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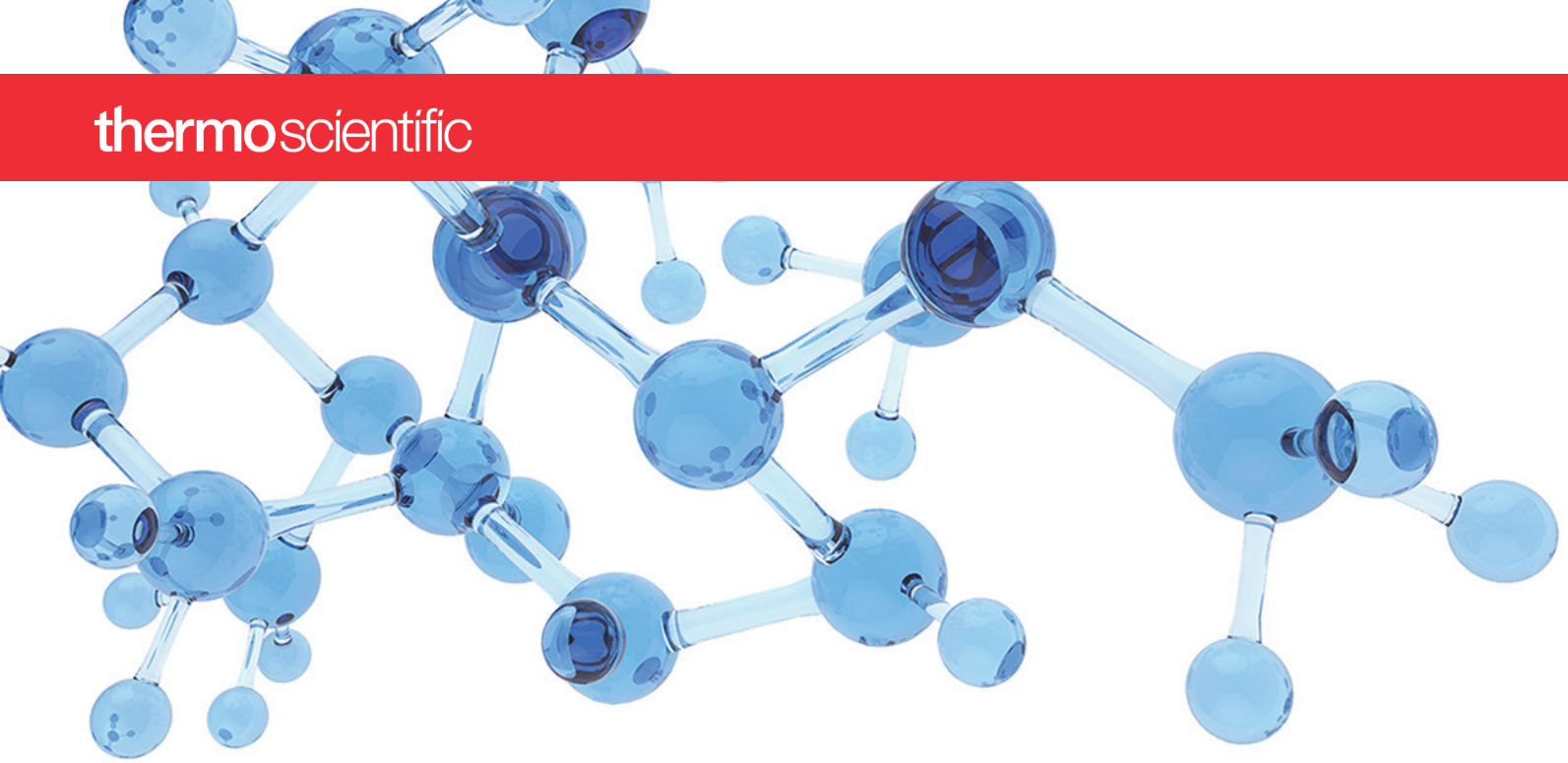

Prelude MD

HPLC for in vitro diagnostic use

Operator Manual

Prelude MD software version 1.2

65000-97045 Revision A • August 2019

DOCUMENTATION SURVEY

ThermoFisher SCIENTIFIC

Release history: Revision A, August 2019.

IVD **In vitro diagnostic medical device.**

CE

thermoscientific

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.

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2012/19/EU

Thermo Fisher Scientific is registered with B2B Compliance (B2Bcompliance.org.uk) in the UK and with the European Recycling Platform (ERP-recycling.org) in all other countries of the European Union and in Norway. If you have questions about recycling this product, contact your local sales representative.

If this product is located in Europe and you want to participate in the Thermo Fisher Scientific Business-to-Business (B2B) Recycling Program, send an email request to weee.recycle@thermofisher.com with the following information:

- WEEE product class
- Name of the manufacturer or distributor (where you purchased the product)
- Number of product pieces, and the estimated total weight and volume
- Pick-up address and contact person (include contact information)
- Appropriate pick-up time
- Declaration of decontamination, stating that all hazardous fluids or material have been removed from the product

For additional information about the Restriction on Hazardous Substances (RoHS) Directive for the European Union, search for RoHS on the Thermo Fisher Scientific European language websites.

IMPORTANT This recycling program is **not** for biological hazard products or for products that have been medically contaminated. You must treat these types of products as biohazard waste and dispose of them in accordance with your local regulations.

Contents

Preface

This guide describes how to use the Thermo Scientific™ Prelude MD™ instrument, which is a liquid chromatography (LC) system that runs high-performance liquid chromatography (HPLC), ultra-high-performance liquid chromatography (UHPLC), and Thermo TurboFlow™ methods to separate components in a sample before analysis with a detector.

The Prelude MD software application controls the Prelude MD instrument components, while interfacing with the detector's control application. This guide describes how to use the software to manage instrument components and to create the LC and autosampler (AS) instrument methods.

Contents

Intended Use

The Prelude 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 intended for in vitro diagnostic use.

Intended Users

To operate the Prelude 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 MD instrument, see "Decontaminating the Instrument" on page 166.

When you use the Prelude 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 MD instrument includes complete documentation. In addition to this manual, you can access the *Prelude MD Preinstallation Requirements Guide* as a PDF file and have two ways to access the Prelude 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 >Prelude MD > Prelude MD Operator Manual**.

❖ To view the Prelude 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 Instruments > Prelude MD > Direct Control**.

   The Direct Control window opens.

2. Choose **Help > Help**.

❖ To view the Prelude MD Help from the instrument method area

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

   The LC Method Editor window opens.

2. Choose **Help > Prelude 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."

This manual uses the following types of cautions and special notices.

CAUTION Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.

CAUTION Highlights electric shock hazards to humans. Each electric shock notice is accompanied by the international high voltage symbol.

CAUTION Highlights chemical hazards to humans, property, or the environment. Each chemical notice is accompanied by the chemical caution symbol.

CAUTION Highlights potentially infectious biological hazardous materials. Each biohazard notice is accompanied by the biohazard caution symbol.

CAUTION Highlights burn hazards to humans. Each burn notice is accompanied by the heat symbol.

CAUTION Highlights puncture hazards to humans. Each puncture notice is accompanied by the sharp object symbol.

IMPORTANT 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 system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

Table 1 defines additional symbols and labels that are used in this manual, marked on the instrument, or both. Symbols marked on the instrument can appear in color or in black and white.

Table 1. Symbols, labels, and their meanings

Symbol or label	Meaning
CE	Manufacturer marking of conformity to the "Essential Requirements" of European Directive 98/79/EC, *In Vitro Diagnostic Medical Devices*.
IVD	For in vitro diagnostic use.
	Manufacturer
EC REP	European Authorized Representative
SN	Serial number
	Consult the instructions for use.

Environmental Conditions

Refer to the system component manuals for information on environmental conditions and specifications.

Good Laboratory Practices

To obtain optimal performance from your LC system and to prevent personal injury or harm to the environment, do the following:

- Keep good records.
- Read the manufacturers' MSDSs for the chemicals you use in your laboratory.
- Remove particulate matter from your samples before injecting them into the liquid chromatograph.
- Use LC/MS-grade solvents or better.
- Connect the drainage tubes from the pump, autosampler, and detector to an appropriate waste receptacle. Dispose of solvents as specified by local regulations.

Keeping Good Records

To help identify and isolate problems with either your equipment or your methodology, keep good records of all system conditions (for example, %RSDs on retention times and peak areas, peak shape, and resolution). At a minimum, thoroughly document a chromatogram of a typical sample and standard mixture, with system conditions, for future reference. Careful comparison of retention times, peak shapes, peak sensitivity, and baseline noise can provide valuable clues to identifying and solving future problems.

Chemical Toxicity

Although the large volume of toxic and flammable solvents used and stored in laboratories can be quite dangerous, do not ignore the potential hazards posed by your samples. Take special care to read and follow all precautions that ensure proper ventilation, storage, handling, and disposal of both solvents and samples. Become familiar with the toxicity data and potential hazards associated with all chemicals by referring to the manufacturers' MSDSs.

Solvent Requirements

Use LC/MS-grade solvents that are free of particulates. Choose a mobile phase that is compatible with the sample and column you have selected for your separation. Be aware that some solvents are corrosive to stainless steel.

CAUTION Do not use solvents containing Freon™ and perfluorinated solvents, such as Fluorinert™ and Fomblin™ perfluoro polyether solvents. They adversely affect the Teflon™ AF degassing membrane.

Solvent Disposal

Make sure you have a solvent waste container or other kind of drain system available at or below the bench top level. Most solvents have special disposal requirements prohibiting disposal directly down a drain. Follow all governmental regulations when disposing of any chemical.

High-Pressure Systems and Leaks

LC systems operate at high pressures. There is little immediate danger from the high pressures in an LC system. However, if a leak occurs, correct it as soon as possible. Always wear eye and skin protection when operating or maintaining an LC system. Always shut down the system and return it to atmospheric pressure before attempting any maintenance.

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 MD instrument at your laboratory. Refer to the *Prelude MD Preinstallation Requirements Guide*, which is located on the Prelude MD DVD.

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 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

Introduction

This topic describes the operating principles of the Prelude MD instrument.

Contents

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

Table 2. Prelude instrument features

Feature	Description
TurboFlow technology	The Prelude MD instrument runs TurboFlow methods, which separate sample components from complex sample matrices prior to separation on an HPLC column.
Cross-sequential optimization	The Prelude MD instrument contains two LC channels that can run simultaneously using one detector, maximizing efficiency.
UHPLC (ultra-high-performance liquid chromatography)	The pumps, valves, and connections contain components optimized to operate at high pressures up to 1000 bar. This allows you to run UHPLC methods, which provide optimal separation and speed.
Method flexibility	The flexibility of the Prelude MD instrument allows you to develop your own methods to run new, unique tests on the instrument.

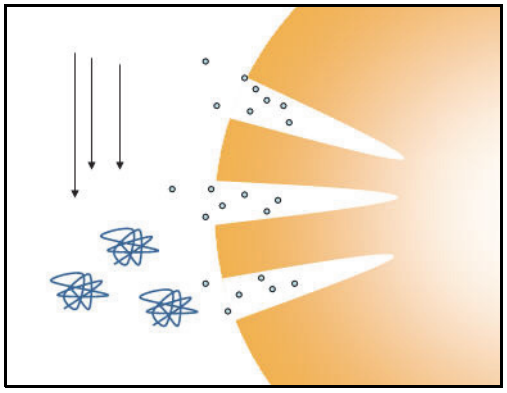
TurboFlow Technology

Using TurboFlow technology, the Prelude MD instrument separates low molecular weight compounds from high molecular weight matrix components in a fraction of the time that it takes manual extraction procedures, such as protein precipitation, liquid-liquid extraction, and solid phase extraction.

The Prelude MD instrument injects the sample directly onto a TurboFlow column. When fluids pass through the narrow TurboFlow column at the recommended flow rates, high linear velocities result.

The TurboFlow particles contain small pores into which sample molecules can enter through diffusion. Small molecules have a faster diffusion rate than large molecules. As the mobile phase flows through the column, the small sample compounds diffuse into the particle pores. The flow of the mobile phase quickly transports the large sample compounds through the column before they have an opportunity to diffuse into the particle pores. Therefore, the differences in diffusion rates among small and large molecular weight molecules initially separate the small molecular weight compounds from the sample matrix. See Figure 1.

Figure 1. Small compounds diffuse into pores. Large compounds flow to waste.



Of the molecules that enter the pores, only those that have an affinity to the chemistry inside the pores bind to the column particles' internal surface. The small molecules that have a lower binding affinity quickly diffuse out of the pores and pass to waste.

A mobile phase change then elutes the small molecules that were bound by the TurboFlow column to the detector or to an analytical column for further separation.

The Prelude MD instrument design and software have been optimized for use with TurboFlow methods, allowing the required flow rates, pump pressures, unique plumbing configuration, and valve changes.

Thermo Fisher Scientific provides TurboFlow columns with a variety of column chemistries to accommodate different analyte types. They can withstand repeated, direct injection of complex samples, such as biological fluids and reaction mixtures, which are often associated with combinatorial chemistry, drug metabolism studies, and clinical diagnostic assays. When TurboFlow columns run on the Prelude MD instrument, which is optimized for their use, fast, efficient separations of complex sample matrices and compounds of interest are achieved.

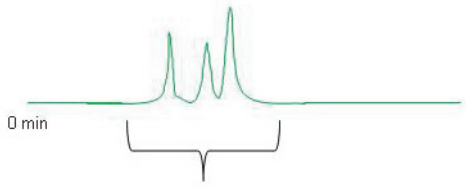
Cross-sequential Optimization

Cross-sequential optimization on the Prelude MD instrument refers to running two LC channels simultaneously with staggered sample starts, which brings the productivity of two separate, parallel LC systems 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.

Using cross-sequential optimization, you can run two different sequences at one time, using the same or different methods.

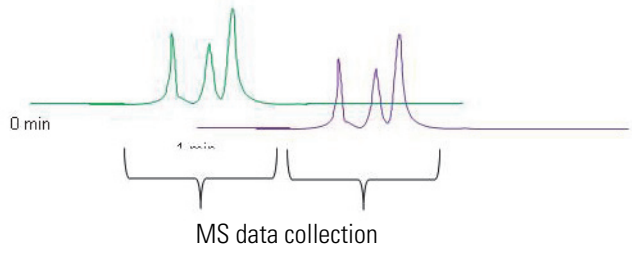
During a method in a single LC system, the detector analyzes samples for only a fraction of the total method time. Figure 2 illustrates the data collection time within the method of a single-channel system.

Figure 2. Single LC/MS system



With the Prelude MD instrument, cross-sequential channels connect to a single detector. Each channel operates independently, permitting two methods to run simultaneously. The Prelude MD instrument generates the throughput of two traditional LC/MS systems, while maximizing the productivity of one detector. Figure 3 illustrates the data collection times when two channels run simultaneously, but with staggered starts so that the data collections do not occur at the same time.

Figure 3. Two LC channels synchronized to a single detector



Samples continue to run, even if one of the channels becomes inoperable.

UHPLC Technology

The Prelude MD instrument can use UHPLC columns, which have smaller diameter tubes and particles than columns used in traditional HPLC and provide ultra-high performance.

3

Instrument Components

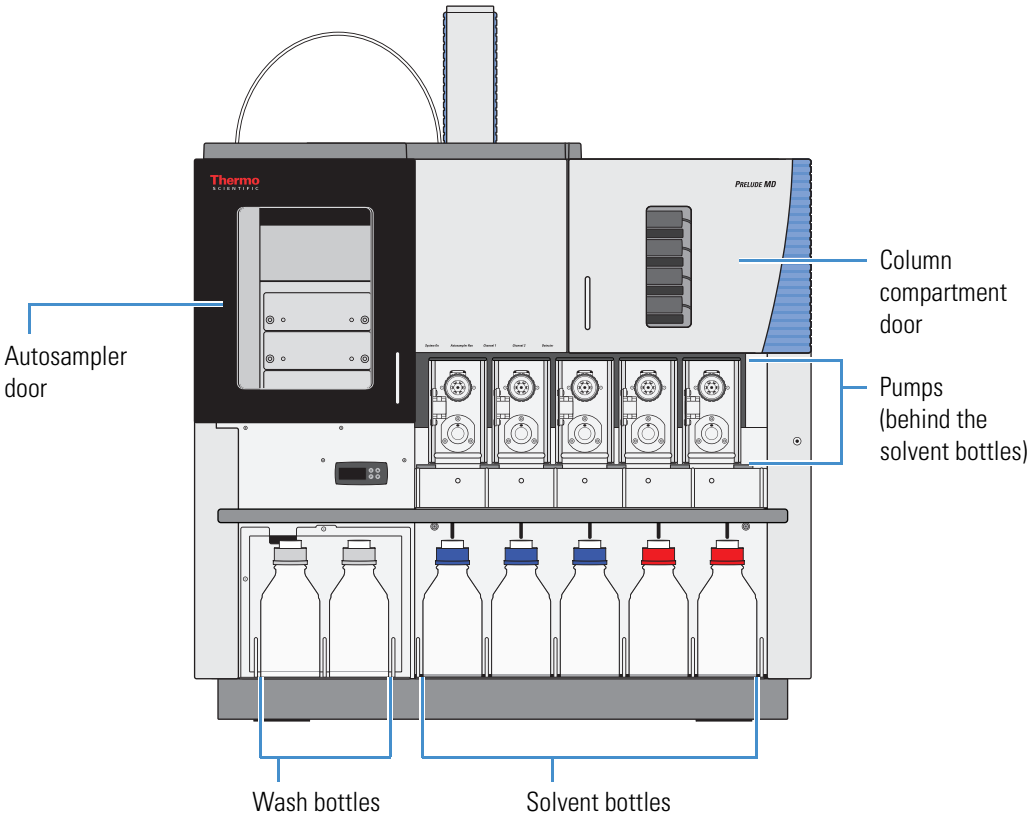
These topics describe the instrument components, including the autosampler, pumps, solvent bottles, and columns.

Contents

Prelude MD System Components

Figure 4 shows the Prelude MD system and its components.

Figure 4. Prelude MD instrument and components



Autosampler

The autosampler automates sample handling tasks, such as getting the sample, injecting the sample, and cleaning the syringe and injectors valves. The following topics describe the components that make up the autosampler.

- Sample Probe
- Cool Stack
- Wash Stations
- Wash Bottles
- Injector Valves

Sample Probe

The sample probe contains a 100 µL syringe for withdrawing sample from the sample vial and dispensing it into the sample injector. The sample syringe can withdraw 1–100 µL of sample volume. Use the recommended sample volume of 10–80 µL. The sample displaces the contents of a loop of tubing called the DLW holding loop so that the sample never enters the syringe.

Cool Stack

The sample cool stack contains three drawers. Each drawer can hold up to two sample trays. The cool stack maintains a temperature between 4 and 40 degrees Celsius. Table 3 lists the 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
96-deep-well plates	96 wells per plate, 2 plates per drawer

Wash Stations

The Prelude instrument uses an advanced syringe wash mechanism called dynamic load wash (DLW). This wash cleans the syringe barrel and syringe components without using the syringe plunger to aspirate liquid, reducing the amount of wear on the syringe and syringe motor. It also 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 biological samples.

Wash Bottles

Two wash bottles store the wash solutions that the instrument uses to wash the sample probe and injector ports. A solvent filter that is attached to the solvent line filters out particulates.

Injector Valves

The Prelude instrument uses four injector valves to inject sample onto the instrument. Each LC channel has two injector valves: one for loading sample onto the TurboFlow column, and one for loading sample onto the HPLC column.

The syringe injects the sample onto 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 TurboFlow or HPLC column. You can select injector valve wash steps to perform before and after the sample injection.

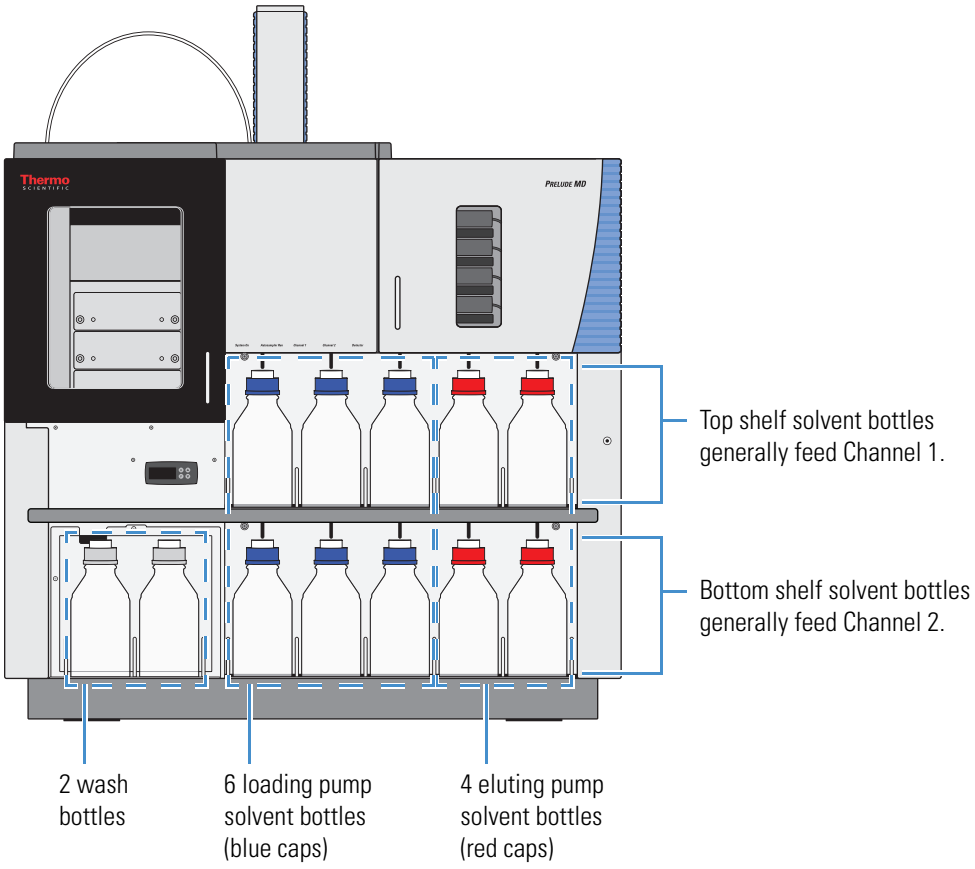
Solvent Bottles

Twelve bottles reside on the instrument. Ten of these bottles contain solvents that transport sample compounds through the columns and instrument. A solvent filter that is attached to the solvent line filters out particulates. A dedicated pump for each solvent draws from the solvent bottle.

Each LC channel contains five solvent bottles. Three bottles feed the three loading pumps, and two bottles feed the two eluting pumps. Generally, the top five bottles feed Channel 1, and the bottom five bottles feed Channel 2. However, to provide flexibility, you can change this arrangement in the method or during priming.

Two bottles contain wash solutions for washing the autosampler probe and injector valves. One bottle generally holds an aqueous solution, and one bottle generally holds a wash solution. Figure 5 shows the solvent bottle locations on the Prelude MD instrument.

Figure 5. Prelude MD instrument showing solvent and wash bottles

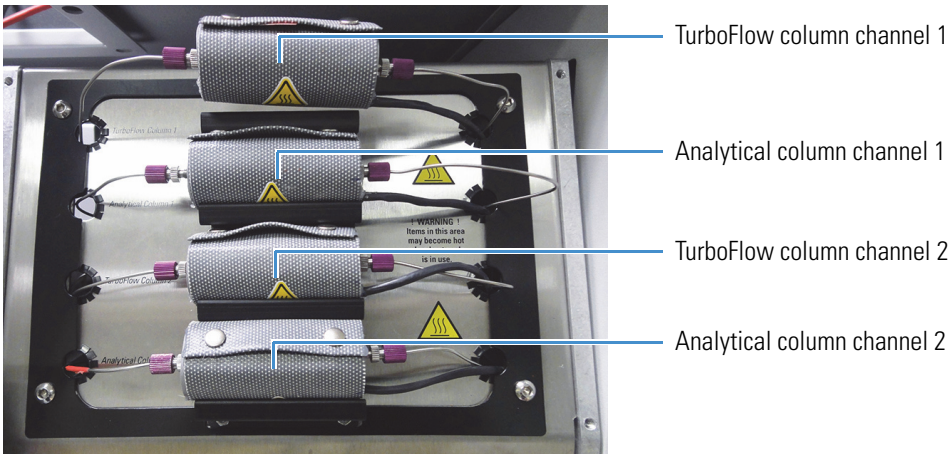


Columns

The instrument contains one TurboFlow and one HPLC column per channel for separating components in a liquid using TurboFlow technology and HPLC or UHPLC technology. The columns are available in a variety of chemistries 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

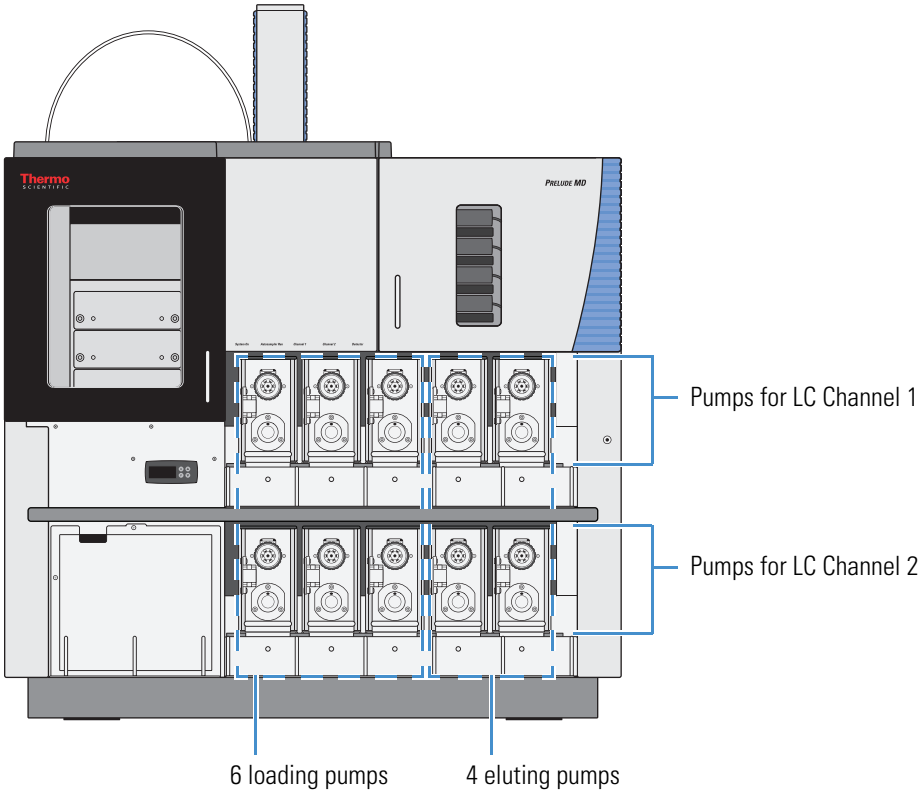


Pumps

The Prelude instrument contains ten pumps that force the solvents through the TurboFlow and HPLC columns, and then to waste or to the detector. The pumps can reach pressures up to 1000 bar. This high pressure, combined with the instrument and column design, provides the instrument environment in which UHPLC is achieved. Each pump operates 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 instrument contains two LC channels that operate independently. Each LC channel uses five pumps: three loading pumps and two eluting pumps. See Figure 7.

Figure 7. Prelude instrument showing pump locations behind the solvent bottles



The fluids from the three loading pumps blend together in each channel after they are dispensed by the pump syringes and before reaching the injector. These three pumps form the flow of mobile phases that load sample compounds onto the TurboFlow column. The solvent bottles that feed these pumps have blue caps. See Figure 5 on page 10. With this arrangement, you can use a combination of one, two, or three solvents to load the sample onto the TurboFlow column. The method determines the selection of solvents and their ratios. The loading pumps also fill the transfer loop, which assists with the transfer of the compounds to the HPLC column.

The two eluting pumps combine their fluids to form the mobile phase flow that elutes sample compounds from the HPLC column. The solvent bottles that feed these pumps have red caps. See Figure 5 on page 10. The method determines the selection of solvents used and their ratios. With this pump arrangement, you can use one or two solvents using complex gradients to change mobile phases during a method.

Prelude MD Control Software

The Prelude MD software controls the LC system pumps, valves, and autosampler. It works within your detector's control application. You work with the software when you view the status of the pumps, pump pressure, valves, and autosampler, and when you create the LC and autosampler method steps.

If you are developing a method, you use the Prelude MD software to create the LC and autosampler method steps. These become part of the instrument method that you create using the detector system.

While the Prelude MD software controls the LC system pumps, valves, autosampler, and detector data window, the detector control application manages the detector data acquisition parameters, sample scheduling, and data collection and processing. For information on your detector's control application, refer to the appropriate documentation or the control application Help.

4

Performance Characteristics

The Prelude MD LC instrument performs online sample cleanup and UHPLC. It separates sample components prior to analysis on a detector. See Figure 8.

Figure 9 shows the front and side views of the instrument, and Figure 10 shows the instrument's placement on a worktable.

