see Figure 57) instructs the autosampler to eject the entire contents of the syringe at its current location. The only option for this step type is Eject Speed, which is the plunger movement speed for the ejection.

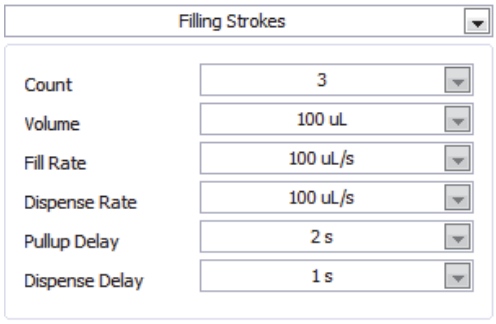
Figure 57. Model A autosampler Eject Syringe step type



Filling Strokes

(Model B autosampler only) The Filling Strokes step type fills the syringe and then dispenses for a set number of counts at specified rates. Precede this step type with the Move to Object step type to move the syringe to the appropriate vial location for filling.

Figure 58. Filling Strokes step type



Infuse Sample

The Infuse Sample step type moves the autosampler arm to the current injector, switches the injector valve into the fluid path, activates the LC pumps to start their methods, and then injects the sample.

Use this feature to infuse the sample into the stream, rather than introduce it into the system as a single injection. You can use the Infuse Sample step type in place of the Inject Sample step type. For a description of the Infuse Sample options, see Table 25 for systems equipped with the Model A autosampler and Table 26 for systems equipped with the Model B autosampler.

Figure 59. Infuse Sample step type (Model A autosampler)

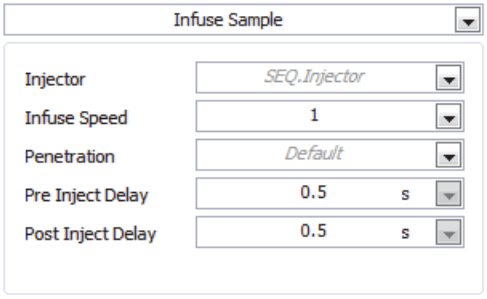


Table 25. Model A autosampler Infuse Sample options

Option	Description
Injector	Specifies the injector where the autosampler infuses the sample. To select the injector in the sample list, select **SEQ.Injector**.
	Note If the selection in this list does not match the selection in the Injector lists for other related step types in the method, a warning message appears when you save the method. Click **Yes** to continue.
Infuse Speed	The speed at which you want the autosampler to inject the sample.
Penetration	Determines the depth at which the needle penetrates the injector. • If this value appears gray, the autosampler uses the default value; otherwise, type a new value. • If this value appears black, it overrides the default value. To return to the autosampler default value, delete the override. • Thermo Fisher Scientific recommends using the parameter defaults when infusing into the LC flow.
	IMPORTANT Changing this value can affect the performance of your system. See "Penetration Value Special Notice (Model A Autosampler)" on page 100.
Pre Inject Delay	Adds a delay before infusion begins. Select the delay time in seconds.
Post Inject Delay	Adds a delay after the sample infusion. Select the delay time in seconds.

Figure 60. Infuse Sample step type (Model B autosampler)

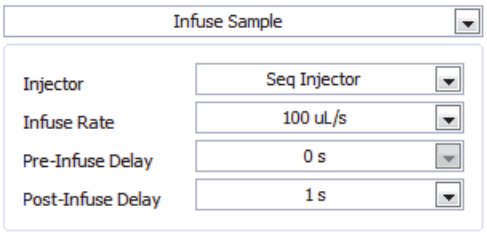


Table 26. Model B autosampler Infuse Sample options

Option	Description
Injector	Specifies the injector that you want to use to inject the sample.
Infuse Rate	The speed at which you want the autosampler to inject the sample.
Pre-Infuse Delay	Adds a delay before infusion begins. Select the delay time in seconds.
Post-Infuse Delay	Adds a delay after the sample infusion. Select the delay time in seconds.

Move to Object

The Move to Object step type (see Figure 61) instructs the autosampler arm to move to a specified location. Use this step type with certain step types that do not automatically move to an object. These include Aspirate Syringe, Dispense Syringe, and Eject Syringe. For a description of the Move to Object options, see Table 27.

Figure 61. Move to Object step type (default view, Model A autosampler)

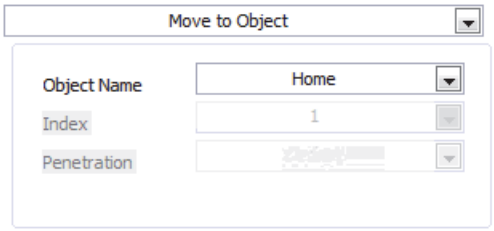


Table 27. Move to Object options (Model A autosampler)

Option	Description
Object Name	The object to which the autosampler moves. • To move the autosampler arm to the current sample vial as determined by the sample list, select **SEQ.Tray**. • To move the autosampler arm to the current AS injector as determined by the sample list, select **SEQ.Injector**. • To move the autosampler arm to a specific location, select an autosampler object position such as the wash station, home (autosampler arm resting position), injector, vial, or tray.
Index	The specific vial location when the object specified is a tray.
Penetration	The depth at which the needle penetrates the object. For the Model A autosampler, if this value appears gray, the autosampler uses the default value. To override the autosampler default value, type a new value. If this value appears black, the autosampler default value has been overridden. To return to the autosampler default value, delete the override. **IMPORTANT** For the Model A autosampler, changing this value can affect the performance of your system. See "Penetration Value Special Notice (Model A Autosampler)" on page 100.

Move Valve

(Model B autosampler only) The Move Valve step type actuates the LC injector valve to the specified position (from Standby to Active). For a description of the Move Valve options, see Table 28.

Figure 62. Move Valve step type (Model B autosampler only)

Table 28. Move Valve options (Model B autosampler only)

Option	Description
Valve	Specifies the valve that you want to use for the method step. Thermo Fisher Scientific recommends using the default setting (Seq Injector).
Position	Specifies the valve position that you want to use for the method step. Two options: • Active • Standby

Rinse Injector

The Rinse Injector step type instructs the probe to move to, and then rinse the injector without pulling up the syringe plunger. See Figure 63 for Model A and Figure 64 for Model B autosamplers respectively.

You can set a needle gap on the Model B autosampler that allows rinsing the outside of the needle without pulling up the syringe plunger. For a description of the Rinse Injector options for both autosampler models, see Table 29.

Figure 63. Rinse Injector step type example (Model A autosampler)

Figure 64. Rinse Injector step type example (Model B autosampler)

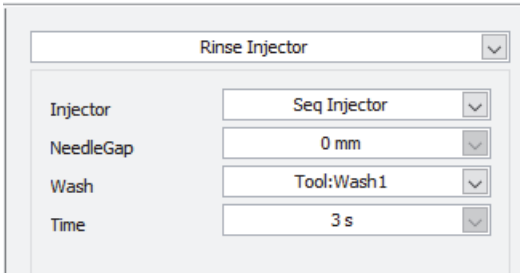


Table 29. Rinse Injector options

Option	Description
Injector	The injector that you want the autosampler to wash.
	Note If the selection in this list does not match the selection in the Injector lists for other related step types in the method, a warning message appears when you save the method. Click **Yes** to continue.
Needle Gap (Model B autosampler)	The height above the normal penetration depth for the injector.
	Tip Use the default value unless you have been instructed to change it by a service engineer.
Wash (Model A autosampler)	The solvent that the autosampler uses to wash the injector. Select **Wash 1** or **Wash 2**.
Wash (Model B autosampler)	The solvent that the autosampler uses to wash the injector. Select **Tool:Wash 1** or **Tool:Wash 2**.
Rinse Time (Model A autosampler)	The time in seconds that the autosampler washes the injector.
	Tip Enter a rinse time equal to **2** or higher.
Time (Model B autosampler)	The time in seconds that the autosampler washes the injector.
	Tip Enter a rinse time equal to **2** or higher.

Rinse Needle

(Model A autosampler only) The Rinse Needle step type (Figure 53) washes the *outside* portion of the needle during the AS method. To effectively wash residual sample from the needle after an Inject Sample or an Infuse Sample step, set the Injector to Waste and the Needle Gap to 3 mm.

Note For the Model B autosampler, the Rinse Injector step type allows exterior needle rinsing when using a 3 mm needle gap and injector rinsing when using a 0 mm needle gap. For more information, see Rinse Injector.

For a description of the Rinse Needle options, see Table 30.

Figure 65. Rinse Needle step type (Model A autosampler)

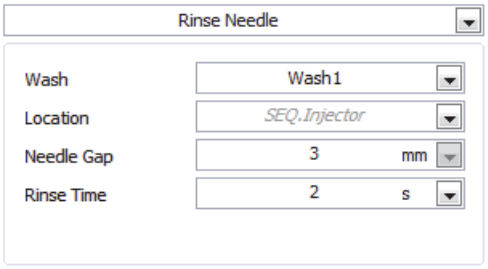


Table 30. Rinse Needle options

Option	Description
Wash Station	Select either **Wash 1** or **Wash 2**, as applicable.
Injector	The location that the autosampler uses to wash the needle.
Needle Gap	The height above the normal penetration depth for the injector.
	Tip Use the default value unless you have been instructed to change it by a service engineer.
Rinse Time	The time in seconds that the autosampler washes the needle.

Set Out Signal

(Model A autosampler only) The Set Out Signal step type (see Figure 66) sets the output signal and signal state of a non-injector autosampler valve. Some external applications use non-injector autosampler valves.

Note You do not typically use this advanced step.

For a description of the Set Out Signal options, see Table 31.

Figure 66. Model A autosampler Set Out Signal step type

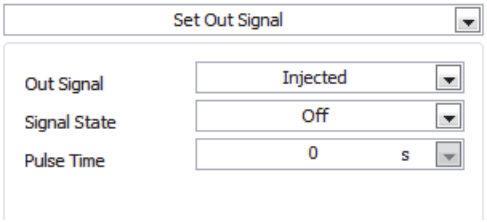


Table 31. Model A autosampler Set Out Signal options

Option	Description
Out Signal	The out signal type to set.
Signal State	The signal state.
Pulse Time	The time in milliseconds that you want the signal pulse to last. To keep the valve in the new state, type **0**.

Switch Injector

(Model A autosampler only) The Switch Injector step type (see Figure 67) actuates the LC injector valve to the specified position. For a description of the Switch Injector options, see Table 32.

Figure 67. Switch Injector step type

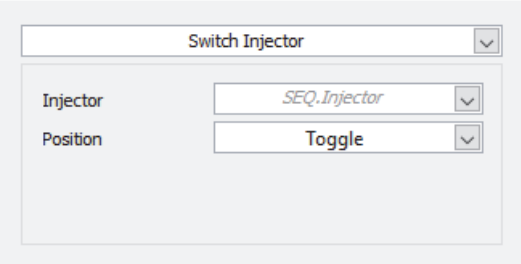


Table 32. Switch Injector options

Option	Description
Injector	Specifies the injector valve that you want to use.
Position	Specifies the injector valve position. For example, if the injector is in Standby, it switches to the Active position, and it remains in the Active position until another step in the method changes it back. The three positions are as follows: • Standby: The sample loop is in line with the fluid path and closed to the injector port and waste. • Active: The sample loop is closed to the fluid path and open to the injector port and waste. • Toggle: Switches the injector valve from current (set) position to the opposite and back to the original (set) position.
	Tip Switching the injector several times during a method might be helpful for optimal cleaning.
	IMPORTANT To prevent the system from shutting down due to increased pressure, always end the method with the valve in the Standby position.

Wait

The Wait step type (see Figure 68) adds a wait time to your autosampler method.

The Time option is the wait time in seconds that you want to add to the method.

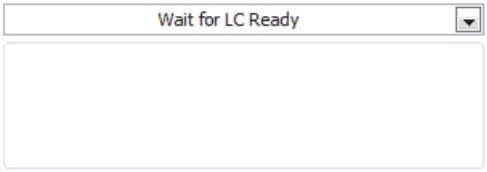
Figure 68. Wait step type

Wait for LC Ready

The Wait for LC Ready step type (Figure 69) instructs the autosampler to pause until it receives a ready signal from the LC system.

This step type is standalone and has no additional parameters.

Figure 69. Wait for LC Ready step type

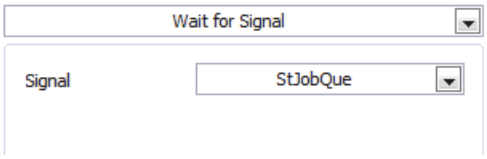


Wait for Signal

(Model A autosampler only) The Wait for Signal step type (see Figure 70) temporarily halts the autosampler method to wait for the occurrence of the specified hardware signal.

Note This option is for advanced users only.

Figure 70. Wait for Signal step type



The Signal option indicates which signal the autosampler will wait for. The signals include the following:

- St Job Que
- Start
- Start 2
- Inject
- Inject 2

- Pause
- Filling Strokes
- Rinse Injector
- Rinse Needle

Penetration Value Special Notice (Model A Autosampler)

The Inject, Infuse, and Move to Object step types for the Model A autosampler provide the option to set the penetration value; however, Thermo Fisher Scientific recommends that you keep the default value.

IMPORTANT A service engineer carefully calibrates the default Penetration value at the time of installation. Override this value only for experimental purposes and only if you have advanced knowledge of the autosampler functions. If you believe that the current default penetration value is faulty, then a service engineer must recalibrate it. Contact Technical Support. See "Contacting Us" on page xv.

Editing the AS Method for Maximum Throughput

To optimize your autosampler method for better throughput, perform one or more of the following procedures:

- Verify that the Allow Cross-Seq Optimization check box is selected in the Logic Settings dialog box. See "Editing Logic Settings" on page 66 and "AS Method Timing Options."
- In the AS Method Editor window, leave the Prior to Sample, Pre-Inject Total, LC Sync, and Post Injection boxes blank. See "AS Method Timing Options."
- Create an autosampler method that is less than or equal to one half of the LC method length.

AS Method Timing Options

Note This option is for advanced users only.

The Prior to Sample, Pre-Inject Total, and Post Injection boxes in the AS Method Editor window represent the time segments of the autosampler method before and after the sample injection. The "LC Sync" and "Prior to LC Sync" timing estimates each represent the time when the LC is ready ahead of switching the injection loop in line to make an injection. The default setting of this synchronization is True for the Inject step of the AS method.

When you run multiple channels, the instrument control application considers the AS method timing when it times the sample starts. The accuracy of the time segments before and after the sample injection affects the timing of the sample starts, which then affects sample throughput. As the accuracy of the time segment values improves, so does the sample throughput.

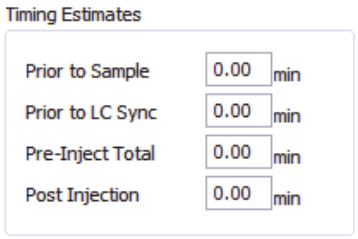
The Optimize AS Methods feature, which is controlled from the Advanced Logic Settings dialog box, maintains the accuracy of these values by recording and averaging the applicable time segments with each method run. However, you can override this feature. For more information on the Logic Settings, see "Editing Logic Settings" on page 66.

Tip Use timing estimates when not using auto-optimization. Doing so helps to normalize inter-sample spacing and/or improves sample processing efficiency when switching between methods. Actual method run times can be reviewed from the event log.

Thermo Fisher Scientific recommends that you use the longest likely time values for your timing estimates.

Figure 71 shows the timing estimates in the AS method editor (see Figure 46).

Figure 71. Autosampler method Timing Estimates area, showing default values



❖ To set the Timing Estimates in the AS Method Editor window

Note Enter values in these boxes only if you are an advanced user.

Do one of the following:

- To adjust the sample starts using AS method timing values calculated from previous runs, leave the boxes at the default value of 0.00 minutes (see Figure 71).

  Note The calculated values do not appear in these boxes. The application stores them internally.

- To override the calculated values for these time segments, clear the **Optimize AS Methods** timing feature (check box) in the Logic Settings dialog box (see "Editing Logic Settings" on page 66), and type values in the four Timing Estimates boxes, based on an average of previous values obtained from the sequence log.

Import and Convert AS Methods

The following sections provide details on importing AS methods from another instrument method and migrating AS methods from legacy Prelude systems that run on instrument control software versions 1.2 or earlier.

Note You cannot convert an AS method created on a Model B autosampler into a Model A autosampler. Imports within the same autosampler model are supported.

Import the AS Method from an Instrument Method

You can import the AS method portion of an instrument method (.meth) into another instrument method. This procedure only imports the AS portion of the method; it does not import the LC method and detector method information.

Note If you want to import the LC method information, see "Importing the LC Method from an Instrument Method" on page 131.

❖ **To import the AS method information from an instrument method**

1. Open the instrument method by using your data processing software.
2. Click Prelude LX-4 MD. The AS Method Editor window opens.
3. Choose **Edit > Import From Inst Method (.meth)**. Navigate to the instrument method file (.meth) that contains the AS method information that you want to import, and select it.
4. Click **Import**.

   The AS method information appears in your open method.

Converting Model A Autosampler Methods

You can convert Model A legacy autosampler methods created on Prelude instruments running instrument control software—version 1.2 or earlier—to Model B autosampler methods running instrument control software version 1.3. Convert your legacy AS method by using the Prelude Autosampler method editor Import feature, which you can access from your data system application.

This feature is useful if your lab is updating its autosampler hardware to the Model B autosampler, or if your lab is using a mix of Model A and Model B hardware.

Note

- Legacy Model A autosampler method steps imported to a Model B autosampler method require review and validation. In certain cases, converted methods might also require modifications to step parameters. Follow the on-screen message instructions when performing a conversion to understand which step parameters might require further editing or modification.
- Model A autosampler methods created on instrument control software version 1.2 or earlier function under instrument control software version 1.3 without further modification on a Model A instrument.

Before you convert a legacy Model A autosampler method to a Model B method, Thermo Fisher Scientific recommends that you do the following:

- Print the legacy AS method report from an existing RAW file for reference. Refer to your data acquisition software documentation for information how to print a RAW data file.
- Create a separate copy of the method file by performing a Save As command of the Model A method file to prevent overwriting a file that might be in use.

The instrument control software notifies you that it will attempt to convert to the current hardware, but some parameters might not translate. Review, make any necessary modifications to the converted method, and then save your changes.

❖ To convert a legacy Model A autosampler method to a Model B autosampler method

1. Open the Instrument Method editor from the data acquisition application that is used in your laboratory.
2. Click **Autosampler**.

   The autosampler method editor window appears (see Figure 72).
3. Choose **Edit > Import from Inst Method (*.meth)**.

   The Import from instrument method dialog box opens.
4. Using Windows Explorer, navigate to and then choose the method file that you want to open and convert, and then click **OK**.

   A method warning message appears regarding the conversion process.
5. Read the message, and then click **OK**.

   The legacy autosampler method is converted and the method steps appear in the Model B step table.

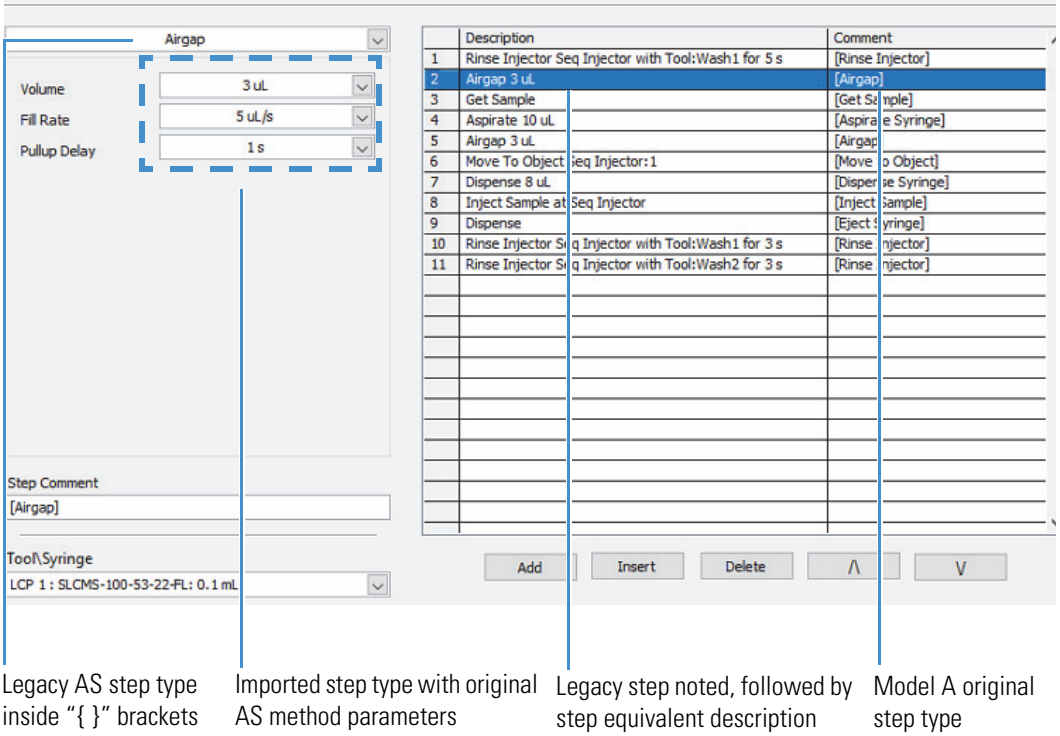
Note Contact Thermo Fisher Scientific Technical Support if you have any questions on the appropriate modifications that your converted method might require

After you have performed the conversion, review the method steps that might require substitution or modification. The Model B autosampler method editor highlights the converted steps as follows (see Figure 72):

- All legacy autosampler (AS) method steps are noted and placed inside "[ ]" brackets in the Model B method step table, Comments column.
- All legacy method steps that require additional modifications are placed inside "{ }" brackets in the Model B method step table Description column. The converted Model B method step immediately follows the bracketed legacy AS step type.
- The converted Model B method step types, when selected in the step table (blue highlighted row in Figure 72), appear in the step type area and *retain the original (legacy) AS method step parameters*.
- The original (legacy) AS method step appears inside "{ }" brackets inside the Model B step type box. Use the step type box to formally change the displayed legacy AS step type to the equivalent Model B step type, but *be aware that default Model B method step parameters replace the original AS method step parameters*.

Note A default Model B autosampler method is included on the Prelude software installation disk. Thermo Fisher Scientific recommends that you review the various step type parameters used in the default method, which can be different between the Model A and Model B autosamplers, and use them as a starting point when making changes to your laboratory's converted legacy methods.

Figure 72. Model B method editor with a converted AS method showing step type substitutions



After importing the legacy autosampler method to a Model B AS method, review the result and then do the following:

- (Recommended) Change the Model A legacy AS step types—highlighted inside "{ }" brackets—to the equivalent Model B step type by choosing it from the step type list (see Figure 72).

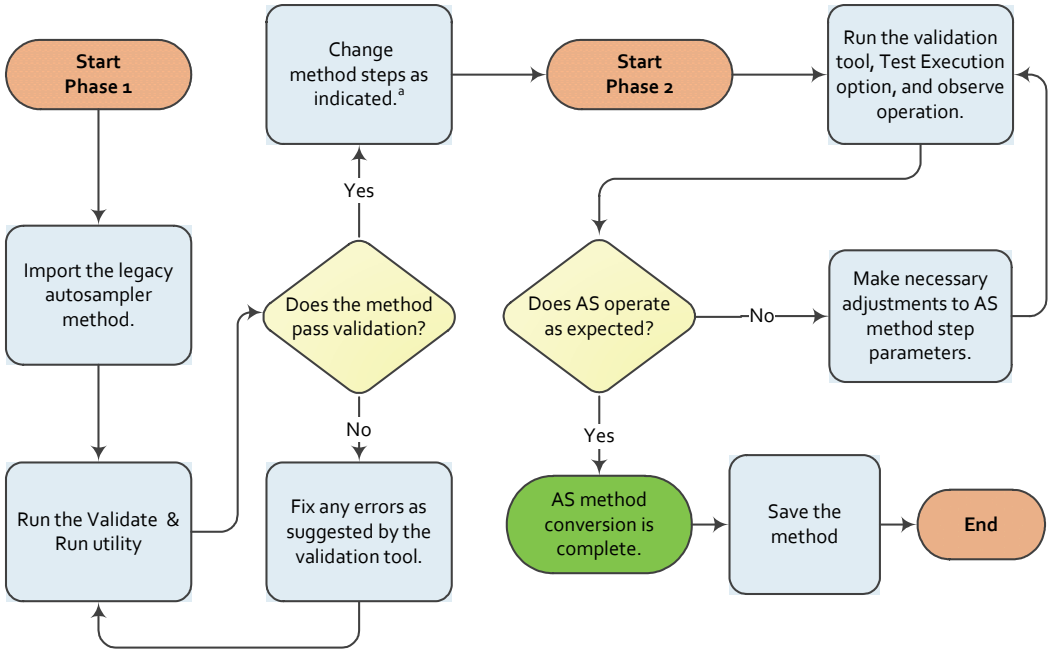
  Note Model B method step parameters are different from the Model A step parameters. Do the following:

  - Carefully review your printed legacy Model A method step options.
  - Double check your Model B step parameters after making the suggested step changes, as applicable.

- Validate the new method using the Direct Control Validate & Run utility, which is completed in two phases (see Figure 73), and then correct any errors or make step type parameter adjustments, as needed. For more information, see "Validate and Run a New AS Method" on page 79.

- (Optional) Change legacy Rinse Injector/Rinse Needle commands to Clean Injector/Clean Syringe commands to access the more versatile Model B autosampler command options. Make a note of legacy Rinse command step parameters before changing to the Clean command step.

Figure 73. Workflow for validating converted Model A (legacy) autosampler methods



[a] Review a copy of the legacy method step parameters before changing step types to Model B version .

Complete the legacy method conversion by validating the new Model B autosampler method. For more information, see "Validate and Run a New AS Method" on page 79.

Related Topics

- Supported Autosampler Models
- Create an Autosampler Method
- Tips for Creating an Autosampler Method
- Validate and Run a New AS Method

Saving the Instrument Method

Saving the instrument method saves the autosampler method.

❖ **To save the instrument method in the Instrument Setup window**

Do one of the following:

- To save the autosampler method and any changes you made to a new file name, choose **File > Save As** in the Instrument Setup window. Then navigate to the folder where you want to save the instrument method, type a name for the method, and click **Save**.
- To save changes made to the autosampler or instrument method, choose **File > Save** in the Instrument Setup window. For information on accessing the Instrument Setup window, refer to the documentation provided with the data processing software.

8

Creating an LC Method

This chapter describes how to enter or edit LC method information, such as adding and deleting steps, and changing the flow rates, valve positions, and mobile phase composition.

Contents

The LC method refers to the portion of the instrument method that controls the pumps, valves, and the data acquisition start and stops. It contains a series of steps that specify valve position, flow rate, and mobile phase composition.

Accessing the LC Method Editor

❖ To access the LC Method Editor

1. Open the instrument method that you want to view.

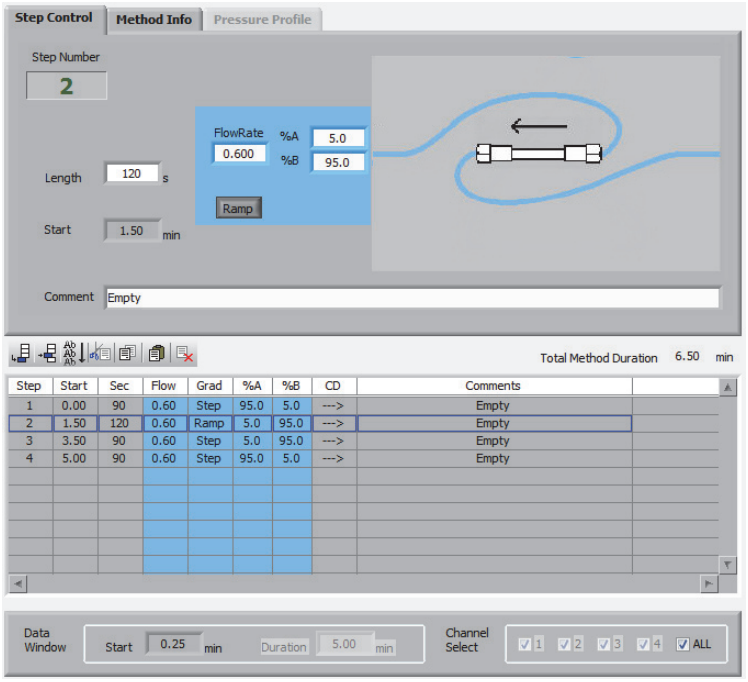
   For information on accessing the instrument method, refer to the documentation provided with the data processing software.

2. Click **Prelude LX-4 MD**.

3. In the left pane, click **LC Method**.

   The LC Method Editor window opens (see Figure 74).

Figure 74. LC Method Editor window



Creating an LC Method

This procedure describes how to enter the LC method information.

❖ To create an LC method

1. Open the LC Method Editor. See "Accessing the LC Method Editor" on page 108.
2. To add a step to your method, click the **Step Control** tab and then click the **Add** button,  . Repeat this process to add more steps.

   The new steps appear with default values for duration, flow rate, and mobile phase composition.
3. On the Step Control page, enter values for the method step duration, flow rate, mobile phase composition, and valve position. See "Modifying Components in an LC Method Step" on page 111.
4. Assign the start time and duration of the data window. See "Assigning the Data Window" on page 115.
5. (Optional) To view a graphical representation of your method, see "Viewing the Graph" on page 119.
6. Verify that the total volume consumed for any one solvent does not exceed 3 mL.

   To view the solvent usage, see "Determining the Method's Solvent Usage" on page 118. The volume of each mobile phase for one sample injection must be less than 3.0 mL.
7. Enter the appropriate column temperature values. See "Setting the Heater Temperature in an Instrument Method" on page 132.
8. Enter the Prelude Prestart pressure values for each pump. See "Entering the Prelude Prestart Values" on page 116.
9. Enter the bottle set that you want the system to use while running this method. See "Assigning Bottle Sets to a Method" on page 117.
10. To enter variables for a method component, such as flow rate or mobile phase composition, see "Allowing Method Variables During a Run" on page 123. Use this feature to vary method components while optimizing a method.
11. Click the **Method Info** tab and enter general information about the LC method, such as the solvents used. See "Entering Information on the Method Info Page" on page 117.
12. Enter the LC method data window duration into the detector portion of the instrument method for the acquisition time.

    Refer to your data processing software documentation for information about entering the detector acquisition time. If the LC method length and the detector acquisition length are not the same, system errors might occur during the run.

Modifying Steps in an LC Method

This section describes how to add, delete, copy, and move steps in the LC Method Editor. Follow these procedures:

- To add a step to the end of the method
- To insert a step within the method
- To delete a step
- To remove a step and paste it to a different position
- To copy one or more steps
- To undo a change you made to the LC method
- To redo the most recent changes that you undid

❖ To add a step to the end of the method

1. Click the **Add** button,  .

   A new step appears at the end of the LC Method Step table with the same information as the previous step.

2. Edit the step information. See "Modifying Components in an LC Method Step" on page 111.

❖ To insert a step within the method

1. Click the row above which you want to add the step.

   The row becomes highlighted.

2. Click the **Insert** button,  .

   A new step with the same information as the highlighted step appears beneath it. The control application sequences the step numbers.

3. Edit the step information. See "Modifying Components in an LC Method Step" on page 111.

❖ To delete a step

1. Click the step that you want to delete.
2. Click the **Delete** button,  .

   The control application removes the highlighted step.

❖ **To remove a step and paste it to a different position**

1. Select the step that you want to move.
2. Click the **Cut** button, , to remove the step.
3. Click the step that is below the position where you want to paste the step.
4. Click the **Paste** button, .

   The step appears above the selected step.

❖ **To copy one or more steps**

1. Select the step or steps that you want to copy.
2. Click the **Copy** button, .
3. Click the step below the position where you want to place the copied step.
4. Click the **Paste** button, .

   The step appears above the selected step.

❖ **To undo a change you made to the LC method**

In the LC Method Editor window, choose **Edit > Undo**.

The LC method appears as it did before the most recent change.

Note You can undo up to ten most recent changes.

❖ **To redo the most recent changes that you undid**

In the LC Method Editor window, choose **Edit > Redo**.

The LC method appears as it did before you selected Undo.

Modifying Components in an LC Method Step

Follow these procedures:

- To activate a step for editing
- To change the duration of the step
- To copy information from one cell to all the selected cells below it (by using Fill Down)
- To change the pump flow rate
- To change the pump flow rate option from a step change to a flow rate ramp
- To change the composition of the mobile phase

- To select a ramp or step mobile phase composition change
- To change the direction of flow through the analytical column

❖ To activate a step for editing

1. In the LC Method Editor window, click the **Step Control** tab.

   The step information appears (see Figure 74 on page 108).

2. In the LC Method Step table, click anywhere in the step that you want to edit to highlight the step.

   The selected step information appears in the upper portion of the window.

   You can now edit the step components by clicking directly in the table cell that you want to edit, or by clicking in the boxes that are located in the upper portion of the window.

> **Note** For a description of the LC Method Step table column headings, see "Working with the LC Method Step Table Columns" on page 123.

❖ To change the duration of the step

Select the Sec value in the LC Method Step table and type the new length.

❖ To copy information from one cell to all the selected cells below it (by using Fill Down)

1. Hold down the mouse button in the column that you want to copy.
2. Drag the cursor to the last entry in the column that you want to edit and release the mouse button to highlight the entries.
3. Click the **Fill Down** button,  .

   The value in the first entry appears in all the selected entries.

> **Tip** You can also use the right mouse button to copy information from one entry in the column to the following entries. To do this, right-click the list of entries. A list of shortcut commands appears. Choose **Fill Down**. The new values appear in the selected entries.

❖ To change the pump flow rate

Enter the new flow rate in the Flow Rate box.

> **Tip** You can also edit flow rate values within the LC Method Step table. To do this, select the value in the table that you want to edit and type the new value.

❖ To change the pump flow rate option from a step change to a flow rate ramp

Note With a ramp flow rate change, the step begins by using the pump flow rate that was entered in the previous step. It then gradually changes to using the pump flow rate that was entered for the current step until it achieves the flow rate at the end of the step.

Select the **Ramp FR** check box (see Figure 75).

Figure 75. Area of LC Method Editor window showing the Ramp FR check box

If the Ramp FR check box does not appear, do the following:

a. In the LC Method Editor window, choose **Edit > Method Configuration**.

The Method Configuration dialog box opens (see Figure 83 on page 120).

b. Select the **Flow Ramping** check box, and then click **OK** to close the dialog box.

❖ To change the composition of the mobile phase

Enter the appropriate percentages of solvent A or B in the %A or %B box.

The control application adjusts values to ensure a total solvent percentage of 100.

Note

You can edit the solvent percentage that is used directly in the LC Method Step table. To do this, select the value that you want to change in the table and type the new value.

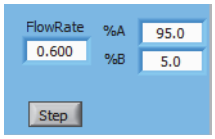
Check that the total volume of any solvent used in the method does not exceed 3 mL.

Each pump uses a 3 mL syringe mechanism to pump fluids through the system and columns. The pump slowly dispenses the syringe volume throughout the method and refills the syringe at the end of the method. As you create your method, check the total volume for each solvent. See “Determining the Method’s Solvent Usage” on page 118.

❖ To select a ramp or step mobile phase composition change

Click **Step/Ramp** to change the way in which the mobile phase composition changes in the method step (see Figure 76).

Figure 76. Area of LC Method Editor window showing the Step button



The following occurs:

- The button display changes to Ramp or Step.
- The entry in the Grad column changes to Ramp or Step.
- If you select Ramp, the instrument executes the mobile phase composition changes for this step during the run as follows:

  The mobile phase conditions in the beginning of the step are the same as in the previous step. Then, they gradually change to the mobile phase conditions that you entered for the step. By the end of the step, the instrument achieves the mobile phase conditions entered for the step.

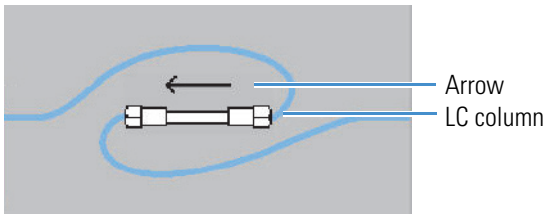
- If you select Step, the instrument executes the mobile phase composition changes for this step during the run as follows:

  The mobile phase conditions change in the beginning of the step to achieve this step's assigned mobile phase composition as soon as possible. When the mobile phase reaches the assigned composition depends on the pump type you are using.

❖ To change the direction of flow through the analytical column

1. In the table in the LC Method Editor window, highlight the step row where you want to make a change (see Figure 74 on page 108).
2. Click anywhere in the pictorial above the table (see Figure 77).

Figure 77. Pictorial in LC Method Editor window



The direction of flow changes in the pictorial and in the step row.

Assigning the Data Window

The data window refers to the method time segment in which the detector records data. If you run more than one LC channel with one detector, set the data window start time and duration to maximize throughput.

❖ To assign the data window

1. Open the LC Method Editor window. See "Accessing the LC Method Editor" on page 108.
2. In the Start box in the Data Window area, enter the time in the method when you want the data collection to start.
3. In the Duration box, enter the length of time that you want to collect the data.

   If you run more than one channel at a time, the data window length can affect your throughput. To maximize throughput, enter a data window length that is equal to or less than one quarter the total LC method time.
4. If you did not select the Sync Data Window to MS check box in the Options page of the Editor Configuration dialog box (see "Changing the LC Method Editor Options" on page 121), or you are not using a Thermo Scientific detector, enter the data window duration in the detector method by using your data processing software.
5. To view a graphical representation of the data window and method timing, choose **Tools > Graph Display**. See "Viewing the Graph" on page 119.

Assigning Channels to the Method

If one or more LC channels are assigned to a method, and no channels are assigned in the sample list, the method runs on the method-assigned channels. Assign the channels that can run this method if solvent or column conditions on any of the channels are not compatible with the method.

❖ To assign channels to the method

1. Open the LC Method Editor window. See "Accessing the LC Method Editor" on page 108.
2. In the Channel Select area, select the channels that you want to use to run this method (see Figure 78).

   Figure 78. Channel Select area in the Method Editor window

   Channels that you select in the sample list or batch file override channels that you select in the LC Method Editor.

Entering the Prelude Prestart Values

During the Prelude Prestart period, the system first pressurizes to the Target Pump Pressure. It then flows at the rates set in the first step of the method for the Prestart Duration, to pressurize the entire system prior to the injection.

❖ To assign the Prelude Prestart values to a method

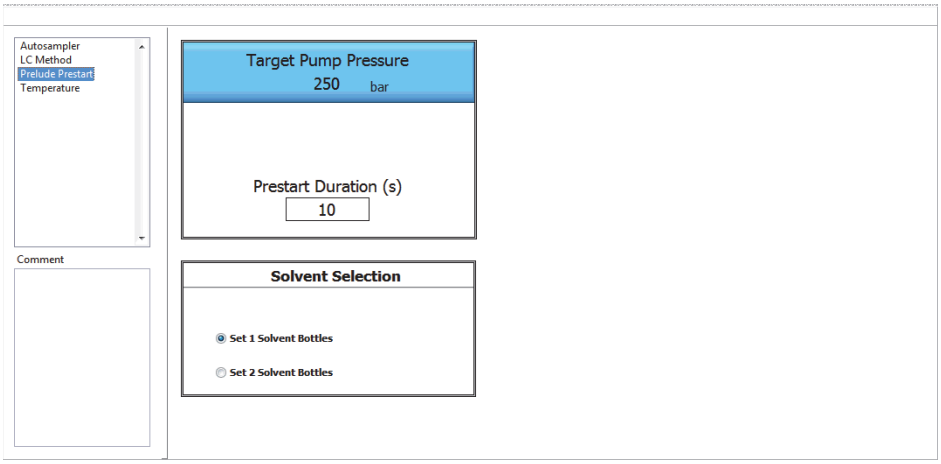
1. From your data processing software's Instrument Setup window, click **Prelude LX-4 MD**.

   A list of Prelude LX-4 MD method types appears in the left pane.

2. Click **Prelude Prestart**.

   The Prelude Prestart window opens (see Figure 79).

   Figure 79. Prelude Prestart window



3. Select the value in the Target Pump Pressure box and enter the new target value.

   Note The Target Pump Pressure value refers to the expected pump pressure during the first step of the method. To determine this value, do the following:

   1. Install the appropriate analytical column.
   2. In the Direct Control window, select the channel and set the flow rates to the flow rates in the first step (step 1) of the method.
   3. Dispense the fluids and observe the pump pressure to determine the target.

4. In the Prestart Duration (s) box, enter the number of seconds that you want the flow rate to be stable at the target pressures before injecting the sample.

   A typical value is 10 seconds.

Assigning Bottle Sets to a Method

❖ **To assign a bottle set to a method**

1. From your data processing software's Instrument Setup window, click **Prelude LX-4 MD**.

   A list of Prelude LX-4 MD method types appears in the left pane.

2. Click **Prelude Prestart**.

   The Prelude Prestart window opens (see Figure 79 on page 116).

3. In the Solvent Selection area, select one of the following options:

   - To assign Bottle Set 1 to this method, select the **Set 1 Solvent Bottles** option.
   - To assign Bottle Set 2 to this method, select the **Set 2 Solvent Bottles** option.

   For information about bottle sets, see "Assigning Bottle Sets" on page 46.

Saving an LC Method

❖ **To save an LC method**

1. Do one of the following:

   - To save the method under the same name, choose **File > Save** in the Instrument Setup window. For information on accessing the Instrument Setup window, refer to the documentation provided with the data processing software.
   - To save the method under a different name, choose **File > Save As**.

   The Select Method File Path dialog box opens.