Specifications for the Prelude MD instrument follow:

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

Figure 8. Prelude MD instrument

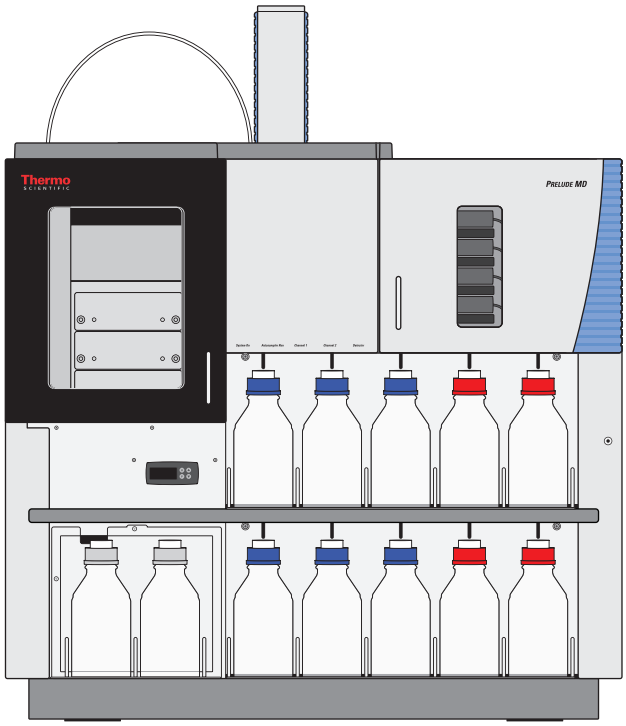
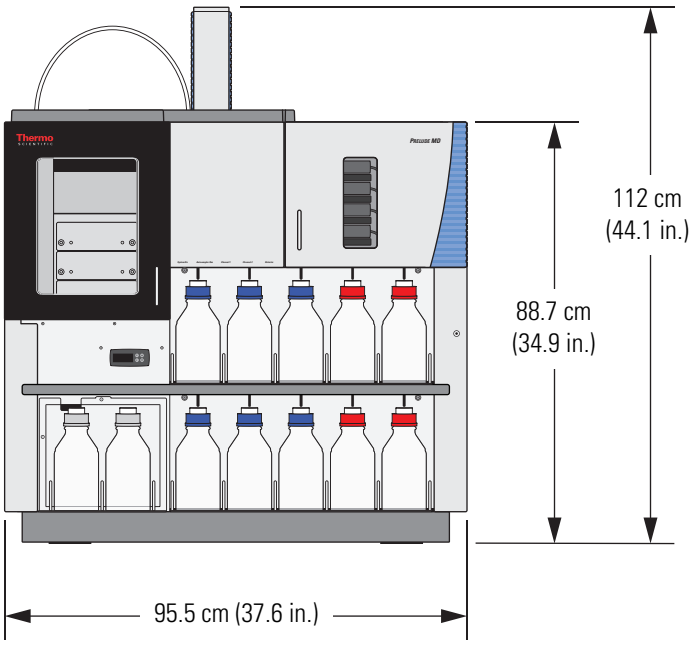


Figure 9. Prelude MD instrument dimensions

Front view



Left-side view

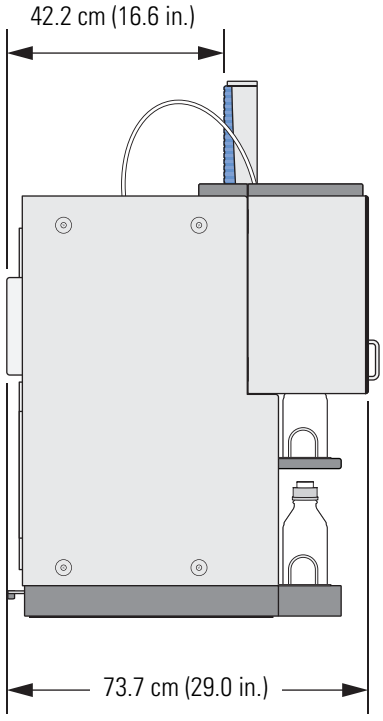


Figure 10. Front, side, and top views with table

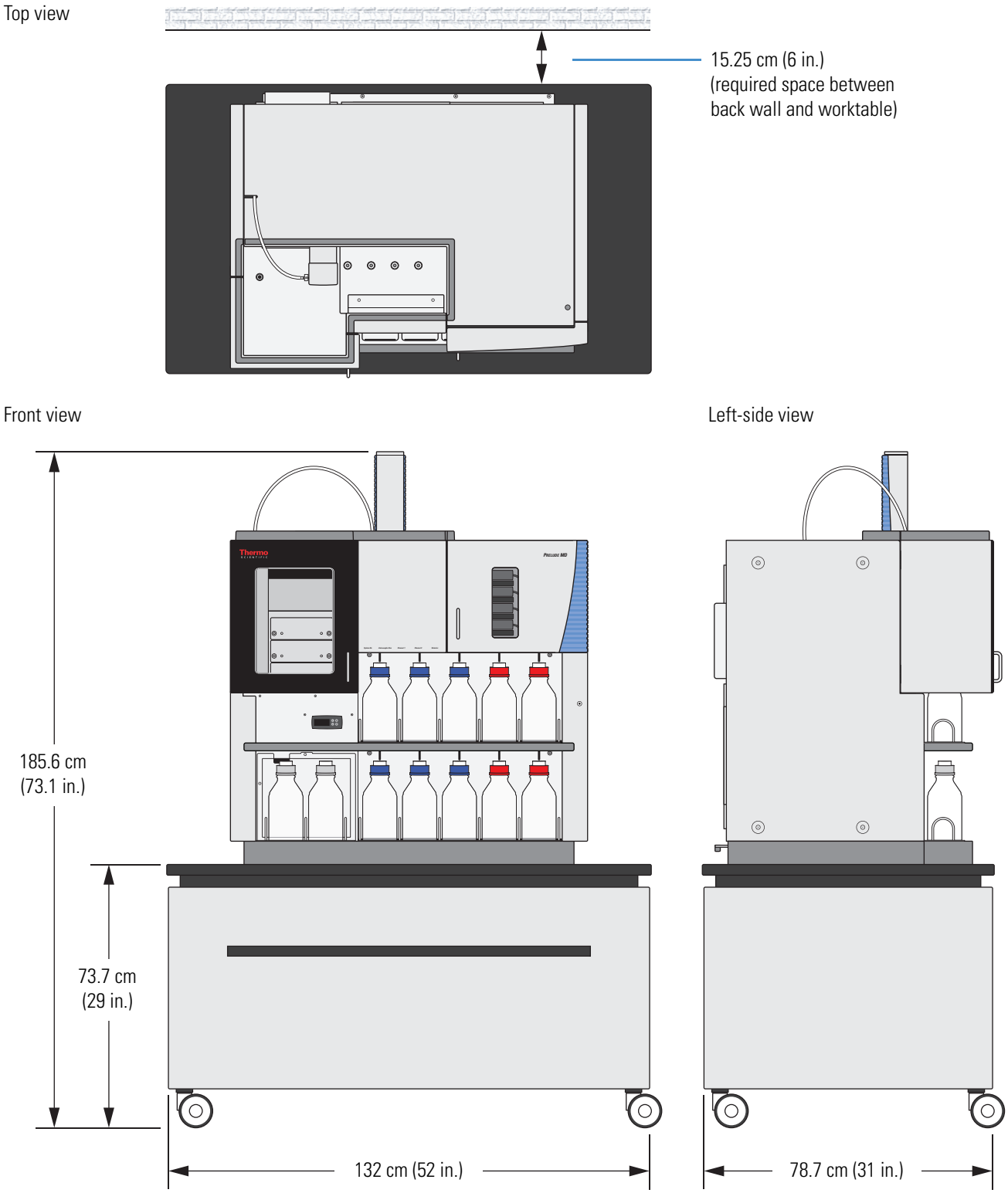


Table 4. Prelude MD instrument and table dimensions

Component	Dimensions
Instrument (without table)	95.5 × 112 × 73.7 cm (*w* × *h* × *d*) (37.6 × 44.1 × 29.0 in.) (*w* × *h* × *d*) See Figure 9 on page 16.
Table	132 × 73.7 × 78.7 cm (*w* × *h* × *d*) (52 × 29.0 × 31.0 in.) (*w* × *h* × *d*) Required space between back wall and table = 15.25 cm (6 in.)
Instrument and table	132 × 185.6 × 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
Temperature	18–27 °C (64.4–80.6 °F)
Maximum relative humidity	40–80% noncondensing

Table 6. Pump operating specifications

Specification	Description
Maximum operating pressure	1000 bar (15 500 psi)
Minimum programmable flow rate	1 µL/min
Flow rate range	1–10 000 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	110–250 Vac, ±20%, 50–60 Hz Voltage fluctuation not to exceed ±10% of the nominal voltage

5

Operating Procedures

These topics describe a workflow and procedures for running samples on the Prelude MD instrument.

Contents

Getting Started

The procedures assume the following:

- You have read and are familiar with the operating hazards for this instrument. See Chapter 13, “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 acquisition software features an instrument setup tool that includes guidance for running methods on the Prelude MD instrument. To create a new method, begin by defining the data acquisition parameters for the detector in the instrument setup application. Then add autosampler and LC instrument method steps by using the Prelude MD control application. See Chapter 10, “Creating an LC Method,” Chapter 9, “Creating an Autosampler Method,” and Chapter 11, “Developing a TurboFlow Method.”

Preparing the Instrument Before Each Run

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

Installing Columns

If your system does not contain a TurboFlow or HPLC column or a column has expired, see "Replacing the TurboFlow Column" on page 189 or "Replacing the Analytical Column" on page 191.

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 distinguish it from the analyte. You can use the IS to monitor or account for sample loss and variations during the TurboFlow and 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 "Changing the Solvent Bottles" on page 186, and "Preparing the Aqueous Mobile Phases" on page 184.

Prime the instrument lines if you installed new mobile phases or the instrument has been idle for more than 12 hours. See "Priming the LC Pumps" on page 167.

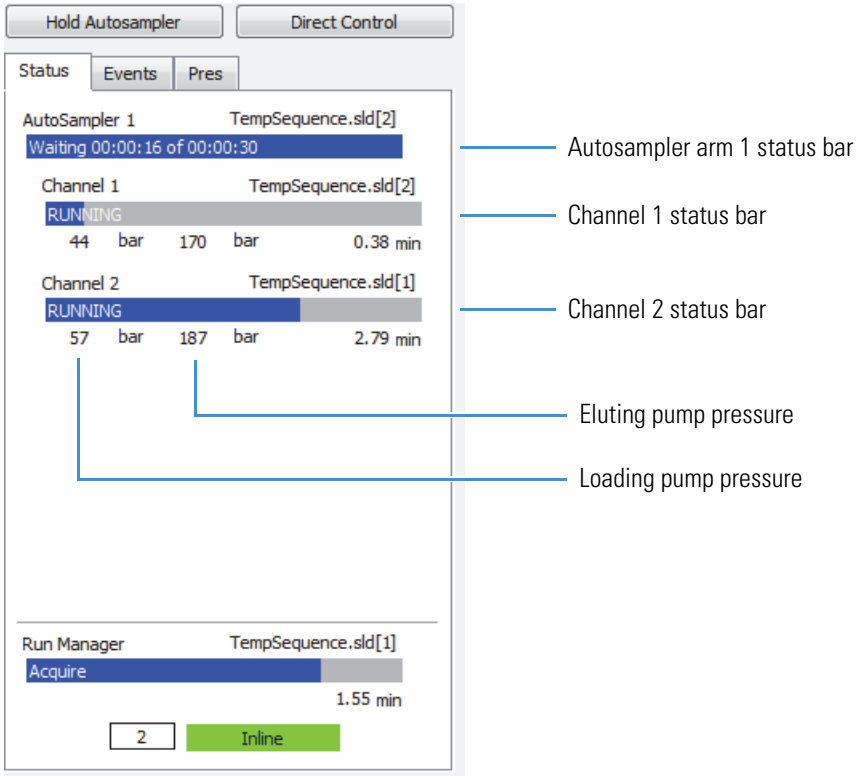
Preparing the Channels

Perform the channel preparation procedure 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

1. Open the status window from the detector control application. See Figure 11.

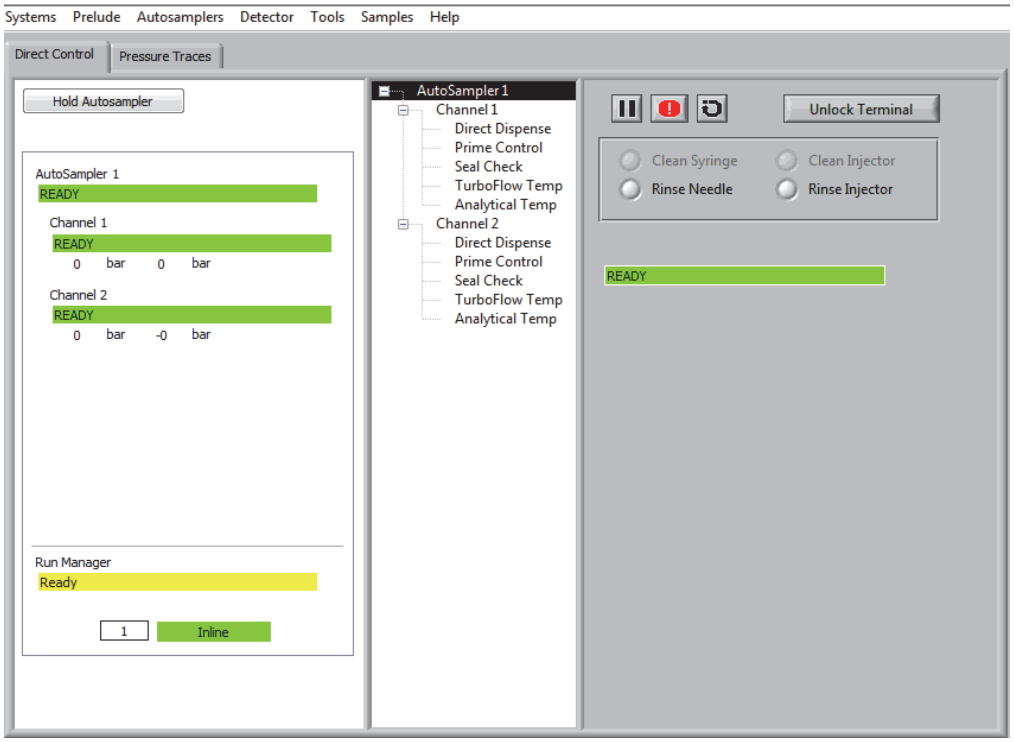
Figure 11. Prelude MD Status page



2. Click **Direct Control**.

   The Direct Control window opens. See Figure 12.

Figure 12. Direct Control window



3. Do one of the following:

   - (Option 1) Prepare all of the LC channels at one time by choosing **Prelude > Prepare All**.

     The systems begins priming all of the LC channels. When the priming operation has finished, a seal check starts.

   - (Option 2) Prepare an individual LC channel by choosing **Prelude > Channel 1** (or **Channel 2) > Prepare**.

     The system begins priming the selected channel. When the priming operation has finished, a seal check starts.

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.

Preheat any configured column heaters once the precondition is set.

Note The LC flow begins after the method sequence starts.

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 "Accessing the Prelude MD Status Window" on page 25.
- Pause the autosampler. See "Controlling the Autosampler" on page 55.
- View the pressure trace. See "Monitoring the Pump Pressure" on page 30.

6

Monitoring the Pumps and Autosampler

These topics describe how to check the pump and probe status, and monitor the pump pressure.

Contents

Accessing the Prelude MD Status Window

❖ **To access the status window**

In the detector control application status window, click **Prelude MD**.

The Prelude MD status page appears. See Figure 13.

Figure 13. Prelude MD Status page, sample processing

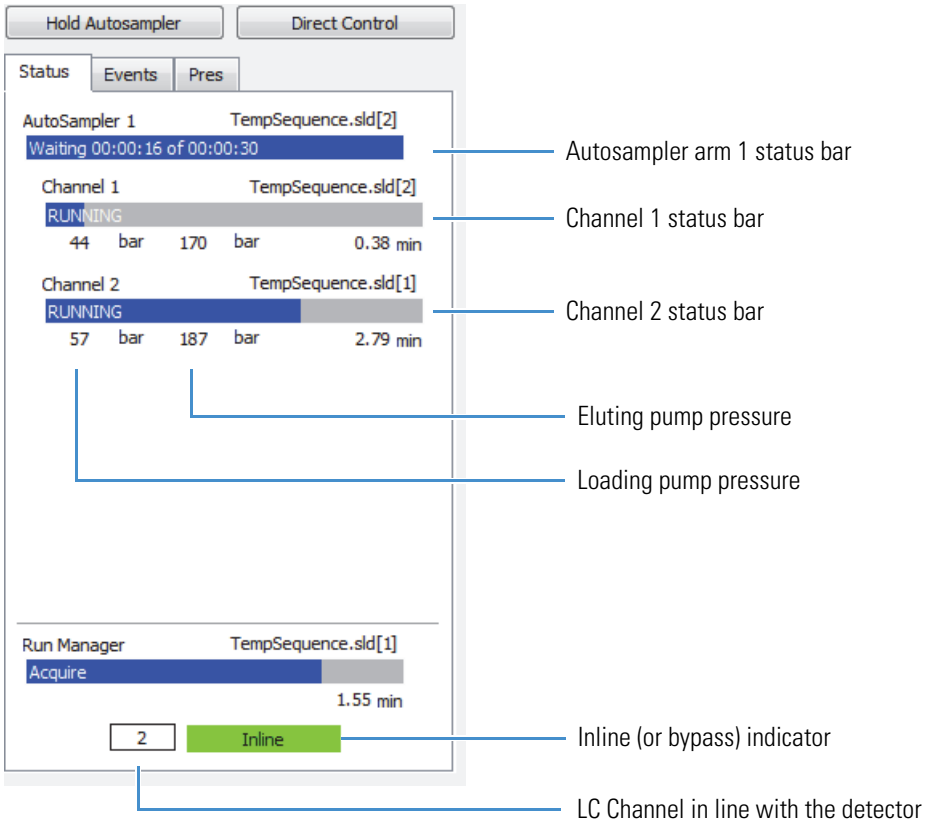


Table 8 describes the Status page.

Table 8. Channel status page information (heater and pump)

Item	Description
Status bars for autosampler and channels	The bar color indicates the pump, heater, or probe status. A status message appears in the bar to provide specific information on the pump, heater, or probe condition. For a definition of the status colors, see Table 9. Right-click a channel status bar to access a list of operating commands.
Pump pressure	Displays the current loading and eluting pump pressure.

Table 9 describes the colors corresponding to the pump's status.

Table 9. Channel status messages

Status	Color	Description
Offline	Gray	The application cannot establish a communication link with one or more pumps or heaters.
Not Ready	Yellow	Communication between the application and the channel has been established, but the pumps or heaters are not ready to begin. The status changes to Ready (green) when the heaters are on and the pumps are ready to run.
Ready	Green	The channel is ready to run methods.
Running	Blue	The channel is currently running a method.
Error	Red	A component is in an error state.
Disabled	Muted	Channel is unavailable for use.

Accessing the Direct Control Window

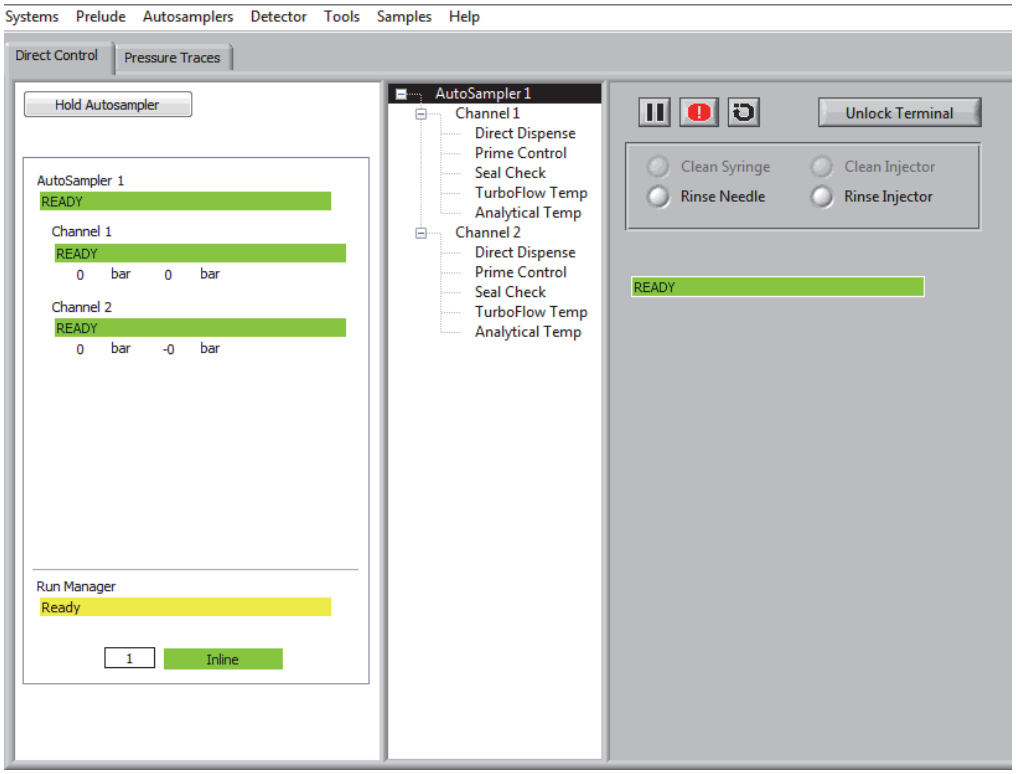
❖ **To access the Direct Control window**

1. Open the system application status window.
2. Click **Prelude MD**.

   The Status information appears (Figure 13).
3. Click **Direct Control**.

   The Direct Control window opens (Figure 14).

Figure 14. Direct Control window

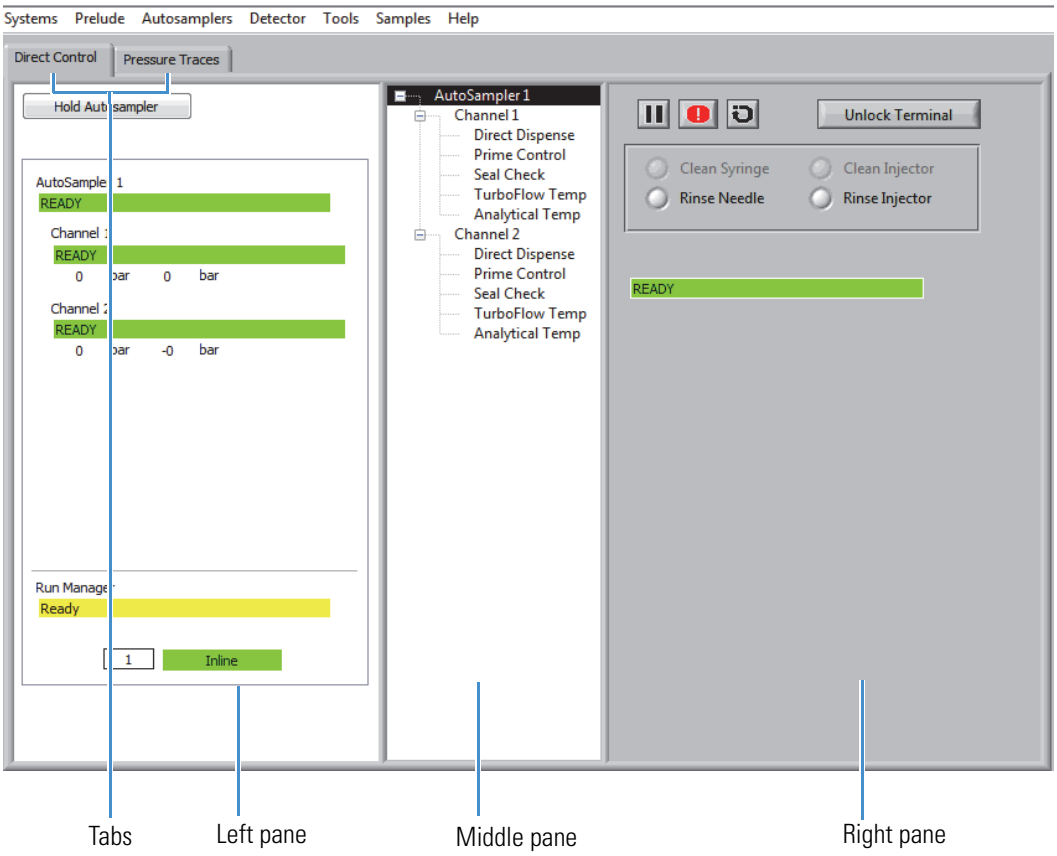


About Direct Control

Direct Control gives you a clear view of the system channels and complete control over the system components.

The Direct Control window is organized into multiple panes and two tabs as shown in Figure 15.

Figure 15. Direct Control window



The panes in the Direct Control window have the following functions:

- The left pane provides the system status.
- The middle pane provides direct control of the autosampler, pump, and heaters that are configured on the data system computer.
- The right pane provides access to additional options or status information depending on the item that you select in the middle pane.

Additionally, you can click the Pressure Traces tab at the top to view and monitor system pressure traces in real time.

Monitoring the Pump Pressure

This topic describes viewing the pump pressures on the Status page, viewing the pressure trace on the Prelude MD Pres page, viewing the pressure trace in the Direct Control window for current and previous samples, and using pressure profiles.

Follow these procedures:

- To view the pump backpressure
- To view the pressure trace for the current sample
- To view a larger image of the pressure trace

❖ To view the pump backpressure

Open the status window.

You can view the current pressure for the loading and eluting pumps on the Prelude MD Status page. See Figure 13 on page 26.

❖ To view the pressure trace for the current sample

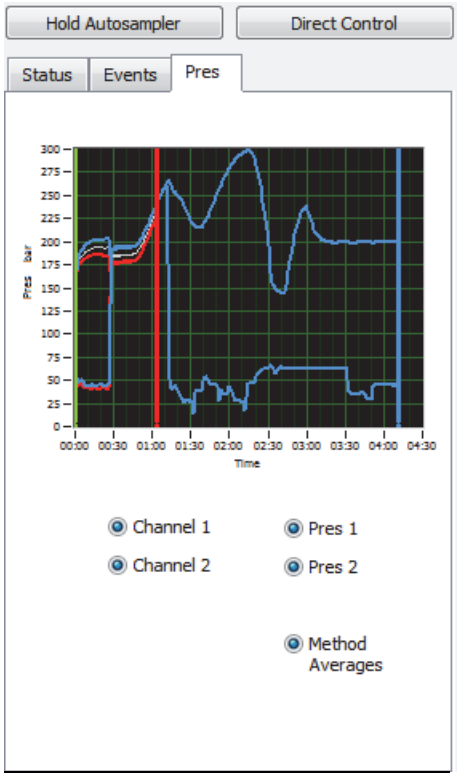
Note

- The backpressure of the LC pumps changes throughout the method as flow rates and mobile phase compositions change, and as the valves change positions. A plot of the backpressure for the loading pump over the duration of the method appears similar from sample to sample if the operating conditions remain the same. Similarly, a plot of the eluting pump over the duration of the method appears similar from sample to sample.
- A fluctuation or change in the pump pressure graph can indicate a change in your chromatography conditions. You can view the pressure trace for the current run from the Pres page of the status window.

1. Open the status window.
2. Click the **Pres** tab.

   The pressure trace for the currently running sample opens. See Figure 16.

Figure 16. Pressure trace on the Pres page



3. Select the channel that you want to view.
4. Select the pumps that you want to view.

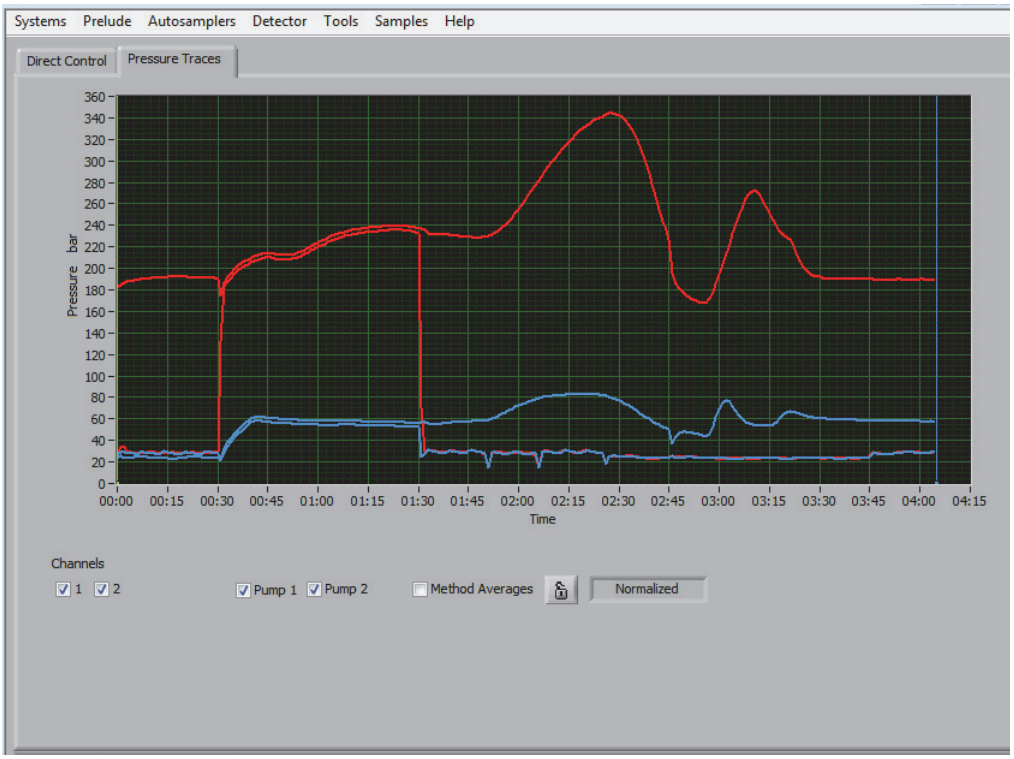
   The pressure trace opens for the selected pumps.

❖ To view a larger image of the pressure trace

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.
2. Click the **Pressure Traces** tab.

   The current pressure trace opens. See Figure 17.

Figure 17. Pressure trace in the Direct Control window



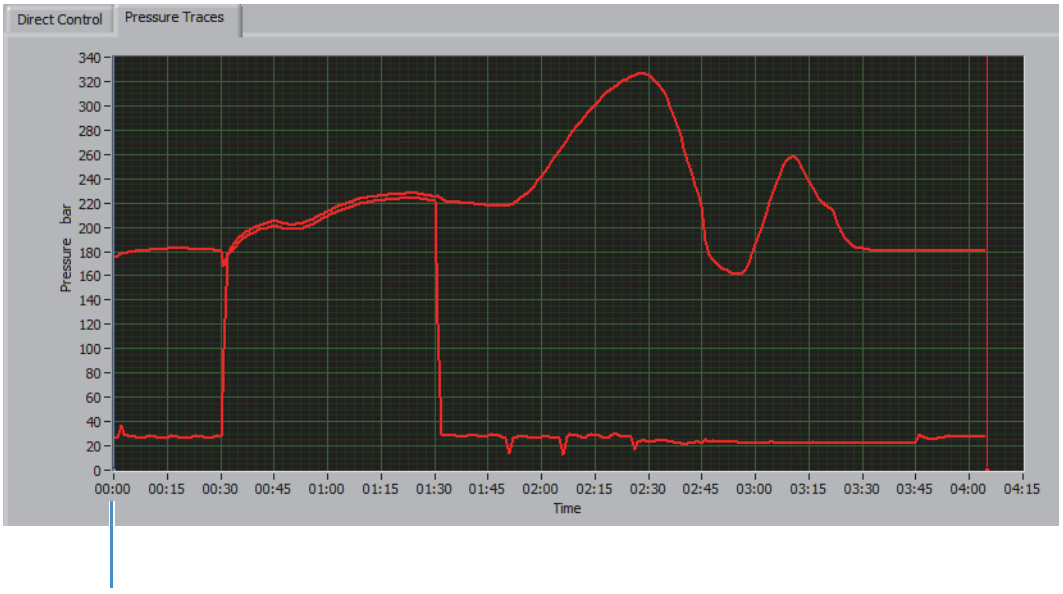
3. Select the channels that you want to view in the Channels area.
4. To view the pressure for the loading pump, select the **Pump 1** check box.
5. To view the pressure for the eluting pump, select the **Pump 2** check box.
6. To view an average trace of the selected channels, select the **Method Averages** check box.
7. Click **Normalized/Real Time** to switch between Normalized and Real-time views. See "Normalized Versus Real Time."
8. To access the pan and zoom features, select the **Lock** icon,  .

   The image no longer updates, and the zoom and pan icons appear. See "Using the Zoom and Pan Tools on the Pressure Trace" on page 36.

Normalized Versus Real Time

If the Normalized button appears, the graph displays 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 18 shows the normalized view of the loading and eluting pumps' backpressures throughout a run on a single channel.

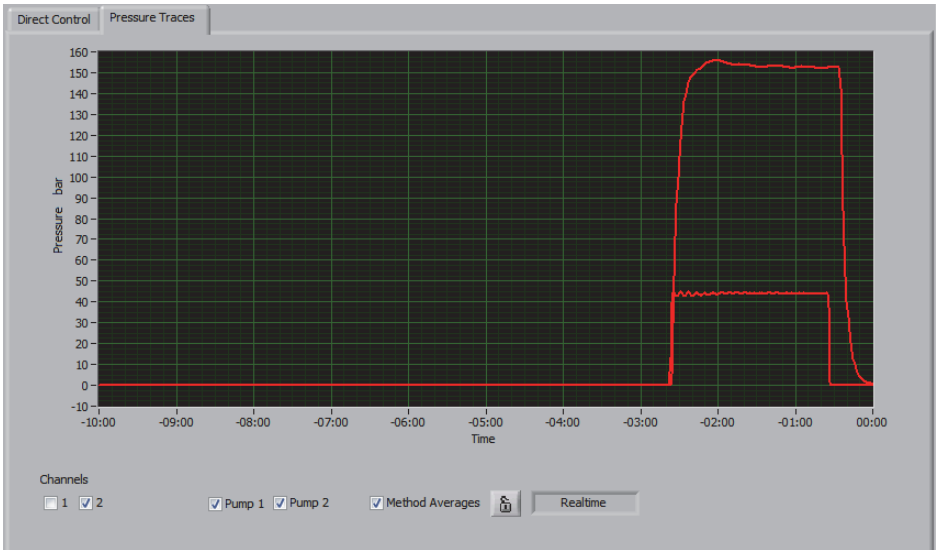
Figure 18. Graphs page showing normalized view of the loading and eluting pumps' backpressures for LC system 2



Method clock is zero for the method start for all pumps that appear on the graph.

If the Real Time button appears, the graph displays the selected pump pressures in the current time and up to 10 minutes of elapsed time, with 0.00 representing the current time. Figure 19 shows the real-time view of the eluting and loading pumps' backpressures for the previous minutes of the run.

Figure 19. Graphs page showing the real-time view of the loading and eluting pumps' backpressures for LC system2



Follow these procedures:

❖ To use the zoom and panning tools on the pressure trace

1. Click the **Lock** icon,  (Figure 19).

   The icon appears locked () and the display stops updating.

2. Continue with "Using the Zoom and Pan Tools on the Pressure Trace" on page 36.

❖ To view the pressure trace for completed samples

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.

2. Choose **Tools > Sequence Log Viewer**.

   The Sequence Log Viewer window opens.

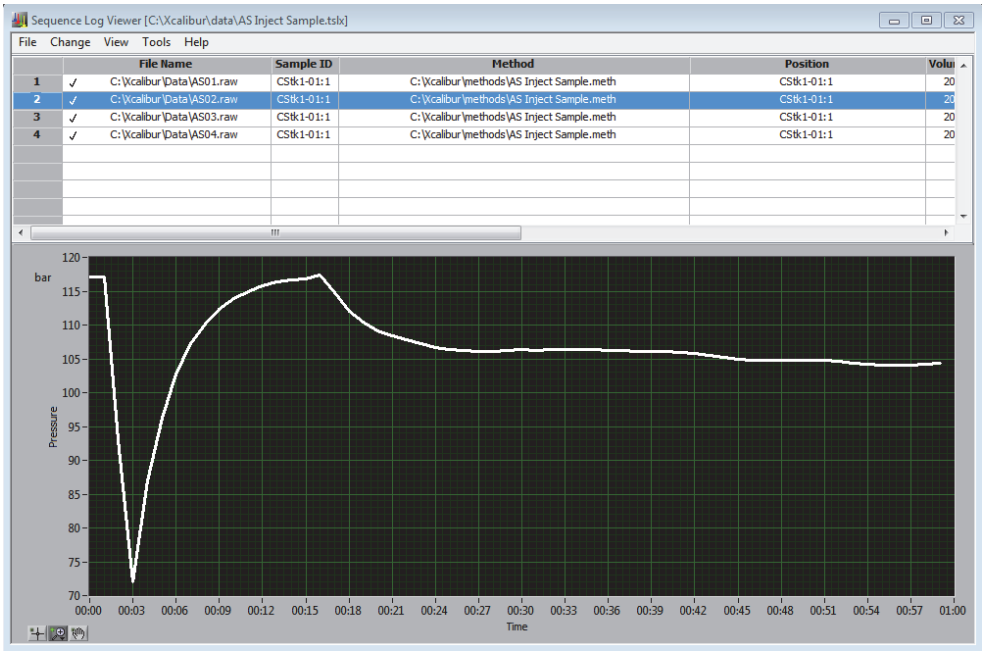
3. Choose **File > Open**, and navigate to the sequence file that you want to view.

   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 (Figure 20).

Figure 20. Pressure graph view



5. Select a sample.

   The sample pressure trace opens.

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.

7. To use the pan or zoom features, see "Using the Zoom and Pan Tools on the Pressure Trace."

❖ To assign pressure profiles to monitor pressure

See "Assigning a Pressure Profile" on page 127.

You can establish a pressure profile for each method, and use the pressure profile to automatically monitor the system for unexpected pump pressure readings. The 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.

Using the Zoom and Pan Tools on the Pressure Trace

❖ To use the zoom and pan tools

1. Do one of the following:
   - If you are viewing the pressure trace in the Direct Control window, click the **Lock** icon,  . The icon appears locked, the display stops updating, and the zoom and panning icons appear. Continue with step 2.
   - If you are viewing the pressure trace in the sequence file viewer, continue with step 2. See Figure 20.
2. To zoom the pressure trace, do the following:
   a. Click the **Zoom** icon,  .
   b. Select the appropriate tool, and complete the procedure as described in Table 10.

Table 10. Zoom tools (Sheet 1 of 2)

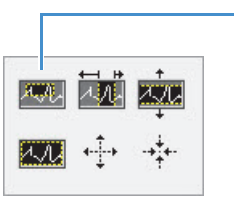
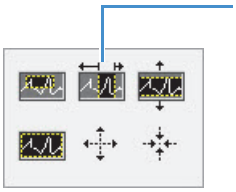
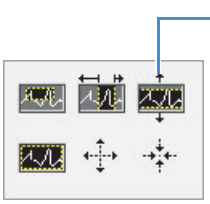
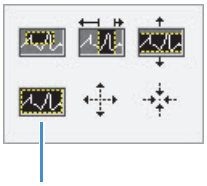
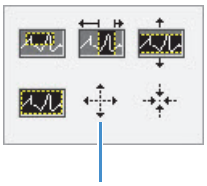
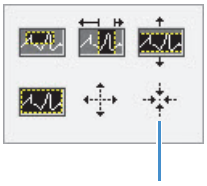
Tool	Description
	Select this tool to change the *x*- and *y*-axis scales. Then do the following: 1. Select the area on the graph that shows the lower end of the *x*- and *y*-axes that you want to view and hold down the mouse button. 2. Drag the cursor to the higher end of the *x* and *y* axes that you 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.

Table 10. Zoom tools (Sheet 2 of 2)

Tool	Description
	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 to adjust the *x* and *y* axes to fit the data into the window.
	Select this tool 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.
	Select this tool 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**

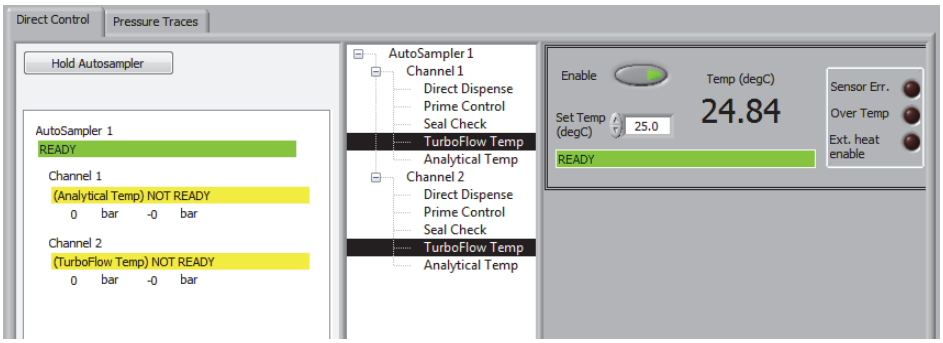
1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.

   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 21 shows the Direct Control window displaying temperature options, and Table 11 describes these options.

Figure 21. Temperature options in the Direct Control window



Heater names in this image are TurboFlow Temp and Analytical Temp for both Channel 1 and Channel 2. The heaters can be configured using different names, so the heater name on your system might be different.

Table 11. Temperature options (Sheet 1 of 2)

Option	Description
Enable	Light green indicates the heater is on. Dark green indicates that the heater is off.
Set Temp	The assigned temperature in Celsius.
Temp (degrees Celsius)	The actual temperature reading in Celsius as indicated by the heater feedback.

Table 11. Temperature options (Sheet 2 of 2)

Option	Description
Sensor Err.	Bright red indicates communication from the heater to the controller has failed. Call Technical Support. See "Contacting Us" on page xviii. Dark red indicates that no sensor error state was detected by the controller.
Over Temp	Bright red indicates an error condition exists. Call Technical Support. See "Contacting Us" on page xviii. If this button appears dark, then no error state has been detected.
Ext. heat enabled	Bright red indicates the heater can be enabled or disabled using the controller, which is not the preferred condition. Call Technical Support. See "Contacting Us" on page xviii. Dark red indicates the heater can be enabled/disabled only in the Direct Control window (preferred).
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 application continuously updates the event log and logs 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

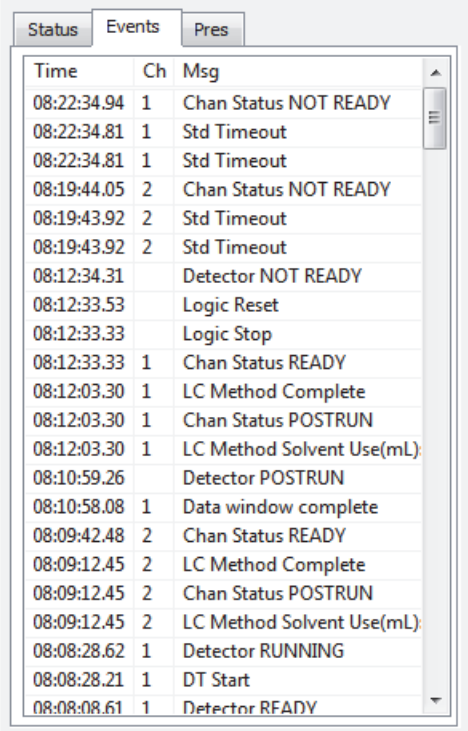
Follow these procedures:

- To view the event log
- To view past event logs
- To view past events by sample

❖ To view the event log

1. Open the status window.
2. Click the **Events** tab.

   The Event Log page opens.



3. To view information on a specific event, point to the event and wait one second.

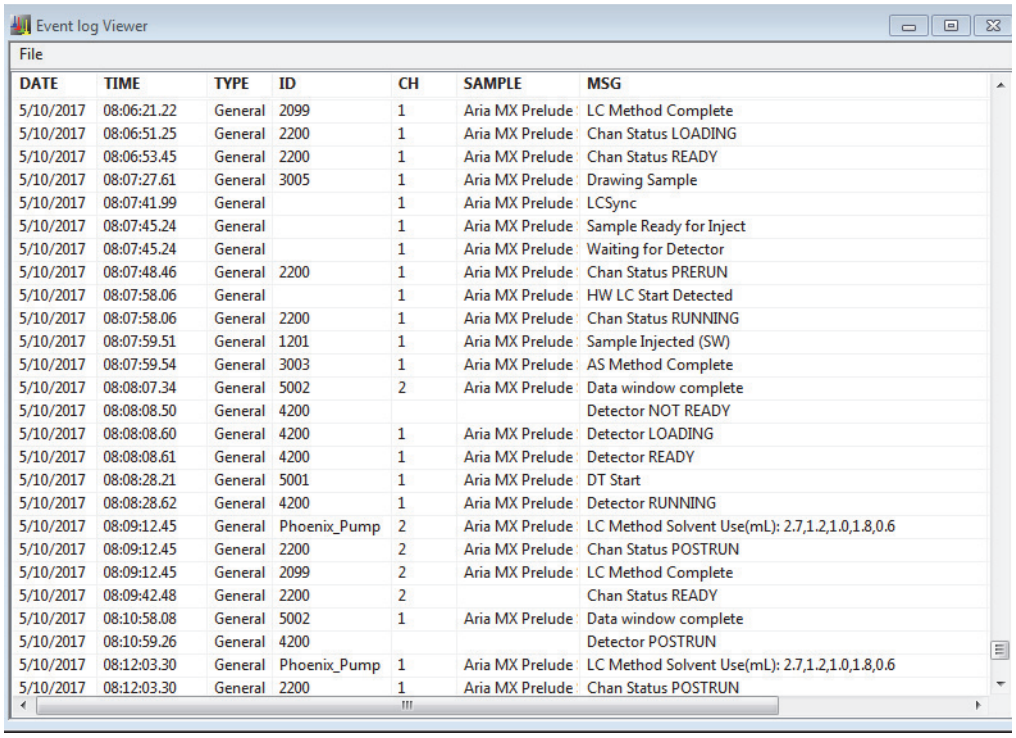
   The event information opens.

❖ To view past event logs

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.
2. Choose **Tools > Event Log Viewer.**

   The Event Log Viewer opens showing the current event log. See Figure 22.

Figure 22. Event Log Viewer window



DATE	TIME	TYPE	ID	CH	SAMPLE	MSG
5/10/2017	08:06:21.22	General	2099	1	Aria MX Prelude	LC Method Complete
5/10/2017	08:06:51.25	General	2200	1	Aria MX Prelude	Chan Status LOADING
5/10/2017	08:06:53.45	General	2200	1	Aria MX Prelude	Chan Status READY
5/10/2017	08:07:27.61	General	3005	1	Aria MX Prelude	Drawing Sample
5/10/2017	08:07:41.99	General		1	Aria MX Prelude	LCSync
5/10/2017	08:07:45.24	General		1	Aria MX Prelude	Sample Ready for Inject
5/10/2017	08:07:45.24	General		1	Aria MX Prelude	Waiting for Detector
5/10/2017	08:07:48.46	General	2200	1	Aria MX Prelude	Chan Status PRERUN
5/10/2017	08:07:58.06	General		1	Aria MX Prelude	HW LC Start Detected
5/10/2017	08:07:58.06	General	2200	1	Aria MX Prelude	Chan Status RUNNING
5/10/2017	08:07:59.51	General	1201	1	Aria MX Prelude	Sample Injected (SW)
5/10/2017	08:07:59.54	General	3003	1	Aria MX Prelude	AS Method Complete
5/10/2017	08:08:07.34	General	5002	2	Aria MX Prelude	Data window complete
5/10/2017	08:08:08.50	General	4200			Detector NOT READY
5/10/2017	08:08:08.60	General	4200	1	Aria MX Prelude	Detector LOADING
5/10/2017	08:08:08.61	General	4200	1	Aria MX Prelude	Detector READY
5/10/2017	08:08:28.21	General	5001	1	Aria MX Prelude	DT Start
5/10/2017	08:08:28.62	General	4200	1	Aria MX Prelude	Detector RUNNING
5/10/2017	08:09:12.45	General	Phoenix_Pump	2	Aria MX Prelude	LC Method Solvent Use(mL): 2.7,1.2,1.0,1.8,0.6
5/10/2017	08:09:12.45	General	2200	2	Aria MX Prelude	Chan Status POSTRUN
5/10/2017	08:09:12.45	General	2099	2	Aria MX Prelude	LC Method Complete
5/10/2017	08:09:42.48	General	2200	2		Chan Status READY
5/10/2017	08:10:58.08	General	5002	1	Aria MX Prelude	Data window complete
5/10/2017	08:10:59.26	General	4200			Detector POSTRUN
5/10/2017	08:12:03.30	General	Phoenix_Pump	1	Aria MX Prelude	LC Method Solvent Use(mL): 2.7,1.2,1.0,1.8,0.6
5/10/2017	08:12:03.30	General	2200	1	Aria MX Prelude	Chan Status POSTRUN

The Event Log Viewer window displays current and past recorded LC, autosampler, user, and detector events that occurred during operation. When the Event Log Viewer window reaches its capacity limit, the 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**.
   b. Navigate to and select the event log that you want to open.
   c. Click **Open**.

Note The application assigns Aria.log as the name of the current event. As the application saves each new event log, it appends the file name with the date and time. The Aria log files reside in the following directory:

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 27.
2. Choose **Tools > Sequence Log Viewer**.

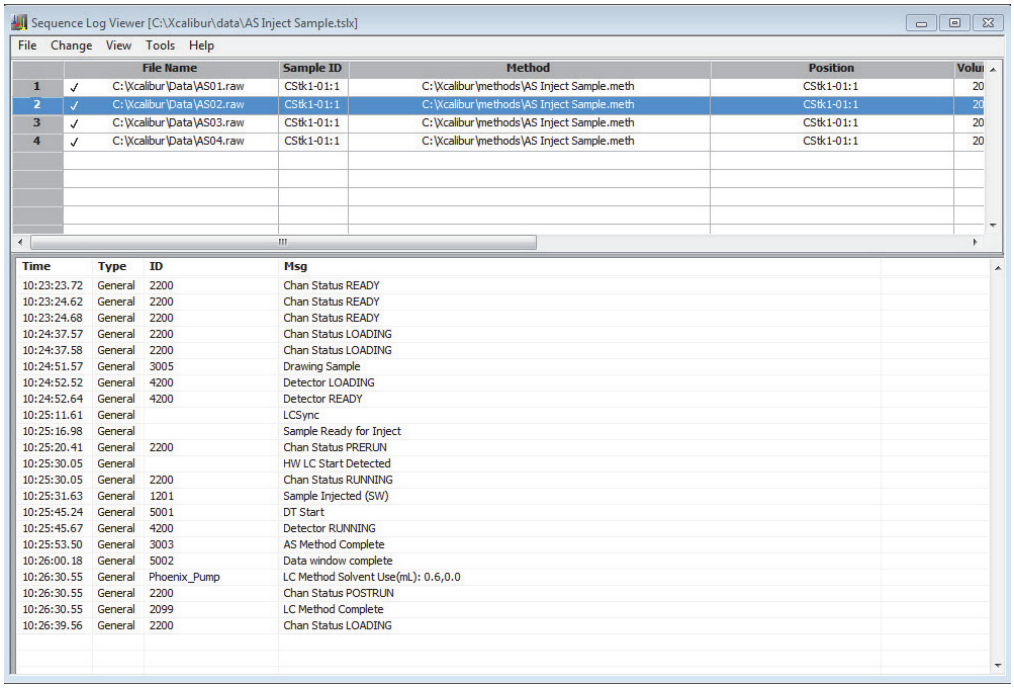
   The Sequence Log Viewer window opens.

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**.

   The sample events appear.



7

Controlling System Components

Use the procedures in this topic to control the pumps, valves, column heater, and autosampler directly using the Prelude MD application rather than through a method.

Note Method settings override direct control settings during a method run.

Contents

Controlling the Pumps

Follow these procedures.

- To enable or disable a channel
- To start or stop all LC pumps
- To dispense solvent through the system
- To prime the pumps

❖ To enable or disable a channel

1. Open the status window. See "Accessing the Prelude MD Status Window" on page 25.
2. Right-click the LC channel that you want to disable or enable.
3. From the shortcut menu, do one of the following:
   - Choose **Disable** to disable the selected LC channel from use during a sequence run.
   - Choose **Enable** to enable the selected LC channel for use during a sequence run.

Note The system does not use a disabled channel during a sequence run.

❖ To start or stop all LC pumps

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.
2. From the middle pane, select **Direct Dispense**, and from the right pane, click **Dispense** for each channel.

   The LC pumps begin pumping for the channel selected.
3. To turn off the pumps for all LC channels, choose **Pumps > All Off**.

❖ To dispense solvent through the system

Note Use this procedure to flow solvent from the solvent bottles to the pumps, valves, and columns, and then to waste.

1. Open the Direct Control window.
2. To dispense fluid from Channel 1 or Channel 2, select **Direct Dispense** in the middle pane.

   The Channel 1 and Channel 2 information opens to the right. See Figure 23.

3. (Optional) To change the valve positions, do either of the following:

Note

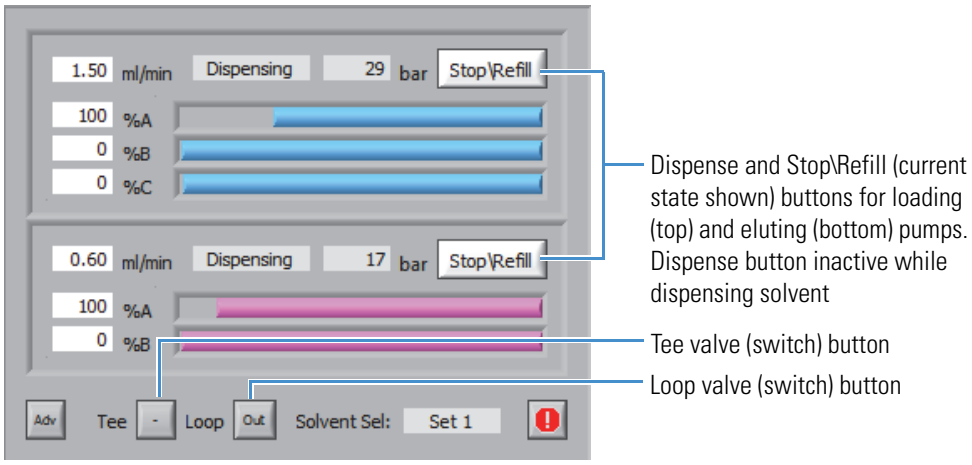
- Either selection affects both loading and eluting pump dispensing.
- You can change valve positions before or during dispensing.

- To change the position of the Tee valve, click the **Tee** button,  .

  The Tee valve switches its position and the Tee button changes its state to  .
- To change the position of the Loop valve, click the **Loop** button,  .

  The Loop valve switches its position and Loop button changes its state to  .

Figure 23. Direct Dispense area (one channel shown)



4. To dispense the loading solvents, do the following in the loading pumps area:
   a. Type the appropriate solvent percentage for each loading solvent in the %A, %B, and %C boxes beside the blue status bars.
   b. To change the flow rate from the set value, type the new flow rate in the ml/min box.

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

   c. Click **Dispense**.

      The pump status bar shows the pump syringe dispensing and filling, and the Dispense button changes its state to Stop\Refill (see Figure 23).

5. To dispense the eluting solvents, do the following in the eluting pumps area:
   a. Type the appropriate solvent percentage for each eluting solvent. The eluting solvents have a pink status bar.

b. To change the flow rate from the set value, type the new flow rate in the ml/min box.

IMPORTANT Do not exceed the recommended flow rate for the analytical column.

c. Click **Dispense**.

The pump status bar shows the pump syringe dispensing and filling, and the Dispense button changes its state to Stop\Refill (see Figure 23).

6. To stop all pump action, click the **Abort** button,  , for any channel.

IMPORTANT The pumps stop dispensing when the LC timeout limit has elapsed unless you press the Abort button.

❖ **To prime the pumps**

See "Priming the LC Pumps" on page 167.

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 Flow Mode, the LC eluting 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 Timeout for Continuous Flow Mode" on page 49).

This feature is useful for method development tasks using a detector and for detector 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.

Channel 1 and Channel 2 eluting pumps are used as the solvent sources for the continuous flow feature.

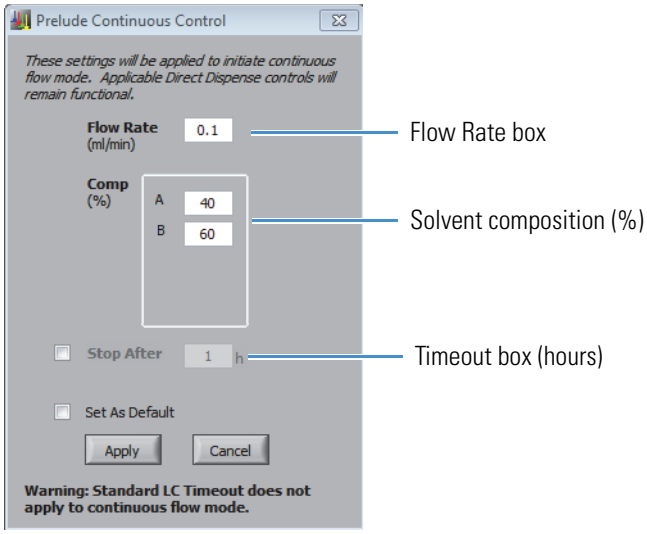
❖ **To flow mobile phase to the detector for manual loop injection or compound optimization (Continuous Flow Mode)**

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.
2. Confirm that the eluting 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 24).

> **IMPORTANT** The Continuous Flow mode does not heat the analytical column. If you want to use column heat to allow a more appropriate flow rate for your method, set both LC Timeout and Continuous Flow timeouts to 1 hour.

Figure 24. 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 manually control the bypass valve" on page 55.

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 Timeout for Continuous Flow Mode).

Setting a Timeout for Continuous Flow Mode

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 24 on page 48).
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

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

Setting a High-Pressure Limit for the System LC Pumps

You can set a high-pressure limit for the Prelude MD LC pumps using the Adv button. When you set a high-pressure limit, a pump that exceeds the set high-pressure limit shuts off and enters an error state until cleared.

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.

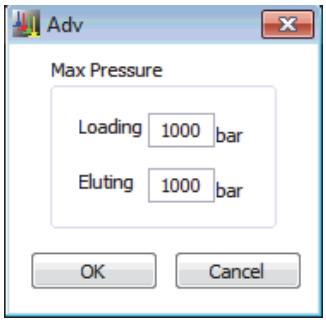
❖ To set a high-pressure limit on the system pumps

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 25).

   Figure 25. 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 either Bottle Set 1 or Bottle Set 2. 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 currently assigned to a channel
- To assign the bottle set for one channel
- To assign the bottle set for all channels

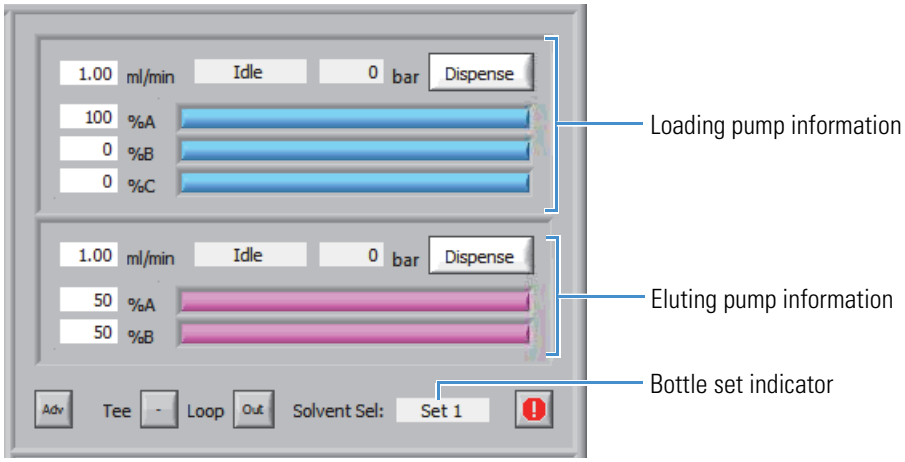
Tip If you are working in a high-throughput environment and running more than one channel under the same method, Thermo Fisher Scientific recommends that the channels share mobile phase bottles to ensure that there is no variation in the dispensed solvents.

❖ To determine which bottle set is currently assigned to a channel

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.
2. In the middle pane, select **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 is currently assigned to the channel—either Set 1 or Set 2. See Figure 26.

Figure 26. Direct Dispense area

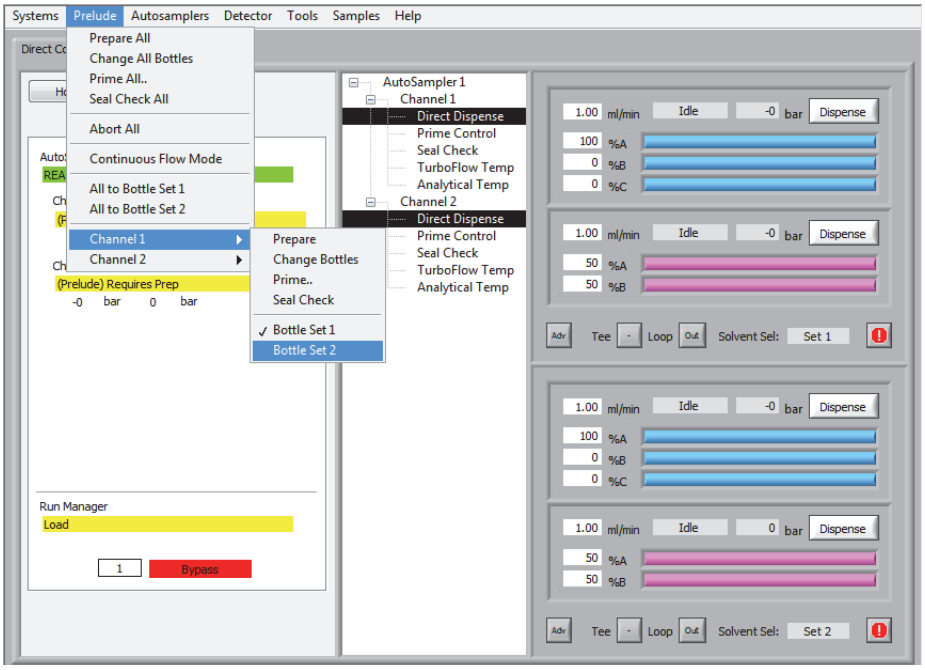


❖ To assign the bottle set for one channel

Note This procedure starts a Change Bottle priming process that uses new solvents. The number of priming cycles is set in the Prelude Configuration dialog box. (The system default is set to 6).

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.
2. Choose **Prelude > Channel *n***, where *n* is the channel that 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 27.

Figure 27. Channel bottle set assignment

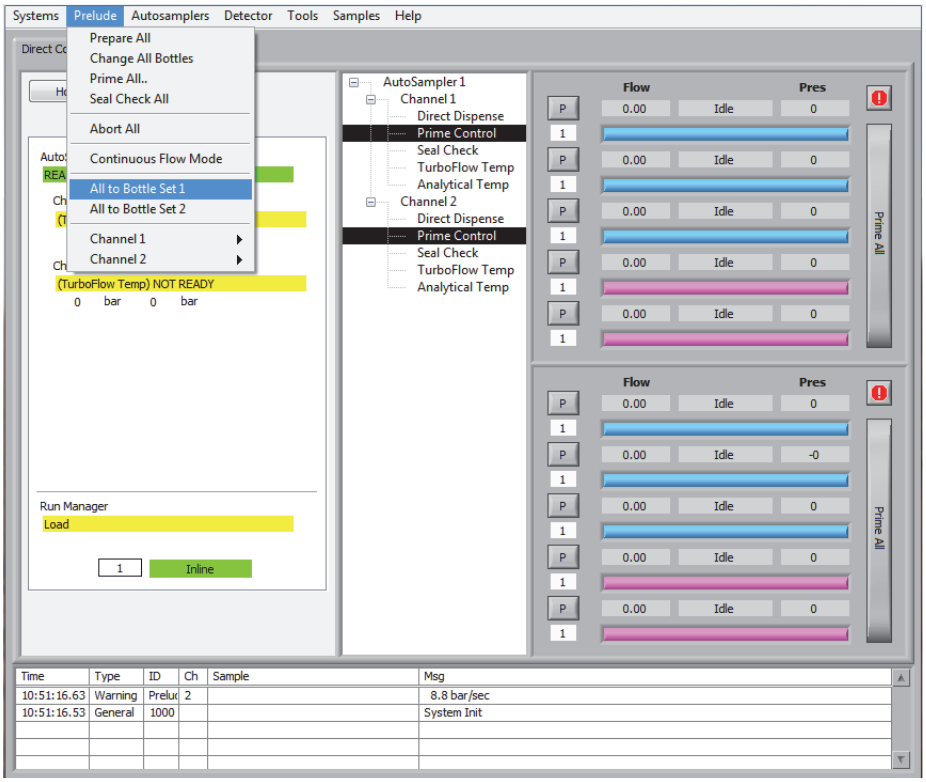


❖ To assign the bottle set for all channels

Note This procedure starts a Change Bottle priming process that uses the new solvents. The number of priming cycles is set in the Prelude Configuration dialog box (the system default is 6).

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.
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**. See Figure 28.

Figure 28. Direct Control window showing All to Bottle Set 1



Controlling the Valves Using Direct Control

You can control the system valves manually from the Direct Dispense area of the Direct Control window. (see Figure 26 on page 51).

Follow these procedures.