2. Type a name for the LC method and click **OK**.

Entering Information on the Method Info Page

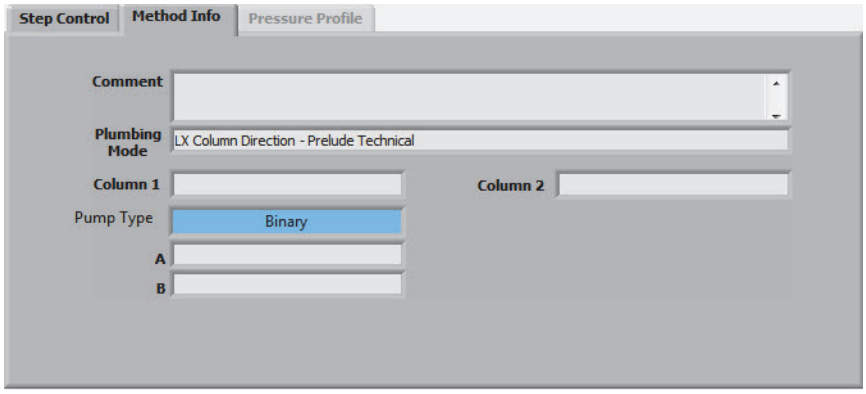
Use the Method Info page to record method information.

❖ **To enter information on the Method Info page**

1. In the LC Method Editor window (see Figure 74 on page 108), click the **Method Info** tab.

   The Method Info page opens (see Figure 80).

Figure 80. Method Info page in the LC Method Editor window



2. In the Comment box, type a description of the LC method.
3. In the Column 1 box, type the column information.
4. In the Column 2 box, type the column information.
5. In the Pump Type boxes, type information that identifies the solvents.

   The number of options that appear depends on the values entered in the LC Method Editor Configuration window.
6. Choose **File > Save** to save the method, or choose **File > Save As** to save the method by using a new name.

Determining the Method's Solvent Usage

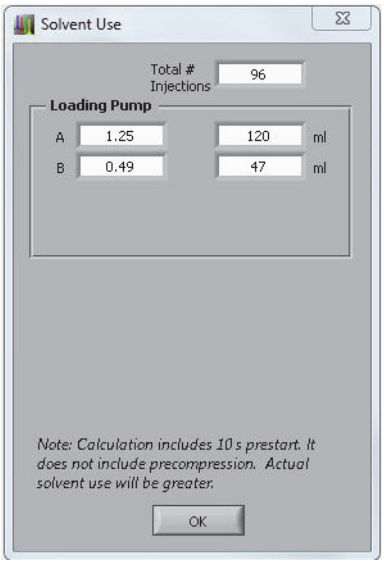
As you develop a method, use this procedure to determine the solvent usage. You must keep the total volume used during a method for any one solvent equal to or less than 3.0 mL.

❖ To view the amount of solvent used by the method

1. In the LC Method Editor window (see Figure 74 on page 108), choose **Tools > Solvent Use**.

   The Solvent Use dialog box opens (see Figure 81).

Figure 81. Solvent Use dialog box



2. To change the number of injections, select the current value in the Total # Injections box, and type a new number.
3. Click anywhere outside the Total # Injections box.

   The solvent volumes change to match the new value.

Note These values do not include volume lost due to solvent compression.

Viewing the Graph

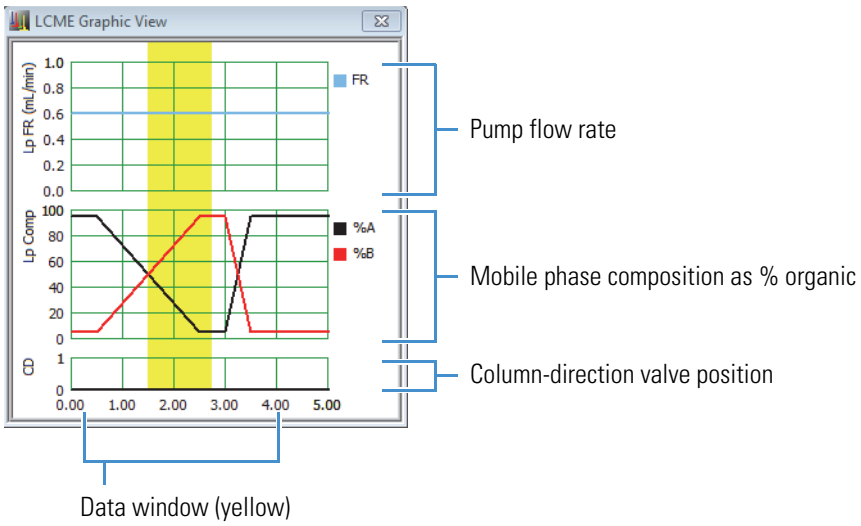
With the method graph, you can see the method component changes in relation to the method timing.

❖ **To view a graph of the LC method**

In the LC Method Editor window (see Figure 74 on page 108), choose **Tools > Graph Display**.

The method graph opens (see Figure 82).

Figure 82. Graphic view of a method



Changing the LC Method Configuration

❖ **To change the LC method configuration**

In the LC Method Editor window (see Figure 74 on page 108), choose **Edit > Method Configuration**.

The Method Configuration dialog box appears showing the configuration of the open LC method (see Figure 83).

Figure 83. Method Configuration dialog box

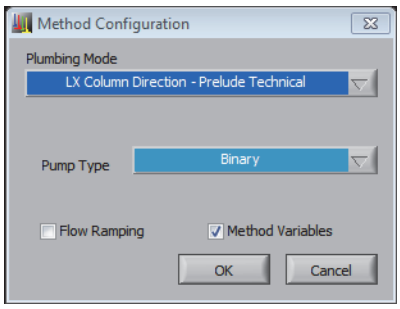


Table 33 describes the settings for the Method Configuration dialog box.

Table 33. LC Method Configuration settings

Setting	Description
Plumbing Mode	Changes the column diagram in the LC Method Editor window. Choose one of the following: • LX Column Direction - Prelude Technical: Shows the actual plumbing, valves, analytical column, and column direction. • LX Column Direction: Shows the analytical column and column direction.
Pump Type	Set to **Binary** to indicate the system uses a binary pump on each channel. This setting must match the system plumbing.
Flow Ramping	Select if you want to edit or create methods in which the flow rate changes gradually over the duration of a step. When you select this option, the Ramp FR check box appears in the Flow Rate area of the LC Method Editor window (see Figure 75 on page 113). Use this option only if you have advanced system and technology knowledge.
Method Variables	Select to allow variables to be assigned to LC methods. If you do not select this option, the Variables tab does not appear in the LC Method Editor window. See "Allowing Method Variables During a Run" on page 123. Use this feature when developing a method.

Changing the LC Method Editor Options

You can make the following changes to the LC Method Editor options:

- Sync the data window and MS (or detector) method duration.
- Change the time format that appears in the LC Method Editor.

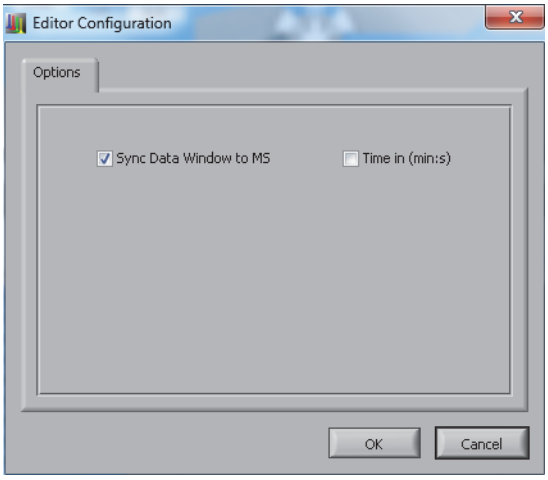
To make these changes, use the Options page of the Editor Configuration dialog box; changes here affect all LC methods.

❖ To change the LC Method Editor options

1. In the LC Method Editor window (see Figure 74 on page 108), choose **Tools > Preferences**.

   The Editor Configuration dialog box opens showing the Options page (see Figure 84).

   Figure 84. Options page of the Editor Configuration dialog box



2. Make entries and selections based on the descriptions in Table 34.

   Table 34. Editor Configuration dialog box parameters

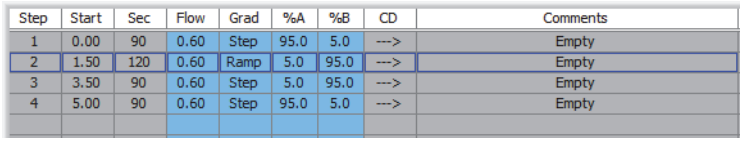
Parameter	Description
Sync Data Window to MS	Select to sync the LC method data window and the detector method time duration. If you do not select this option and you enter a data window in the LC method that is smaller than the instrument method, you must enter the data window duration into the detector method time duration. This option applies only if your LC instrument is connected to a Thermo Scientific detector. Otherwise, you must enter the data window duration into the detector method time duration box if you enter a data window that is smaller than the instrument method.
Time In (min:s)	Select to view the method times in the minute:seconds format. Otherwise, the method times appear in the minutes format, with partial minutes appearing as a decimal.

3. Click **OK** to save your changes.

Working with the LC Method Step Table Columns

Table 35 describes the columns in the LC Method Step table of the LC Method Editor window (see Figure 85).

Figure 85. LC Method Step table



Step	Start	Sec	Flow	Grad	%A	%B	CD	Comments
1	0.00	90	0.60	Step	95.0	5.0	--->	Empty
2	1.50	120	0.60	Ramp	5.0	95.0	--->	Empty
3	3.50	90	0.60	Step	5.0	95.0	--->	Empty
4	5.00	90	0.60	Step	95.0	5.0	--->	Empty

Table 35. Step table column headings

Heading	Description
Step	Step number.
Start	Starting time for the step (minutes/decimal minutes).
Sec	Length of the step (seconds).
Flow	Flow rate of the pump (mL/s).
Grad	Type of gradient used: Step or Ramp. Step means that both the flow rate and composition change immediately to the appropriate value. Ramp means that the flow rate and composition change gradually over the length of the step to the value you indicated.
%A, %B	Composition of the mobile phase. Columns for %A and %B appear in the table when selected in the LC Method Configuration window.
CD	Direction of flow for the column.
Comments	Note about a particular step.

Allowing Method Variables During a Run

When you optimize method conditions during method development, you run a method several times, varying only one component in the method at a time to determine the optimal value for the analyte. For example, to determine the best solvent strength to start the elution, you vary the percentage of the eluting solvent for each sample.

Add a method variable to the LC method as a convenient way to vary a component in a method. When you create the method variable, specify the method component that you want to change, the step number in which the change occurs, and an acceptable value range. Then, enter the values that you want to use for each sample in the batch. By creating a method variable, you can use one method with varying values for a component.

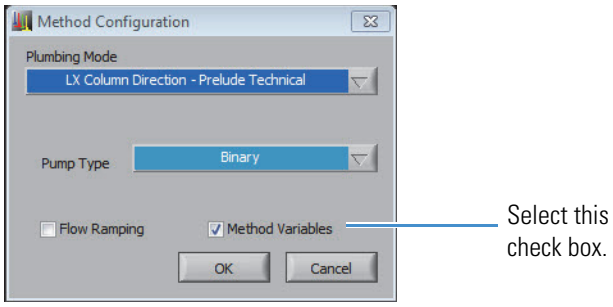
Adding Method Variables to an LC Method

❖ To add a method variable to an LC method

1. Open the LC method where you want to add a variable.
2. In the LC Method Editor window (see Figure 74 on page 108), click the **Variables** tab.

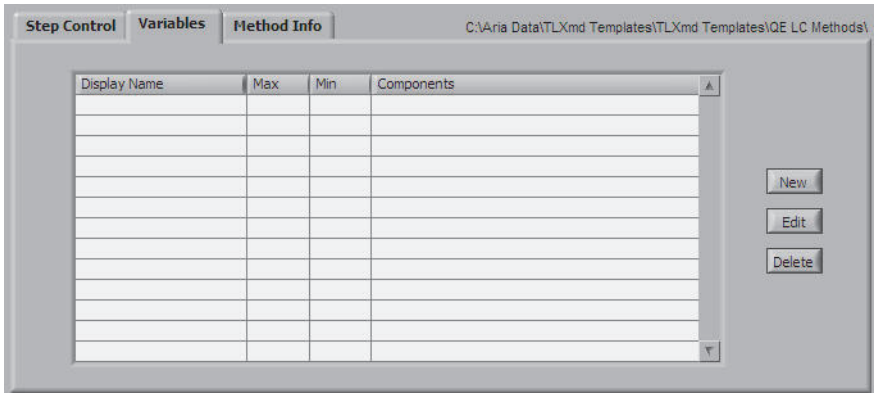
Tip If the Variables tab does not appear, select the **Method Variables** check box in the Method Configuration dialog box. See "Changing the LC Method Configuration" on page 120 and Figure 86.

Figure 86. Method Configuration dialog box



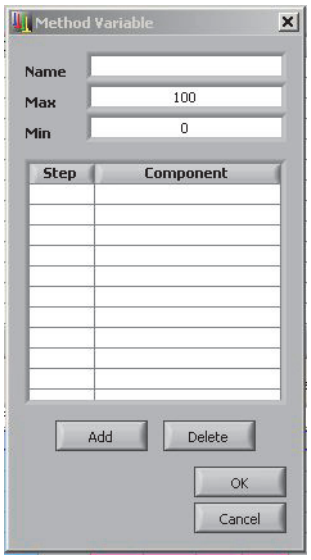
The Variables page opens (see Figure 87).

Figure 87. Variables page



3. Click **New**. The Method Variable dialog box opens (see Figure 88).

Figure 88. Method Variable dialog box



4. In the Name box, type a name that identifies the method variable.

> **Tip** The name you enter in this step will also be the name you enter into the sample list to identify the variable.

5. In the Max box, type the maximum value for the variable. For example, if you want to run your method with an eluting organic concentration of 20, 40, 60, and 80%, type **80**.
6. In the Min box, type the minimum value for the variable range. For example, if you run your method with an eluting organic concentration of 20, 40, 60, and 80%, type **20**.
7. Click **Add**. A default step number and method component appear.
8. In the Step column, type the step number that you want to change.
9. To show a list of method components, click in the Component column.
10. Select the method component that you want to change.
11. If you want variables for additional steps, repeat step 7 through step 9.

    Add steps and components if the component you want to change appears in more than one step in the method. For example, if you are changing the eluting mobile phase composition for an isocratic method, include all the relevant steps.
12. Click **OK** to close the Method Variables dialog box. The method variable that you entered appears on the Variables page.

13. Choose **File > Save As** and type a name for the new LC method.
14. Click **Save**, and then click **OK**.
15. If you are creating an LC method to vary mobile phase composition, see "Changing the Mobile Phase Composition by Using Method Variables."

Entering Values into the Sample List

Once a method variable exists for the method, create a column in the sample list, and enter the method component value that you want to use for each sample. If your variables involve mobile phase composition, see "Changing the Mobile Phase Composition by Using Method Variables."

❖ To enter values into the sample list

1. Create a sample list by using the data processing software.
2. Create a custom column in the sample list for each method variable that you created by using the data processing software. Name the column the same name as it appears in the Method Variable dialog box (see Figure 88 on page 125).
3. In the sample list, enter the values that you want to use for each sample (see Figure 89).

Figure 89. Example of a sample list showing the method variable column

	Sample ID	SampleName	Position	Inj Vol	Sample Type	Comment	Inst Meth	Path	File Name	LC_elution_B
1	Sample001	elution100 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\			100
2	Sample002	elution100std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\			100
3	Sample003	elution100std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\			100
4	Sample004	elution100 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\			100
5	Sample005	elution90 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\			90
6	Sample006	elution90std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\			90
7	Sample007	elution90std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\			90
8	Sample008	elution90 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\			90
9	Sample009	elution80 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\			80
10	Sample010	elution80std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\			80
11	Sample011	elution80std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\			80
12	Sample012	elution80 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\			80

Method variable column

Changing the Mobile Phase Composition by Using Method Variables

To vary the mobile phase composition of a pump by using method variables, do the following to ensure that the system uses the appropriate mobile phase composition.

❖ To change the mobile phase composition by using method variables

1. Open the LC Method Editor window for the LC method that you want to edit. See "Accessing the LC Method Editor" on page 108.

2. Enter **100** in the %A column of the appropriate pump in all the steps where you want to change solvent composition. Do this even if you intend to use 0% solvent from Channel A.
3. Open the Method Variable dialog box (see Figure 88 on page 125) and select the solvent that you want to change, for example, **Solvent B**. Enter minimum and maximum values and a method variable name. See "Adding Method Variables to an LC Method" on page 124.
4. In the sample list, create a custom column for each method variable you created. See "Entering Values into the Sample List" on page 126.
5. For each sample, enter the new percentage value of the channel that you want to change.
6. Verify that the total value in the method variable columns does not exceed 100.

When the method runs, the value of A automatically decreases as the value of B increases.

Note The control application changes solvent composition according to the rules described in "Rules for Changing Mobile Phase Composition."

Rules for Changing Mobile Phase Composition

The Prelude LX-4 MD control application changes mobile phase composition based on the following rules. These rules apply to changes specified in the Method Variable dialog box, the LC Method Editor window, and the Direct Control window.

- The total solvent percentage (A + B) must equal 100 for each channel.
- When you increase the solvent B percentage, the control increases solvent B to the specified value and decreases the percentage of solvent A by the same amount to maintain a total percentage of 100.

In the Method Variable dialog box (see Figure 88 on page 125), the control application changes the solvent proportions based on the value specified in the method variable column for each sample in the sample list. For an example, see Table 36.

Table 36. Example of mobile phase composition changes due to method variables

Example	Percentage of solvents dispensed
The LC method indicates 100% solvent A. The method variable specifies solvent B as the variable. The value in the method variable column in the sample list indicates 25.	The pumps dispense 75% solvent A and 25% solvent B.

Assigning a Pressure Profile

You can create a pressure profile from the recorded pressures of a previously run sample (or the average pressures of a group of samples) that represents a typical pressure profile for your method. The Prelude LX-4 MD control application compares the pump pressures of the currently running method to the pressures in the stored profile. The control application flags values that fall outside specified limits, or the system shuts down depending on the preferences you select.

You can use the pressure profile feature to monitor pressure changes that might indicate a system malfunction or an aging column. Choose a profile that accurately represents a typical run for the method, and view the pressure profiles of previously run samples. Choose a method from a batch that was run by using the same method and solvent conditions as the method to which you are assigning the profile. Consider normal fluctuations observed from batch to batch as well as from sample to sample, and enter limits that are not too tight or too wide.

❖ To establish a pressure profile

1. In the LC Method Editor window (see Figure 74 on page 108), choose **Tools > Pressure > Add Profile**.

   A new window opens showing a list of files.

2. Navigate to the sequence file (.tlsx extension) that contains the representative pressure profile of your LC method and select it.

   The Profile Select dialog box opens.

   Note Choose a batch that was run by using the same method and solvent conditions as the method that you are assigning the profile to. The sequence files (.tlsx) appear in the batch folder within the project or subproject folder.

3. Select a sample.

   If you want to select more than one sample, hold down the CTRL key and then select additional samples. When you select more than one sample, the control application averages the pump pressure values.

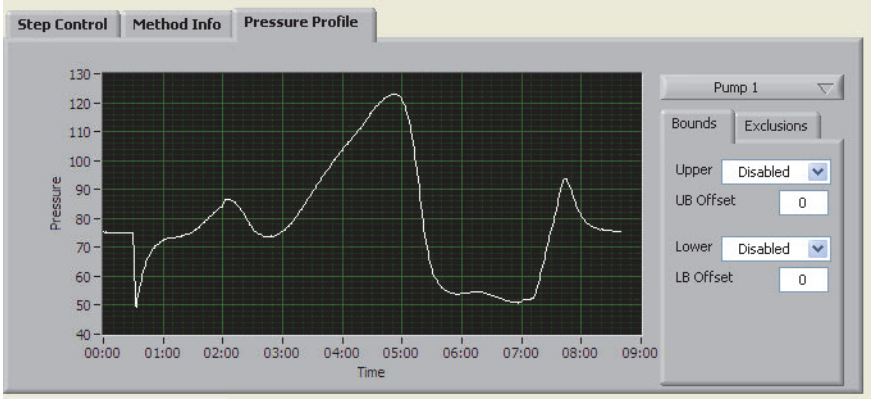
4. Click **OK**. The pressure profile opens on the Pressure Profile page.

❖ To view the pressure profile and set limits

1. In the LC Method Editor window (see Figure 74 on page 108), click the **Pressure Profile** tab.

   The graph shows the pressure profile you assigned to this method (see Figure 90).

Figure 90. Pressure Profile page



2. Select or edit any of the following limits.

Table 37. Pressure Profile page limits (Sheet 1 of 2)

Limit	Description
Upper (visible if you click the Bounds tab)	Select one of the following options: • **Disabled**: Take no action when the pressure exceeds the upper limit of the profile. • **Sample Error**: Flag samples that have pressures that exceed the upper limit of this profile. • **System Error**: Flag LC instruments with pressures that exceed the upper limit. The flagged LC instrument stops running samples and shuts down.
UB Offset (visible if you click the Bounds tab)	Type the upper boundary limit in bar. For example, if the UB Offset value indicates 10, values that fall beyond 10 bar (145 psi) higher than the profile value are considered outside the limit. Action taken depends on the option selected in the Upper list.