- To manually control the Tee and Loop feature
- To manually control the selector valve
- To manually control the bypass valve

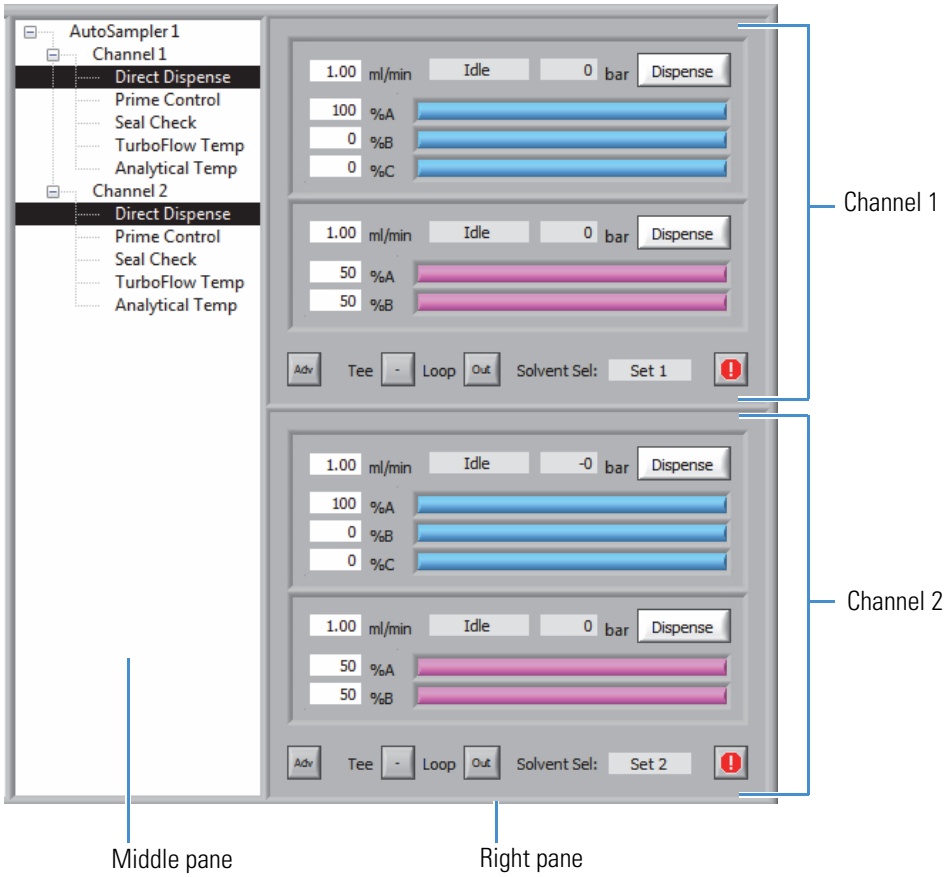
❖ To manually control the Tee and Loop feature

1. Open the Direct Control window.
2. In the middle pane, select **Direct Dispense** for any channel (see Figure 29).

   The Direct Dispense controls for each channel appear in the right pane.

Tip Tree items selected in the Direct Control window's middle pane apply to both channels by default. You can change the default so that your selections only apply to one channel; choose **Tools > Tree Selection > Individual**.

Figure 29. Direct Control window showing middle pane (Direct Dispense selected) and right pane, showing channel 1 and 2 pump information



3. Do one of the following:
   - To change the position of the Tee valve, click the **Tee** button,  .

     The Tee valve switches its position and the Tee button changes its state to  .
   - To change the position of the Loop valve, click the **Loop** button,  .

     The Loop valve switches its position and Loop button changes its state to  .

For more information on setting valve positions, see "Recommendations for Setting Valve Positions" on page 111.

❖ To manually control the selector valve

1. Open the Direct Control window.
2. Choose **Detector > Source**, and select the system channel that you want to flow to the detector.

❖ To manually control the bypass valve

Note The bypass valve directs the flow exiting the in-line channel column to the detector or to waste. To switch the position of the bypass valve, follow this procedure.

1. Open the Direct Control window.
2. Choose **Detector > Bypass** to switch the position of the bypass valve.

   The Bypass indicator in the Direct Control window and status window switches to indicate 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 Autosampler

Follow these procedures:

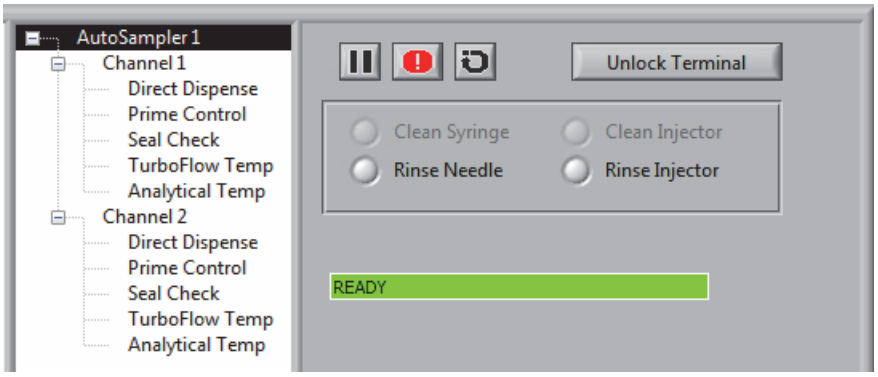
- To access the manual control features of the autosampler
- To pause the autosampler
- To stop the autosampler
- To reset the autosampler
- To clean the autosampler needle
- To clean an autosampler injector
- To view autosampler objects
- To change the tray type configuration for a sample tray

❖ To access the manual control features of the autosampler

1. Open the Direct Control window.
2. Select the autosampler in the middle pane.

   The autosampler options appear. See Figure 30.

Figure 30. Autosampler options



❖ To pause the autosampler

Click the **Pause** icon, .

The autosampler completes the method for the current sample, but does not draw additional samples. The Pause icon changes its color to red.

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

❖ To stop the autosampler

Click the **Abort** icon, .

The autosampler stops the current method.

❖ To reset the autosampler

Click the **Reset** icon, .

The autosampler arm returns to the origin (0,0,0) position and resets the XYZ coordinates based on the origin position.

❖ To clean the autosampler needle

1. Select the **Rinse Needle** option.

   The Rinse Needle dialog box appears.

2. In the Wash list, select the wash solution that will clean the syringe.
3. In the Injector list, select the injector in which to rinse the needle.
4. In the Needle Gap box, leave the value in the default setting, unless you have been instructed to change it by a service engineer.
5. In the Rinse Time list, select the number of seconds to rinse the needle, and then click **OK**.

Tip Thermo Fisher Scientific recommends 5 seconds for optimal cleaning.

The dialog box closes. The autosampler cleans the outside of the needle while flushing the wash through the needle.

❖ To clean an autosampler injector

1. Select **Rinse Injector** option.

   The Rinse Injector dialog box opens.

2. In the Wash list, select the wash that will clean the injector.

3. In the Injector list, select the injector that you want to clean.

4. In the Rinse Time list, select the number of seconds to rinse the injector, and click **OK**.

   Tip Thermo Fisher Scientific recommends 5 seconds for optimal cleaning.

   The dialog box closes and the autosampler cleans the injector.

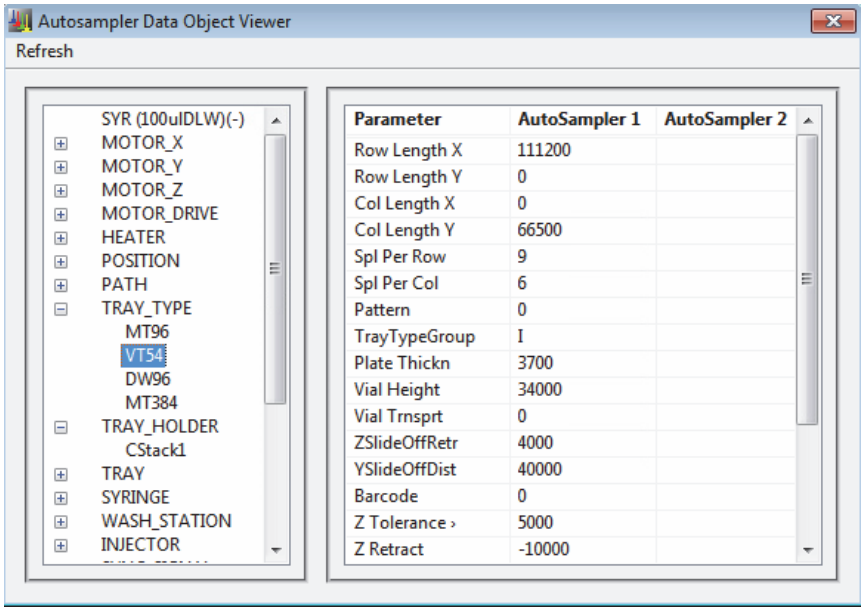
❖ To view autosampler objects

1. Open the Direct Control window.

2. Choose **Autosamplers > AS Object Viewer**.

   The list of autosampler objects opens. See Figure 31.

Figure 31. Autosampler Data Object Viewer dialog box

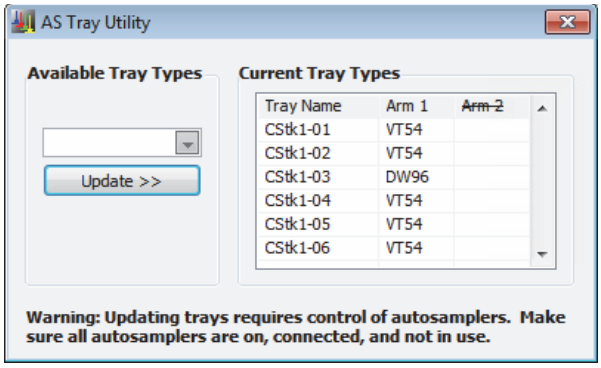


❖ To change the tray type configuration for a sample tray

1. In the Direct Control window, choose **Autosamplers > AS Tray Utility**.

   The AS Tray Utility dialog box opens.

Figure 32. 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 to which you want to configure the tray.
4. Click **Update**, and then close the AS Tray Utility.

   The AS objects are updated.

Controlling the Column Heater Temperature

During a run, the LC method controls the column temperature. Use this procedure if you want to change the column temperature and you do not want to run a method.

CAUTION Do not use high heat on the columns for an extended period of time when the pumps are not dispensing fluids.

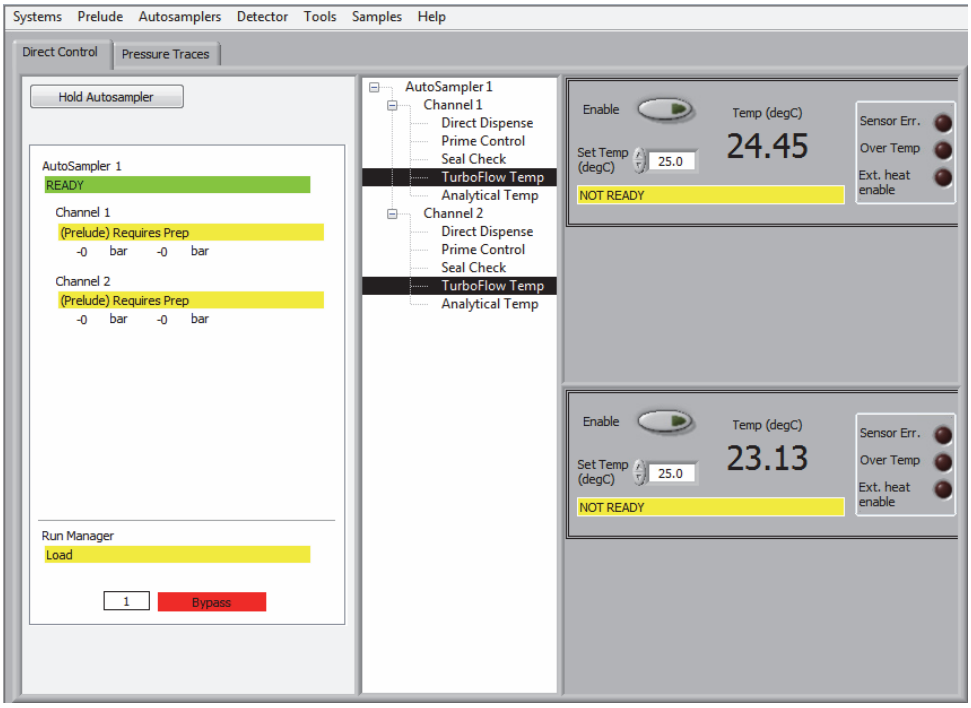
❖ To change a column heater temperature

1. Open the Direct Control window.
2. In the middle pane, select the heater that you want to control, either **TurboFlow Temp** or **Analytical Temp**.

   The heater options appear to the right for the selected heater.

Note The names of your heaters might be different from the names of the heaters in Figure 33 because they can be changed during the heater configuration.

Figure 33. Temperature options in the Direct Control window showing the selected (TurboFlow Temp) heater on Channel 1 and (TurboFlow Temp) heater on Channel 2



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, turn it on. 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.
2. To turn off the heater, click the **Enable** button again.
   - When the heater is on, the Enable button appears light green.
   - When the heater is off, the Enable button appears dark green. See Figure 34.

Figure 34. Enable button showing on and off states

Heater turned on (enabled)

Heater turned off (disabled)

Changing the LC Timeout Value

You can set the LC timeout value according to how long that you want the pumps and/or column heaters to remain on when you use the Direct Dispense controls.

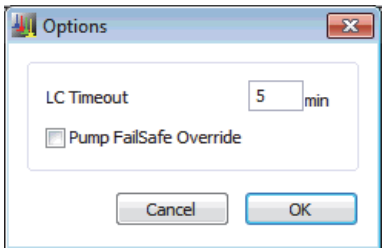
Note The LC timeout applies only to how long the column heaters remain turned on after a sequence completes.

❖ To change the LC Timeout value

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

   The Options dialog box opens.

   Figure 35. 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 LC pumps stop pumping and the heaters turn off.
4. (Optional) Select the **Pump Failsafe Override** check box if you want to prevent pump channel devices (heaters) from turning off when a channel has not run for over 1 hour.

   Note The 1 hour fail safe time period conserves system resources when a channel is not in use—or a system issue occurs—but it might also cause problems for certain workflows.

See Table 12 for details on the Pump Failsafe Override check box and other Prelude MD logic settings.

Editing Logic Settings

The service engineer configures the Prelude MD application at the time of the installation visit. You can change the logic settings to better meet your laboratory's needs.

❖ To change the logic settings

1. Close all Thermo 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 dialog box opens.

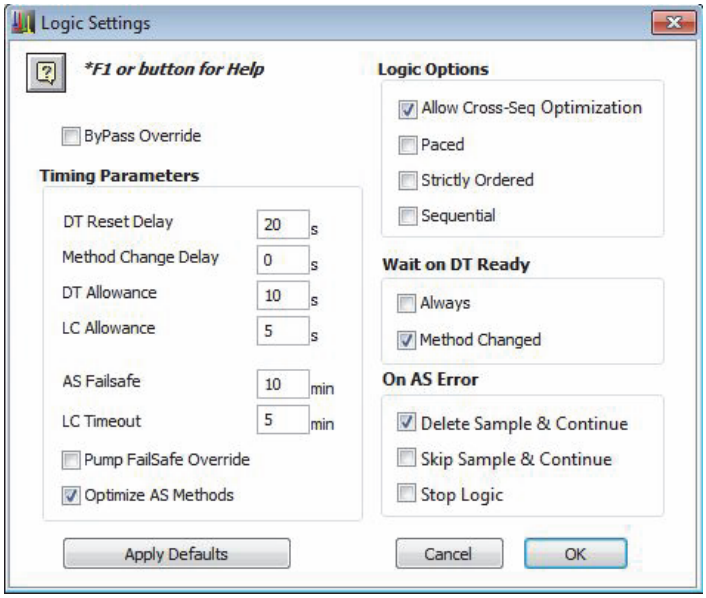
3. On the right side of the dialog box, select the **Prelude MD** icon and click **Configure**.

   The Configurations dialog box opens.

4. Choose **Logic > Advanced**.

   The Logic Settings dialog box opens. See Figure 36.

   Figure 36. Logic Settings dialog box



5. Edit any of the options described in Table 12.

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**.
   - To discard your changes and return to the factory-set selections, click **Apply Defaults**.

Table 12. Logic Settings dialog box options (Sheet 1 of 3)

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 during the method, the fluid flows to waste. • Leave this check box cleared if you want fluid exiting the HPLC column to flow to the detector throughout the LC method duration unless diverted to waste by the selector valve (when multi-channeling methods).
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 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 time that you want the system to wait before sampling whenever a new instrument method runs. This time allows the columns' and detector's conditions to equilibrate before the system runs the next method.
DT Allowance	Type the time allowed for the detector to respond to a command.
LC Allowance	Type the time allowed for the LC pumps to respond to a command.
AS Failsafe	Type the time allowed to elapse (idle time) before the autosampler shuts down.
LC Timeout	Type the time allowed to elapse after the method completes before the heaters turn off or the pumps stop dispensing when the system is under direct control.
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 heaters do not shut off when the LC Timeout has elapsed.

Table 12. Logic Settings dialog box options (Sheet 2 of 3)

Option	Description
Optimize AS Methods	Select this check box if you want the application to calculate the optimal system starts based on stored AS method timing values from previous runs of the autosampler method. If you leave this check box cleared, the application uses the values you entered into the Prior to Sample and Pre-injection Total times in the AS Method Editor window.
Allow Cross-Seq Optimization	If you select this check box, the Sequential check box becomes available, where you can indicate your preference for running samples in order. The following lists the possible combinations of these options: • If you select both Allow Cross-Seq Optimization and Sequential, the application attempts to run batches and samples in the order in which they were submitted under most circumstances, but runs them out of sequence if it cannot proceed in order for any reason, such as a disabled channel. • If you select Allow Cross-Seq Optimization and do not select Sequential, batches or sequences can run out of the order in which you submitted them, which allows batches to run concurrently. This option generally improves throughput and allows you to run different methods cross sequentially. • If you do not select Cross-Seq Optimization, the batches run in the order they were submitted under all circumstances. If a batch cannot run for any reason, batches that were submitted subsequently do not run.
Paced	This logic style calculates the maximum sample throughput rate, and paces sample aspirations so that little variation exists in the amount of time between sample starts. This results in less variation in the data collection intervals, which improves detector efficiency. It also equalizes the equilibration times for all samples.
Strictly Ordered	Select this check box if your system uses cross-sequential optimization, and you want to run the channels in numerical order. Throughput might be slower with this check box selected. Selecting this check box provides equal wear and tear on all channels.

Table 12. Logic Settings dialog box options (Sheet 3 of 3)

Option	Description
Sequential	This check box appears when you select Allow Cross-Seq Optimization. When you select this check box, the batches generally run in the order in which you submitted them. Leave this check box cleared if you prefer that the samples and batches run out of order. See "Allow Cross-Seq Optimization."
Wait on DT Ready: Always	Select this check box if you want the application to receive the detector ready signal before starting the next sample in all conditions. Thermo Fisher Scientific recommends that you keep this check box cleared.
Wait on DT Ready: Method Changed	Select this check box if you want the application to receive the detector ready signal before starting the next sample if the next sample involves a different instrument method.
On AS Error: Delete Sample and Continue	Select this check box if you want the application to delete any sample with an autosampler error, and to continue with the next sample. The sample remains in the batch but the application deletes it from the system application queue.
On AS Error: Skip Sample and Continue	Select this check box if you want the application to skip any sample with an autosampler error, and to continue with the next sample. The sample remains in the batch and the system application queue.
On AS Error: Stop Logic	Select this check box if you want the application to stop sampling whenever a sample has an autosampler error.

Assigning Values Using the Sample List

This topic 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 using the same method and batch. All of the values described in this topic, 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 injector in the sample list
- To assign the column temperature in the sample list

- To assign values to a method variable in the sample list
- To assign the LC channel in the sample list

❖ To assign the injector in the sample list

Note To select the injector where you want to dispense the sample by using the sample list, the instrument method must indicate "SEQ.Injector" in the Injector option for any task that specifies an injector. See "Assigning the Injector in the Sample List" on page 96.

1. Create a custom column in the sample list named **AS_Injector**.
2. Enter one of the following into the AS_Injector column in the sample list:
   - For a laminar HPLC method, type **LX** in the AS_Injector column for each sample. The system injects the sample using the LX injector.
   - For a TurboFlow method, type **TX** in the AS_Injector column for each sample. The system injects the samples using the TX injector.

❖ To assign the column temperature in the sample list

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

- 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.
- 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 detector control application. View the Direct Control window on your system for the name of your column heaters.
2. In the new column, for each sample, type the temperature that you want to set.

   Figure 37 shows an example of a sample list with temperature values set for the TurboFlow and Analytical column heaters.

Figure 37. Sample list showing set temperatures for column heaters named TurboFlow and Analytical

Type	File Name	Sample ID	Path	Inst Meth	Proc Meth	Position	Inj Vol	Level	TurboFlow	Analytical
	Test01	Stk1-01:1	C:\Xcalibur\Dat	C:\Xcalibur\metho		Stk1-01:1	0.00		25	25
	Test02	Stk1-01:2	C:\Xcalibur\Dat	C:\Xcalibur\metho		Stk1-01:2	0.00		30	30
	Test03	Stk1-01:3	C:\Xcalibur\Dat	C:\Xcalibur\metho		Stk1-01:3	0.00		35	35
	Test04	Stk1-01:4	C:\Xcalibur\Dat	C:\Xcalibur\metho		Stk1-01:4	0.00		40	40
	Test05	Stk1-01:5	C:\Xcalibur\Dat	C:\Xcalibur\metho		Stk1-01:5	0.00		45	45
	Test06	Stk1-01:6	C:\Xcalibur\Dat	C:\Xcalibur\metho		Stk1-01:6	0.00		50	50
	Test07	Stk1-01:7	C:\Xcalibur\Dat	C:\Xcalibur\metho		Stk1-01:7	0.00		55	55
	Test08	Stk1-01:8	C:\Xcalibur\Dat	C:\Xcalibur\metho		Stk1-01:8	0.00		60	60
	Test09	Stk1-01:9	C:\Xcalibur\Dat	C:\Xcalibur\metho		Stk1-01:9	0.00		65	65
	Test10	Stk1-01:10	C:\Xcalibur\Dat	C:\Xcalibur\metho		Stk1-01:10	0.00		70	70
							0.00			

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

1. Create a custom column. Name the column the same name as the method variable. For information on method variables, see "Allowing Method Variables During a Run" on page 122. For information on creating custom columns, refer to the documentation that comes with the detector control application.
2. Enter the variable value for each sample in the new column.

> **Tip** Use this feature to evaluate different loading step solvent compositions in a method. With this feature, you do not have to create a new method for each loading step solvent composition you evaluate.

❖ To assign the LC channel in the sample list

1. Create a custom column named "**ChannelSelect**".
2. In the ChannelSelect column, enter the number of the LC channel that you want to run the sample.

> **Tip** You can enter more than one channel by using a separator. For example, enter **12** to specify Channels 1 and 2.

The system runs the samples using the first available channel that you enter.

8

Prelude MD Instrument Methods

This topic describes how the AS and LC methods function on the Prelude MD instrument. It provides starting methods and a protocol for optimizing the methods to your analyte.

Contents

Autosampler Methods

The autosampler method 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 dispense steps for the autosampler.

For instructions and tips on entering autosampler method information and tips on selecting autosampler commands, see Chapter 9, “Creating an Autosampler Method.”

LC Methods

The LC method controls the LC channels, including the pumps, valves, and heater. When you create an LC method, you can maximize sample extraction and recovery by determining the best flow rates, mobile phase compositions, and step durations to use in your TurboFlow method.

For instructions on entering LC method information into the instrument method, see Chapter 10, “Creating an LC Method.”

For instructions on optimizing LC method steps, see Chapter 11, “Developing a TurboFlow Method.” This topic provides starting methods and a protocol for optimizing the methods to your analyte.

For information on TurboFlow methods, see TurboFlow Methods.

TurboFlow Methods

An LC method contains the flow rate values, mobile phase compositions, and valve positions used throughout the sample run. A TurboFlow method uses the TurboFlow column to separate large sample matrix molecules, salts, and sugars from the analytes. After the TurboFlow separation, the compounds transfer to the analytical column, where they are separated using HPLC analysis.

Typical TurboFlow method separations involve at least five steps. The number of steps in each method can vary depending on the optimal conditions established for the analyte during method development. Table 13 summarizes the five method steps.

Table 13. Method steps

Step	Description
1. Loading step	The sample flows through the column at a relatively high linear velocity. The compounds of interest remain on the TurboFlow column and the matrix debris flows to waste.
2. Transfer step	The LC uses the organic contents of a transfer loop to remove the compounds retained by the TurboFlow column. The compounds are then transferred and focused onto the head of the analytical column.
3. Eluting step	The analytes elute off the analytical column and flow to the detector. The pumps flow mobile phase through the TurboFlow column to wash it.
4. Loop-filling step	The pumps flow mobile phase through the loop to fill it for the next sample and through the analytical column to wash it.
5. Equilibrating step	The pumps flow mobile phase through the system to equilibrate the lines and columns with solvent conditions for the next sample.

To learn about the LC system activities during each step, read these topics:

- Loading Step
- Transfer Step
- Eluting Step
- Loop-Filling/Wash Step
- Equilibrating Step
- Laminar Methods

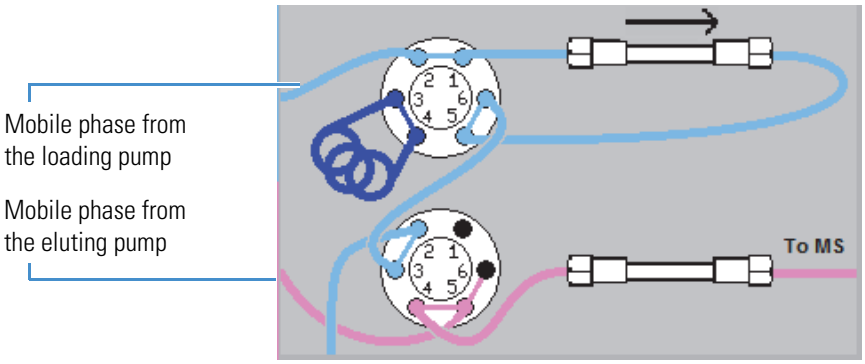
Loading Step

During the loading step, the loading pump delivers an aqueous mobile phase to the TurboFlow column. The TurboFlow column separates the analytes from the sample matrix components. The analytes remain on the column while the matrix components pass to waste.

The eluting pump's aqueous mobile phase flows through the analytical column and then to the detector, but it has had no contact with the sample, and therefore, contains no analytes.

The transfer loop and Tee are out of the fluid path. Figure 38 shows the position of valves A and B during this step.

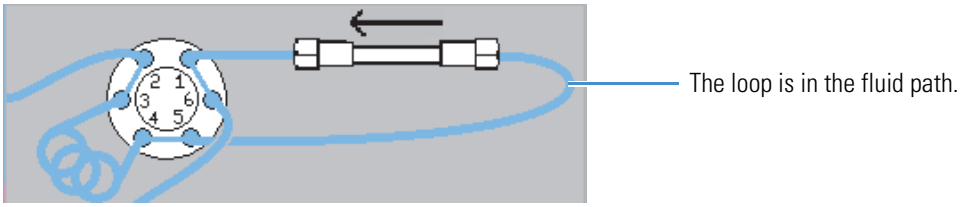
Figure 38. Flow of mobile phase through the TurboFlow column and then to waste



Transfer Step

At the beginning of this step, Valves A and B rotate so that the transfer loop and Tee are in the fluid path. Figure 39 shows a diagram of valve A positioned with the loop in the fluid path.

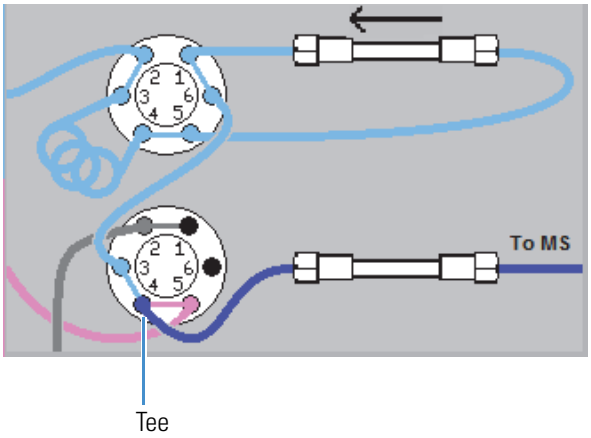
Figure 39. Valve A: Loop in the fluid path



Flow from the loading pump pushes the organic loop contents through the TurboFlow column and removes the analytes from the column.

The analytes traveling with the organic loop contents reach the Tee. The Tee combines the flow of the loading pump, which currently carries the loop contents, with an aqueous flow from the eluting pump. Figure 40 shows valve B positioned with the Tee in the fluid path, combining the flows from the loading and eluting pumps.

Figure 40. Valves A and B showing the Tee

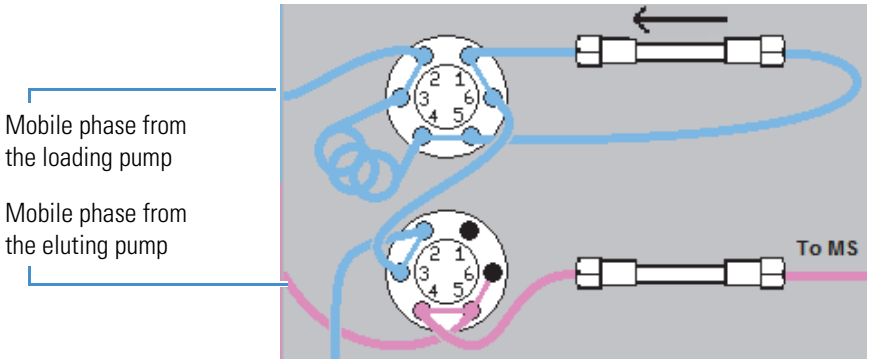


With the Tee in the fluid path on Valve B, the organic mobile phase flow (loop contents) combines with an aqueous flow from the eluting pump after exiting the TurboFlow column. The aqueous flow lowers the strength of the solvent from the loop so that the analytes remain on the analytical column when they reach it.

Eluting Step

In the eluting step, Valve B turns and takes the Tee out of the fluid path. Figure 41 shows Valve B with the Tee out of the fluid path.

Figure 41. Loop in the fluid path/Tee out of the fluid path



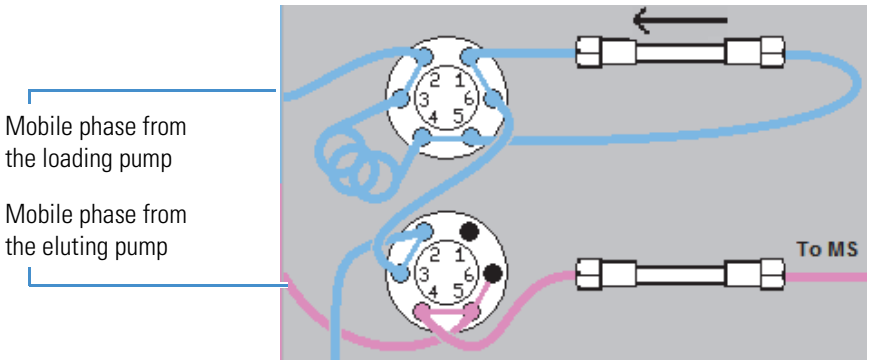
The eluting pump flow becomes increasingly organic throughout this step. Once it reaches a critical percentage of organic solvent, the analytes elute off the analytical column and flow to the detector.

The flow from the loading pump washes the TurboFlow column using an organic solvent, which removes the more highly retained, unwanted compounds.

Loop-Filling/Wash Step

At the beginning of this step, the valves remain in position with the loop still in the fluid path and the Tee out of the fluid path. Figure 42 shows the positions of the valves during the Loop-filling/Wash step.

Figure 42. Loop in the fluid path and Tee out of the fluid path

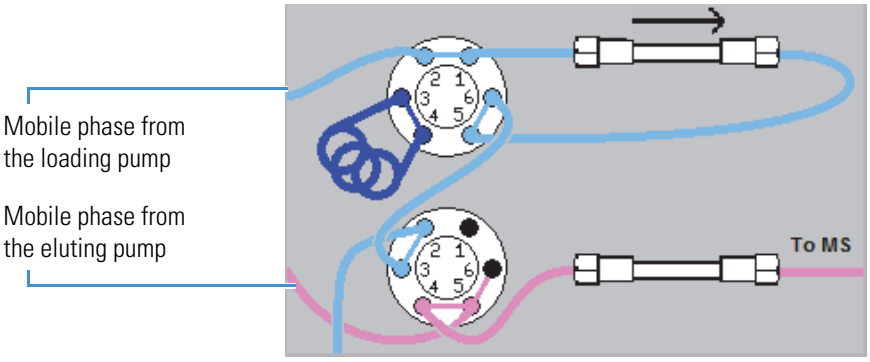


The mobile phase compositions change. The loading pump delivers an organic solvent combination, and fills the loop for the next sample. Organic solvent from the eluting pump washes the analytical column, removing any highly retained compounds.

Equilibrating Step

At the start of the equilibrating step, Valve A turns to take the loop out of the fluid path so that the organic solvent remains in the loop for the next sample. Figure 43 shows the position of the valves during the equilibrating step.

Figure 43. Loop and Tee out of the fluid path



The mobile phase compositions change so that the loading and eluting pumps deliver mobile phases appropriate for the initial conditions of the next sample.