Table 37. Pressure Profile page limits (Sheet 2 of 2)

Limit	Description
Lower (visible if you click the Bounds tab)	Select one of the following options: • **Disabled**: Take no action when the pressure exceeds the lower limit of this profile. • **Sample Error**: Flag samples that have pressures that exceed the lower limit of this profile. • **System Error**: Flag LC instruments with pressures that exceed the lower limit. The flagged LC instrument stops running samples and shuts down.
LB Offset (visible if you click the Bounds tab)	Type the lower boundary limit in bar. For example, if the LB Offset value indicates 10, values that fall beyond 10 bar (145 psi) lower than the profile value are considered outside the limit. Action taken depends on the option selected in the Lower list.

3. As applicable, set the times in the method when you do not want the limits to apply. See "To exclude time segments in the method."

❖ To exclude time segments in the method

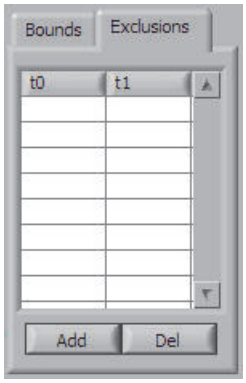
Tip You can assign time segments within the method when the profile limits do not apply. For example, you might want to exclude the moments when a valve change occurs to avoid flagging the system or sample unnecessarily.

1. In the LC Method Editor window (see Figure 74 on page 108), click the **Pressure Profile** tab.

   The Pressure Profile page opens (see Figure 90 on page 129).

2. Click the **Exclusions** tab (see Figure 91) to open the Exclusions table.

Figure 91. Exclusions tab



3. Click **Add**.

   Values appear in the T0 and T1 columns in the Exclusions table and two yellow lines appear on the left side of the graph. Do one of the following:

   - Click the exclusion time segment that you want to edit. The row becomes highlighted. Select the value in the T0 column and type the time when you want to begin excluding. Select the value in the T1 column and type the time when you want to end the exclusion.
   - Use the cursor to drag the yellow lines until they border the time in the method that you want to exclude.

4. If you want to add another exclusion time segment, repeat step 3.

❖ **To include a time segment that has been excluded (to remove an exclusion)**

Click the time segment exclusion that you want to remove and click **Delete**.

Importing an Aria OS LC Method

When you import an Aria OS method, you import only the LC method. The detector and autosampler methods remain intact.

❖ **To import an LC method**

1. Open the instrument method where you want to import the LC method.
2. In the Instrument Setup window, click **Prelude LX-4 MD**.

   The LC Method Editor window opens (see Figure 74 on page 108).

3. Choose **Edit > Import Aria Method (*.htc)**.

   A list of files opens.

4. Navigate to and select the method to import.

   The LC method information appears.

Importing the LC Method from an Instrument Method

You can import the LC method portion of an instrument method (.meth) into another instrument method. The AS method and detector method information do not import by using this procedure. To import the AS method portion of the instrument method, see "Import the AS Method from an Instrument Method" on page 102. To copy or use the detector portion of the method, open the applicable method, save it by using a different name, and then import the AS and LC method information that you want into the method.

❖ To import the LC method information from an instrument method

1. Open the instrument method where you want to add the LC method information.
2. Click **Prelude LX-4 MD**.

   The LC Method Editor window opens (see Figure 74 on page 108).
3. Choose **Edit > Import from Inst Method (*.meth)**.
4. Navigate to the instrument method with the LC method information that you want to import, and select the method.

   The LC method information appears in your open method.

Note The Prelude prestart and column temperature parameters do not import from a .meth file.

Setting the Heater Temperature in an Instrument Method

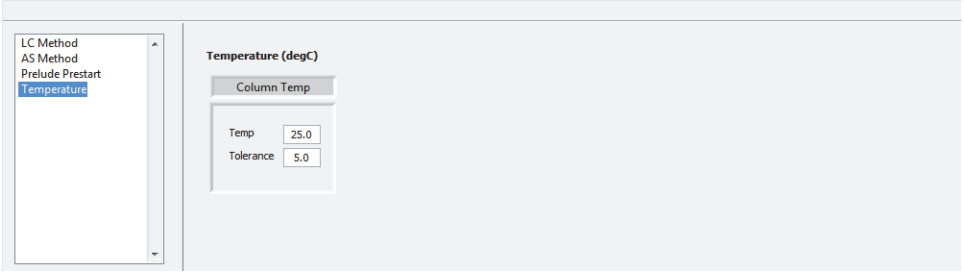
This section describes how to set the optional column heater temperature in a method. If you want to manage the temperature control on demand, see "Controlling the Column Heater Temperature" on page 60.

❖ To set the column heater temperature in a method

1. Open the Temperature area of the instrument method as follows:
   a. Open the LC Method Editor window. See "Accessing the LC Method Editor" on page 108.
   b. In the left pane, click **Temperature**.

      The Temperature options appear (see Figure 92).

      Figure 92. LC method temperature information



2. In the Temp box, type the preferred temperature setting for the column heater during the instrument method.
3. In the Tolerance box, type the tolerance limit.

   The maximum value you can enter in the Tolerance box is 5.

Note The tolerance value sets a temperature range above or below the set temperature value. If the heater temperature exceeds this range or falls below it, the following occurs:

- A warning appears in the Prelude LX-4 MD event log.
- The system continues to run the injected sample.
- The system does not inject another sample until the temperature has returned to a value that falls within the tolerance range.

The temperature might fall outside the tolerance range due to a sudden change in laboratory temperature or the method flow rate, or a malfunctioning component in the heating mechanism or thermostat.

9

Operating Hazards

This chapter describes the operation hazards and the location of hazard labels on the instrument.

Contents

Before Operating the Instrument

Observe the precautions listed in this section to ensure the safe operation and longevity of the instrument.

IMPORTANT Placing samples in the wrong tray position can result in inaccurate data. Verify that the sample vial position matches the assigned position in the batch or sequence file, and that the sample trays are placed correctly into the sample drawers.

IMPORTANT When you use the system, follow the generally accepted procedures for quality control and method development. If you observe a change in retention time of a particular compound, in the resolution between two compounds, or in peak shape, immediately determine the reason for the changes. Until you determine the cause of the change, do not rely on separation results.

CAUTION Do not use a damaged or expired HPLC column on the system. Run a preview batch at regular intervals to evaluate the quality of the HPLC columns.

CAUTION Use equipment only in the manner specified by its manufacturer to avoid impairing protections provided by the equipment and potential voiding of product warranties.

CAUTION Follow the maintenance procedures in this manual when replacing or repairing customer serviceable components. Never try to repair or replace components not described in this manual without the assistance of a Thermo Fisher Scientific service engineer.

CAUTION Do not service any part of the instrument while the instrument is powered on. The power plug should be removed from the facility power outlet.

CAUTION Do not remove or open the instrument front panels while the instrument is performing an operation. Do not remove any panel that requires a tool to open or remove it.

CAUTION The instrument contains voltage lines. Switch off the power and disconnect the power cable prior to servicing any component on the system.

CAUTION To prevent personal injury, take personal protection measures—including the wearing of personal protective equipment—and safety training for hazardous chemicals, when handling solvents, changing tube lines, or both. Consult the pertinent safety data sheets (SDSs) for the solvents you use for HPLC analysis.

CAUTION Do not run the system with the autosampler door open. The autosampler contains a sharp moving part, which can cause injury if you open the door during operation.

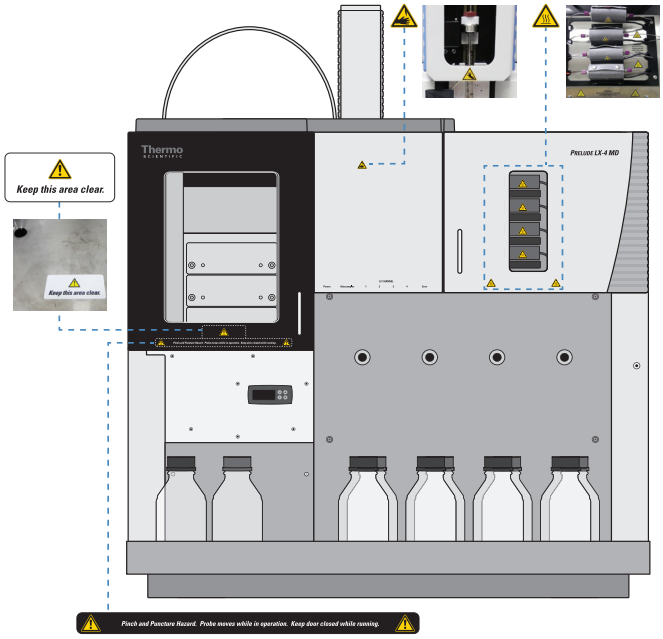
CAUTION The system might have been exposed to potentially infectious biological hazardous materials by the samples that were introduced to the instrument. To avoid infection, refer to the biohazard safety precautions described by your company's standard operating procedures. This might include, but is not limited to, wearing protective clothing, and hand, eye, nose, and mouth protection.

Instrument Caution Label Placement

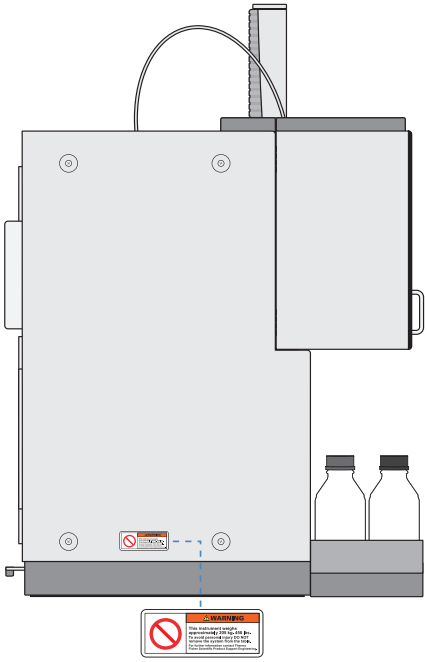
Figure 93 shows the placement of various caution labels on the instrument.

Figure 93. Placement of caution labels on the instrument

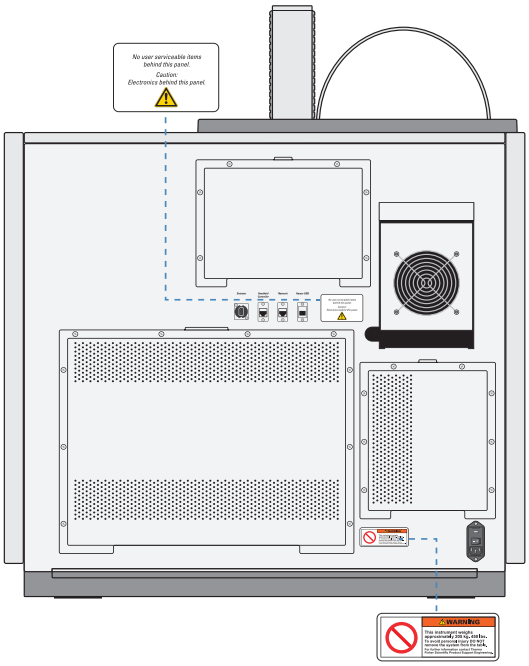
Front view



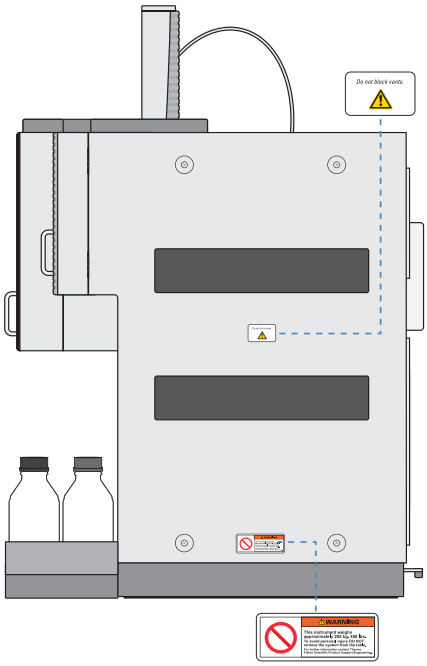
Left-side view



Back view



Right-side view



Residual Product Risks

This chapter describes residual product risks that are associated with the Prelude LX-4 MD instrument. Residual product risks are those risks that still remain despite all measures taken to mitigate them.

- This instrument contains column heaters. Be aware that heated surfaces can cause burns on contact.
- Open the sample drawer only when the autosampler LED is off. The autosampler needle might cause an injury if you attempt to access the sample drawer while the needle is moving.
- To reduce the risk of instrument malfunction due to sample mismatching, verify that you have entered the correct sample location.
- To reduce the risk of instrument malfunction from accidental cross tubing, make sure to reconnect the plumbing correctly after replacing a column.
- To reduce the risk of instrument malfunction due to temperature variations, make sure to set the correct cooled stack temperatures.
- To reduce the risk of instrument malfunction due to contamination, make sure sufficient wash solvents are available throughout the run.
- To reduce the risk of instrument malfunction, always use clean, undamaged, in-date columns.
- To reduce the risk of instrument malfunction, always use a sufficient amount of the correct, clean, and in-date mobile phase.
- To reduce the risk of instrument malfunction, operate the instrument according to specified environmental requirements.
- To increase awareness of potential errors, use the pressure profiling functionality.
- To reduce the risk of personal injury or damage to the instrument or installed environment, the instrument is to be used as specified in these instructions.
- If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment might be impaired.

11

Maintenance Procedures

This chapter describes the procedures that will help maintain your system's operation quality.

Contents

Recommended Maintenance Schedule

Table 38 provides the recommended schedule for performing system maintenance procedures.

Table 38. Maintenance schedule

Frequency	Procedure
Daily	Track the Number of Injections—System Counts Tab
	Prepare the Mobile Phase Solvents
	Record Pump Pressures
	Prime the Autosampler Wash
	Clean the Syringe
Weekly	Change a Needle or a Syringe (Model A Autosampler)
	Change a Needle or a Syringe (Model B Autosampler)
	Prepare the Mobile Phase Solvents
	Change the Solvent Bottles
Every 2000 injections	Replace the Analytical Column
Every 10 000 injections	Preventive maintenance performed by a qualified service engineer

Prepare the Mobile Phase Solvents

Prepare mobile phase solvents according to the following guidelines:

- Prepare fresh solvent mobile phases weekly in clean bottles. Do not refill or top off standing bottles.
- Use solvents that are LC/MS-grade or higher. Fisher Scientific™ offers mobile phase blends for your convenience. Refer to the Prelude LX-4 MD *Preinstallation Requirements Guide*.
- Make solvents in quantities that you will use weekly.
- Do not use a thermoplastic sealing film, such as Parafilm, as a mobile phase reservoir cover. The film reacts with the organic solvents and leaches polymers into the liquids. Use an appropriate bottle cap that accommodates the solvent lines. If you do not have these caps, use aluminum foil to secure the solvent lines in the bottle and protect the solvent from dust. Make sure that each solvent line reaches the bottom of its intended solvent reservoir.
- Wherever possible, include 2% acetonitrile in aqueous mobile phases to inhibit microbial growth. The addition of 2% LC/MS-grade acetonitrile has minimal impact on the chromatography.

- Do not use any mobile phases that have visible particulates or look foggy. Before each batch, vigorously swirl the mobile phase bottles and look for particulates that might be floating or moving in the liquid. Check the fluid lines and filters for particulates or slime. If you find particulates or foggy mobile phases, discard the liquid and thoroughly clean or replace the bottles. Replace the solvent filters and purge the lines fully with new, clean LC/MS-grade mobile phase.

Record Pump Pressures

On each day, make a note of the pump pressure about 15 seconds after the sample injection. Keep a log of the daily pressures and column installations. Record the pressures on each channel with the columns in place.

View the pressure trace of a recently run sample and compare it with a baseline pressure trace. If you observe signs of high pressure anywhere on the system, contact Technical Support. See "Contacting Us" on page xv.

Use the Maintenance Dialog Box

The Maintenance dialog box helps you optimize instrument performance by tracking various system and device health metrics, such as the number of injections made by each probe or system, and general system health items available on the Model B autosampler.

See these topics:

- Track the Number of Injections—System Counts Tab
- Device Health Tab (Model B Autosampler)

Track the Number of Injections—System Counts Tab

❖ **To view and track the total number of injections on a probe, system, or detector**

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Tools > Maintenance**.

   The Maintenance dialog box opens.
3. Click the **System Counts** tab (see Figure 94).

Figure 94. Maintenance dialog box, System Counts tab

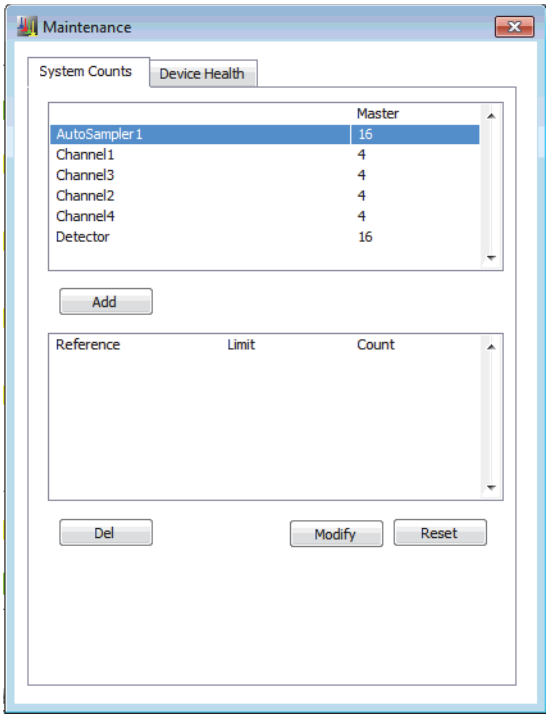


Table 39 describes the System Counts tab options.

Table 39. Maintenance dialog box, System Counts tab options

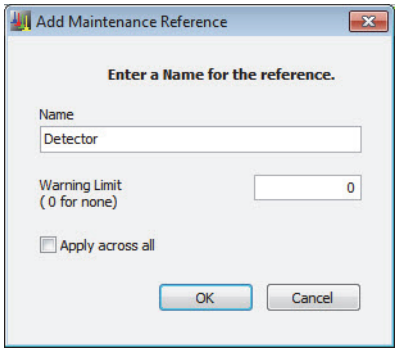
Option	Description
Master	The total, cumulative number of injections for each component.
Add	Opens the Add Reference dialog box.
Reference	A user-defined interval of injections for tracking the number of injections for a system component. For example, define a reference named “Analytical column”, and install a new column. At the start of the reference, the count shows 0. Reset the count to zero each time you change the column. You can use this reference to determine the number of injections since the column was changed.
Count	The number of injections that have elapsed since the reference was created or reset to 0.
Limit	The number of injections that must elapse before the system alerts you with a message.
Del	Deletes the selected reference.
Reset	Resets the count of the selected reference to 0.
OK	Saves the changes.

❖ To create a reference for tracking injections

1. Click the system component that you want to track in the upper portion of the dialog box to highlight the component name.
2. Click **Add**.

   The Add Maintenance Reference dialog box opens (see Figure 95).

   Figure 95. Add Maintenance Reference dialog box

   A reference is the component or group of components that you want to track.
3. Type a name for the reference that reflects the component that you are tracking, for example, **Analytical Column**, **AS Syringe,** or **Detector**, and so on.
4. In the Warning Limit box, do one of the following:
   - To have the system notify you when a specific number of injections that involve the selected component have elapsed, type the number of injections in the Warning Limit box.
   - To not have the system notify you when a number of injections have elapsed involving the component, leave the box value set to **0**.
5. To apply the settings across all channels for similar components, select the **Apply Across All** check box.

   With this check box selected, an injection on any of the channels updates the count for this reference.
6. Click **OK**.

❖ To reset a reference count to zero

1. In the Maintenance dialog box, select the reference name at the bottom of the window.

   The reference name becomes highlighted.
2. Click **Reset**.

   A confirmation box opens.

3. Click **Yes** to the message.

   The box closes, and the count value for the selected reference resets to 0.

Device Health Tab (Model B Autosampler)

You can set, review, and track the health of autosamplers on Prelude instruments that are equipped with the Model B autosampler by using the Device Health Tab. This configurable device health feature is available on Model B autosamplers that use a “Smart Syringe” and injection valve drives. See Figure 96.

When configured, the Prelude software indicates when a specific autosampler module that you are tracking has exceeded its limit. This gives your lab and Thermo Fisher Scientific field service engineers valuable information to help keep your system operating at peak performance.