Laminar Methods

The Prelude MD instrument system can run HPLC methods that do not use the TurboFlow column. In laminar methods, the autosampler injects the sample directly onto the HPLC or UHPLC column. The flow bypasses the TurboFlow column. Table 14 describes the four steps in a laminar method.

Table 14. Laminar method steps

Step	Description
1. Loading step	A pump delivers mobile phase to the HPLC column. The HPLC column captures the analytes.
2. Eluting step	Mobile phase becomes increasingly organic. Analytes elute off the column when the mobile phase composition reaches a critical percentage of organic solvent.
3. Wash step	A strong organic solvent washes the column.
4. Equilibrating step	Mobile phase compositions change to those of the loading step to prepare for the next sample.

IMPORTANT The instrument does not remove the sample matrix when you run a laminar method. Use only samples that have been previously treated to remove the matrix, or use samples that do not require pretreatment.

9

Creating an Autosampler Method

The autosampler executes all tasks associated with drawing the sample from the sample vial, injecting it into the LC system, and washing the needle and injectors. These topics describe how to create or edit an autosampler method.

Contents

Accessing the Autosampler Method Editor

❖ To open the Autosampler 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 detector control application.

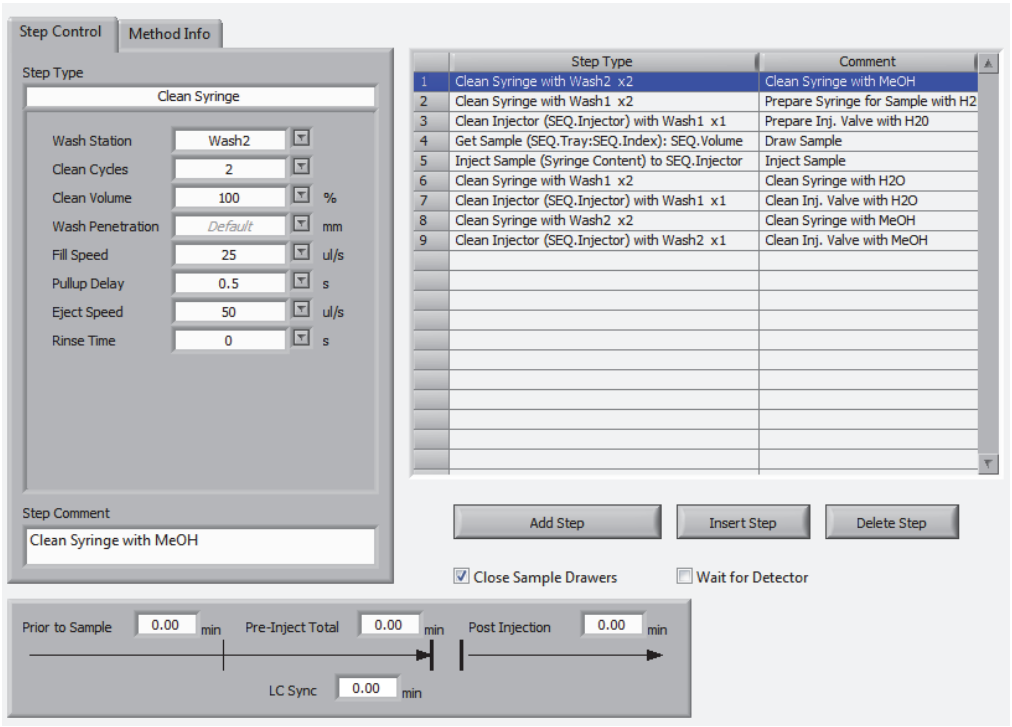
2. Click **Prelude MD**.

   The LC Method Editor window opens.

3. Click **AS Method**.

   The Autosampler Method Editor window opens.

Figure 44. Autosampler Method Editor window



Creating an Autosampler Method

The Prelude MD application saves the autosampler method you create as part of an instrument method in the detector control application. Instrument methods have a .meth file extension.

During the autosampler method, the autosampler draws sample from the vial and injects it onto the column. To program this action, you use two method step types: Get Sample and Inject Sample.

To wash the injector and syringe, add wash steps. If you have biological samples, insert the sampling between two or more aqueous washes to avoid precipitating the sample with an organic wash.

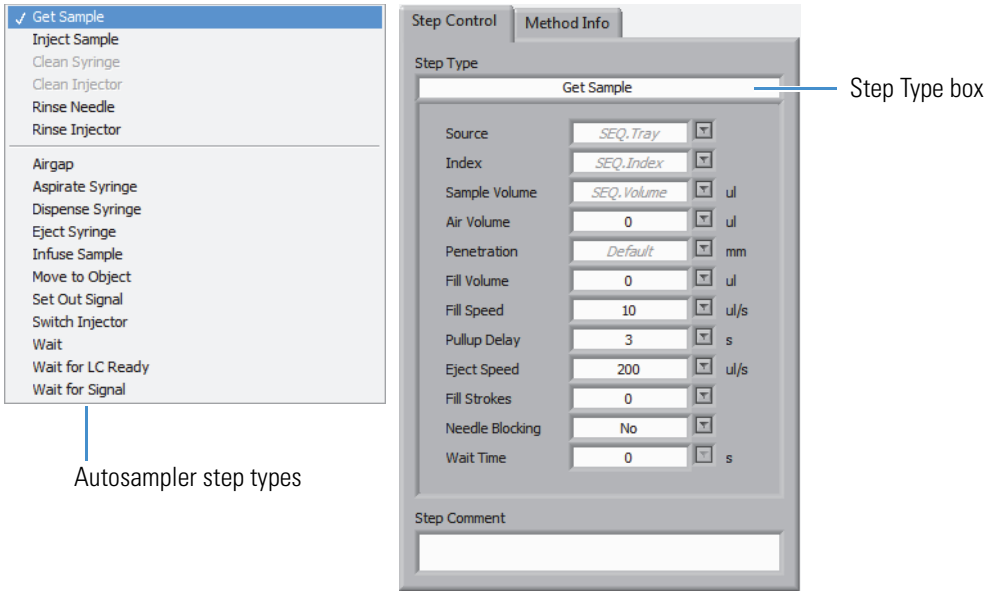
❖ To create an autosampler method

1. Open the AS Method Editor. See "Accessing the Autosampler Method Editor" on page 74.
2. Click **Add Step**.

   A new step appears in the step table. See also "Tips for Creating an Autosampler Method."
3. Click the Step Type box to open a list of Step types.
4. Choose the step type that describes the action you want the autosampler to perform in this step, for example, **Get Sample** in Figure 45.

   For information on the step types, see "Autosampler Step Types" on page 79.

Figure 45. Step Types



The step types appear in the Step Type table on the right, and step type options appear in the Step Control pane on the left. See Figure 44 on page 74 and Figure 45.

Tip You can create a useful autosampler method using only these five step types:

- Get Sample
- Inject Sample
- Rinse Injector
- Rinse Needle
- Airgap

Use the remaining step types only when these five do not meet your requirements. For a description of the step types, see "Autosampler Step Types" on page 79.

5. Type the appropriate parameters for the step types options or use the default values. For more information on these parameters, see "Autosampler Step Types" on page 79.
6. In the Step Comment box, type a description of the step for your reference.
7. Repeat step 2 through step 6 until the method actions meet your needs.
8. Leave the Prior to Sample, Pre-Inject Total, LC Sync, and Post Injection boxes blank. For more information on these parameters, see "AS Method Timing Options" on page 94.
9. To close the sample drawer after each injection, select the **Close Sample Drawers** check box.

   Tip If you have a cool stack, select this check box to keep the temperature of the cool stack regulated.

10. If you want the autosampler to wait for a signal from the detector that indicates the detector is ready to accept a new sample, select the **Wait for Detector** check box.

    Tip To improve throughput, leave this check box blank if you are running more than one LC channel.

11. Click the **Method Info** tab.

    The Method Info page opens.

12. Type a description of the wash solutions to keep for your records. See "Entering Information on the Method Info Page" on page 78.
13. Do one of the following:

    - To save the method, choose **File > Save**.
    - To save the method using a new name, choose **File > Save As**, type the new name, and click **Save**.

Tips for Creating an Autosampler Method

Follow these tips while you create an autosampler method.

- Thermo Fisher Scientific recommends the following wash solutions for Wash 1 and Wash 2.
  - Wash 1: Water with 2% acetonitrile or 0.1% formic acid (to prevent microbial growth in the reservoir).
  - Wash 2: A mixture of acetonitrile/isopropanol/acetone, 45:45:10.
- 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 93.

The following tips refer to the AS Method Editor window. See Figure 44.

- Always wash the injector and syringe with aqueous solution before and after a step where a biological sample has contacted the injector and syringe. This prevents the proteins in the biological sample from precipitating in the organic wash solution. Figure 44 shows an example of an AS method. Notice that an aqueous Wash 1 appears before and after the Get Sample and Inject Sample step types.
- Enter **2** or higher in the Rinse Time box for the Rinse Needle and Rinse Injector step types. "Rinse Needle" on page 84 and "Rinse Injector" on page 85.
- In the Rinse Injector step type, ensure that you specify the appropriate injector in the Injector list. Injector options are TX and LX. See "Rinse Injector" on page 85.
- Use the Air Gap step types before and after the Get Sample step types to separate the sample and solvent. See "Airgap" on page 93.
- When you use viscous samples, enter a slower fill speed and longer pull-up delay in the Get Sample and Aspirate Sample step types. See "Get Sample" on page 81 and "Inject Sample" on page 83.

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. For example, the method in Figure 45 has nine steps.

❖ To add or delete steps

1. Open the AS method. See "Accessing the Autosampler Method Editor" on page 74.
2. Do one of the following:
   - To add a step at the end of the method, click **Add Step**. A copy of the last step 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 Step**. A copy of the selected step appears below it.

3. Edit the step type as necessary. See "Editing Autosampler Step Types" on page 79.

❖ **To delete a step**

Select the step and click **Delete Step**.

Entering Information on the Method Info Page

Use the Method Info page to record information about the method. For example, record the wash solution 1 and 2 composition or add comments. Figure 46 shows the Method Info page.

Figure 46. Method Info page in the AS Method Editor window

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

1. In the Comment box, type a description of the autosampler method.
2. In the Wash 1, Wash 2, and Loop boxes, type a description of the wash solutions 1 and 2, and indicate the size of the sample loop.
3. In the Syringe box, select your system's syringe type. The Prelude MD instrument comes with the DLW syringe.

Editing Autosampler Step Types

❖ **To edit an autosampler step type**

1. Open the AS Method Editor. See "Accessing the Autosampler Method Editor" on page 74.
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.

Autosampler Step Types

This topic 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 15.

Table 15. AS method step types (Sheet 1 of 2)

Step types	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.
Rinse Needle	The syringe needle enters a specified wash port. The specified wash solution rinses the inside and outside of the needle. This option appears only when the DLW is present.
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. This option only available when the DLW present.
Airgap	The syringe moves from the sample or wash and draws in air.
Aspirate Syringe (Intended for advanced users and applications)	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 (Intended for advanced users and applications)	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.

Table 15. AS method step types, continued (Sheet 2 of 2)

Step types	Function
Eject Syringe (Intended for advanced users and applications)	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.
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.
Move to Object (Intended for advanced users and applications)	Instructs the autosampler arm to move to a specified position.
Set Out Signal (Intended for advanced users and applications)	Controls the output signal of a non-injector autosampler valve.
Switch Injector (Intended for advanced users and applications)	Instructs the injector valve to change position.
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 (Intended for advanced users and applications)	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 (Figure 47) instructs the probe to move to the sample vial and draw the sample. For a description of the Get Sample options, see Table 16.

Figure 47. Get Sample step type

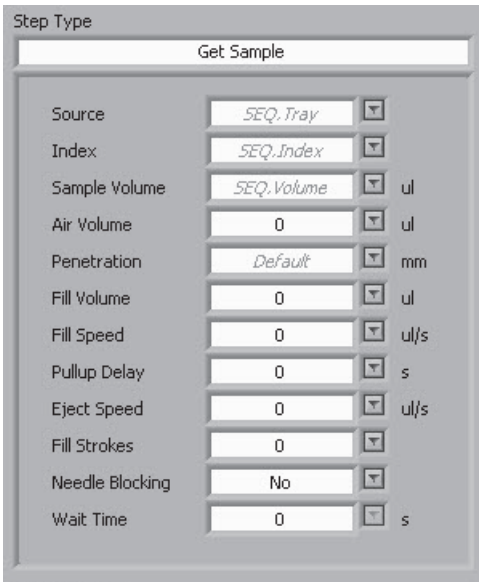


Table 16. Get Sample options (Sheet 1 of 2)

Option	Description
Source	The tray number that contains the sample. To specify the tray in the sample list, select **SEQ. Tray**. Otherwise, select the tray number.
Index	The vial location for the sample that you want to draw. To specify the vial in the sample list, select **SEQ. Index**. Otherwise, select the vial location.
Sample Volume	The sample volume that you want the syringe to aspirate. To specify the volume in the sample list, select **SEQ. Volume**. Otherwise, type the sample volume.
Air Volume	The volume of air that you want the needle to draw in after the needle moves out of the sample.

Table 16. Get Sample options (Sheet 2 of 2)

Option	Description
Penetration	The depth at which you want the needle to enter the vial. If this value appears gray, the autosampler uses the default value. To override the default value, type a new value. If this value appears black, the autosampler default value has been overridden. If you want to return to the autosampler default value, delete the override.
	IMPORTANT Changing this value can affect the performance of your system. See "Penetration Value Special Notice" on page 93.
Fill Volume	The total amount of sample that you want drawn into the needle while it performs fill strokes. This value does not affect final sample volume.
Fill Speed	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 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.
Eject Speed	The plunger movement speed for all ejection movement while the syringe performs fill strokes.
Fill Strokes	The number of aspiration cycles in the sample vial.
Needle Blocking	When you select Yes, the application temporarily locks the needle-guide in place as the syringe extracts the sample. The system unlocks the needle guide in the end of the Get Sample step type.
Wait Time	The time in seconds that the autosampler waits before going to the next step.

Inject Sample

The Inject Sample step type (Figure 48) instructs the autosampler to inject the sample into the specified autosampler injector. For a description of the Inject Sample options, see Table 17.

Figure 48. Inject Sample step type

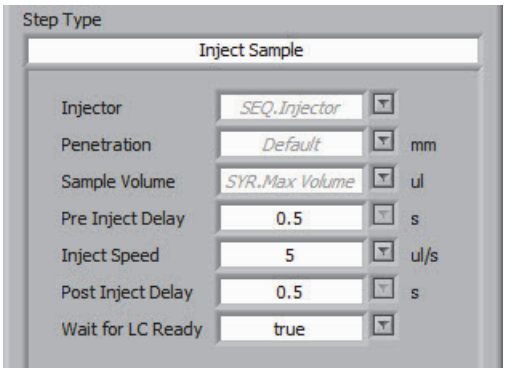


Table 17. Inject Sample options (Sheet 1 of 2)

Option	Description
Injector	Specifies the injector into which the autosampler injects the sample. If you want to enter the injector in the sample list, select **SEQ.Injector**. Otherwise, select the injector. If the selection in this box does not match the selection in the Injector boxes for other related step types in the method, a warning message appears when you save the method. Click **Yes** to continue.
Penetration	Depth at which the needle point enters the LC injector. • If this value appears gray, the autosampler uses the default value. Type a new value if you want to override the autosampler default value. • If this value appears black, the autosampler default value has been overridden. If you want to return to the autosampler default value, delete the override. **IMPORTANT** Changing the Penetration value can affect the performance of your system. See "Penetration Value Special Notice" on page 93.

Table 17. Inject Sample options (Sheet 2 of 2)

Option	Description
Sample Volume	Specifies the sample volume to inject. • If SYR.Max Volume appears, the syringe injects the entire syringe contents. • If SEQ.Volume appears, the syringe injects the volume specified in the sample list.
Pre Inject Delay	The delay time prior to sample injection.
Inject Speed	The plunger speed for sample injection.
Post Inject Delay	The delay time after 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).

Rinse Needle

The Rinse Needle step type (Figure 49) washes the needle during the AS method. If you select the Rinse Needle step type, the robotic arm moves to the specified location during the method, and flushes both the interior and exterior of the needle with the specified wash solution. For a description of the Rinse Needle options, see Table 18.

Figure 49. Rinse Needle step type

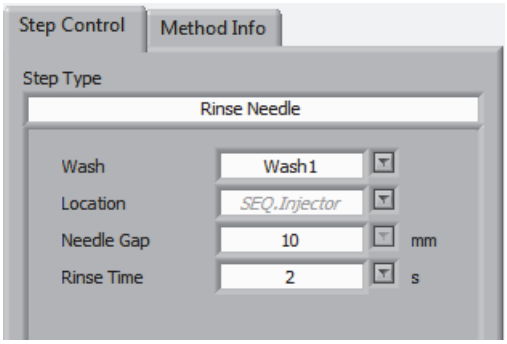


Table 18. Rinse Needle options (Sheet 1 of 2)

Option	Description
Wash	Do one of the following: • To use Wash 1 for the wash, select **Wash 1**. • To use Wash 2 for the wash, select **Wash 2**.
Location	The location that the autosampler uses to wash the needle.

Table 18. Rinse Needle options (Sheet 2 of 2)

Option	Description
Needle Gap	The height above the normal penetration depth for the injector.
	Tip Leave this value at the system default unless you have been instructed to change it by a service engineer.
Rinse Time	The time in seconds that the autosampler washes the needle in the wash station.
	Tip Select **2** or higher.

Rinse Injector

Use the Rinse Injector step type (Figure 50) to wash the injector during the AS method. For a description of the Rinse Injector options, see Table 19.

Figure 50. Rinse Injector step type

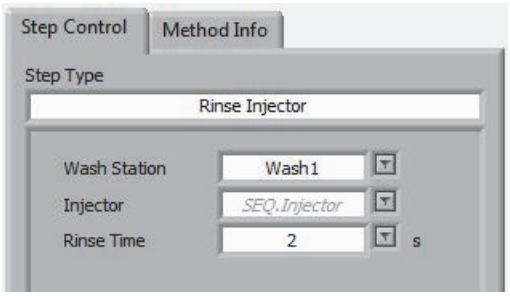


Table 19. Rinse Injector options

Option	Description
Wash Station	The solvent that the autosampler uses to wash the injector. Select **Wash 1** or **Wash 2**.
Injector	The injector that you want the autosampler to wash. To select the injector in the sample list, select **SEQ.Injector**.
	Note If the selection in this box does not match the selection in the Injector boxes for other related step types in the method, a warning message appears when you save the method. Click **Yes** to continue.
Rinse Time	The time in seconds that the autosampler washes the injector.
	Tip Enter a rinse time equal to **2** or higher.

Infuse Sample

The Infuse Sample step type (Figure 51) 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 20.

Figure 51. Infuse Sample step type

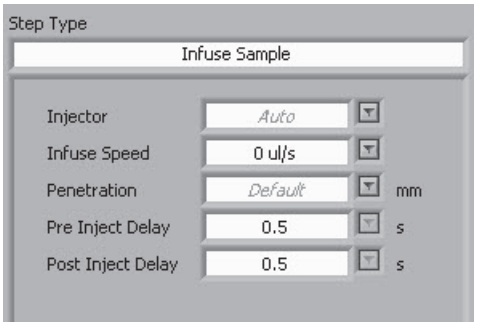


Table 20. Infuse Sample options (Sheet 1 of 2)

Option	Description
Injector	Specifies the injector into which the autosampler infuses the sample. To enter the injector in the sample list, select **SEQ.Injector**. **Note** If the selection in this box does not match the selection in the Injector boxes 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 point penetrates into the sample. • If this value appears gray, the autosampler uses the default value. Type a new value if you want to override the autosampler default value. • If this value appears black, it overrides the default value. If you want to return to the autosampler default value, delete the override. **IMPORTANT** Changing this value can affect the performance of your system. See "Penetration Value Special Notice" on page 93.

Table 20. Infuse Sample options (Sheet 2 of 2)

Option	Description
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.

Aspirate Syringe

The Aspirate Syringe step type (Figure 52) 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 21.

Figure 52. Aspirate Syringe step type

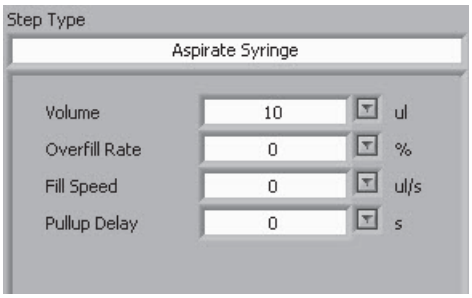


Table 21. Aspirate Syringe options

Option	Description
Volume	Amount of volume to aspirate.
Overfill Rate	Additional percentage to aspirate and return to the sample vial.
Fill Speed	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. When you use viscous samples, enter a longer delay time than the default value.

Dispense Syringe

The Dispense Syringe step type (Figure 53) delivers a specified volume at the needle's current location. For a description of the Dispense Syringe options, see Table 22.

Figure 53. Dispense Syringe step type

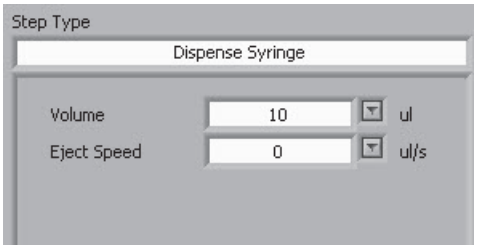


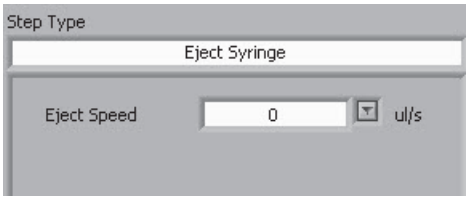
Table 22. Dispense Syringe options

Option	Description
Volume	The volume in the syringe that you want to eject.
Eject Speed	The plunger movement speed for the ejection movement.

Eject Syringe

The Eject Syringe step type (Figure 54) 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 54. Eject Syringe step type



Switch Injector

The Switch Injector step type (Figure 55) actuates the LC injector valve to the specified position. For a description of the Switch Injector options, see Table 23.

Figure 55. Switch Injector step type

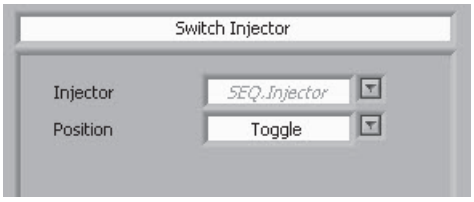


Table 23. Switch Injector options

Option	Description
Injector	The injector that contains the valve that you want to switch. To enter the injector in the sample list, select **SEQ.Injector**. Otherwise, select the injector.
	Note If the selection in this box does not match the selection in the Injector boxes for other related step types in the method, a warning message appears when you save the method. Click **Yes** to continue.
Position	Specifies the injector valve position. For example, if the injector was 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 two positions are as follows: • Standby: The sample loop is in line with the fluid path and closed to the injector port. • Active: The sample loop is closed to the fluid path and open to the injector port.
	Tip Switching the injector several times during a method might be helpful for optimal cleaning.
	Note To prevent the system from shutting down due to increased pressure, always end the method with the valve in the Standby position.

Move to Object

The Move to Object step type (Figure 56) 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 24.

Figure 56. Move to Object step type

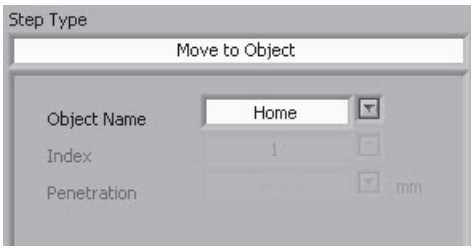


Table 24. Move to Object options

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 in the tray.
Penetration	The depth at which the needle penetrates the object. 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** Changing this value can affect the performance of your system. See "Penetration Value Special Notice" on page 93.

Wait for Signal

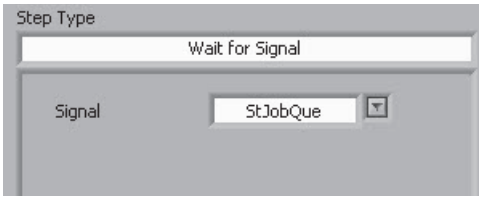
The Wait for Signal step type (Figure 57) temporarily halts the autosampler method to wait for the occurrence of the specified hardware signal.

Note Typical users do not use this advanced option.

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

Figure 57. Wait for Signal step type

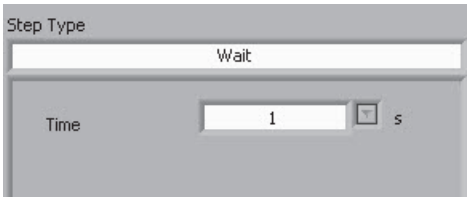


Wait

The Wait step type (Figure 58) adds a wait time to your autosampler method.

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

Figure 58. Wait step type



Wait for LC Ready

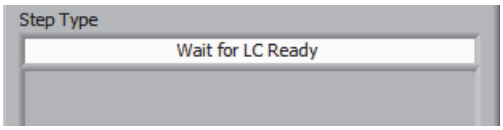
The Wait for LC Ready step type (Figure 59) instructs the autosampler to pause until it receives a ready signal from the LC system.

There are two options:

- True—Instructs the autosampler to pause for an LC ready signal.
- False—Instructs the autosampler to continue regardless of the LC status.

This step type has no additional parameters.

Figure 59. Wait for LC ready step type



Set Out Signal

The Set Out Signal step type (Figure 60) sets the output signal and signal state of a non-injector autosampler valve. Non-injector autosampler valves are used in some external applications.

Note Do not use this advanced step type for TurboFlow or laminar methods.

For a description of the Set Out Signal options, see Table 25.

Figure 60. Set Out Signal step type

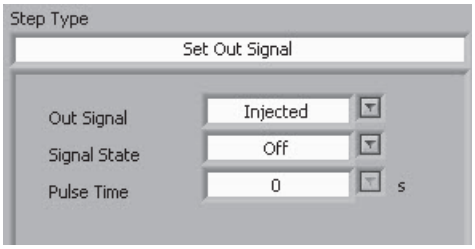


Table 25. Set Out Signal options

Option	Description
Out Signal	The autosampler signal type that triggers the valve to change.
Signal State	The signal state that changes the valve to the position you want.
Pulse Time	The time in milliseconds that you want the valve to remain in the new state. To keep the valve in the new state, type **0**.

Airgap

The Airgap step type (Figure 61) removes the syringe from the injector or wash station 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, see Table 26.

Figure 61. Airgap step type

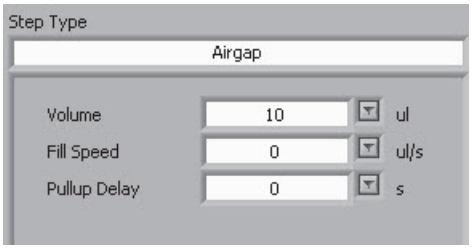


Table 26. Airgap options

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

Penetration Value Special Notice

The Inject, Infuse, and Move to Object step types provide the option to set the penetration value; however, Thermo Fisher Scientific recommends that you keep this value the same.

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 xviii.

Editing the AS Method for Maximum Throughput

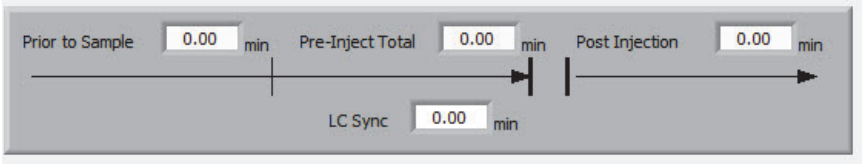
To optimize your autosampler method for better throughput, perform one or more of the following procedures:

- Verify that the Allow Cross-Seq Optimization option is selected. See "Logic Settings dialog box" on page 61 and "AS Method Timing Options."
- Leave the Prior to Sample, Pre-inject total, LC Sync, and Post injection boxes in the AS Method Editor window 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

The Prior to Sample, Pre-inject Total, LC Sync, and Post Injection boxes in the AS Method Editor window represent the time segments of the autosampler method before and after the sample injection. See Figure 62.

Figure 62. AS Method timing options



When you run multiple channels, the Prelude MD 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 maintains the accuracy of these values by recording and averaging the applicable time segments with each method run. However, you can override this feature.

❖ To set the timing estimates in the AS Method Editor window

Do one of the following:

- If you want the Prelude MD application to adjust the sample starts using AS method timing values calculated from previous runs, leave these boxes blank.

  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 Prelude MD Logic Settings dialog box, and type values in these fields. See "Logic Settings dialog box" on page 61.

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

Importing 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 to which you want to add the AS method information.
2. Click **Prelude MD**.

   The LC Method Editor window opens.
3. Click **AS Method**.

   The AS Method Editor window opens.
4. Choose **Edit > Import**, and then navigate to the instrument method (.meth) that contains the AS method information that you want to import, and then select it.
5. Click **OK**.

   The AS method information appears in your open method.
6. Do one of the following:
   - Choose **File > Save** to save and overwrite the file with your changes.
   - Choose **File > Save As** to save the changes with a new file name.

Assigning the Injector

The Prelude MD instrument has two injectors on each LC channel. The TX injector injects sample onto the TurboFlow column, and the LX injector injects sample onto the analytical column. You can select the injector in the autosampler method or in the sample list.

Assigning the Injector in the AS Method Editor

You can assign an injector in each step type in the AS method that is associated with an injector; these include the Inject Sample, Rinse Injector, and Infuse Sample step types. If an injector is assigned in the Injector box for the step type, the autosampler uses that injector to complete the step.

❖ **To assign the injector in the AS method**

1. In the AS Method Editor window, select a step type that is associated with an injector, such as I**nject Sample**, **Rinse Injector**, and **Infuse Sample**.
2. Open the Injector list, and select the injector that you want the system to use.
   - To inject into the TurboFlow column, select **TX**.
   - To inject into the analytical column, select **LX**.
3. Choose **File > Save** to save the method.

Assigning the Injector in the Sample List

If SEQ.Injector appears in the Injector box, the autosampler uses the injector specified in the sample list. The following procedure describes how to use the sample list to assign a specific injector to a sample or a set of samples.

❖ **To assign the injector in the sample list**

1. In the AS Method Editor, select **Inject Sample**.
2. Open the Injector list, and select **SEQ.Injector**.
3. Repeat step 2 for other injector step types in your method, such as the Rinse Injector, or Infuse Sample step type.
4. In the sample list, create a custom column named **AS_Injector**. Refer to the appropriate detector application documentation.
5. Do one of the following:
   - To inject into the analytical column, type **LX** in the AS_Injector column for each sample.

     The system injects the sample using the LX injector.
   - To inject onto the TurboFlow column, type **TX** in the AS_Injector column for each sample.

     The system injects the samples using the TX injector.

Saving the Method

Saving the instrument method saves the autosampler method.

❖ **To save the instrument method in the Autosampler Method Editor window**

Do one of the following:

- To save changes made to the autosampler or instrument method, choose **File > Save** in the Autosampler Method Editor window.
- To save the autosampler method and any changes you made to a new file name, choose **File > Save As**. Then navigate to the folder where you want to save the instrument method, type a name for the method, and then click **Save**.

10

Creating an LC Method

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.

The LC method can be a TurboFlow method or a laminar method. A TurboFlow method uses a TurboFlow column to remove the large compounds from the sample matrix, and then uses the HPLC column for further separation. An HPLC method uses only the HPLC or UHPLC column.