❖ To view, monitor, and set maintenance reminders for a Model B autosampler

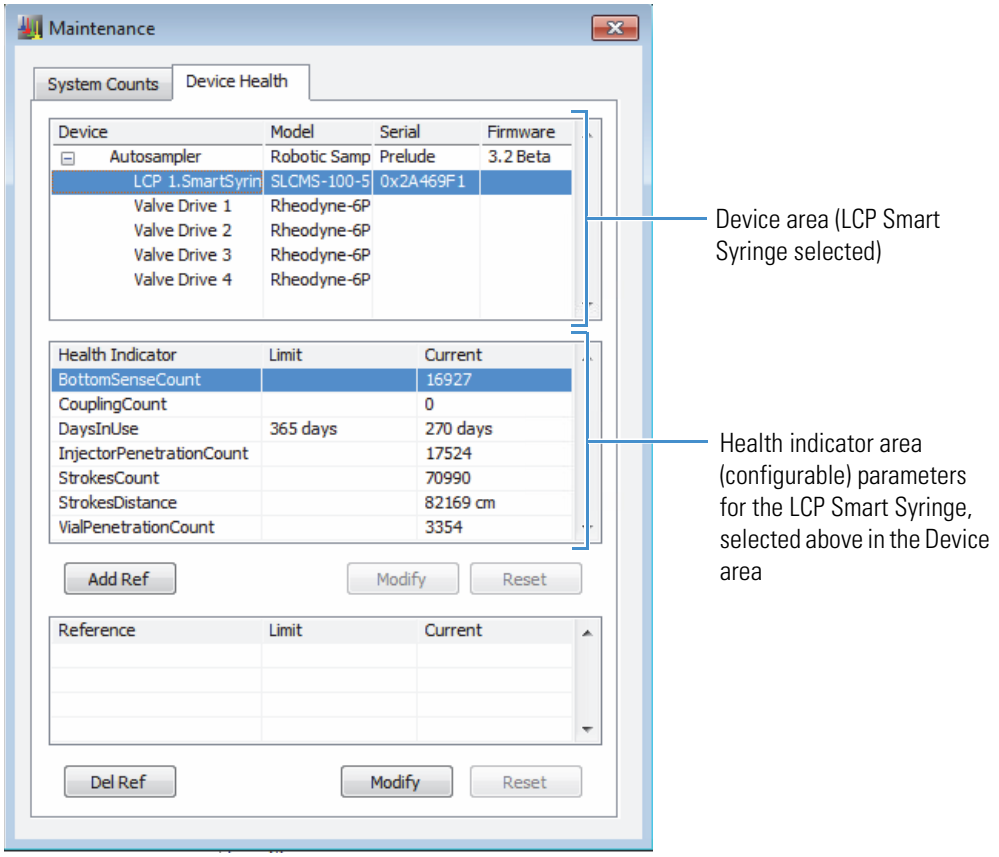
1. From Direct Control window, click **Tools > Maintenance**.

   The Maintenance window opens (Figure 96).

2. Click the Device Health tab (if not already active).

   The Device Health tab opens and displays the available device health parameters.

Figure 96. Direct Control Maintenance window, Device Health tab

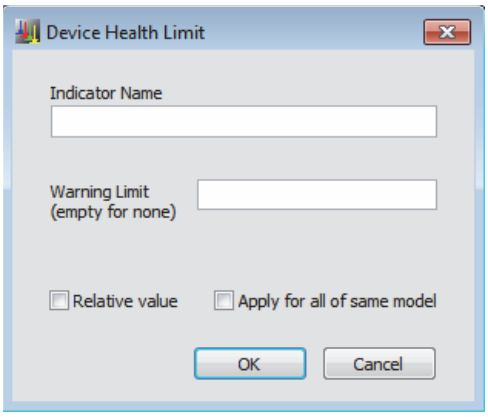


Tip You can obtain additional health information on the autosampler. Select a device from the device area, right-click, and then click **More Info**.

3. Click the parameter that you want to view, modify or add a custom reference for maintenance tracking, and then do one of the following:

Note Some parameters cannot be customized in version 1.3 of the Prelude software.

- Review the available parameters for the current status, as needed.
- To add a custom reference, click **Add Ref**. The Device Health Limit dialog box appears.



Type an indicator name and warning limit in the appropriate labeled box.

Tip Move the mouse pointer over any parameter in the Device Health Limit dialog box for additional information.

Prepare the Wash 1 and Wash 2 Autosampler Wash Solution Bottles

The following are common Wash 1 and Wash 2 solutions.

- Wash 1: Water with 2% acetonitrile. The acetonitrile prevents microbial growth in the reservoir.
- Wash 2: A mixture of acetonitrile/isopropanol/acetone, 45:45:10.

Note Use wash solutions that are based on your lab's developed and validated methods.

❖ To prepare and change the autosampler wash solutions

1. Prepare the autosampler wash solutions as directed in your laboratory's standard operating procedure.
2. Remove the cap of the installed wash solution bottle and set it aside.
3. Place the cap onto the new wash solution bottle.

   IMPORTANT Make sure you place the wash solution bottles in the appropriate locations. Improper locations of Wash 1 and Wash 2 can affect data quality.

4. Prime the autosampler wash system. See "Prime the Autosampler Wash" on page 149.

Prepare the Autosampler Organic Wash Solution

Prepare a 45:45:10 acetonitrile/isopropanol/acetone solution in a clean, 2-liter bottle by mixing these solvents:

- 100 mL of LC/MS-grade acetone
- 450 mL of LC/MS-grade isopropanol
- 450 mL of LC/MS-grade acetonitrile

Try this solution to use as your autosampler organic wash cleaning solvent. The solution is stable for 30 days at room temperature.

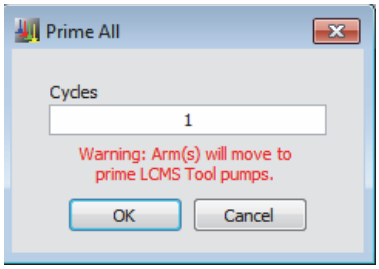
Prime the Autosampler Wash

Prime the autosampler wash according to your laboratory's maintenance schedule by using the Direct Control window.

❖ To prime the wash

1. Open the Direct Control window.
2. From the menu, click **Autosampler > Prime All Wash Lines**

   The Prime All window appears.



3. Type the number of prime cycles that you want in the Cycles box, and then click **OK**.

   Tip Thermo Fisher Scientific recommends that you run 6 to 8 prime cycles. This ensures that there is sufficient aqueous solution in the syringe loop (recommended for biological sampling).

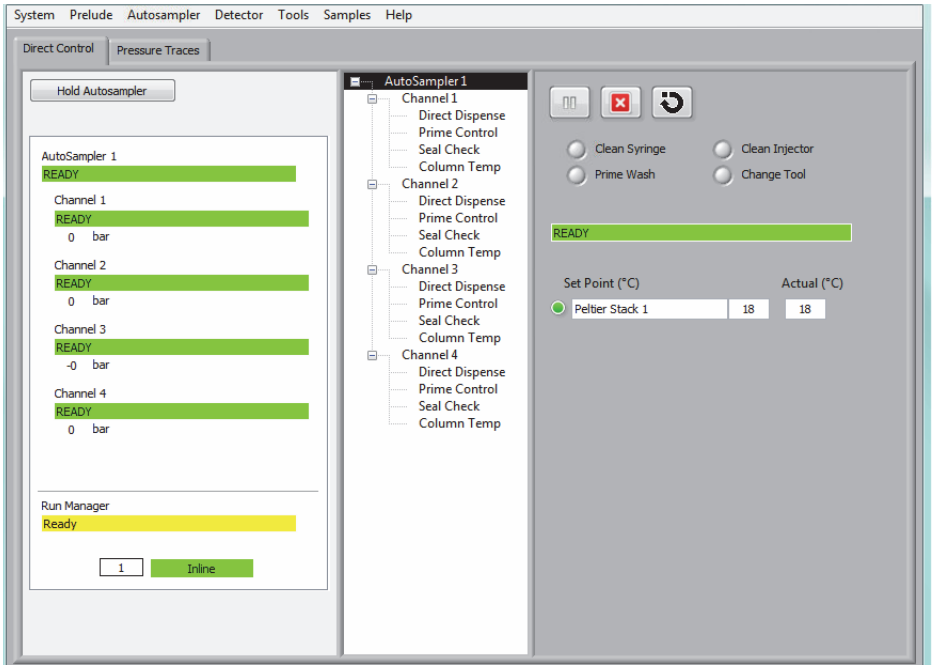
   The system starts the priming operation and runs until it is completed (the status bars return to green "Ready" state). Wash 2 is primed first and is then followed by Wash 1.

Clean the Syringe

❖ To clean the syringe

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. In the middle pane, select **Autosampler 1** if the autosampler options are not already displayed in the right pane (see Figure 97).

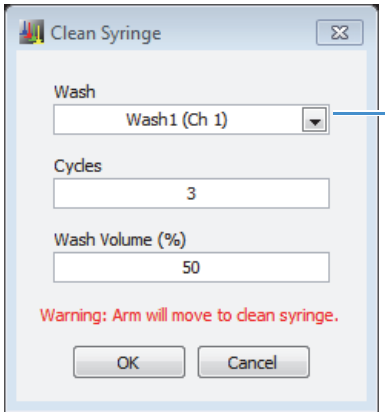
Figure 97. Direct Control window showing autosampler options (Model B autosampler)



3. Select the **Clean Syringe** option.

The Clean Syringe dialog box opens (see Figure 98).

Figure 98. Clean Syringe dialog box



Wash list, showing Wash1, Channel 1 selected.

4. In the Wash list, choose the wash that you want to use to clean the syringe—**Wash1** or **Wash2**—together with the associated channel number (see Figure 98).
5. In the Cycles box, type the number of cycles that you want to run.
6. In the Wash Volume (%) box, type the wash volume that you want to use to clean the syringe.
7. Click **OK** to start the wash operation.

Tip To push and remove a blockage from the needle, Thermo Fisher Scientific recommends that you use 100% Wash Volume of Wash1.

The dialog box closes. The autosampler flushes the wash through the needle and into the wash cup waste.

Change a Needle or a Syringe (Model A Autosampler)

Use the Change Tool command to change a Model A autosampler syringe barrel or needle.

❖ **To change a syringe barrel or needle on a Model A autosampler**

1. Open the Direct Control window, and then click **Autsoampler1** in the middle pane (see Figure 24).
2. Click the Unlock/Lock Terminal button,  from the right pane of the Direct Control window, and then select **Change Tool**.

   The AS arm moves to its maintenance position inside the AS cabinet. You can now safely change the syringe barrel or needle.
3. Click **OK** when finished.
4. Click the Unlock/Lock Terminal button to return the AS to a ready state.

Change a Needle or a Syringe (Model B Autosampler)

Use the Change Tool command to change a Model B autosampler LCP tool, syringe barrel, or needle. When you run the Change Tool command, the Syringe Exchange wizard appears and takes you through the step-by-step procedure (see Figure 39).

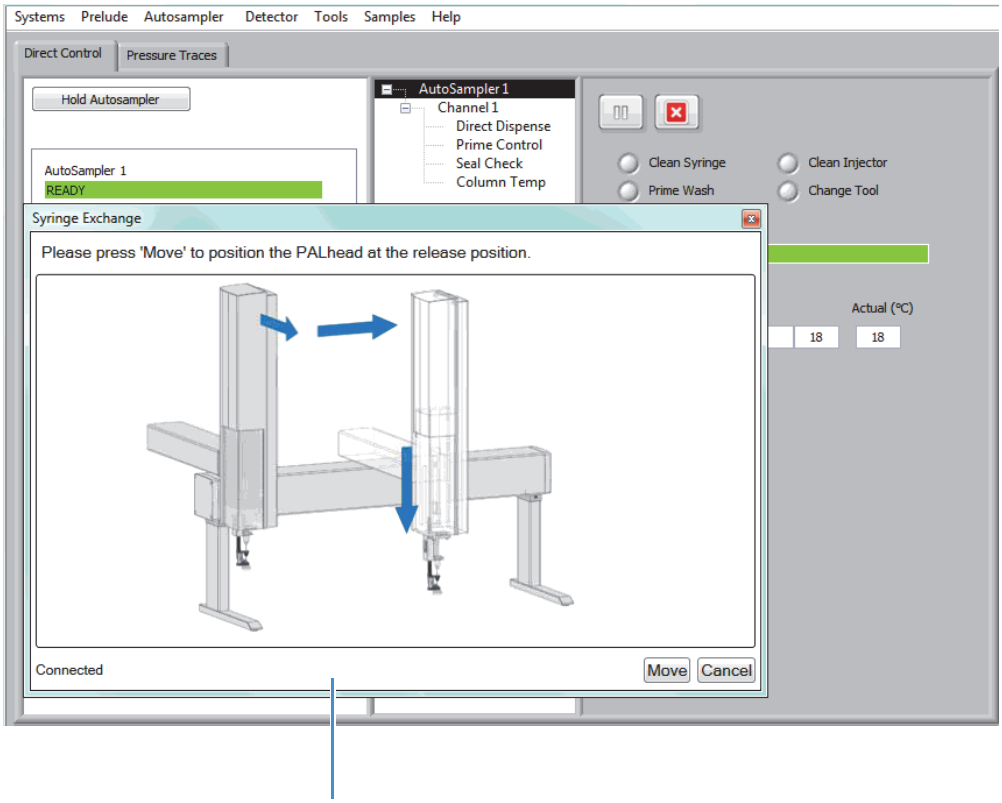
❖ To change an LCP tool, syringe barrel, or needle

CAUTION The autosampler arm moves to perform the following operation. To prevent personal injury, make sure that you wait until the autosampler arm stops moving before you open the autosampler door. Carefully follow the on-screen Syringe Exchange wizard instructions. Always wait until the autosampler arm stops moving before opening the autosampler compartment door.

1. Open the Direct Control Window.
2. From the middle pane, click **Autosampler1**,, and then open the autosampler compartment door.
3. In the right pane, select the **Change Tool** option.

   The Syringe Exchange wizard appears.

Figure 99. Syringe Exchange wizard from the Direct Control window



Syringe Exchange wizard

Follow the on-screen wizard instructions to change the LCP tool, syringe barrel, or needle.

Change the Solvent Bottles

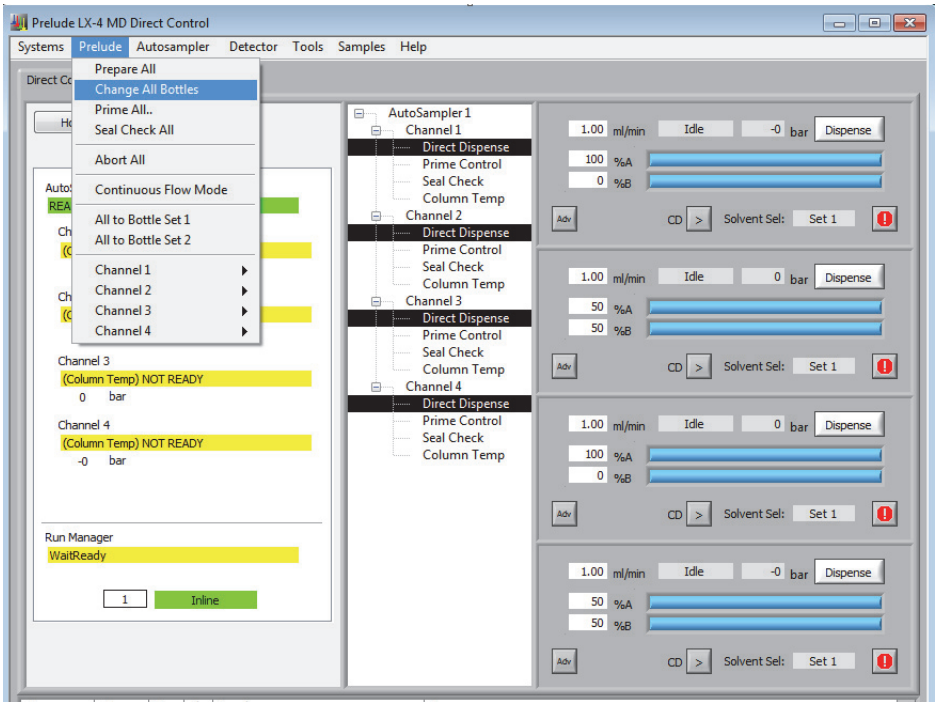
This section describes how to replace the solvent bottles.

IMPORTANT When replacing solvent bottles, be sure that you place the new solvent bottle into the appropriate location. Incorrect solvent bottle locations can affect the data quality.

❖ To replace the solvent bottles

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Do one of the following:
   - If you refilled the bottles and you want all of the channels to draw fresh solvent without changing bottle selections, choose **Prelude > Change All Bottles** from the main menu (see Figure 100). This runs the predefined Change Bottles macro consisting of a number of prime cycles and the optional seal check.

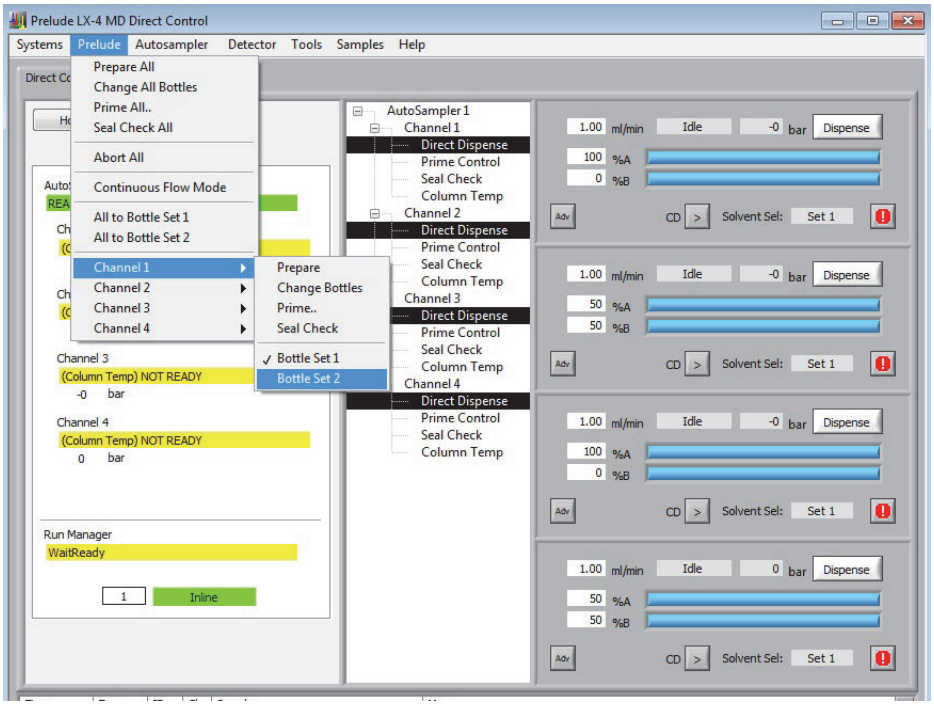
     Figure 100. Changing all bottles

   - If you want all of the channels to use Bottle Set 1 or Bottle Set 2, choose **Prelude > All to Bottle Set 1** or **All to Bottle Set 2**, respectively. This changes all channels to the specified bottle set. The system runs the Change Bottles macro automatically for the channels that did not previously draw from that bottle set.

- For one or more channels, if you want to change a to a specific bottle set, or you have just refilled or changed the bottles, choose **Prelude > Channel *n* > Bottle Set 1** or **Bottle Set 2** to select a specific bottle set (see Figure 101), or choose **Prelude > Channel *n* > Change Bottles** to draw fresh solvent from the changed bottles. The system runs the Change Bottles macro automatically for the selected channel or channels.

Figure 101. Changing bottles for a specific channel



The purpose of the Change Bottles macro is to draw solvent through the full length of the supply tubing. This macro helps ensure that the pumps are supplied with the new solvents, similar to purging supply lines on a traditional LC instrument.

Autosampler Wash Pump Evaluation Tool

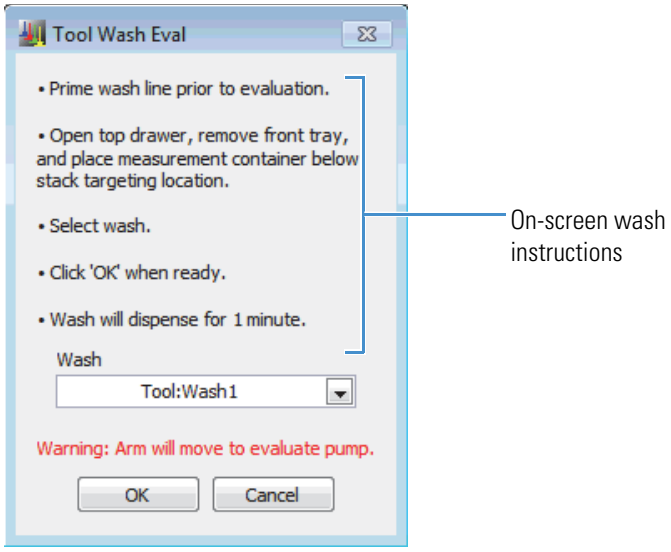
Use the Tool Wash Eval command to evaluate the flow rate of the autosampler wash pumps (Wash1 or Wash2). The wash pump flow rates vary based on the composition of wash solution used. After you prime a wash pump of interest, collect and measure the volume of produced during 60 seconds of pumping.

❖ To check the quality of the autosampler wash tool system

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. From the File menu, click **Autosampler > Tool Wash Eval**.

   The Tool Wash Evaluation dialog box opens (Figure 102).

Figure 102. Tool Wash Evaluation dialog box



3. Follow the dialog box on-screen instructions.

Replace the Analytical Column

Replace the analytical column every 2000 injections. Use the Maintenance dialog box to track injection numbers. See "Track the Number of Injections—System Counts Tab" on page 143.

CAUTION Replace the column with the column type and size specified in your laboratory's standard operating procedures. Column types and sizes must be compatible with the method you are running.

CAUTION To keep the new column from drying out, do not remove the end caps from a new column until you are ready to install it onto the system.

CAUTION Column heaters can become extremely hot and, therefore, unsafe to handle. Allow heated components to cool before you touch them. If removed from their heating sleeves, columns may be hot enough to burn skin. Allow the column and tubing to cool to below 50 °C before handling the column—approximately 15 minutes—before attempting to remove columns from heaters.

CAUTION If you use solvents that emit hazardous vapors, take appropriate precautions in response to chemical and hazardous vapor when you remove the analytical column from the system. Wear gloves, protective clothing, and eye wear as indicated in your laboratory's chemical safety operating procedures.

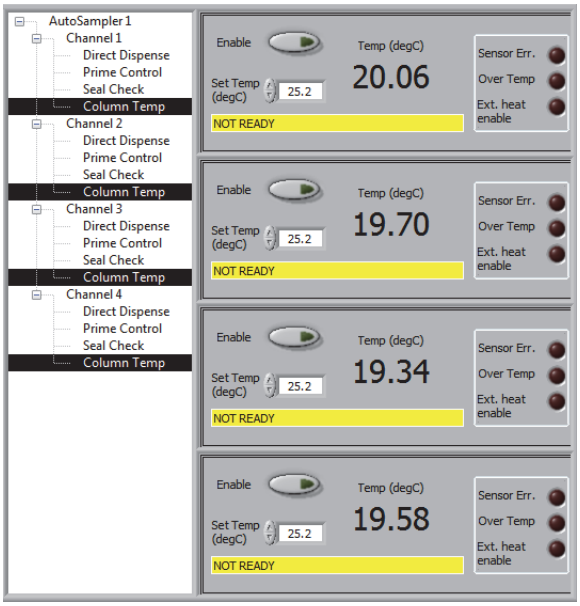
❖ To replace the analytical column

1. Turn off the column heater as follows and allow the column heater to cool to room temperature:

   a. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.

   b. In the middle pane, click a column heater for the appropriate channel (see Figure 103).

   Figure 103. Direct control window showing column heater controls

   c. Click the **Enable** button until the bright green icon becomes dark green.

   d. Repeat step b through step c for each additional column heater if applicable.

2. When the column heater has cooled, unwrap the column heater from the column.

3. Replace the column as follows:

   Note Use only your fingers to manipulate the tubing fittings. Do not use tools to loosen or tighten the fittings.

   a. Using your fingers, loosen the purple cap by turning it counterclockwise, and then slide the purple grip away from the column.

   b. Loosen the silver grip on the fitting, and remove the tubing from the column.

   c. Repeat the steps to remove the tubing from the other side of the column, and dispose of the column according to your laboratory's standard operating procedure.

   d. Remove the end cap from one end of the new column.

   e. Insert the tubing tip into the column port.

   f. Fingertighten the silver grip.

g. Fingertighten the purple cap.

h. Remove the end cap from the other end of the column, and insert the tubing into the column port. Tighten the fitting as you did on the other end.

4. Dispense fluid through the instrument (see "To dispense solvent through the system" on page 40) and monitor the pump pressure (see "To view the pressure trace in the Direct Control window" on page 163).

12

Troubleshooting

This chapter describes how to access system information that is useful for troubleshooting system issues. It provides possible solutions and diagnostic and corrective procedures for some of the most common instrument and control application issues.

Contents

Accessing the Status Page from the Data Processing Software

❖ To access the Prelude LX-4 MD Status Page

On the Status page of the data processing software, click Prelude LX-4 MD if it is available.

The Prelude LX-4 MD Status page appears (see Figure 104).

Figure 104. Prelude LX-4 MD Status page

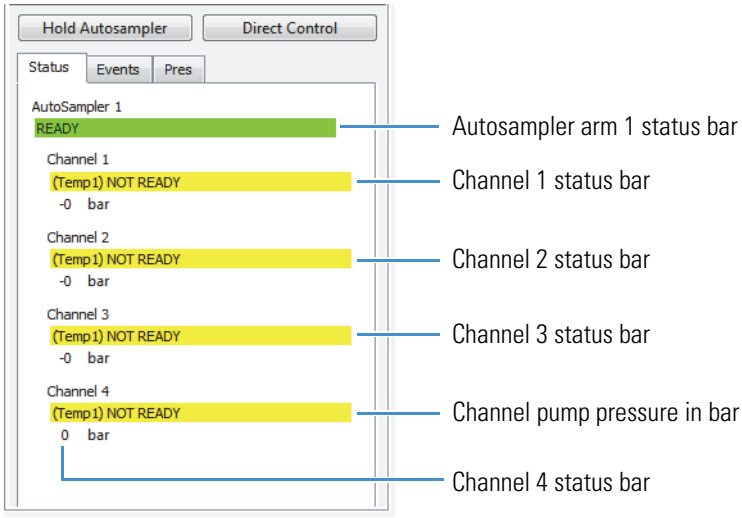


Table 40 describes the Prelude LX-4 MD Status page.

Table 40. Status page information and commands (Sheet 1 of 2)

Item	Description
Tabs	
Status	
Status bars for autosamplers and channels	The bar color indicates the pump or probe status. A status message appears in the bar to provide specific information on the pump or probe condition. For a definition of the status colors, see Table 11.
Channel pressure	The channel pressure appears below the status bar for each channel. It displays the current pump pressure in bar. The channel pressure is measured after the solvent mixing.

Table 40. Status page information and commands (Sheet 2 of 2)

Item	Description
Right-click menu	Accessed by right-clicking any of the channel status bars. The menu commands apply to the selected channel and are as follows: • On: Turns on the column heater and changes the channel status to Ready. • Off: Turns off the column heater and changes the channel status to Idle. • Standby: Turns on heaters. • Enable/Disable: Turns off the column heater. The status bar is grayed out. The channel will not run during a method and cannot be selected to perform any task in the Direct Dispense window.
Events	Accesses the Events page, which displays the system events. See "To view the event log" on page 172.
Pres	Accesses the Pump Pressure graph. See "To view the pressure trace for the current sample on the Pres page" on page 162.
Buttons	
Hold Autosampler	Pauses the autosampler. The autosampler finishes the current sample and then stops sampling. The LC method continues. To bring the autosampler back online, click **Hold Autosampler** again. See "To pause the autosampler" on page 51.
Direct Control	Opens the Direct Control window.

Monitoring the Pump Pressure

Follow these procedures:

- To view the pump backpressure in bar
- To view the pressure trace for the current sample on the Pres page
- To view the pressure trace in the Direct Control window
- To view the pressure trace for completed samples
- To assign pressure profiles to monitor pressure
- Applying Color-coded Overlays to Pressure Plots

❖ To view the pump backpressure in bar

Open the Status pane in the Direct Control window (see "Accessing the Direct Control Window" on page 28) or the Prelude LX-4 MD Status page from the data processing software (see "Accessing the Status Page from the Data Processing Software" on page 160).

The status area shows you the current pressure in bar for each pump. See Figure 104 on page 160.

❖ To view the pressure trace for the current sample on the Pres page

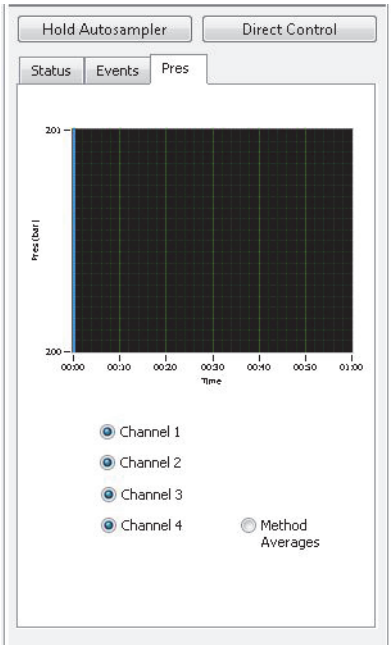
Note The backpressure of the LC pump changes throughout the method as flow rates and mobile phase compositions change. A plot of the backpressure for the pump over the duration of the method appears similar from sample to sample if the operating conditions remain the same.

A fluctuation or change in the pump pressure graph can indicate a change in the chromatography conditions. On the Pres page on the Prelude LX-4 MD Status page, you can view the pressure trace for the current method.

1. Open the Prelude LX-4 MD Status page from the data processing software. See "Accessing the Status Page from the Data Processing Software" on page 160.
2. Click the **Pres** tab.

   The most recent pressure traces for each channel appear (see Figure 105).

   Figure 105. Pressure trace on the Pres page



3. Select the channel that you want to view.

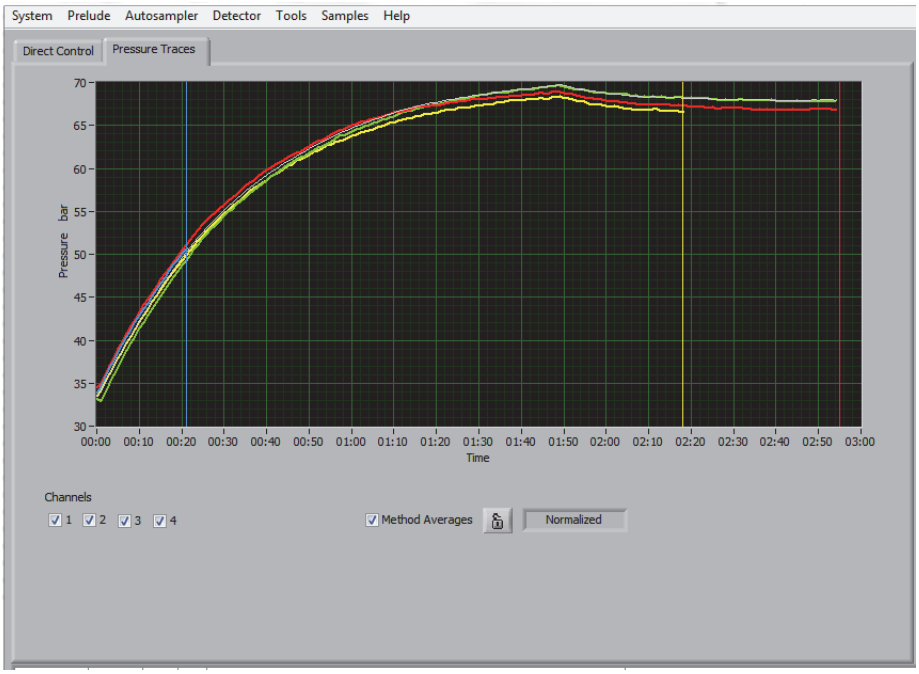
   You can select multiple channels to compare pressures.

❖ To view the pressure trace in the Direct Control window

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Click the **Pressure Traces** tab.

   The current pressure trace opens (see Figure 106).

   Figure 106. Pressure trace graph window in the Direct Control window



3. Select the channels that you want to view in the Channels area.
4. To view an average trace of the selected channels, select the **Method Averages** check box.
5. Click **Normalized/Real Time** to switch between Normalized and Real-time views. See "Normalized Versus Real Time" on page 165.
6. To access the pan and zoom features, select the **Lock** icon,  .

   The trace no longer updates, and the Zoom and Pan icons appear. See "" on page 167.

❖ To view the pressure trace for completed samples

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Tools > Sequence Log Viewer**.

   The Sequence Log Viewer window opens.
3. Choose **File > Open**, and navigate to the sequence file that you want to view.

The sample information for the samples associated with the sequence appears in the upper portion of the window.

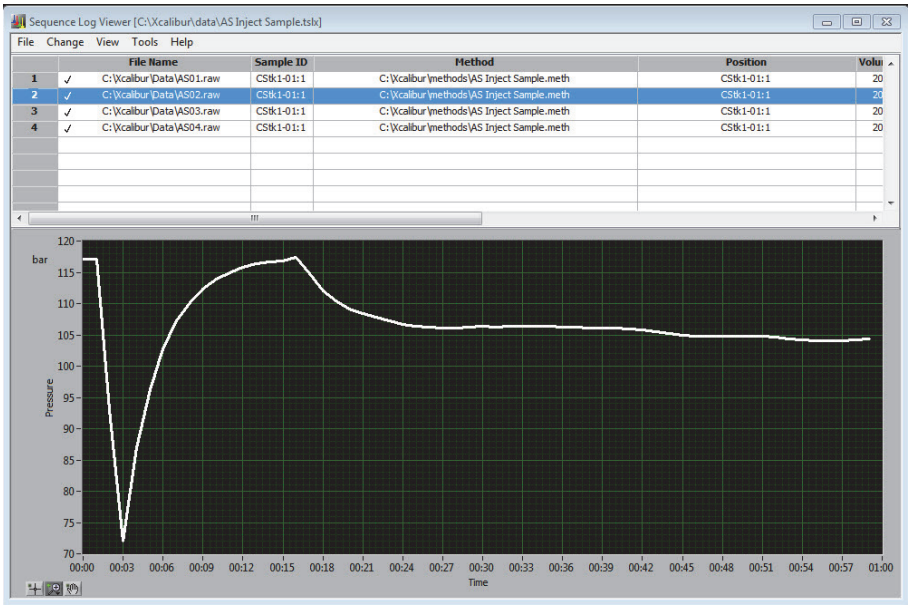
4. Choose **View > Pressure View**.

   The pressure graph view opens.

5. Select a sample.

6. To view the pressure trace of more than one sample at a time, select a sample name, hold down the CTRL key, and then select the additional samples that you want to view (see Figure 107).

   Figure 107. Pressure trace view



7. To use the pan or zoom features, see "Pan and Zoom Tools" on page 167.

❖ To assign pressure profiles to monitor pressure

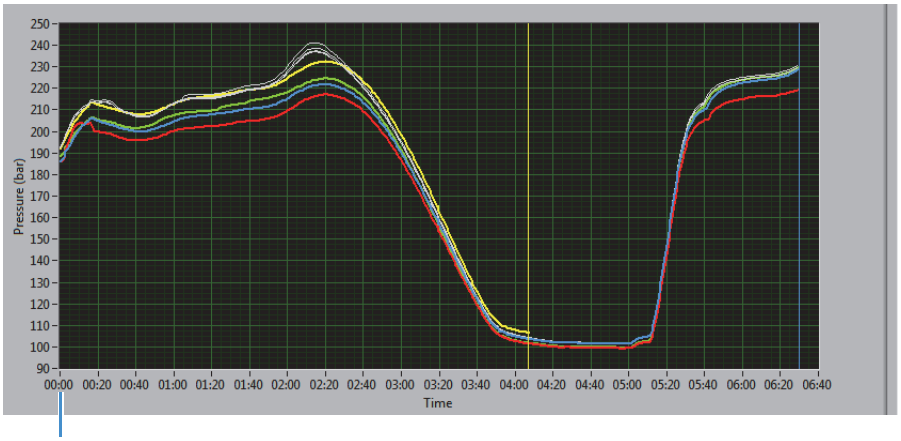
See "Assigning a Pressure Profile" on page 128.

You can establish a pressure profile for each method and use the pressure profile to automatically monitor the channel for unexpected pump pressure readings. The control application compares the current pump pressure and method time to that of the method's saved pressure profile. The system flags samples or stops the channel's pumps when the pump pressure falls outside assigned limits.

Normalized Versus Real Time

If the Pressure Traces page of the Direct Control window displays the Normalized button (see Figure 106 on page 163), the graph shows the selected pump pressures with the method clock normalized to zero. Even if the methods did not run at the same time, the zero on the graph represents the start of all the methods displayed. Figure 108 shows the normalized view of the pumps' backpressures throughout a run.

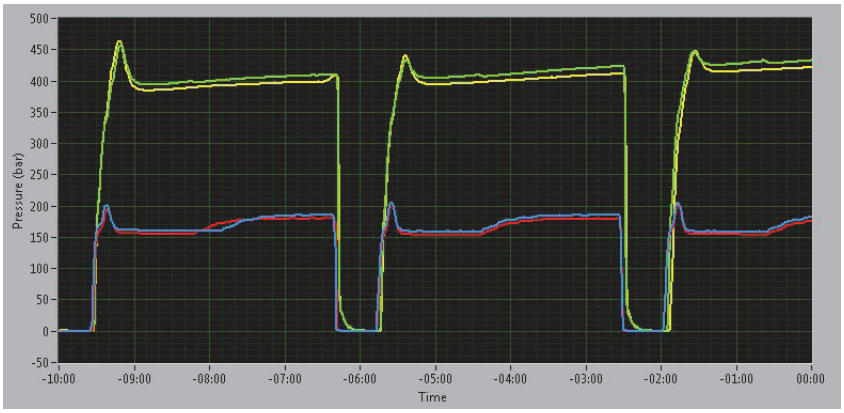
Figure 108. Normalized view of the pumps' backpressures for LC channels 1, 2, 3, and 4



Method clock is zero for the method start for all channels that appear on the graph.

If the Pressure Traces page displays the Real Time button, the graph shows the selected pump pressures in the current time and up to 10 minutes of elapsed time, with 0.00 representing the current time. Figure 109 shows the real-time view of the pumps' backpressures for the previous minute of the run.

Figure 109. Real-time view of the pumps' backpressures for LC channels 1, 2, 3, and 4

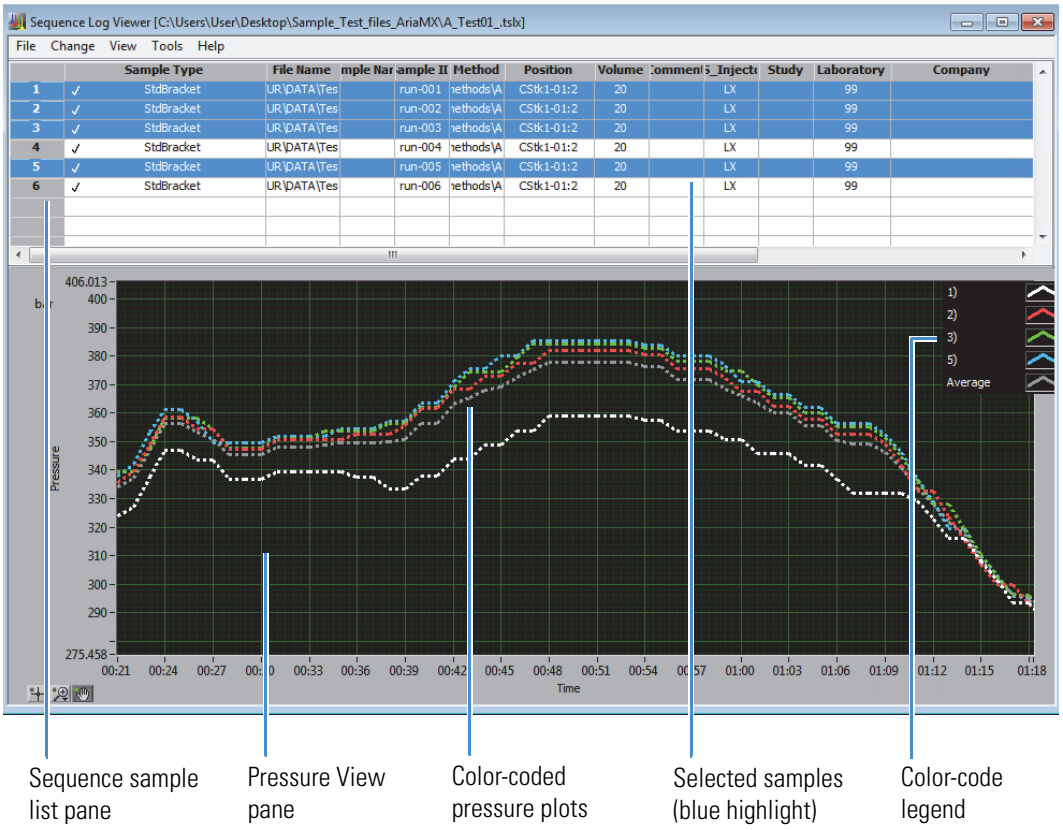


Applying Color-coded Overlays to Pressure Plots

You can apply a color-coded overlay for the pressure plots of up to 10 sequence samples in the Sequence Log Viewer and distinguish them from one another using the color-code legend. Color-coded pressure plots help you to focus on sequence samples of interest and quickly compare them to each other. The plot colors are automatically assigned in the order selected, beginning with white (see Figure 110).

Note The colors applied to the selected sequence samples repeat if you select more than 10 samples to view.

Figure 110. Selected sample color-coding in the Sequence Log Viewer



General attributes of the color-coding feature are as follows:

- The Pressure View pane displays individual pressure plots and their associated colors (see Figure 110).
- The color-code legend appears in the upper right corner of the Pressure View pane and displays the corresponding sample name or sample number. Right-click inside the Pressure View pane to change between the use of Sample ID or Sample Name in the legend.

- The color-code legend is on by default. Turn this feature off by right-clicking inside the Pressure View pane and then clearing the check mark next to the legend.

  Tip Right-click the Pressure View pane to see additional options for display of the color-code legend and for exporting.

- The gray plot line represents the average plot from all of the selected sequence samples.

❖ To select specific sequence samples to view with the color-coded legend

1. Open the Direct Control window.
2. Choose **Tools > Sequence Log Viewer**.

   The Sequence Log Viewer opens.
3. Choose **File > Open**.
4. Navigate to the TSLX file that you want to review, and then click **Open**.

   The TSLX file opens and displays the sequence log data (top) pane.
5. Choose **View > Pressure View**.