These topics describe 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

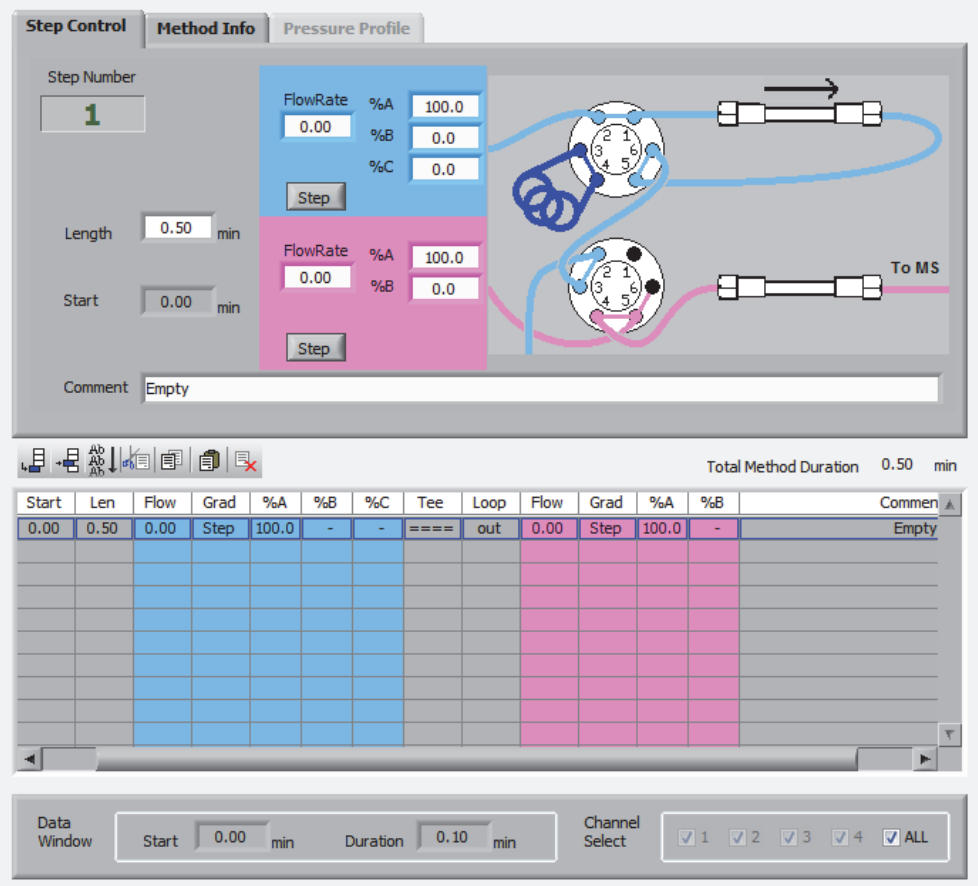
Accessing the LC Method Editor

❖ **To access the LC Method Editor**

1. Open the instrument method that you want to view using the detector control application.
2. Click **Prelude MD**.
3. Click **LC Method**.

   The LC Method Editor window opens (see Figure 63).

Figure 63. LC Method Editor window



Creating an LC Method

This topic describes how to enter the LC method information.

❖ To create an LC method

1. Open the LC Method Editor window using the detector control application.
2. Click the **Step Control** tab and then click the **Add** icon, , to add steps to your method. See "Modifying Steps in an LC Method."
3. On the Step Control page, enter the appropriate method step duration, flow rate, mobile phase composition, and valve positions. See "Modifying Components in an LC Method Step" on page 104.
4. Assign the start time and duration of the data window. See "Assigning the Data Window" on page 112.
5. If you want to view a graphical representation of your method, see "Viewing the Graph" on page 118.

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 117. The volume of each mobile phase for one sample injection must be less than 3.0 mL.

7. Enter the Prelude Prestart pressure values for each pump. See "Entering the Prelude Prestart Value" on page 113.

8. To enter variables for a method component, such as flow rate or mobile phase composition, see "Allowing Method Variables During a Run" on page 122. Use this feature to vary method components while optimizing a method.

9. Click the **Method Info** tab and enter general information about the LC method, such as solvents used. See "Entering Information on the Method Info Page" on page 116.

10. Enter the LC method data window duration into the detector portion of the instrument method for the acquisition time.

    Refer to your application user guide for information on 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

These topics describe how to add, delete, copy, and move steps in the LC Method Editor. Follow these procedures:

- To change the columns displayed in the method step table
- 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 change the columns displayed in the method step table

Edit the method steps using the method step table.

The method step table lists the method steps, the step duration, solvent compositions, flow rates, and valve positions. See Figure 64.

Figure 64. Method step table

Step	Start	Sec	Flow	Grad	%A	%B	%C	%D	Tee	Loop	Flow	Grad	%A	%B
1	0.00	30	1.50	Step	100.0	-	-	-	====	out	0.80	Step	100.0	-
2	0.50	60	0.40	Step	100.0	-	-	-	T	in	0.40	Step	100.0	-
3	1.50	60	1.50	Step	-	-	-	100.0	====	in	0.80	Ramp	5.0	95.0
4	2.50	90	1.50	Step	20.0	-	-	80.0	====	in	0.80	Step	5.0	95.0
5	4.00	60	1.50	Step	100.0	-	-	-	====	out	0.80	Step	100.0	-

❖ 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 104.

❖ 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 application sequences the step numbers.

3. Edit the step information. See "Modifying Components in an LC Method Step" on page 104.

❖ To delete a step

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

   The 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 change the Valve A position
- To change the Valve B position
- To copy information from one cell to all the selected cells below it (using Fill Down)
- To change the pump flow rate
- To redo the most recent changes that you undid
- 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 change

❖ To activate a step for editing

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

   The step information appears.

2. In the 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 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 "LC Method Step Table Columns" on page 121.

❖ To change the duration of the step

Do one of the following:

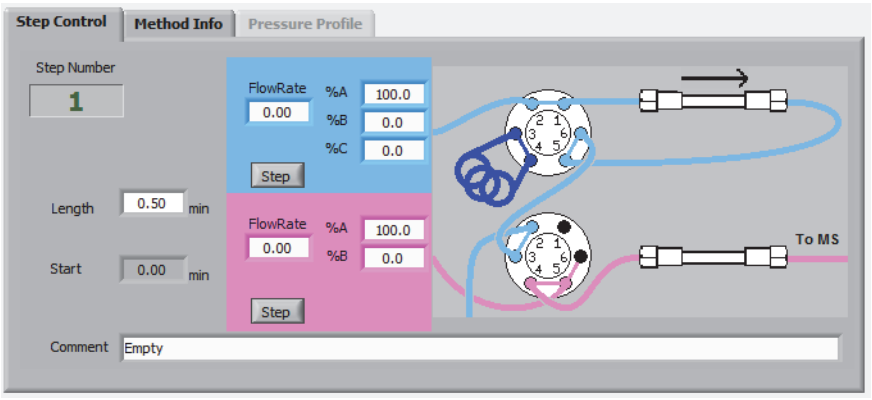
- Click the up or down arrow of the Length box, located in the top portion of the window, until the desired length appears.
- Click the sec value in the LC Method Step table and type the new length.

❖ To change the Valve A position

Note Change the Valve A position to move the transfer loop in or out of the fluid path. The organic solvent in the loop elutes the compounds retained on the TurboFlow column. The loop must be in the fluid path during both the transfer step and the loop-filling step.

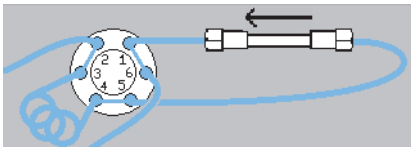
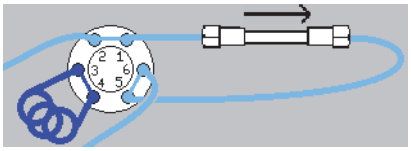
In the LC Method Editor window, click any part of Valve A. See Figure 65.

Figure 65. LC Method Editor window



The graphical display in the Method Editor window changes to show the new valve position, and the value in the Loop column switches to "In" or "Out." Table 27 summarizes the Valve A position change.

Table 27. Valve A positions

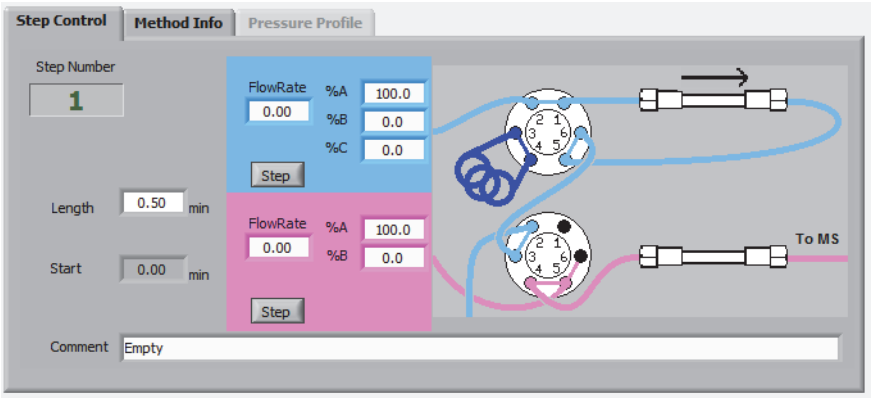
Diagram	Loop column value	Description
	In	Loop is in the fluid path. When the loop is in the fluid path, the mobile phase from the loading pump flows through the loop to the TurboFlow column.
	Out	Loop is out of the fluid path. When the loop is out of the fluid path, the mobile phase from the loading pump bypasses the loop and flows directly to the TurboFlow column.

❖ To change the Valve B position

Note Change the Valve B position to move the Tee in or out of the fluid path. When the Tee is in the fluid path, the aqueous mobile phase from the eluting pump combines with and dilutes the eluent from the TurboFlow column before loading onto the analytical column. The Tee must be in the fluid path during the transfer step.

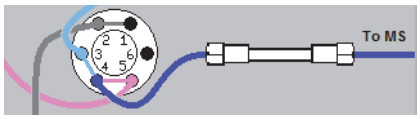
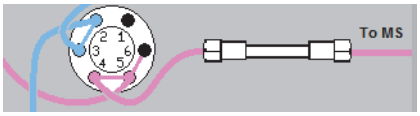
In the LC Method Editor window, click any part of Valve B. See Figure 66.

Figure 66. LC Method Editor window



The graphical display in the Method Editor window changes to show the new valve position, and the value in the Tee column switches to "T" or "====." Table 28 summarizes the Valve B position change.

Table 28. Valve B positions

Diagram	Tee column value	Description
	T	Tee is in the fluid path. When the T is in the fluid path, the flow from the TurboFlow column combines with the aqueous flow from the eluting pump, passes through the analytical column, and then enters the detector.
	====	Tee is out of the fluid path. When the T is out of the fluid path, the flow from the TurboFlow column flows to waste. The eluting pump mobile phase flows undiluted through the analytical column and to the detector.

❖ **To copy information from one cell to all the selected cells below it (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.

   The entries become highlighted.
3. Click the **Fill Down** button,  .

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

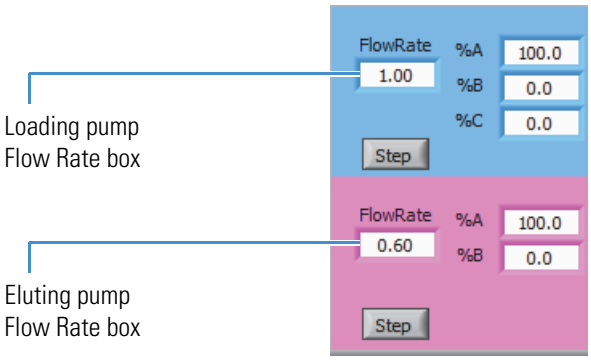
Tip You can also use the right mouse button to copy information from one entry in the column to the following entries. To do this, highlight the list of entries with the right mouse button. A list of options appears. Click the **Fill Down** button. The new values appear in the selected entries.

❖ To change the pump flow rate

Do any of the following:

- To change the loading pump flow rate, enter the new flow rate in the Flow Rate box in the blue Loading Pump area (Figure 67).
- To change the eluting pump flow rate, enter the new flow rate in the Flow Rate box in the pink Eluting Pump area (Figure 67).

Figure 67. Flow rate boxes, loading and eluting pumps



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

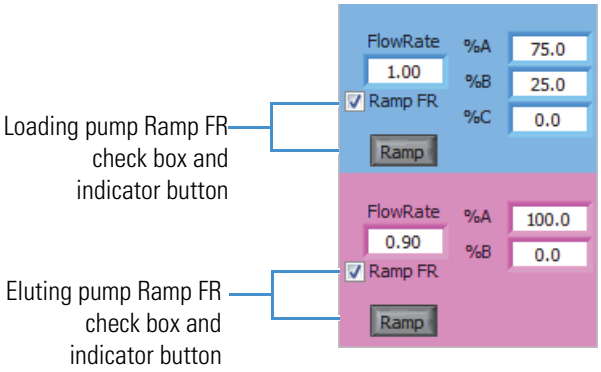
❖ To change the pump flow rate option from a step change to a flow rate ramp

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

Do one of the following:

- To change the loading pump flow rate to a ramp, select the **Ramp FR** check box in the loading pump area (Figure 68).
- To change the eluting pump flow rate to a ramp, select the **Ramp FR** check box in eluting pump area (Figure 68).

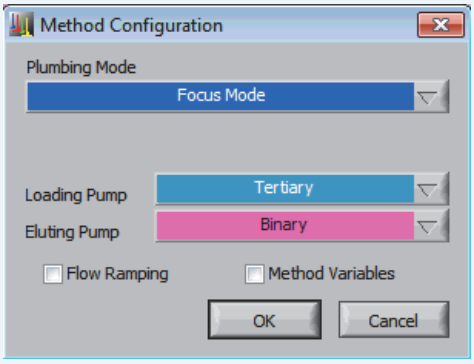
Figure 68. Loading and eluting pump Ramp FR check boxes and Ramp indicator buttons



Tip If the Ramp indicator button does not appear, do the following:

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

   The Method Configuration window appears.



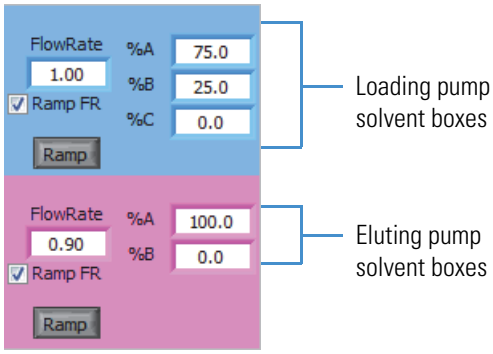
2. Select **Flow Ramping**.
3. Click **OK** to close the window.

❖ To change the composition of the mobile phase

Do either of the following (Figure 69):

- To change the composition of the loading mobile phase, enter the desired percentages of solvent A, B, or C, in the %A, %B, and %C boxes in the Loading Pump area. The application adjusts values to ensure a total solvent percentage of 100.
- To change the composition of the eluting mobile phase, enter the desired percentage of solvent A in the %A box. The value in the % B box adjusts so that the total solvent percentages equal 100.

Figure 69. Loading and eluting pump Ramp FR check boxes and Ramp indicator buttons



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 in 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 117.

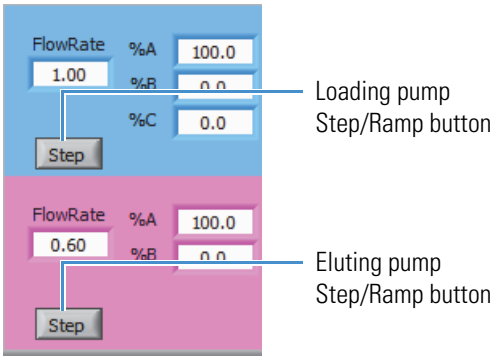
❖ To select a ramp or step mobile phase change

Click **Step/Ramp** to change the way in which the mobile phase composition changes. See Figure 70.

The following occurs:

- The button display changes to Ramp or Step.
- The entry in the Grad column changes to Ramp or Step.

Figure 70. Loading and eluting pump Step/Ramp buttons with "Step" showing



Note If you select Ramp, the mobile phase conditions in the beginning of the method step are the same as in the previous step. Throughout the length of the method, they gradually change to the mobile phase conditions entered for the current step. It will achieve the new conditions at the end of the step.

If you select Step, the mobile phase conditions change in the beginning of the step. The time at which the mobile phase reaches the assigned composition for the current method step depends on the pump type you are using. Gradient delay times are usually provided with the pump specifications.

Recommendations for Setting Valve Positions

Table 29 lists the recommendations for Valve A and B positions for each method step.

Table 29. Valve position recommendations (Sheet 1 of 2)

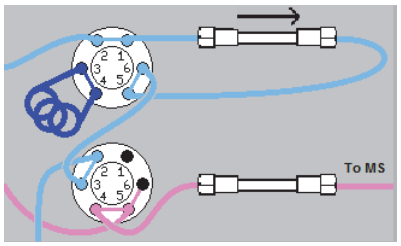
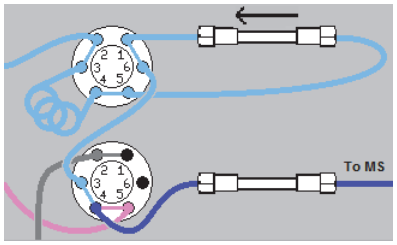
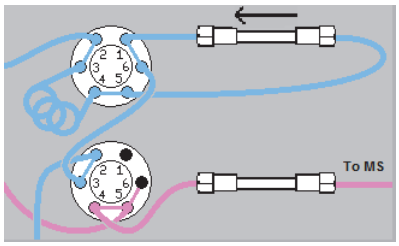
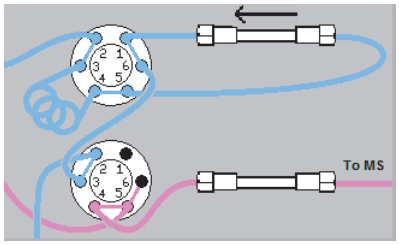
Step	Valve position recommendation	Figure
Loading	Loop—Out of the fluid path Tee—Out of the fluid path When loading sample onto the TurboFlow column in a Focus Mode method, change the Valve A and Valve B positions so that the Loop and Tee, respectively, are out of the fluid path. The loading pump mobile phase bypasses the loop and flows directly to the TurboFlow column and then to waste.	
Transfer	Loop—in the fluid path Tee—in the fluid path When eluting the analytes off the TurboFlow column and transferring them to the analytical column, change the Valve A and Valve B positions so that the loop and Tee are in the fluid path. The loading pump mobile phase flows through the loop to the TurboFlow column. The flow from the TurboFlow column combines with aqueous flow from the eluting pump, flows through the analytical column, and then enters the detector.	

Table 29. Valve position recommendations (Sheet 2 of 2)

Step	Valve position recommendation	Figure
Elution	Loop—in the fluid path Tee—out of the fluid path When eluting the analytes off the analytical column, change the Valve A position so that the loop is in the fluid path. Change the Valve B position so that the Tee is out of the fluid path and the eluting mobile phase does not combine with the loading mobile phase.	
Loop-filling	Loop—in the fluid path Tee—out of the fluid path The loop is in the fluid path to fill with mobile phase for the next sample. The Tee is out of the fluid path so that the organic loading mobile phase, which washes the TurboFlow column, does not pass through the analytical column.	
Equilibrate	Same as the loading step to prepare for the next sample.	

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 100.
2. In the Start box in the Data Window area, enter the time in the method that 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 the instrument channels cross sequentially, the data window length can affect your throughput. To maximize throughput, enter a data window length that is equal to or less than half the total LC method time.
4. To view a graphical representation of the data window and method timing, choose **Tools > Graph Display**. See "Viewing the Graph" on page 118.

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 100.
2. In the Channel Select area, select the channels that you want to use to run this method.

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 Value

The Prelude Prestart value equals the expected pump pressure when the autosampler injects the sample. The system pressurizes the solvent in the syringe to this value before the method starts to achieve the correct flow rate.

❖ To assign the Prelude Prestart values to a method

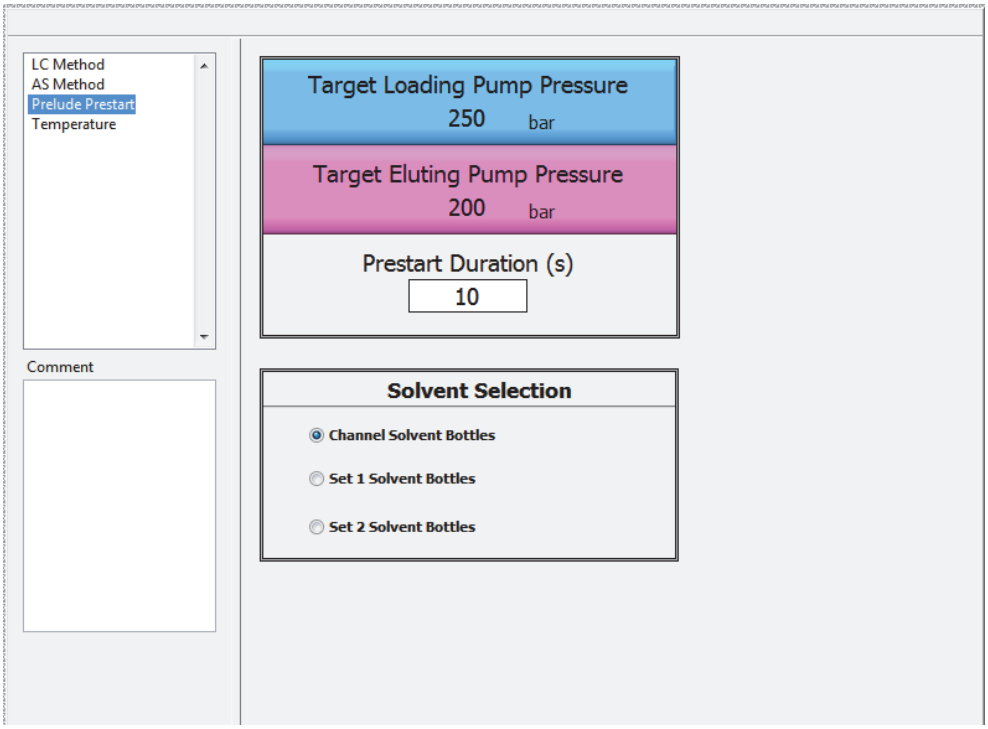
1. From your application's Instrument Setup window, click **Prelude MD**.

   A list of Prelude MD method types opens.

2. Click **Prelude Prestart**.

   The Target loading pump pressures appear (Figure 71).

Figure 71. Prelude Prestart Target Loading and Eluting Pump Pressure controls



3. Click the value in the Target Loading Pump Pressure (bar) box, and enter the new target value.

Note The target loading pump pressure value refers to the expected loading pump pressure during the loading step of the method. To determine this value, do the following:

1. Install the appropriate TurboFlow and analytical columns.
2. In the Direct Control window, select the channel and set the flow rates to the flow rates in the loading step of the method.
3. Dispense the fluids and observe the loading pump pressure. This is the target loading pump pressure.

4. Click the value in the Target Eluting Pump Pressure (bar) box, and type the new target value.

Note The target eluting pump pressure value refers to the expected eluting pump pressure during the loading step of the method. To determine this value, do the following:

1. Install the appropriate TurboFlow and analytical columns.
2. In the Direct Control window, select the channel and set the flow rates to the flow rates in the loading step of the method.
3. Dispense the fluids and observe the eluting pump pressure. This is the target eluting pump pressure.

4. Click the value in the Prestart Duration box, and enter the number of seconds that you want the flow rate to be stable at the target pressures before injecting the sample. Ten seconds is a typical value in this box.

Note The total volume that is dispensed for the method displayed in the Solvent Use dialog box does not include the volume of solvent dispensed during the prestart. See "Determining the Method's Solvent Usage" on page 117.

5. In the Solvent Selection area, choose one of the following:

- Select the **Channel Solvent Bottles** option if you want this method to use the bottles assigned to the channel that runs the sample.

  Channel 1 uses the Set 1 solvent bottles, and Channel 2 uses the Set 2 solvent bottles.

  Note If you run the same method on both channels, Thermo Fisher Scientific suggests that you do not select this option. Using different solvent bottles might add variability to your run. Select the **Set 1 Solvent Bottles** or the **Set 2 Solvent Bottles** option so that all samples in the run use the same solvent bottles.

- Select the **Set 1 Solvent Bottles** option if you want this method to always run using the solvents on the top shelf of the Prelude instrument system.
- Select the **Set 2 Solvent Bottles** option if you want this method to always run using the solvents on the bottom shelf of the Prelude instrument system.

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 the **Prelude MD** icon in the left pane.

   The list of Prelude MD method types appears in the middle pane.

2. Click **Prelude Prestart**.

   The Prelude Prestart window opens (see Figure 71 on page 114).

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 50.

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**.
   - 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 if needed and click **OK**.

Entering Information on the Method Info Page

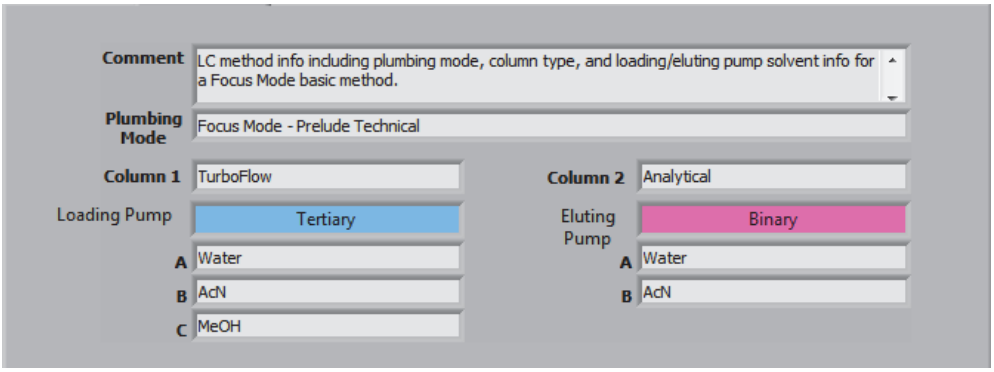
Use the Method Info page to record method information.

❖ To enter information on the Method Info page

1. In the LC Method Editor, click the **Method Info** tab.

   The Method Info page opens (Figure 72).

Figure 72. 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 TurboFlow column information.
4. In the Column 2 box, type the analytical column information.
5. In the Loading Pump boxes, type information that identifies the loading pump solvents.
6. In the Eluting Pump boxes, type information that identifies the eluting pump solvents.
7. Choose **File > Save** to save the method, or choose **File > Save As** to save the method using a new name.

Determining the Method's Solvent Usage

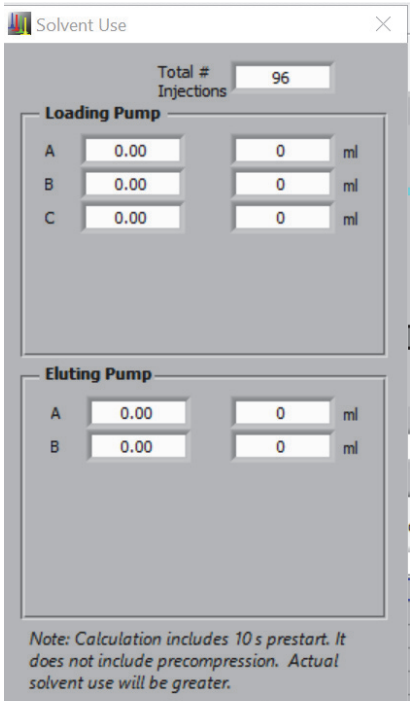
As you develop a method, use this procedure to determine the solvent usage. You must keep the total volume used during a method for any one solvent equal to or less than 3.0 mL.

❖ **To view the amount of solvent used by the method**

1. In the LC Method Editor window, choose **Tools > Solvent Use**.

   The Solvent Use dialog box opens (Figure 73).

Figure 73. 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 The total volume dispensed for the method displayed in the Solvent Use dialog box does not include the volume of solvent dispensed during the prestart. See "Entering the Prelude Prestart Value" on page 113.

Viewing the Graph

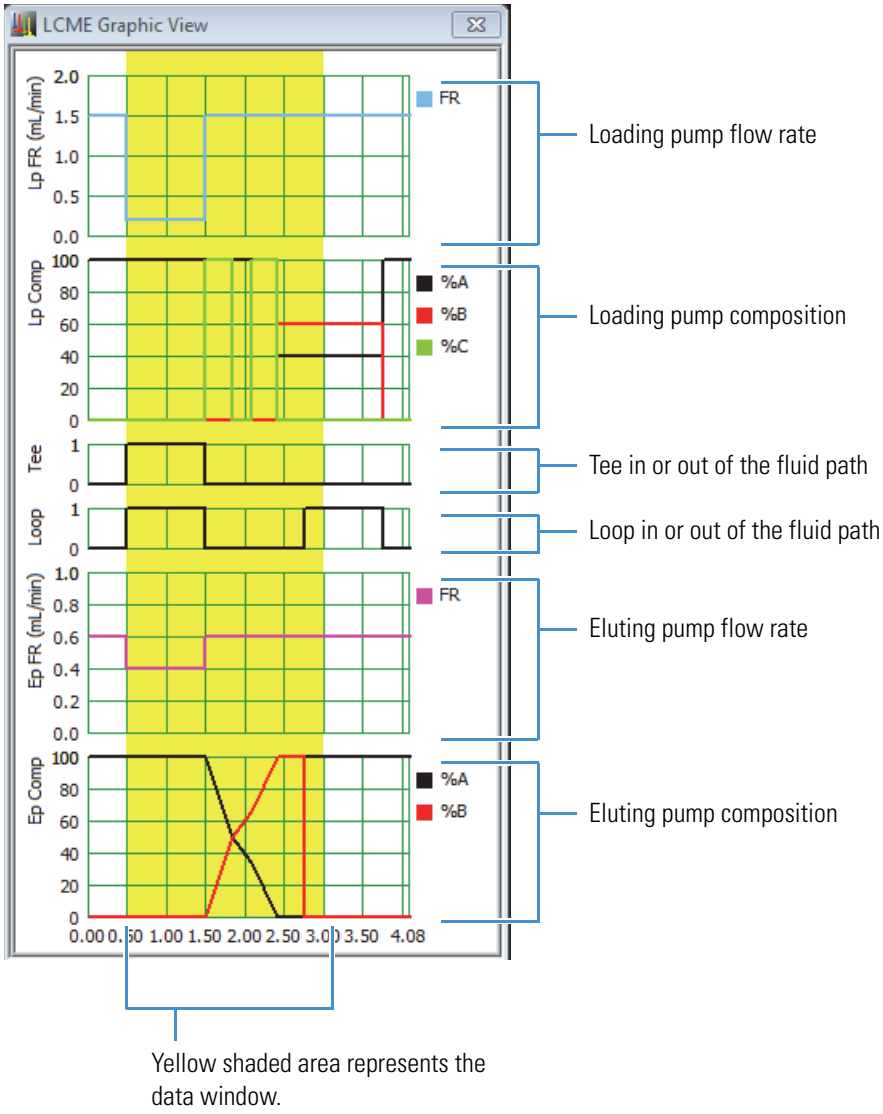
With the method graph, you can see the method component changes in relation to the method timing.

❖ To view a graph of the LC method

In the LC Method Editor window, choose **Tools > Graph Display**.

The method graph opens (Figure 74).

Figure 74. Graphic view of the method



About Changing the LC Method Configuration

Do not change the settings in the Method Configuration window. The boxes in this window must appear with the settings shown in Table 30.

Table 30. LC method configuration options (Sheet 1 of 2)

Parameter	Required setting
Plumbing Mode	Focus Mode - Prelude Technical
HTLC Style	This box appears with a check mark to indicate that the method uses the TurboFlow column.

Table 30. LC method configuration options (Sheet 2 of 2)

Parameter	Required setting
Loading Pump	Tertiary
Eluting Pump	Binary

Changing the LC Method Editor Options

Edit the LC Method Editor options if you want to change any of the following editing features for all LC methods.

- Change the time format that appears in the LC Method Editor.
- Change the headings that appear in the LC Method Editor.
- Select—or clear—the **Sync Data Window to MS** check box.