   The pressure plot pane is displayed below the sequence log data pane.
6. From the sequence log pane, select the sequence samples that you want to color code.

   Each sequence sample selected is assigned a unique color for its pressure plot.

Pan and Zoom Tools

❖ To use the pan and zoom tools on the pressure trace

1. On the Pressure Traces page of the Direct Control window (see Figure 106 on page 163), click the **Lock** icon,  .

   The icon appears locked, the display stops updating, and the zooming and panning icons appear (see Figure 111).

   Figure 111. Lock, panning, and zooming icons

2. To zoom in on the pressure trace, do the following:
   a. Click the **Zoom** icon,  .
   b. Select the appropriate tool and complete the procedure as described in Table 41.

Table 41. Zoom tools (Sheet 1 of 2)

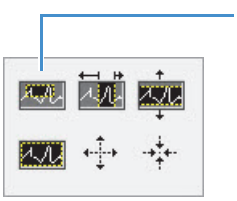
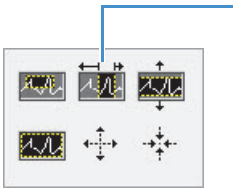
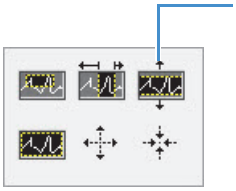
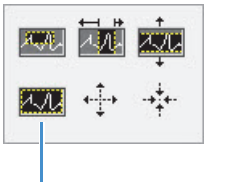
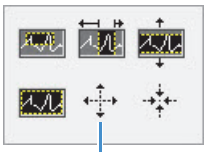
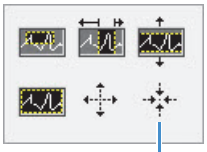
Tool	Description
	Select this tool to change the *x*- and *y*-axis scales. Then do the following: 1. Select the area on the graph that shows the lower end of the *x* and *y* axes that you want to view and hold down the mouse button. 2. Drag the cursor to the higher end of the *x* and *y* axes that you want to view, and release the mouse button. The highlighted area appears on the graph.
	Select this tool to change the *x*-axis scale to enlarge the data. Then do the following: 1. Click the lower end of the range that you want to view, and hold down the mouse button. 2. Drag the cursor to the higher end of the range that you want to view, and release the mouse button. The scale changes to reflect the highlighted range.
	Select this tool to change the *y*-axis scale to enlarge the data. Then do the following: 1. Click the lower end of the range that you want to view, and hold down the mouse button. 2. Drag the cursor to the higher end of the range that you want to view, and release the mouse button. The scale changes to reflect the highlighted range.
	Select this tool if you want to adjust the *x* and *y* axes to fit the data into the window.

Table 41. Zoom tools (Sheet 2 of 2)

Tool	Description
 Zoom-in tool	Select this tool if you want to zoom in on an area of the data. Then do the following: 1. Click the point on the graph that you want to position in the center of your graph, and hold down the mouse button. The data enlarges around the point you clicked. 2. Release the mouse button when the data appears the size that you want.
 Zoom-out tool	Select this tool if you want to zoom out on an area of the data. Then do the following: 1. Click the point on the graph that you want to position in the center of your graph and hold down the mouse button. The data decreases in size around the point you clicked. 2. Release the mouse button when the data appears the size that you want.

3. To view an area of the pressure trace that falls outside the viewing area, do the following:
   a. Click the **Pan** icon,  .
   b. Hold down the left mouse button in the pressure trace and drag the cursor up or down.
4. To return to the standard cursor, click the **Standard Cursor** icon,  .

Viewing the Heater Status

❖ To view the heater status

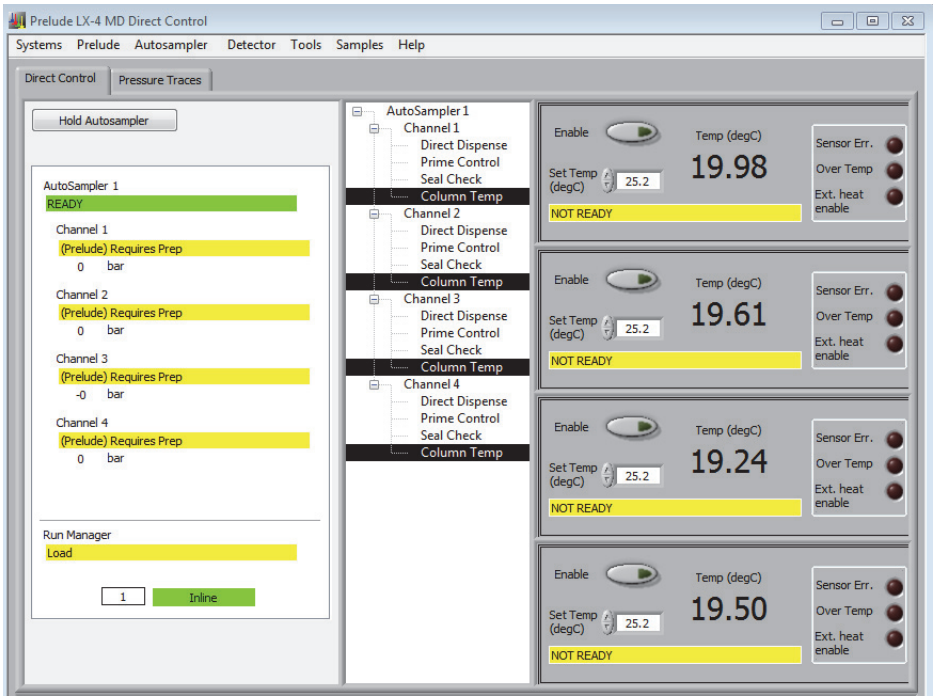
1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.

   The heater name appears below its associated channel in the middle pane.
2. In the middle pane, select the heater to which you want to assign a temperature.

   The heater options appear.

   Figure 112 shows the Direct Control window displaying temperature options, and Table 42 describes the temperature display.

Figure 112. Temperature display in the Direct Control window



The heater name, Column Temp, might be different on your system. You can enter custom names to configure the heaters.

Table 42. Temperature display parameters (Sheet 1 of 2)

Parameter	Description
Enable	Light green indicates the heater is on. Dark green indicates that the heater is off.
Set Temp	View the temperature in Celsius.
Temp (degrees Celsius)	The actual temperature reading in Celsius as indicated by the heater feedback.
Sensor Err.	Bright red indicates communication from the heater to the controller has failed. Call Technical Support. See "Contacting Us" on page xv. Dark red indicates that the controller found no sensor error state.
Over Temp	Bright red indicates an error condition exists. Call Technical Support. See "Contacting Us" on page xv. If this button appears dark, then the controller detected no error state.

Table 42. Temperature display parameters (Sheet 2 of 2)

Parameter	Description
Ext. heat enabled	Bright red indicates an internal hardware issue. Call Technical Support. See "Contacting Us" on page xv. Dark red indicates the normal state.
Status bar	Green indicates that the heater temperature is within the tolerance range. Yellow indicates the heater temperature is outside the tolerance range.

Viewing the Event Log

The Event Log displays the most recent events that occurred on the system. The control application continuously updates the event log and records any significant event that occurs on the system. Examples of events that might appear in the Event Log follow:

- Adding a batch for analysis
- Running a specific sample in a particular batch
- Current system triggering for a specific sample
- Assigned probe for sample pickup
- Assigned valve for sample injection
- Arrival of sample at a particular channel for analysis

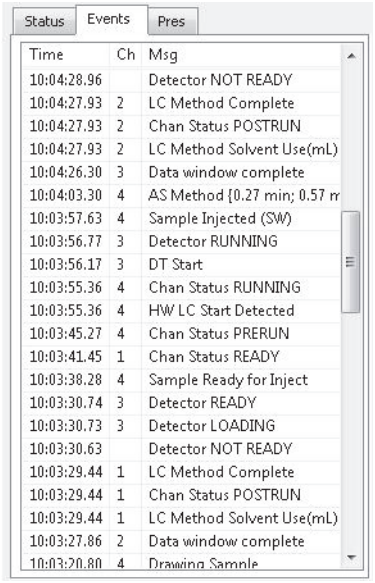
Follow these procedures:

- To view the event log
- To view events by the Event Log Viewer
- To view past events by sample

❖ To view the event log

1. Open the Prelude LX-4 MD Status page from the data processing software. See "Accessing the Status Page from the Data Processing Software" on page 160.
2. Click the **Events** tab (Figure 113).

Figure 113. Event Log



3. To view information on a specific event, point to the event, and wait one second.

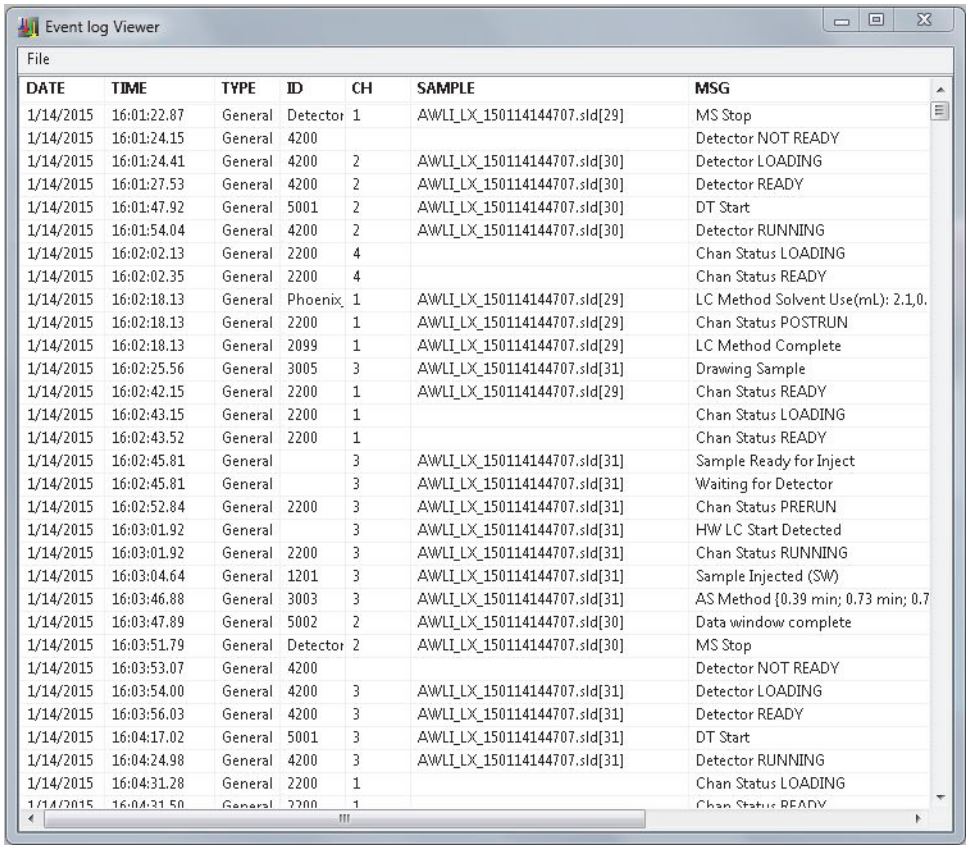
   The event information opens.

❖ To view events by the Event Log Viewer

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Tools > Event Log Viewer.**

   The Event Log Viewer opens showing the current event log (see Figure 114).

Figure 114. Event Log Viewer window



DATE	TIME	TYPE	ID	CH	SAMPLE	MSG
1/14/2015	16:01:22.87	General	Detector	1	AWLI_LX_150114144707.sld[29]	MS Stop
1/14/2015	16:01:24.15	General	4200			Detector NOT READY
1/14/2015	16:01:24.41	General	4200	2	AWLI_LX_150114144707.sld[30]	Detector LOADING
1/14/2015	16:01:27.53	General	4200	2	AWLI_LX_150114144707.sld[30]	Detector READY
1/14/2015	16:01:47.92	General	5001	2	AWLI_LX_150114144707.sld[30]	DT Start
1/14/2015	16:01:54.04	General	4200	2	AWLI_LX_150114144707.sld[30]	Detector RUNNING
1/14/2015	16:02:02.13	General	2200	4		Chan Status LOADING
1/14/2015	16:02:02.35	General	2200	4		Chan Status READY
1/14/2015	16:02:18.13	General	Phoenix	1	AWLI_LX_150114144707.sld[29]	LC Method Solvent Use(mL): 2.1,0.
1/14/2015	16:02:18.13	General	2200	1	AWLI_LX_150114144707.sld[29]	Chan Status POSTRUN
1/14/2015	16:02:18.13	General	2099	1	AWLI_LX_150114144707.sld[29]	LC Method Complete
1/14/2015	16:02:25.56	General	3005	3	AWLI_LX_150114144707.sld[31]	Drawing Sample
1/14/2015	16:02:42.15	General	2200	1	AWLI_LX_150114144707.sld[29]	Chan Status READY
1/14/2015	16:02:43.15	General	2200	1		Chan Status LOADING
1/14/2015	16:02:43.52	General	2200	1		Chan Status READY
1/14/2015	16:02:45.81	General		3	AWLI_LX_150114144707.sld[31]	Sample Ready for Inject
1/14/2015	16:02:45.81	General		3	AWLI_LX_150114144707.sld[31]	Waiting for Detector
1/14/2015	16:02:52.84	General	2200	3	AWLI_LX_150114144707.sld[31]	Chan Status PRERUN
1/14/2015	16:03:01.92	General		3	AWLI_LX_150114144707.sld[31]	HW LC Start Detected
1/14/2015	16:03:01.92	General	2200	3	AWLI_LX_150114144707.sld[31]	Chan Status RUNNING
1/14/2015	16:03:04.64	General	1201	3	AWLI_LX_150114144707.sld[31]	Sample Injected (SW)
1/14/2015	16:03:46.88	General	3003	3	AWLI_LX_150114144707.sld[31]	AS Method {0.39 min; 0.73 min; 0.7
1/14/2015	16:03:47.89	General	5002	2	AWLI_LX_150114144707.sld[30]	Data window complete
1/14/2015	16:03:51.79	General	Detector	2	AWLI_LX_150114144707.sld[30]	MS Stop
1/14/2015	16:03:53.07	General	4200			Detector NOT READY
1/14/2015	16:03:54.00	General	4200	3	AWLI_LX_150114144707.sld[31]	Detector LOADING
1/14/2015	16:03:56.03	General	4200	3	AWLI_LX_150114144707.sld[31]	Detector READY
1/14/2015	16:04:17.02	General	5001	3	AWLI_LX_150114144707.sld[31]	DT Start
1/14/2015	16:04:24.98	General	4200	3	AWLI_LX_150114144707.sld[31]	Detector RUNNING
1/14/2015	16:04:31.28	General	2200	1		Chan Status LOADING
1/14/2015	16:04:31.50	General	2200	1		Chan Status READY

The Event Log Viewer window displays current and past recorded LC, autosampler, user, and detector events that occurred during system operation. When the Event Log Viewer window reaches its capacity, the control application saves all the event information as a sequential file and then creates a new file.

3. To open a previously stored event log, do the following:
   a. Choose **File > Browse**, and then navigate to and select the appropriate log file.
   b. Click **Open**.

      The stored event log opens.

Note

- The control application assigns Aria.log as the name of the current event. When the file size of Aria.log reaches 10 MB, it changes the file name to Aria.old.log, and creates a new file named Aria.log. If a file named Aria.old.log already exists, the older one is deleted.
- Aria.log files are located in C:\ProgramData\Thermo\Aria MX

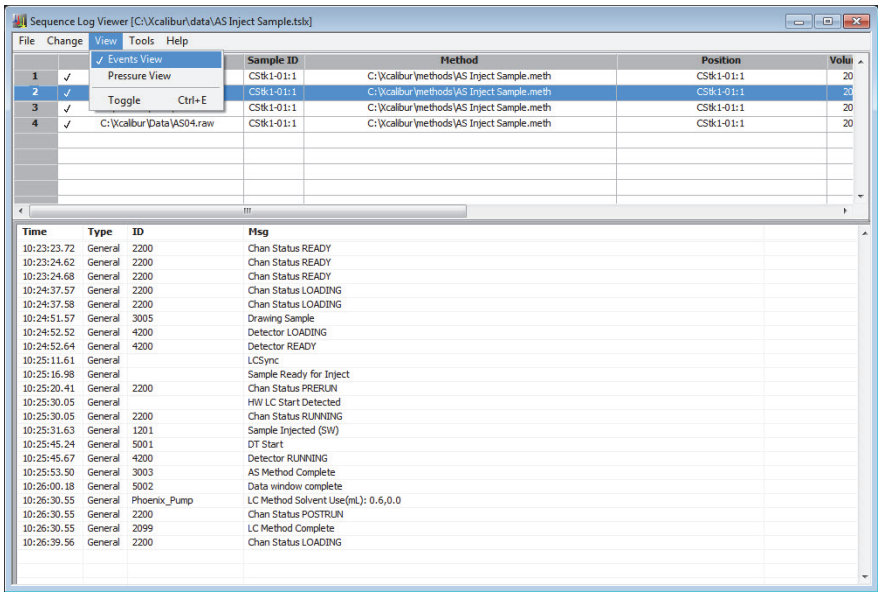
❖ To view past events by sample

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Tools > Sequence Log Viewer**.

   The Sequence Log Viewer window opens.
3. Choose **File > Open** and navigate to the sequence file that contains the sample that you want to view.

   The sample information for the samples associated with the sequence appears in the upper portion of the window.
4. Choose **View > Events View** (see Figure 115).

   Figure 115. Sample events



Accessing the Sequence Log Viewer

The Sequence Log Viewer shows the stored run information, such as the samples, events, and LC method information.

❖ To access the Sequence Log Viewer

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Tools > Sequence Log Viewer**, and navigate to the sequence log you want to open.

Note The Sequence Log Viewer is a separate application. You can also open it by choosing **Start > All Apps** (Windows 10) **or All Programs** (Windows 7) **> Thermo Prelude LX-4 MD > Sequence Log Viewer**.

3. Select the appropriate sequence log.

   The Sequence Log Viewer window opens (see Figure 116).

Figure 116. Sequence Log Viewer window

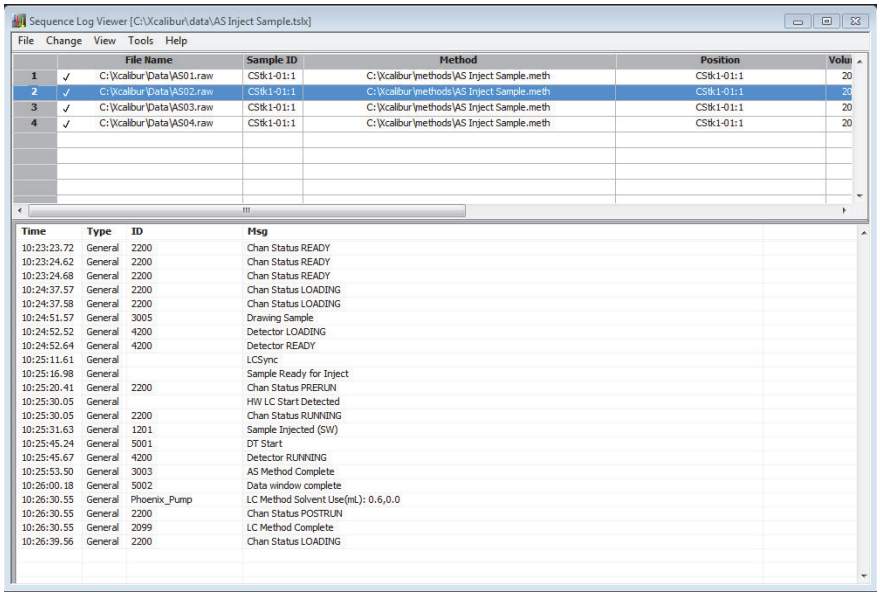


Table 43 describes the Sequence Log Viewer window.

Table 43. Sequence Log Viewer display parameters

Parameter	Description
Menu bar	The Sequence Log Viewer window has the following menus: • File: Shows the File commands. • Change: Shows the Column Arrangement dialog box. Customize the columns that you want to view and their display order. • View: Shows these commands: – Events View: Shows the sample list information in the upper portion of the window and the sample events for the selected sample in the lower portion of the window. – Pressure View: Shows the pressure trace for the selected sample. – Toggle: Changes the view from the Events View to the Pressure View or from the Pressure View to the Events View. • Tools: Provides the option to extract a method from a raw data file. • Help: Provides access to the Prelude LX-4 MD application Help.
Sequence (batch) information	Shows the batch information for the selected sequence, such as the samples, location of the raw data file, sample name, sample ID, method location and name, vial location (Position), volume of sample drawn, and channel that ran the sample. When you select a sample and then right-click the sequence file, the LC method that ran the sample appears.
Event information	When you choose Event View from the View menu, the sample event information for the selected sample appears at the bottom of the window. This includes the time the method started, the sample, the message ID, and the message text.
Pressure profile	When you choose Pressure View from the View menu, a graph appears showing the stored pump pressure values that were recorded during the run of the selected sample.

Using the Sequence Log Viewer

You can view details regarding logged sample acquisition events—including pressure traces—using the Sequence Log Viewer.

Sequence log files have a .tslx file name extension.

❖ To view a sequence log file

From Windows Explorer, navigate to the TSLX file that you want to view and double-click it.

The sequence log opens in the Sequence Log Viewer.

Tip You can also open TSLX files directly from the Sequence Log Viewer, which displays recently created and viewed log files.

Solutions to Common Issues

Table 44 lists common issues you might have while operating the Prelude LX-4 MD instrument, their possible solutions, and diagnostic procedures.

Table 44. Troubleshooting common issues (Sheet 1 of 3)

Issue	Possible solution or diagnostic test
Controls are out of range.	Do one or more of the following: • Verify that the controls and tray reside in the appropriate location on the autosampler. See "Preparing the Samples" on page 22. • Verify that mobile phases and wash solutions reside in the appropriate locations. See "Prepare the Mobile Phase Solvents" on page 142. • Prepare fresh controls and calibrators, and re-run the controls and calibrators. Refer to the appropriate control and calibrator documentation. • Verify that mobile phases and wash solutions are particulate-free. See "Prepare the Mobile Phase Solvents" on page 142. • Observe the data or perform a run to determine which LC channels are affected. If the problem appears in only one channel, focus your troubleshooting efforts on that channel. • Observe the chromatograms for all samples and look for trends in peak shape and response. • Observe the pressure trace for the samples in the run and compare the pressure data of the run with that of a previous run. Look for shifts or sudden changes in the pump pressures. • Perform the channel-preparation procedure and observe the test data for the pump seal check. See "Preparing the Channels" on page 23.
You observe a leak, or the instrument fails the pump seal check.	Do the following: 1. To stop the autosampler, click **Hold Autosampler** in the Direct Control window. 2. Hand-tighten all column and tubing connections. 3. If the problem persists, call Technical Support. See "Contacting Us" on page xv.

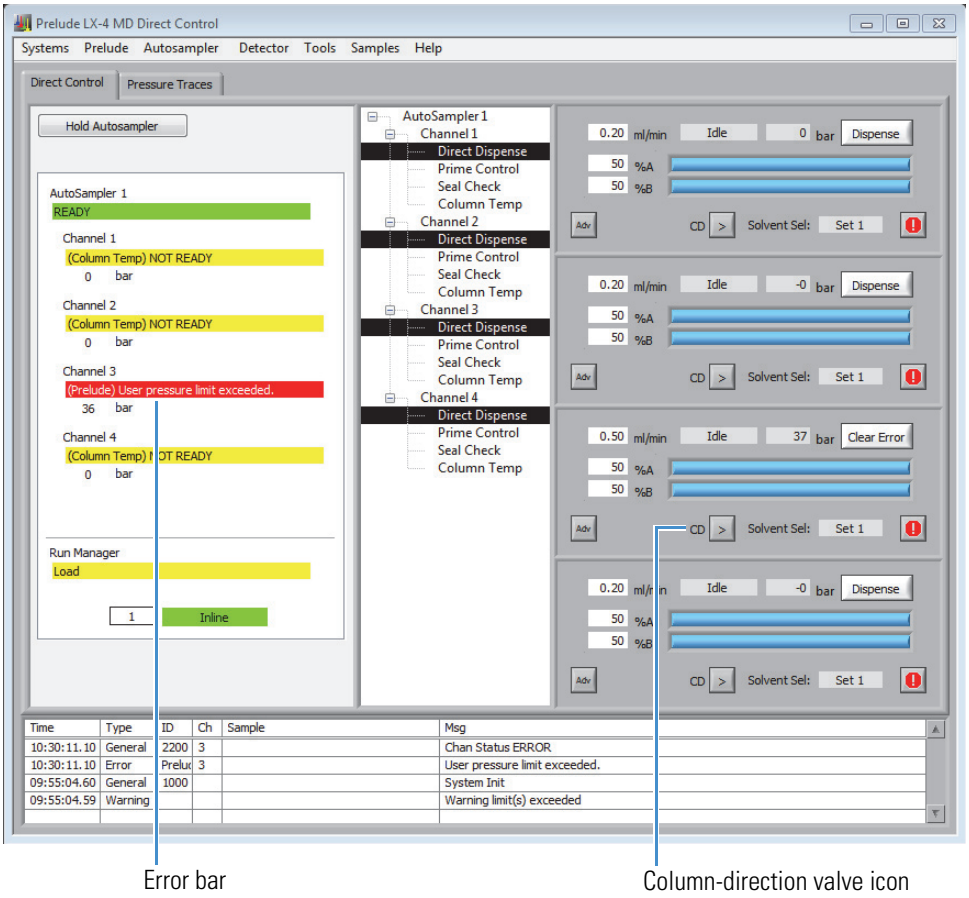
Table 44. Troubleshooting common issues (Sheet 2 of 3)

Issue	Possible solution or diagnostic test
A pump issue causes an error, such as "Pump 1 Over Pressure" (see the example in Figure 117 on page 181).	Do the following: 1. To recover from the error, right-click the red error bar in the Direct Control window and choose **On**. 2. If the pump backpressure heads towards an overpressure again, reverse the flow direction through the column to attempt removing a possible blockage. Click the **CD** (column-direction) valve icon,  , at the right side of the Direct Control window to reverse the flow. 3. If the issue persists, replace the column with a union and watch for eventual stability of the pressure readback. If the pressure stabilizes, replace the bad column with a new one. 4. If the problem persists, call Technical Support. See "Contacting Us" on page xv.
Low response from one channel.	Do one or both of the following: • Verify that mobile phases and wash solutions are particulate-free and in the appropriate locations. • Replace the HPLC column. See "Replace the Analytical Column" on page 155.
Low response from all channels. (All channels share mobile phases.)	Do one or more of the following: • Perform the cleaning procedures for the injector and needle. See "Clean the Syringe" on page 150 and "Cleaning the Injector" on page 186. • Perform detector instrument maintenance. Refer to the appropriate detector instrument documentation. • Recalibrate the detector. Refer to the appropriate detector instrument documentation. • Verify that autosampler Wash 1 and Wash 2 solutions are in the appropriate locations. See "Prepare the Wash 1 and Wash 2 Autosampler Wash Solution Bottles" on page 148.

Table 44. Troubleshooting common issues (Sheet 3 of 3)

Issue	Possible solution or diagnostic test
The pressure trace shows a change or trend from previous runs.	Do one or more of the following: • If the pressure trace shows an overall upward trend in pressure, replace the HPLC column. • If the pressure trace shows a change in one area of the method, call Technical Support. See "Contacting Us" on page xv.
The problem occurs in only one LC channel.	Focus your troubleshooting efforts on these components: • Mobile phases (if channels do not share mobile phase bottles) • HPLC column • Instrument tubing
The problem occurs in all LC channels, and the channels share mobile phases.	Focus your troubleshooting efforts on these components: • Autosampler • Wash solutions • Detector
You hear an unusual noise when the autosampler withdraws or dispenses sample.	A service engineer must calibrate the autosampler components. Call Technical Support. See "Contacting Us" on page xv.
The internal standard (IS) result falls out of range for all samples.	Verify that you added the IS to all samples. See "Preparing the Samples" on page 22.
The IS falls out of range for one sample.	Observe the chromatogram at the same retention time as the quantitative ion. If extra peaks are present, the sample might contain compounds that interfere with the detection.

Figure 117. Pump 3 Over Pressure error



Performing the Pump Seal Check Test

Perform the pump seal check procedure if you are requested to do so as part of a troubleshooting process.

Follow these procedures:

- To perform the pump seal check as part of the channel-preparation procedure
- To perform the pump seal check by using controls in the Direct Control window

❖ To perform the pump seal check as part of the channel-preparation procedure

See "Preparing the Channels" on page 23.

When the test finishes, the pump status color changes to green if the test passes and to red if the test fails.

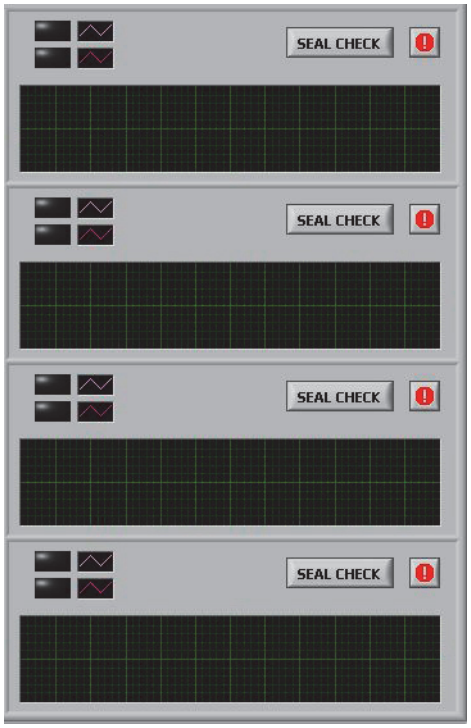
❖ **To perform the pump seal check by using controls in the Direct Control window**

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. Choose **Prelude > Channel *n* > Seal Check** for the channel that you want to check.

   The seal check begins immediately.

Tip Click **Seal Check** in the middle pane of the Direct Control window to view the seal check pressure trace in real time (see Figure 118).

Figure 118. Seal check and pressure trace information



Priming the LC Pumps

If you observe fluctuations in pump pressure, change the solvent bottles, and then prime the pump. If the instrument has been idle for more than 24 hours, prime the pumps.

This procedure flows fluid from the solvent bottle to the pump and then to waste. The fluids do not reach the columns.

Prime the channels at least six times by using Bottle Set 1 and at least six times by using Bottle Set 2. Prime the pumps by using the mobile phases you use for sample analysis, unless a service engineer instructs you to use a cleaning solvent or water during a troubleshooting or maintenance procedure.

You can prime the system pumps in two ways:

- Channel-preparation
- Direct Control window, which provides three priming options

❖ To prime the pumps by using the channel-preparation procedure

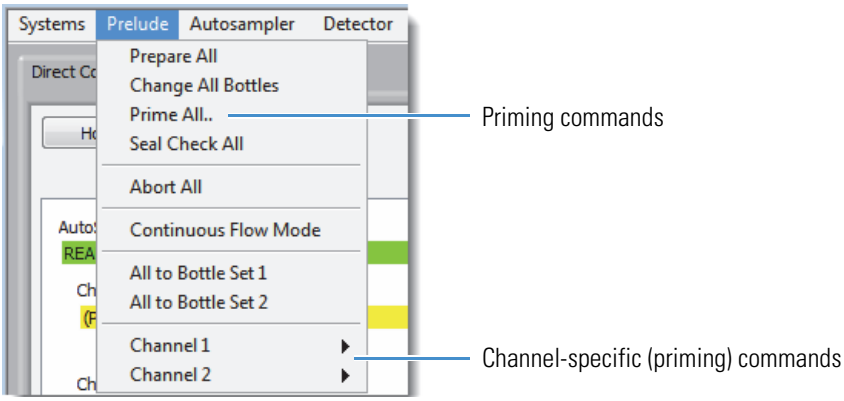
See "Preparing the Channels" on page 23.

❖ To prime the pumps using the controls in the Direct Control window

1. Open the Direct Control window. See "Using the Direct Control Window" on page 28.
2. Open the Prelude menu.

   The Prelude menu shows the available priming commands and options.

   Figure 119. Prelude menu options



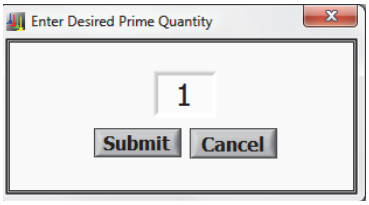
   Use one of the following options to initiate the priming operation.

❖ (Option 1) To prime all the pumps at the same rate by using the controls in the Direct Control window

1. Open the Direct Control window. See "Using the Direct Control Window" on page 28.
2. Choose **Prelude > Prime All**.

   The Enter Desired Prime Quantity dialog box opens (see Figure 120).

   Figure 120. Enter Desired Prime Quantity dialog box



3. Type the number of prime cycles that you want to perform, and then click **Submit**.

   All of the system pumps begin the priming operation for the number of times that you specified.

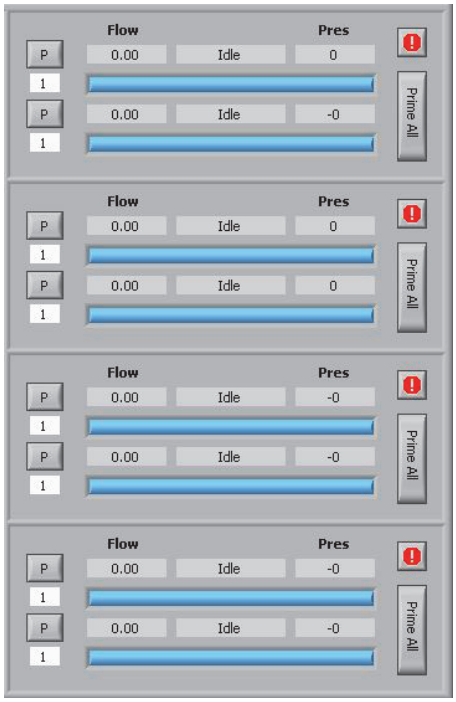
Note The priming function uses the mobile phase bottles that are already assigned to a channel. For information on how to change bottle assignments, see "Assigning Bottle Sets" on page 46.

❖ (Option 2) To prime individual pumps in the Direct Control window

1. Open the Direct Control window. See "Using the Direct Control Window" on page 28.
2. In the middle pane, select **Prime Control** for any available channel.

   The Direct Control priming options appear to the right (Figure 121).

   Figure 121. Direct Control window showing priming options



3. Determine which pump you want to prime, and then click in the box directly below the P button.
4. Type the number of prime cycles that you want to perform on the pump specified, and then click the **P** button.

   The priming operation begins for the number of times that you specified.

Note The priming function uses the mobile phase bottles that are already assigned to a channel. For information on how to change bottle assignments, see "Assigning Bottle Sets" on page 46.

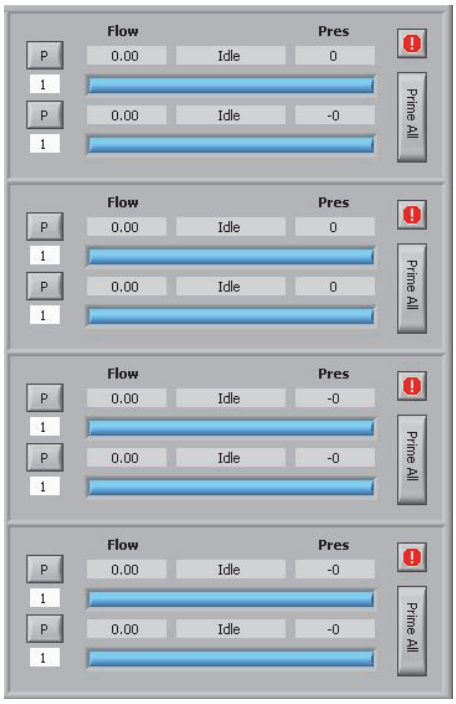
❖ (Option 3) To prime both pumps for a specific channel in the Direct Control window

1. Open the Direct Control window. See "Using the Direct Control Window" on page 28.

2. In the middle pane, select **Prime Control** for any available channel.

   The Direct Control priming options appear to the right (Figure 122).

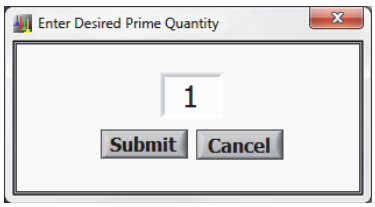
   Figure 122. Direct Control window showing priming options



3. Determine the channel with the pumps that you want to prime, and then click **Prime All**.

   The Enter Desired Prime Quantity dialog box opens (see Figure 123).

   Figure 123. Enter Desired Prime Quantity dialog box



4. Type the number of prime cycles that you want to perform, and then click **Submit**.

   The two pumps for that channel begin the priming operation for the number of times that you specified.

Note The priming function uses the mobile phase bottles that are already assigned to a channel. For information on how to change bottle assignments, see "Assigning Bottle Sets" on page 46.

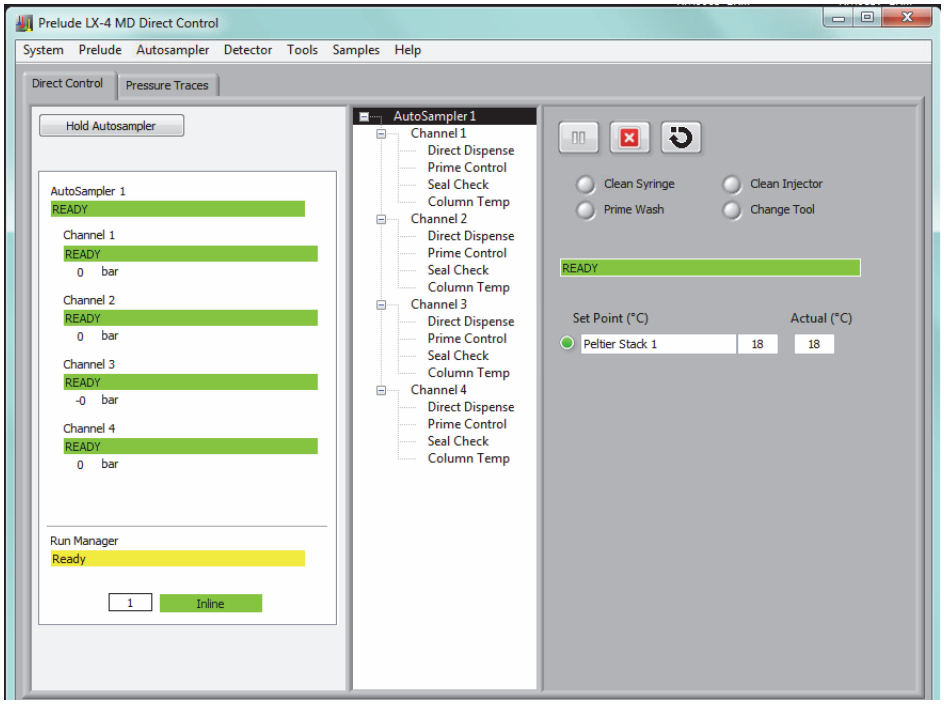
Cleaning the Injector

❖ To clean an injector

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. In the middle pane, click the autosampler.

   The Autosampler options appear on the right (see Figure 124).

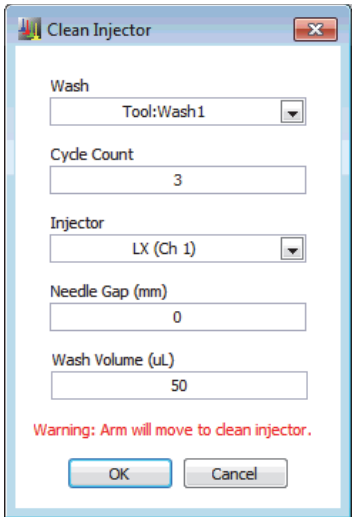
   Figure 124. Direct Control window showing autosampler options



3. Select the **Clean Injector** option.

   The Rinse Injector dialog box opens (see Figure 125).

Figure 125. Clean Injector dialog box



4. In the Wash list, select the wash that you want to use to clean the injector.
5. In the Injector list, select the injector that you want to clean.
6. In the Rinse Time box, select how long you want to wash the injector (in seconds), and click **OK**.

Tip Thermo Fisher Scientific recommends that you select at least five seconds for optimal cleaning.

The dialog box closes and the autosampler cleans the injector.

Note For information about cleaning the needle, see "Clean the Syringe" on page 150.

Resetting the XYZ Positions

Reset the XYZ positions if you accidentally bump the autosampler arm.

CAUTION Do not perform this procedure while the autosampler is performing an operation as this could result in personal injury.

❖ To reset the XYZ position of the autosampler probe

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 28.
2. In the middle pane, click the autosampler.

   The autosampler options appear in the right pane.

3. Click the **Reset** icon,  .

   The instrument resets the positions, injectors, and syringe. The autosampler then moves to the home position.

Note When an instrument component resets a position, it moves to the zero position, which is a fixed reference point that the instrument recognizes as the zero position. Then it resets the X, Y, and Z coordinates to 0.

Decontaminating the Instrument

Before shipping the instrument to another location, or returning it to Thermo Fisher Scientific, you must decontaminate the instrument of chemicals and biohazards.

CAUTION Observe the appropriate biohazard and chemical safety precautions defined by your laboratory while performing the decontamination procedure.

❖ To decontaminate the Prelude LX-4 MD instrument

1. Wipe down the table and external surfaces of the Prelude LX-4 MD instrument with a soft cloth moistened with 100% isopropanol. Do not use bleach or a bleach alternative.
2. Place all mobile phase lines into a container of 45:45:10 acetonitrile/isopropanol/acetone.
3. Prime all the pumps 10 times. See "Priming the LC Pumps" on page 182.
4. Schedule five samples to run as follows:
   a. Install a sample blank in the autosampler.
   b. Start the run on each of the four LC channels by using any method.
5. Remove all samples and trays from the autosampler drawers. Wipe down drawers with 100% isopropanol. Discard all samples. Soak the trays in isopropanol for 5 minutes.
6. For a decontamination form, contact Technical Support. See "Contacting Us" on page xv. Complete the decontamination form and include it with the instrument before shipping.

A

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