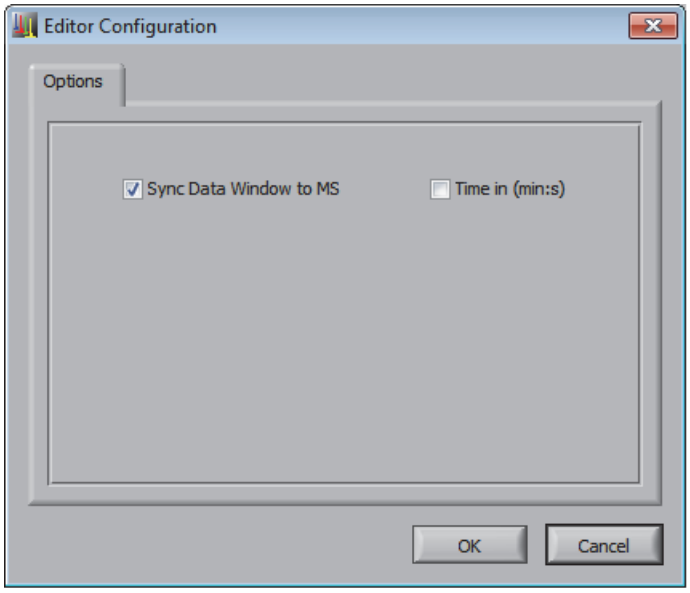
Changes that you make in the Editor Configuration dialog box affect all LC methods.

❖ To change the LC Method Editor options

1. In the LC Method Editor, choose **Tools > Preferences**.

   The Editor Configuration dialog box opens.

   Figure 75. Editor Configuration dialog box

2. Make entries and selections as shown in Table 31.

Table 31. Editor Configuration dialog box parameters

Parameter	Description
Sync Data Window to MS	Synchronizes the LC method to the detector run time if it is supported by the configured detector device driver. You must manually synchronize the LC method to the detector if its driver does not support the Sync Data Window to MS feature. For more information, see "Assigning the Data Window" on page 112.
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.

LC Method Step Table Columns

Table 32 describes the columns in the method step table.

Note The columns that appear in the LC method step table depend on the settings entered in the LC Method Configuration window. Table 32 describes columns that appear in the LC method step table when you select typical LC method configurations. See "Changing the LC Method Editor Options" on page 120.

Table 32. Method step table headings (Sheet 1 of 2)

Heading	Description
Step	Indicates the step number.
Start	Starting time for the step (minutes/decimal minutes)
Len	Length of the step (seconds)
Flow (appears blue)	Flow rate of the loading pump
Loop	Loop valve position (In or Out), determined by the position of Valve A This column appears in the LC method table.
Tee	T valve position (In or Out), determined by the position of Valve B This column appears in the LC method table.

Table 32. Method step table headings (Sheet 2 of 2)

Heading	Description
Flow (appears in pink)	Flow rate of the elution pump
Grad	Type of gradient used: Step or Ramp. Step means that the flow rate and composition change immediately to the desired value. Ramp means that the flow rate and composition change gradually over the length of the step to the desired value.
%A, %B, %C	Composition of the mobile phase. Columns for %A, %B, and %C, appear in the method step table when selected in the LC Method Configuration window. The loading pump information appears in the blue area of the table, and eluting pump information appears in the pink area of the table.
Comments	Add a 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 fill the transfer loop, you vary the percentage of the solvent that fills the loop each time.

Add a method variable to the instrument 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 you want to vary the component, 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.

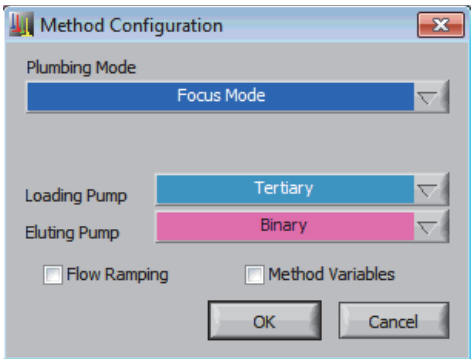
Adding Method Variables to an LC Method

❖ **To add a method variable to an LC method**

1. Open the LC Method Editor.
2. Choose **Edit > Method Configuration**.

The Method Configuration dialog box appears.

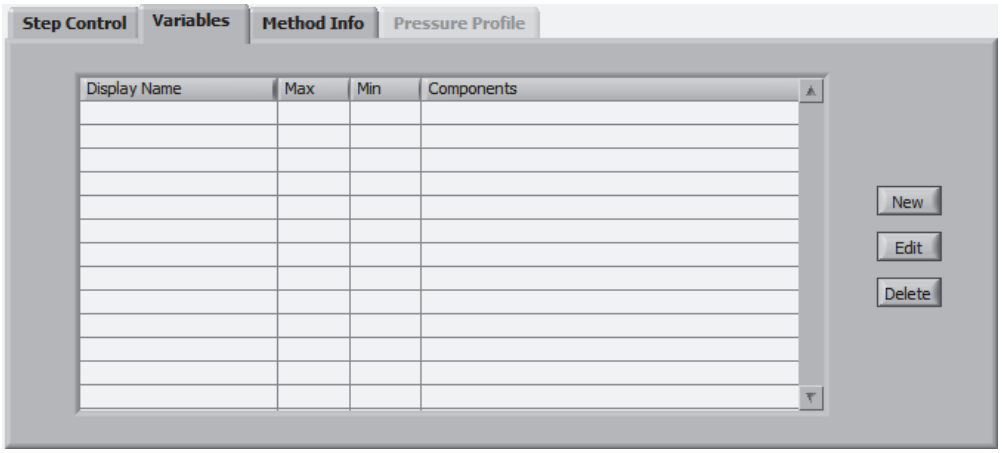
Figure 76. Method Configuration dialog box



3. Select the **Method Variables** check box, which activates the Method Variables tab.
4. Open the LC method where you want to add a variable.
5. In the LC Method Editor window, click the **Variables** tab.

   The Variables page opens (see Figure 77).

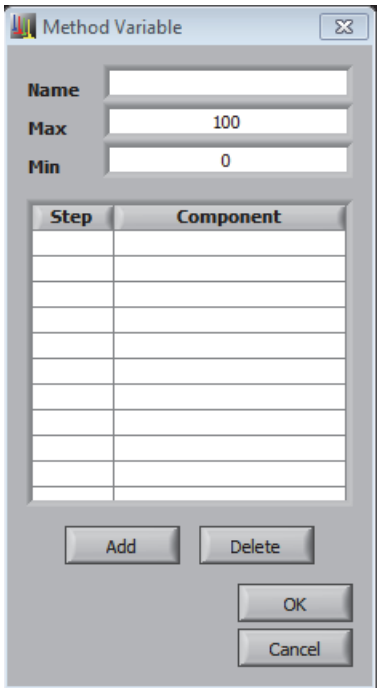
Figure 77. Variables page



6. Click **New**.

   The Method Variable dialog box opens.

   Figure 78. Method Variable dialog box



7. In the Name box, type a name that identifies the method variable.
8. 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**.
9. 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**.
10. Click **Add**.

    A default step number and method component appear.

11. Select the step number and type the step number that you want to change. For example, if you want to use a variable to evaluate the transfer loop contents, type **4** (for the pump filling the loop in the fourth step).
12. Click the default method component.

    A list of method components appears. Select the method component that you want to change. For example, if you want to change the transfer loop contents, and the organic resides on the B channel for the loading pump, select **Loading B**.

13. If you want variables for additional steps, repeat step 10 through step 12. For example, if you are changing the eluting mobile phase composition for an isocratic method, include all the relevant steps.

14. Click **OK**.

    The Method Variables dialog box closes and the method variable that you entered appears on the Variables page.

15. Choose **File > Save As**, type a name for the new LC method, and click **Save**.

16. If you are creating an LC method to vary mobile phase composition, see "Changing the Mobile Phase Composition 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 Using Method Variables."

❖ To enter values into the sample list

1. Create a sample list using the detector control application.

2. Create a custom column in the sample list for each method variable that you created using the detector control application. Name the column the same name as it appears in the Method Variable dialog box.

3. In the sample list, enter the values that you want to use for each sample. See Figure 79.

Figure 79. 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

Changing the Mobile Phase Composition Using Method Variables

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

❖ To change the mobile phase composition using method variables

1. Open the LC Method Editor for the LC method that you want to edit. See "Accessing the LC Method Editor" on page 100.

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 and select the pump channel that you want to change, for example, **Loading B**. Enter minimum and maximum values and a method variable name. See "Adding Method Variables to an LC Method" on page 122.

   Note If you want to vary the proportions of more than one channel (other than Channel A) for a sample, for example, Channels B and C, create a method variable for each channel that you want to vary.

4. In the sample list, create a custom column for each method variable you created. See "Entering Values into the Sample List" on page 125.
5. For each sample, enter the new percentage value of the channel that you want to change.
6. If you have more than one method variable column for a sample, verify that the total value in the method variable columns does not exceed 100 for any pump.

When the method runs, the value of A automatically decreases as the value of the channel selected in the method variable increases.

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

Rules for Changing Mobile Phase Composition

The Prelude MD 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, and C) must equal 100 for each of the loading and eluting pumps.
- When you increase a solvent percentage through the Method Variable dialog box, the LC Method Editor window, or the Direct Control window, the application changes the solvent percentage to the specified value and reduces the solvent A percentage by the same amount to maintain a total pump percentage of 100.
- If the application reduces solvent A to zero, and the total pump percentage is still greater than 100, the application reduces solvent B by the overage.
- The application reduces the solvent in order: A, B, and C.
- When you decrease a pump channel percentage, the application decreases the solvent to the specified value and increases the percentage of solvent A by the same amount.

In the Method Variable dialog box, the application changes the solvent proportions based on the value specified in the method variable column for each sample in the sample list. For examples, see Table 33.

Table 33. Examples of mobile phase composition changes due to method variables

Example	Percentage of solvents dispensed
The LC method indicates 100% Loading A. The method variable specifies Loading C as the variable. The value in the method variable column in the Batch Editor window indicates 25.	The pumps dispense 75% Loading A and 25% Loading C.
The LC method indicates 10% Loading A and 90% Loading B. The method variable specifies Loading C as the variable. The method variable column in the Batch Editor window indicates 25.	The pumps dispense 0% Loading A, 75% Loading B, and 25% Loading C.
The LC method indicates 20% Loading A and 80% Loading C. The method variable specifies Loading C as the variable. The value in the method variable column in the Batch Editor window indicates 40.	The pumps dispense 60% Loading A and 40% Loading C.

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 MD application compares the pump pressures of the currently running method to the pressures in the stored profile. The 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. To be most effective, 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 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.

Follow these procedures:

❖ To establish a pressure profile

1. In the LC Method Editor, choose **Tools > Pressure Profile > Add Profile**.

   The Select Profile Source window opens showing a list of files.

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

   The Profile Select dialog box opens.

   Note Choose a sequence that was run using the same method and solvent conditions as the method that you are assigning the profile to.

   The sequence files (.tslx) appear in the sequence 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 application averages the pump pressure values.

4. Click **OK**.

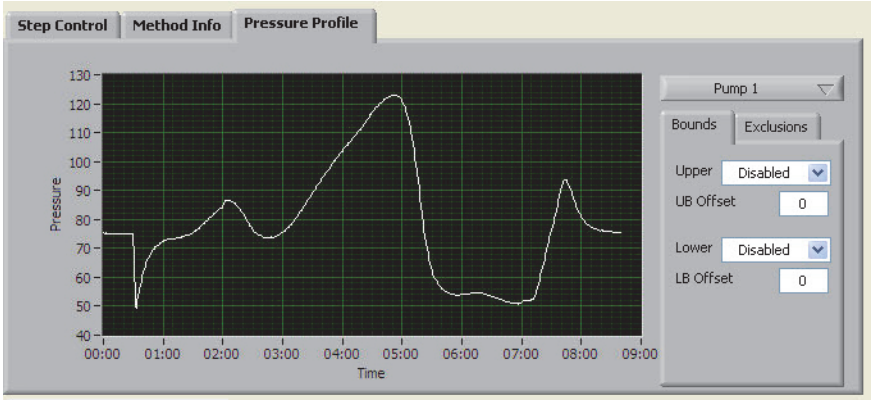
   The pressure profile opens on the Pressure Profile page.

❖ To view the pressure profile and set limits

1. In the LC Method Editor, click the **Pressure Profile** tab.

   The graph shows the pressure profile you assigned to this method (see Figure 80).

Figure 80. Pressure Profile page



2. Select or edit any of the following limits.

Table 34. Pressure Profile page limits

Limit	Description
Pump 1/Pump 2	Do one of the following: • To view or set limits for the loading pump, click **Pump 1**. • To view or set limits for the eluting pump, click **Pump 2**. The graph shows the pressure profile for the selected pump. The options you select on this page affect the selected pump.
Upper (visible if you click the Bounds tab)	Select one of the following options: • **Disabled:** Take no action taken 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 systems with pressures that exceed the upper limit. The flagged LC system 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 higher than the profile value are considered outside the limit. Action taken depends on the option selected in the Upper list.
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 systems with pressures that exceed the lower limit. The flagged LC system 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 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

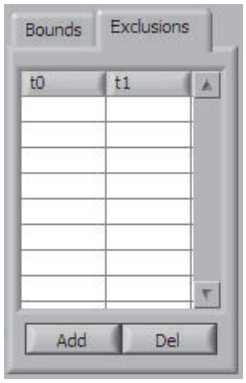
Tip You can assign time segments within the method when the profile limits do not apply. For example, you might want to exclude the moments when a valve change occurs to avoid flagging the system or sample unnecessarily.

1. In the LC Method Editor, click the **Pressure Profile** tab.

   The Pressure Profile page opens.

2. Click the **Exclusions** tab to open the Exclusions table.

   Figure 81. Exclusions tab

3. Click **Add**.

   Values appear in the Start and End 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 Start column and type the time when you want to begin excluding. Select the value in the End 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

You might want to import an Aria OS LC method into an instrument method when you use the development methods. 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 MD**.

   The LC Method Editor opens.
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.

Note If the Aria OS method was developed using a different system type than your own, edit the method to accommodate your system hardware.

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 using this procedure. If you want to import the AS method portion of the instrument method, see "Importing the AS Method from an Instrument Method" on page 95. If you want to import the detector portion of the method, open the applicable method, save it 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 MD** to open the LC Method Editor.
3. Choose **Edit > Import from Inst Method (*.meth)**.

   IMPORTANT The Prelude prestart and temperature portions of the associated LC method are not imported.
4. Navigate to the instrument method with the LC information that you want to import, and select the method.

   The LC method information appears in your open method.

Setting the Heater Temperature in an Instrument Method

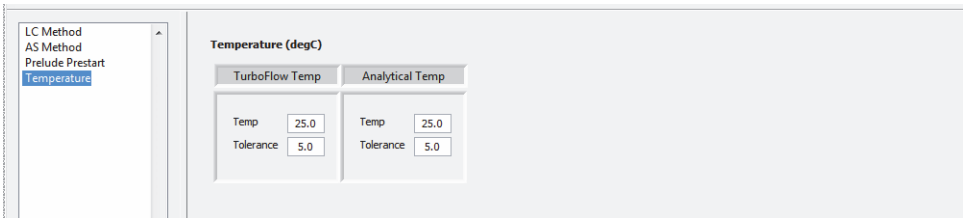
This topic describes how to set the optional column heater temperature in a method. If you want to manage the temperature control on demand, see "Controlling the Column Heater Temperature" on page 58.

❖ To set the column heater temperature in a method

1. Open the Temperature area of the instrument method by doing the following:
   a. Open the LC Method Editor for the instrument method where you want to add temperature control. See "Accessing the LC Method Editor" on page 100.
   b. In the left pane, click **Temperature**.

      The Temperature options appear.

      Figure 82. Temperature options in the instrument method showing two heaters

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

4. Repeat step 2 and step 3 for the second heater.

11

Developing a TurboFlow Method

These topics describe how to optimize the conditions of each method step in the TurboFlow method. It suggests experiments to perform and variables to change to determine the optimal chromatography conditions for your analytes.

Contents

Preparing for TurboFlow Method Development

Consider the following suggestions before performing the experiments in these topics:

- Use aqueous samples spiked with analyte to perform the procedures described in these topics. Use a concentration of analyte that produces an acceptable response (peak height or area counts) using a 10 microliter sample volume.
- A TurboFlow LC method requires at least five steps to complete the TurboFlow and HPLC separations. Most TurboFlow methods contain additional steps for improved washing and separations, which you can add after you optimize these five key steps:
  - Step 1: Loading step
  - Step 2: Transfer step
  - Step 3: Eluting step
  - Step 4: Loop Filling step
  - Step 5: Equilibrating step

  For more information on these five steps, see "TurboFlow Methods" on page 68.
- For a summary of the method development experiments and workflow, see Table 35.
- For a list of common mobile phases used in TurboFlow methods, see Table 36.
- For examples of LC method parameters, open the Default_TX_DLW.meth instrument method using the detector control application.

Table 35. Overview of method development protocol (Sheet 1 of 2)

Method step	Step component	Values to evaluate	Optimal values
Step 1: Loading step	Determine the optimal TurboFlow column.	Choose columns based on column chemistry.	Choose the column and mobile phase combination that provides the greatest peak at transfer step, the smallest peak at loading step, and the least amount of carryover.
	Determine the optimal loading (aqueous) mobile phase.	pH 3, 6, and 8	
Step 2: Transfer step	Determine the optimal percent organic solvent in the transfer loop.	20, 30, 40, 50, 60, 70, and 80% organic solvent	Choose the lowest percent organic solvent that provides complete transfer (no peak in the wash step, greatest peak in the transfer step).
	Record data on transfer step timing as a function of the loading pump flow rate.	0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, and 0.8 mL/min	Use this data later to determine the transfer step duration after you determine the loading pump flow rate.
	Determine the optimal analytical column.	Choose columns based on column chemistry, and that can withstand a flow rate up to 0.8 mL/min.	Choose the column that provides the best separation of analytes.
	Determine the optimal transfer dilution.	Loading/eluting pump flow rate combinations: • 0.1/0.7 mL/min • 0.2/0.6 mL/min • 0.3/0.5 mL/min • 0.4/0.4 mL/min	Choose the eluting flow rate combination that provides complete transfer (peak during the eluting step, no peak during the transfer step).

Table 35. Overview of method development protocol (Sheet 2 of 2)

Method step	Step component	Values to evaluate	Optimal values
Step 3: Eluting step	Determine the optimal elution ramp starting and ending solvent strength.	Starting/ending organic percentage combinations: • 5/50 • 5/75 • 5/95 • 10/50 • 10/75 • 10/95 • 20/50 • 20/75 • 20/95 • 30/50 • 30/75 • 30/95	Choose the eluting starting and ending mobile phase compositions that provide the best peak shape.
Step 4: Loop-filling step	Optimize the loop mobile phase as part of the transfer step (step 2), which is when the loop mobile phase is used.		
Step 5: Equilibrating step	Determine the length of the equilibrating step.	Monitor the pump pressure at the start and end of the method.	If the starting and ending pump pressures are not equal, increase the length of the equilibrating step.

Suggested Mobile Phases for Developing TurboFlow Methods

Table 36. Suggested mobile phases for TurboFlow methods (Sheet 1 of 2)

Mobile phase	Purpose in methods	Recommendation	Location on system
Loading pump aqueous mobile phase	Loads the analytes onto the TurboFlow column.	Choose three aqueous loading mobile phases to evaluate. The following are recommended: • 0.1% formic acid in water (1 mL formic acid/L distilled H_2O) (pH 3) • 10 mM ammonium acetate in water (0.77g ammonium acetate/L H_2O) (pH 6) • 10 mM ammonium acetate in water (0.77g ammonium acetate/L H_2O) (Adjust to pH 8 using ammonium hydroxide.)	While you are optimizing the loading step, install two of the three aqueous mobile phases on the loading pump: one as solvent A and the other as solvent B. Once you have run solvents A and B, you can replace a solvent with the third aqueous mobile phase.
Loading pump organic mobile phase	Washes the TurboFlow column and mixes with loading aqueous mobile phase to fill the loop.	Choose one of the following: • Acetonitrile • Methanol • Acetonitrile/Methanol 50:50	Install this mobile phase on the loading pump as solvent C while optimizing the loading step. Then move it to solvent B.
Loading pump organic cocktail (Do not use while evaluating the loading step.)	(Optional) Use as a strong solvent to wash the TurboFlow column. Do not use this mobile phase to fill the loop. Do not use this mobile phase to fill the transfer loop.	Acetonitrile/isopropanol/acetone 45:45:10	Install this mobile phase on the loading pump as solvent C only after you have optimized the loading step.

Table 36. Suggested mobile phases for TurboFlow methods (Sheet 2 of 2)

Mobile phase	Purpose in methods	Recommendation	Location on system
Eluting pump aqueous mobile phase	Dilutes the loop contents and eluting mobile phase.	Aqueous mobile phase pH 3	Install this mobile phase on the eluting pump as solvent A.
Eluting pump organic mobile phase	Elutes the analytes off the analytical column.	Choose one of the following: • Acetonitrile • Methanol • Acetonitrile/Methanol 50:50	Install this mobile phase on the eluting pump as solvent B.

Optimizing the Loading Step

Optimize the loading step to determine the optimal TurboFlow column and loading mobile phase. The loading step is step 1 in Table 13.

During the loading step, the autosampler injects the sample into the loading pump mobile phase flow. The TurboFlow column retains the analytes and other small molecules, and the large sample molecules flow to waste.

If the TurboFlow column cannot retain your analytes well, the analytes flow to waste during this step. If the column retains them too tightly, they flow to waste during the eluting step, when the loading pump washes the TurboFlow column. This topic describes how to choose the TurboFlow column and loading mobile phase combination that provides optimal retention of your analyte.

Follow these procedures:

- To prepare the system for optimizing the loading step
- To determine the required length of tubing
- To evaluate the data for optimizing the loading step

❖ To prepare the system for optimizing the loading step

1. Choose which TurboFlow column you want to evaluate based on column geometry and chemistry, and install the column. For a description of TurboFlow column chemistries and sizes, see Appendix A, "Columns."
2. Prepare the following mobile phases and install them onto the system in the pump location indicated in "Suggested Mobile Phases for Developing TurboFlow Methods" on page 137.
   a. Two aqueous mobile phases installed as loading pump solvent A and loading pump solvent B (one for each method)
   b. One organic mobile phase installed as loading pump solvent C
   c. One aqueous mobile phase installed as eluting pump solvent A
   d. One organic mobile phase installed as eluting solvent B
3. Install the TurboFlow column that you want to evaluate onto the instrument. See "Replacing the TurboFlow Column" on page 189.
4. Remove the analytical column and install a T union in its place. See "Replacing the Analytical Column" on page 191.

   Use a T to split the flow so that the eluting mobile phase flows to both the detector and to waste. This sends the analytes directly to the detector as they elute from the TurboFlow column so that you can detect the elution of the analytes at all steps in the method. Splitting the flow to the detector and to waste reduces the volume of fluid flowing into the detector.

Note When you split the flow, you can control the flow rate sent to each direction by altering the diameter or length of the tubing. If the length and diameter of the tubing going to the detector and to waste are the same, the flow rates are the same. If you increase the length or decrease the diameter of one tubing, the flow rate decreases due to the higher backpressure.

❖ **To determine the required length of tubing**

1. Determine the flow rate that you want to use to send the fluid to waste based on the capacity of the source and the total flow rate. For example, if the total flow rate equals 2 mL/min and the source capacity equals 0.5 mL/min, then you want 1.5 mL/min to go to waste:

   (2.0 mL/min) – (0.5mL/min) = (1.5 mL/min)

2. Calculate the following ratio, where FR equals flow rate:

   FR to waste ÷ FR to detector. For example, (1.5 mL/min) ÷ (0.5 mL/min) = 3/1

   In this example, make the length of tubing that goes to the detector three times longer than the length of tubing that goes to waste.

3. Using the detector control application, open the detector instrument method that you want to use for creating a TurboFlow method.

4. From the Prelude MD DVD, import the AS method portion of the Default_TX_DLW.meth instrument method into the instrument method that you want to use for method development. See "Importing the AS Method from an Instrument Method" on page 95.

5. Create an LC method for evaluating each mobile phase with the following specifications.

   - See "Suggested Mobile Phases for Developing TurboFlow Methods" on page 137.
   - Use a loading pump flow rate of 1.5 mL/min in the loading step (method step 1).
   - Ensure that the Tee is in line with the fluid path in each method step so that you can measure the eluting analyte throughout the LC method.
   - Use 50% organic solvent in the eluting mobile phase.
   - Fill the loop with 80% organic solvent.
   - Type **100** in the %A column.

6. Prepare standards and blanks and install them onto the appropriate autosampler tray.

   Prepare standards in as much aqueous solution as possible with a range of concentrations. Use standards that result in an acceptable peak size with 10 microliter injections. Include one blank sample.

Create and submit a sample list for each LC method, and start the run. Include 10 blank injections in the beginning of the sample list to condition the column to the new pH. Include one blank injection at the end of the sample list to test carryover. Schedule standards as unknowns.

7. To evaluate other TurboFlow columns, remove the column, install a new one, and rerun the same samples and batches.
8. To evaluate other loading mobile phases, replace the loading A and B solvent bottles. See "Changing the Solvent Bottles" on page 186.

❖ To evaluate the data for optimizing the loading step

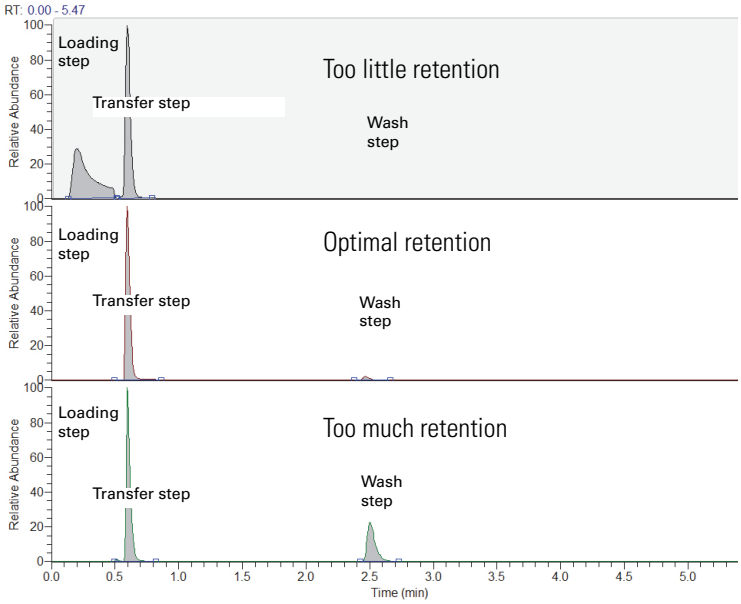
With the optimal column-mobile phase combination, the TurboFlow column retains the analytes during the loading step, and a peak appears during the transfer step. No peak appears during the wash or eluting steps.

Consider the following:

- Results that show a peak during the loading step of the method indicate that the TurboFlow column poorly retains the analytes.
- Results that show a peak during the Wash step indicate that the TurboFlow column retains the analytes too tightly.
- Results that show a peak during the transfer step, and no peak or a very small peak during the loading and wash steps, show optimal retention.
- Any carryover for the peak on blank samples.

Figure 83 shows chromatograms with too little, optimal, and too much retention in a Focus Mode method.

Figure 83. Retention of analytes on the TurboFlow column in a Focus Mode method



The first chromatogram, labeled "Too little retention," shows that the analytes elute during the loading step. This indicates that the TurboFlow column does not retain the analytes well.

The second chromatogram, labeled "Optimal retention," shows that the analytes elute during the transfer step in this method, which is ideal. In a complete method, the analytical column is present, and the analytes transfer from the TurboFlow column to the analytical column in this step.

The third chromatogram, labeled "Too much retention," shows that many of the analytes elute during the wash step. This indicates that the TurboFlow column retains the analytes too tightly. You can expect to observe some elution during the wash.

If the data shows too little or too much retention, or shows carryover in the blank, repeat the run using a different TurboFlow column or a loading mobile phase with a different pH. Try different combinations of columns and loading mobile phase until you achieve data that shows optimal retention.

Optimizing the Transfer Step

The transfer step is step 2 in Table 13. Optimize the transfer step to determine the following:

- The optimal loop contents for transferring the analytes from the TurboFlow column to the analytical column
- The duration of the transfer as it relates to the loading pump flow rate
- The optimal dilution of the loop contents for loading analytes onto the analytical column
- The optimal analytical column

In the transfer step, the analytes elute from the TurboFlow column and transfer to the analytical column by the contents of the transfer loop. If the transfer loop mobile phase is too weak, the analytes remain on the TurboFlow column when they should elute from it. If the mobile phase is too strong, the analytes pass through the analytical column when they should remain on it.

If the transfer loop solvent is strong enough, the analytes elute from the TurboFlow column and reach the Tee. At the Tee, the eluting pump aqueous mobile phase dilutes the loop solvent strength before the analytes enter the analytical column.

If the eluting pump mobile phase does not sufficiently dilute the loop solvent, the analytes flow to waste during the transfer step.

This topic describes how to choose the optimal solvent strength of the loop contents and how to optimize the dilution of the solvent strength before the analytes enter the analytical column.

Determining the Optimal Loop Contents

Follow these procedures to optimize the organic concentration of the loop contents. The loading pump fills the loop in step 4 (see Table 13), and the method uses the loop contents in step 2.

Follow these procedures:

- To set up the run for determining the optimal loop contents
- To evaluate the data for optimizing the loading step

❖ To set up the run for determining the optimal loop contents

1. Create an LC method for evaluating the loading step.

   Ensure your LC method has a method variable that varies the percentages of the solvents used to fill the loop in the loop-filling step (method step 4). See "Adding Method Variables to an LC Method" on page 122.

2. Verify that the duration and flow rate in the loop-filling step (method step 4) are sufficient to fill the loop.

Note

- In general, 15 seconds for 1ml/min is sufficient to fill the loop. Increase this value for lower flow rates. For better accuracy, calculate the value.
- To calculate the time required to fill the loop, use the following equation:

  (Total volume up to and including valve A) ÷ (loading pump flow rate in step 1)
- To calculate the total volume including valve A, use the following equation:

  (The pump's dwell volume) + (the transfer-loop volume) + (the injection-valve sample-loop volume) + (the connective tubing volume)
- To calculate the volume of the connective tubing, use the following equation:

  Volume in tubing = π (½ in. ID tubing)^2 (length of tubing)

3. Install the mobile phases that you want to use to fill the loop. See "Suggested Mobile Phases for Developing TurboFlow Methods" on page 137.
4. Install the optimal loading aqueous mobile phase onto the loading pump solvent A.
5. Install the optimal TurboFlow column onto valve A.
6. Create a sample list for evaluating the loop contents.

   Add a custom column for entering the loop-contents variables. See "To enter values into the sample list" on page 125. Include **20**, **30**, **40**, **50**, **60**, **70**, and **80**% organic. For a sample list example, see Figure 84.

Figure 84. Sample list for evaluating the loop contents

	Sample ID	SampleName	Position	Inj Vol	Sample Type	Comment	Inst Meth	Path	File Name	LC_loop_conc
1	Sample001	loop80 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			80
2	Sample002	loop80std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			80
3	Sample003	loop80std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			80
4	Sample004	loop80 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			80
5	Sample005	loop70 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			70
6	Sample006	loop70std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			70
7	Sample007	loop70std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			70
8	Sample008	loop70 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			70
9	Sample009	loop60 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			60
10	Sample010	loop60std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			60
11	Sample011	loop60std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			60
12	Sample012	loop60 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			60
13	Sample013	loop50 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			50
14	Sample014	loop50std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			50
15	Sample015	loop50std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			50
16	Sample016	loop50 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			50
17	Sample017	loop40 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			40
18	Sample018	loop40std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			40
19	Sample019	loop40std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			40
20	Sample020	loop40 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			40
21	Sample021	loop30 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			30
22	Sample022	loop30std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			30
23	Sample023	loop30std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			30
24	Sample024	loop30 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			30
25	Sample025	loop20 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			20
26	Sample026	loop20std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			20
27	Sample027	loop20std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\meth			20
28	Sample028	loop20 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\meth			20
*				0.00						

Note Because the loading pump fills the loop at the end of the method for the next injection, the first injection of each set uses the loop percentage from the previous set.

❖ To evaluate the data for determining the optimal loop contents

1. Using the detector control application, view the data from the experiment to evaluate the loop contents.
2. Determine the lowest solvent strength that resulted in complete transfer.

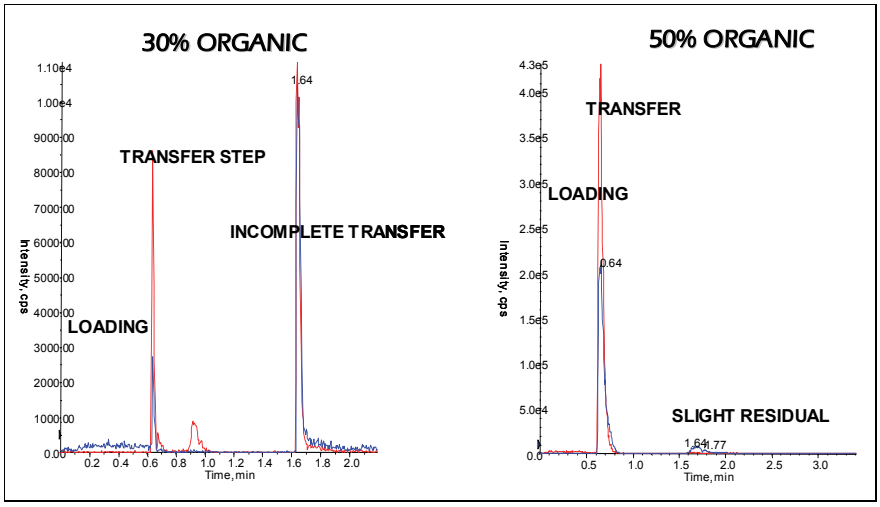
   The best loop mobile phase maximizes the elution during the transfer step and minimizes the elution during the wash step.

Note Reverse-phase TurboFlow columns usually need 30–80% organic solvent to achieve the necessary recovery of the analyte and optimal focusing on the analytical column. Occasionally, a method requires 100% organic mobile phase. However, to optimize selectivity, choose the lowest possible percentage of organic mobile phase.

Figure 86 shows an example with 30% and 50% organic mobile phase in the transfer loop. When the loop contains 30% organic mobile phase, many of the analyte molecules elute during the wash step when the mobile phase flowing through the column is higher than the loop contents. When the loop contains 50% organic mobile phase, the analyte molecules elute during the transfer step. In this example, the optimal loop mobile phase is 50% organic.

Although a higher organic mobile phase usually results in a more complete transfer, a low organic mobile phase in the loop requires less dilution in the transfer step and, therefore, optimization of the transfer step becomes easier. To optimize selectivity, choose the lowest possible percentage of organic mobile phase that results in complete transfer.

Figure 85. Example showing 30 and 50% organic mobile phase



Choosing the Analytical Column

The analytes are loaded onto the analytical column in method step 2, and they elute in method step 3 (see Table 13). Analytical columns come in a variety of chemistries and sizes. Choose an analytical column as you would choose one for an HPLC method, but consider the flow rate requirements for use in a TurboFlow method. Choose an analytical column that can withstand a flow rate up to 0.8 mL/min. Also consider the pump pressure limits of your system. The Prelude instrument can withstand pressures of at least 1000 bar, and can therefore accommodate analytical columns designed for UHPLC. For a partial list of suggested columns, see "Analytical Column Description" on page 196.

This topic describes how to evaluate an analytical column by injecting the sample directly onto the analytical column.

❖ To evaluate one or more analytical columns

1. Install the analytical column that you want to evaluate on Valve B between port 4 and the detector.
2. Create an LC method.
3. In the AS method, change the injector to the LX injector for each step that contains the Injector option.

   With this change, the autosampler bypasses the TurboFlow column and injects the sample onto the analytical column.
4. Choose **File > Save As**, type a new name, and click **Save**.
5. Create a sample list for evaluating the analytical column, and start the run.
6. Choose the analytical column that produces the best separation of analytes, and then install the column onto the system.

Evaluating the Transfer Step Timing

Determine the length of time required to transfer the analytes from the TurboFlow column to the analytical column using various loading pump flow rates.

In the transfer step, the loading pump pushes the loop mobile phase through the TurboFlow column, which causes the analytes to transfer to the analytical column. The loading pump flow rate affects the time required for the transfer. This procedure evaluates the transfer time using various loading pump flow rates. Use this data to determine the time required for the transfer if you change the loading pump flow rate later on during method development.

❖ To set up the run for evaluating the transfer step timing

1. Create an LC method for evaluating the transfer step timing.

   Verify that the LC method contains a method variable for changing the loading pump flow rate in the transfer step (step 2).

2. Verify that the loading pump flow rates are appropriate for the TurboFlow column diameter you selected, and save the method.

3. Change the loading pump composition in the loop-filling step (step 4) to fill the loop at the optimal solvent strength, which you determined in "Determining the Optimal Loop Contents" on page 143.

4. Create a sample list.

   Include a custom column for entering the loading pump flow rate variables. Include **0.1**, **0.2**, **0.3**, **0.4**, **0.5**, **0.6**, **0.7**, and **0.8**. For a sample list example, see Figure 86.

   Figure 86. Sample list for changing the loading pump flow rate in the transfer step (step 2)

	Sample ID	SampleName	Position	Inj Vol	Sample Type	Comment	Inst Meth	Path	File Name	LC_transfer
1	Sample001	trans0.1 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.1
2	Sample002	trans0.1std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.1
3	Sample003	trans0.1std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.1
4	Sample004	trans0.1 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.1
5	Sample005	trans0.2 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.2
6	Sample006	trans0.2std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.2
7	Sample007	trans0.2std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.2
8	Sample008	trans0.2 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.2
9	Sample009	trans0.3 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.3
10	Sample010	trans0.3std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.3
11	Sample011	trans0.3std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.3
12	Sample012	trans0.3 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.3
13	Sample013	trans0.4 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.4
14	Sample014	trans0.4std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.4
15	Sample015	trans0.4std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.4
16	Sample016	trans0.4 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.4
17	Sample017	trans0.5 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.5
18	Sample018	trans0.5std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.5
19	Sample019	trans0.5std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.5
20	Sample020	trans0.5 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.5
21	Sample021	trans0.6 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.6
22	Sample022	trans0.6std01	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.6
23	Sample023	trans0.6std02	Tray01:2	10.00	Std Update	N/C	C:\Xcalibur\r			0.6
24	Sample024	trans0.6 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.6
25	Sample025	trans0.7 blank	Tray01:1	10.00	Blank	N/C	C:\Xcalibur\r			0.7

5. Start the run.

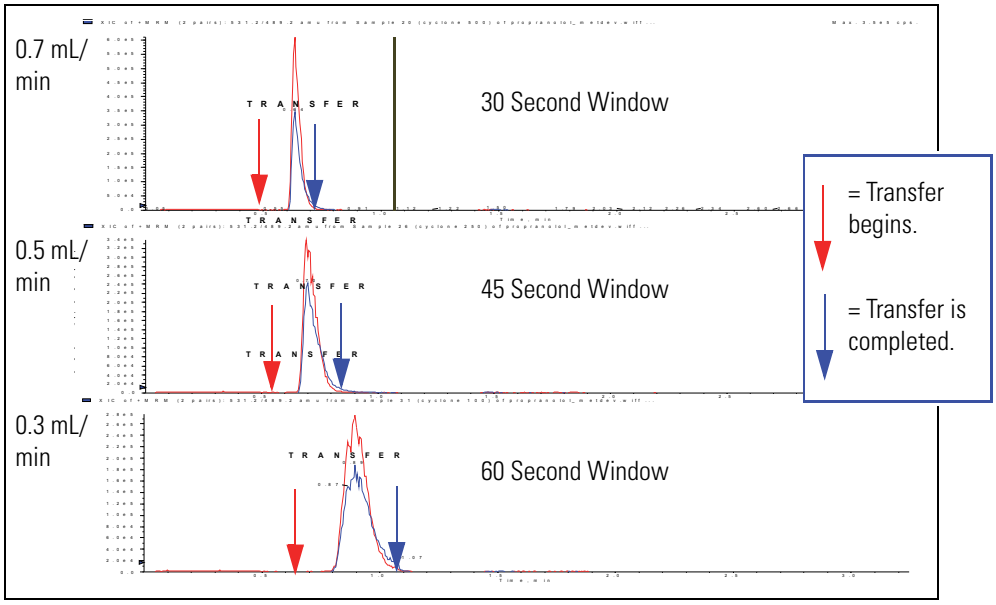
❖ To evaluate the data for evaluating the transfer step timing

1. Observe the transfer flow-rate data.

2. Use this data to determine the transfer step timing if you change the loading pump flow rate in the transfer step. See Optimizing the Transfer Dilution Ratio.

   Figure 87 shows the various effects of transfer flow rates.

Figure 87. Effects of flow rate on transfer timing



The faster flow rate (top chromatogram) delivers a tighter band of analytes to the analytical column. As the flow rate decreases (middle and bottom chromatograms), the band broadens and takes longer to transfer from the TurboFlow column. Since the instrument does not use an analytical column at this time, the peak shape can appear broad with fronting or tailing.

3. After performing the experiment in "Optimizing the Transfer Dilution Ratio," locate the data that you generated for the optimal loading pump flow rate.
4. Based on the peak in the chromatogram, note the end of the transfer, and record the total time required for the transfer to complete.

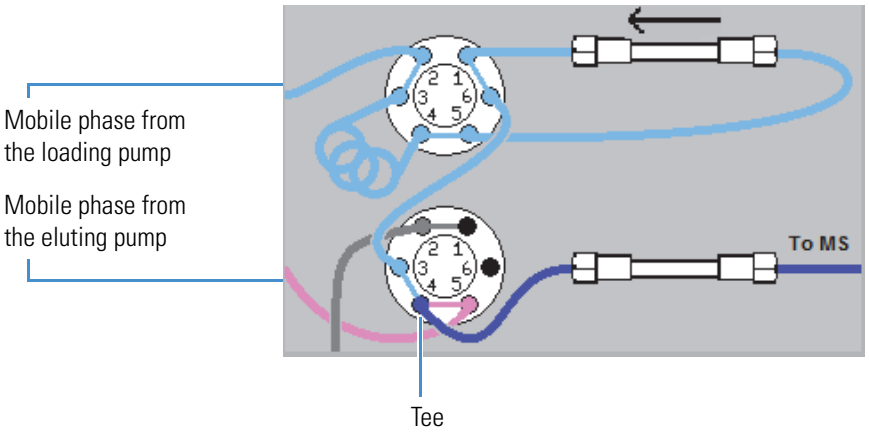
Optimizing the Transfer Dilution Ratio

Determine the optimal dilution of the solvent from the transfer loop. With the optimal dilution, the analytical column retains the analytes.

If the organic concentration of the mobile phase is too high, the analytical column cannot retain the analytes, which leads to breakthrough (peak during transfer), fronting (broad band on analytical column), or both.

In the transfer step, the Tee combines the aqueous eluting pump mobile phase with the organic loop mobile phase. This reduces the solvent strength of the flow into the analytical column. Figure 88 shows the plumbing and valve positions of the transfer step.

Figure 88. System plumbing showing the tee



The following factors affect the solvent strength as it enters the analytical column:

- The organic concentration of the loop contents

  Selecting a loop mobile phase with a high organic concentration requires a high dilution.

- The organic concentration of the eluting pump flow

  Decreasing the organic concentration of the eluting pump mobile phase decreases the solvent strength of the mobile phase entering the analytical column.

- The eluting pump flow rate

  Increasing the eluting pump flow rate in the transfer step decreases the solvent strength of the mobile phase entering the analytical column.

- The loading pump flow rate

  Decreasing the loading pump flow rate in the transfer step decreases the solvent strength of the mobile phase entering the analytical column.

To keep the total flow rate entering the analytical column the same for each run, this experiment varies the eluting and loading pump flow rate ratios. In the examples in Table 37, the eluting pump flow rate increases by 0.2 mL/min and the loading pump flow rate decreases by 0.2 mL/min, keeping the total flow rate constant.

Table 37. Example of pump flow rates during optimization of the transfer dilution

Eluting pump flow rate	Loading pump flow rate	Combined flow rate
0.4 mL/min	0.4 mL/min	0.8 mL/min
0.6 mL/min	0.2 mL/min	0.8 mL/min

This experiment also varies the eluting pump mobile phase composition. Run each flow rate ratio with each mobile phase composition so that you change only one variable at a time.

Note The combined flow rates of the eluting (aqueous) and loading (organic) pumps cannot exceed the capacity of the analytical column.

❖ To set up the run for optimizing the transfer dilution ratio

1. Verify that the instrument contains the optimal analytical column.
2. Disconnect the union and connect the tubing from the analytical column to the detector.
3. Open the instrument method that you used to change the transfer step loading pump flow rate.

   Note Do not open the instrument method for optimizing the analytical column because the AS method uses LX injections.

4. Create the LC method to evaluate the transfer dilution.
   a. Verify that the LC method contains three method variables, one for each of the following variables:
      - The loading pump flow rate
      - The eluting pump flow rate
      - The eluting pump mobile phase solvent strength
   b. Verify that the method uses a ramp elution.
5. Edit the flow rates if necessary, and save the method.
6. Create a sample list for evaluating the transfer dilution and the starting eluting pump mobile phase composition. Include three custom columns for entering the variables. See Figure 89.

   Figure 89. Sample list for evaluating the transfer dilution and starting eluting pump mobile phase composition

	Sample ID	SampleName	Position	Inj Vol	Sample Type	Comment	ist Me	Path	LC_Eluting_B	LC_dilution	LC_transfer
1	Sample001	0%B-blank	Tray01:1	10.00	Blank	N/C	C:\Xcal		0	0.1	0.7
2	Sample002	0.1:0.7std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		0	0.1	0.7
3	Sample003	0.2:0.6std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		0	0.2	0.6
4	Sample004	0.3:0.5std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		0	0.3	0.5
5	Sample005	0.4:0.4std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		0	0.4	0.4
6	Sample006	2%B-blank	Tray01:1	10.00	Blank	N/C	C:\Xcal		2	0.4	0.4
7	Sample007	0.1:0.7std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		2	0.1	0.7
8	Sample008	0.2:0.6std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		2	0.2	0.6
9	Sample009	0.3:0.5std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		2	0.3	0.5
10	Sample010	0.4:0.4std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		2	0.4	0.4
11	Sample011	5%B-blank	Tray01:1	10.00	Blank	N/C	C:\Xcal		5	0.4	0.4
12	Sample012	0.1:0.7std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		5	0.1	0.7
13	Sample013	0.2:0.6std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		5	0.2	0.6
14	Sample014	0.3:0.5std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		5	0.3	0.5
15	Sample015	0.4:0.4std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		5	0.4	0.4
16	Sample016	10%B-blank	Tray01:1	10.00	Blank	N/C	C:\Xcal		10	0.4	0.4
17	Sample017	0.1:0.7std	Tray01:2	10.00	Std Update	N/C	C:\Xcal		10	0.1	0.7

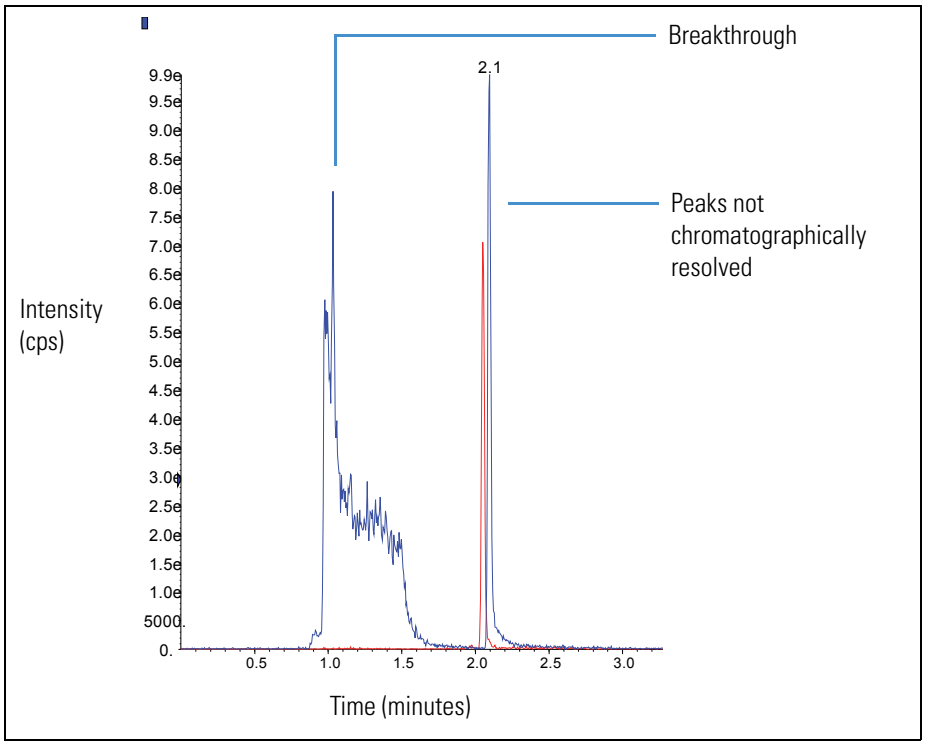
7. Start the run.

❖ To evaluate the data for optimizing the transfer dilution ratio

1. Observe the results from your experiment and identify the data that shows the best results.

   If the organic concentration of solvent flowing through the analytical column is too high, you see an early peak, representing the breakthrough. Figure 90 shows an example of a chromatogram with an early peak due to a high organic concentration.

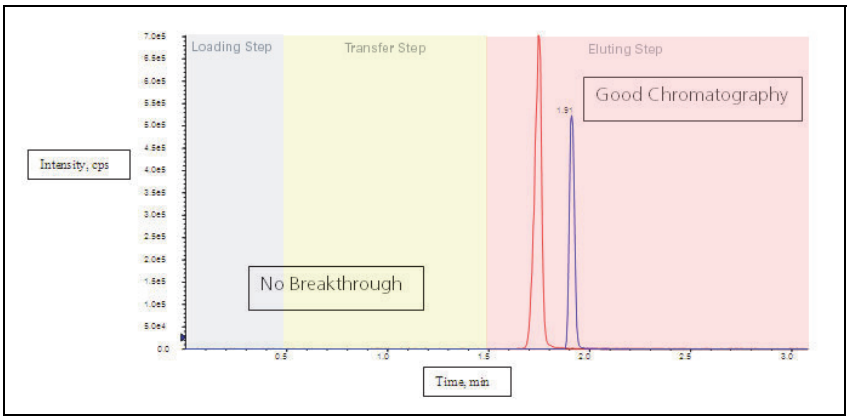
   Figure 90. Breakthrough due to insufficient dilution



   With the analytical column in place, a peak must not appear during the transfer step. A peak present during the transfer step indicates that the analytical column does not fully retain the analyte. The organic concentration of the transfer solvent is too high.

   The peak must be visible in the eluting step, which is when the analytes elute off the analytical column and flow to the detector. Figure 91 shows a chromatogram where analytes elute during the eluting step, with no breakthrough.

Figure 91. Analytes eluted from the analytical column during the eluting step showing good chromatography with no breakthrough



2. Record the following conditions for the run that generated the best data:
   - Transfer step loading pump flow rate
   - Transfer step eluting pump flow rate
   - Transfer step eluting pump solvent strength
3. Compare the peak in the data with the method timing to determine the length of time required to complete the transfer using the selected flow rate. Record this value.
4. If your results show fronting, breakthrough, or carryover, continue optimizing the transfer step. See "Additional Techniques for Optimizing the Transfer Dilution."
5. If your results show good chromatography, continue with "Optimizing the Eluting Step" on page 154.

Additional Techniques for Optimizing the Transfer Dilution

If you observe breakthrough, fronting, or carryover in the data for all combinations of the transfer dilution experiments, try the possible solution described in Table 38.

Table 38 describes unacceptable results that you might observe and possible solutions.

Table 38. Transfer dilution problems and possible solutions

Problem	Possible solution
Breakthrough (peak during transfer)	Increase the aqueous eluting pump flow and lower the loading pump flow by increments of 100 µL/min.
Fronting (broad band)	Increase the aqueous eluting pump flow by increments of 100 µL/min.
No breakthrough, good peak shape, but carryover	Increase the organic concentration of the loop by increments of 100 µL/min.

If you want to calculate the dilution that occurs during the transfer, use the following equation:

$[(\text{LP flow} \div \text{T flow}) \times \%B_{loop}] + [(\text{EP flow} \div \text{T flow}) \times \%B_{EP}] = \%$ organic solvent after Tee

Table 39 describes each component in the equation.

Table 39. Equation components

Equation component	Description
T flow	Total flow rate; the combined flow rate of the loading and eluting pumps during the transfer step
LP flow	Loading pump flow rate during the transfer step
EP flow	Eluting pump flow rate during the transfer step
$\%B_{loop}$	Percent organic of the loop contents
$\%B_{EP}$	Percent organic pumped by the eluting pump during transfer (often 0)

Once you have data with no breakthrough, fronting, or carryover, continue with "Optimizing the Eluting Step."

Optimizing the Eluting Step

The eluting step is step 3 in typical TurboFlow methods (see Table 13). During the eluting step, the analytes elute from the analytical column and flow to the detector for analysis. The speed at which the eluting mobile phase composition changes affects the quality of separation from other small sample molecules and among the analytes themselves.

This topic describes how to optimize the rate of the gradient of the eluting mobile phase composition during the eluting step by altering the starting and ending mobile phase compositions.

This experiment varies the eluting pump composition at the start of the ramp, while holding the final eluting pump concentration constant. It also varies the eluting step's final eluting pump organic concentration, while holding the starting concentration constant.

❖ To set up the run for optimizing the eluting step

1. Create the LC method for evaluating the separation.
2. Verify that the LC method contains two method variables for the percentage of organic solvent in the eluting mobile phase: one for the start of the elution and one for the end of the elution.

   When you select a ramp elution, assign the starting eluting mobile-phase conditions in the method step that occurs before the eluting step. To do this, create a new step after the Transfer step (step 2), and assign the starting eluting conditions to the new step 3.

   Assign the ending eluting step mobile phase conditions to the eluting step (now step 4). The mobile phase conditions during the elution gradually change from the starting mobile phase conditions to the ending mobile phase conditions. Therefore, create the variable for the starting eluting conditions for method step 3, and create the variable for the ending condition in method step 3.
3. In step 2 (transfer step), change the loading and eluting pump flow rates to the optimal dilution ratio that you determined in "Optimizing the Transfer Dilution Ratio" on page 148.
4. Verify that the duration of step 2 (transfer step), is sufficient for the transfer. To determine this, use the loading pump flow rate you entered in step 3 and the data from "Evaluating the Transfer Step Timing" on page 147.
5. Create a sample list for optimizing separation.

   Create two custom columns: one for the starting eluting mobile phase (step 3), and one for the ending eluting mobile phase (step 4). Name the column the same as you named the method variables. See Table 40.

Table 40. Percentages of organic solvent for starting and ending the elution

Eluting pump %B in step 3	Eluting pump %B in step 4
5	50
5	75
5	95
10	50
10	75
10	95
20	50
20	75
20	95
30	50
30	75
30	95

6. Start the run.
7. Evaluate the results and enter the optimal starting and ending mobile phase composition %B values.
8. Remove the method variables from the method.
9. Save the method using a new name.

Adding Optional Wash Steps

During the eluting step, the loading pump mobile phase washes the TurboFlow column, moving highly retained compounds to waste. If necessary, you can add wash steps to reduce carryover. Evaluate the blank data in the previous experiment and determine if you need to reduce the carryover.

To reduce carryover, add wash steps to the method after the transfer step. Use several wash steps to switch the loop in and out of the fluid path. The valve movement helps wash the TurboFlow column.

Optimizing the Equilibrating Step

The equilibrating step is the last step in typical TurboFlow methods (see Table 13). All method parameters in the Equilibrating step must be the same as those in the loading step, with the exception of the step duration. Ensure that the equilibrating step is long enough to return the TurboFlow and analytical columns to the loading conditions.

To verify that the equilibrating step duration is sufficient, view the pressure trace at the beginning and end of the method. If the pressures at the beginning and end of the method differ, or if pressure values trend up or down over several runs, lengthen the equilibrating step.

Entering Data Window Start Time and Duration

If you run both channels at the same time (cross-sequential optimization), calculate and enter the optimal data window start time for better throughput.

❖ To calculate the optimal data window start time and duration

1. Review the data from the previous experiments in method development and determine the time in the method at which the analytes begin to elute to the detector. Enter this time in the Start Data Window box in the LC Method Editor window.
2. Review the data from previous experiments to determine the end time for the elution of the analytes from the analytical column. Enter this time in the Data Window Length box in the LC Method Editor window. See "Assigning the Data Window" on page 112.
3. To allow for variations in retention time, expand the data window on each end. Start the data window several seconds before the actual start of the elution (the value you entered into the Start Data Window box in step 1), and lengthen by several seconds the actual transfer duration (the value you entered in the Data Window Length box in step 2).
4. Enter this new data window value into the detector portion of the instrument method.

Evaluating Matrix Effects

Test the method using sample matrix to determine the impact that the sample matrix has on the method data.

❖ To evaluate the method's ability to reduce sample matrix effects

1. Prepare a set of spiked matrix samples to match the neat aqueous standards that you used to develop the method. Use the following criteria when preparing your matrix:
   - The sample must be free of particulates and precipitates. Centrifuge or filter the samples as necessary.
   - The sample must be in a liquid form when entering the instrument and remain in liquid form until it leaves.

- Consider the stability of both the sample and the analyte for the initial collection, storage, and handling of the samples. Antimicrobial agents, anticoagulant agents, and sample containers can interfere with the assay by binding significant amounts of analyte.

2. Run the neat standards and the spiked matrix samples with the developed method.
3. Prepare a plot of the peak area versus the concentration of the sample for both standards and the spiked matrix.
4. Compare the absolute area values for the standards and matrix samples. Any differences in these values are most likely due to sample matrix effects in the form of the following:
   - Lower retention or recovery of analytes on and off the TurboFlow column.
   - Suppressed or enhanced ionization of the analytes in the ion source of the detector. If results are unsatisfactory, see "Tips for Reducing Matrix Effects."

Tips for Reducing Matrix Effects

If you are not satisfied with the results from "Evaluating Matrix Effects," use the following tips to help reduce matrix effects:

- Add a step that rinses the TurboFlow column before the eluting step. Use a solvent that is at least 20% less organic than the solvent used as the eluting mobile phase in the transfer step.
- Alter the rinse step by doing any of the following:
  - If you added a rinse step after the loading step (method step 1), increase the time of the rinse step (the new method step 2).
  - Increase the strength of the solvent used for rinsing the TurboFlow column. Keep the level of organic 20% lower than the eluting mobile phase.
- Increase the time for the loading step (Step 1).
- Change the pH of the loading mobile phase. Changing the pH might affect the retention and recovery of many compounds. Determine if the matrix retention can be reduced while having a minimal effect on the analyte. An acidic mobile phase helps reduce loss of analyte due to protein binding.
- Rinse with a mobile phase of a different pH. Review sample pretreatment techniques.
- If direct injection of biological samples, raw or diluted, does not provide acceptable results, perform protein precipitation prior to installing the sample on the Prelude instrument. Hydrophobic analytes, such as lipid-soluble vitamins, partition into lipoproteins of blood plasma or serum. To release such analytes, subject the sample to a protein-precipitation or other extraction procedure followed by centrifugation.

- Some analytes, such as the immunosuppressant sirolimus, partition significantly into red blood cells. To assay such compounds from whole blood, the sample preparation procedure must involve cell rupture.
- Protein precipitation or other extraction methods cannot be used reliably, unless the analytes are soluble in the supernatant. To compensate for any loss of analyte throughout the sample handling procedures, add a suitable internal standard to the sample as early as possible. The ideal internal standard is chemically identical to the analyte of interest, yet easily distinguished from it. Stable isotopes of analytes are the most reliable internal standards for assays involving mass spectrometry.

12

Troubleshooting

These topics provide possible solutions and diagnostic procedures for instrument and application issues, and provides other diagnostic and corrective procedures.

Contents

Solutions to Common Problems

Table 41 lists problems, their possible solutions, and diagnostic procedures.

Table 41. Troubleshooting (Sheet 1 of 3)

Problem	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 20. • Verify that mobile phases and wash solutions reside in the appropriate locations. See "Preparing the Autosampler Wash Solutions" on page 186. • Prepare fresh controls and calibrators, and recalibrate the LC method. Refer to the appropriate calibrator and control documentation. • Verify that mobile phases and wash solutions are particulate-free. See "Preparing the Solvent Mobile Phases" on page 188. • 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. See "Monitoring the Pump Pressure" on page 30. • Perform the channel-preparation procedure and observe the test data for the pump seal check. See "Preparing the Channels" on page 21.
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 connections. 3. If the problem persists, call Technical Support. See "Contacting Us" on page xviii.

Table 41. Troubleshooting (Sheet 2 of 3)

Problem	Possible solution or diagnostic test
Low response from one channel.	Do one or more of the following: • Verify that mobile phases and wash solutions are particulate-free and in the appropriate locations. • Replace the TurboFlow column. See "Replacing the TurboFlow Column" on page 189. • Replace the HPLC column. See "Replacing the Analytical Column" on page 191.
Low response from both channels (and mobile phases are shared by both channels).	Do one or more of the following: • Perform the cleaning procedures for the injector and needle. See "Rinsing the Needle" on page 185 and "Rinsing the Injector" on page 170. • 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. "Preparing the Autosampler Wash Solutions" on page 186.
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 trend in pressure, replace the TurboFlow and or HPLC column. • If the pressure trace shows a change in one area of the method, call Technical Support. See "Contacting Us" on page xviii.
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) • TurboFlow column • HPLC column • Instrument tubing

Table 41. Troubleshooting (Sheet 3 of 3)

Problem	Possible solution or diagnostic test
The problem occurs in both 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 xviii.
The internal standard result (IS) falls out of range for all samples.	Verify that you added the IS to all samples. See "Preparing the Samples" on page 20.
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.
The TurboFlow columns need to be changed more frequently due to the compounds you analyze.	Do one or more of the following: • Verify that you are using LC/MS-grade solvents. See "Preparing the Solvent Mobile Phases" on page 188. • Verify that you are using a preservative in the aqueous mobile phase. • To increase the TurboFlow column life, perform a protein-precipitation procedure on the samples before installing them onto the Prelude instrument. To each sample, add a protein precipitating reagent, such as acetonitrile, when you add the IS solution. Then, centrifuge the samples, and load the supernatant onto the Prelude instrument.

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 27.
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 Instruments > Prelude MD> Sequence Log Viewer**.

3. Select the appropriate sequence log.

   The Sequence Log Viewer window opens (see Figure 92).

Figure 92. Sequence Log Viewer

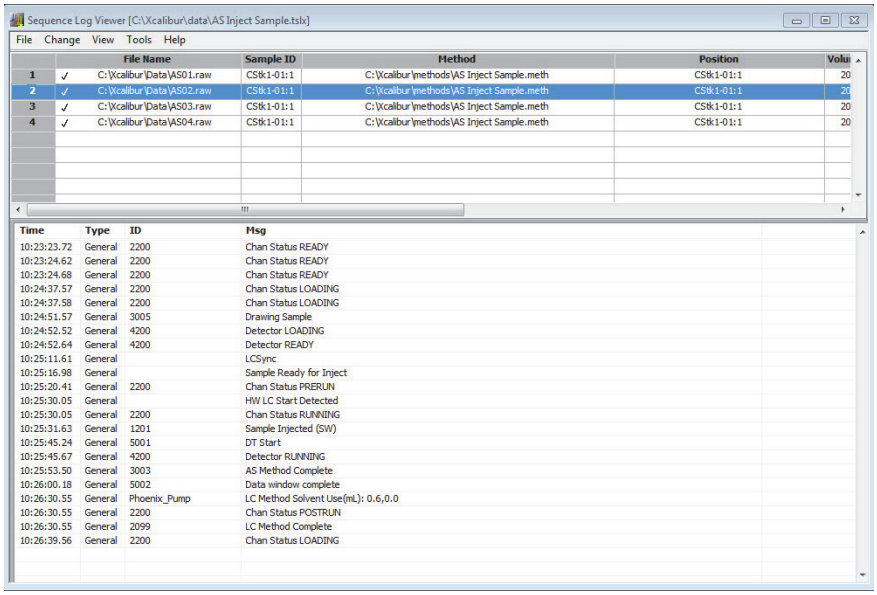


Table 42 describes the parameters in the Sequence Log Viewer window.

Table 42. 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 command. You can rearrange the columns and items displayed using the Column Arrangement dialog box. • 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 MD software 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 graph	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.

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 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 21.

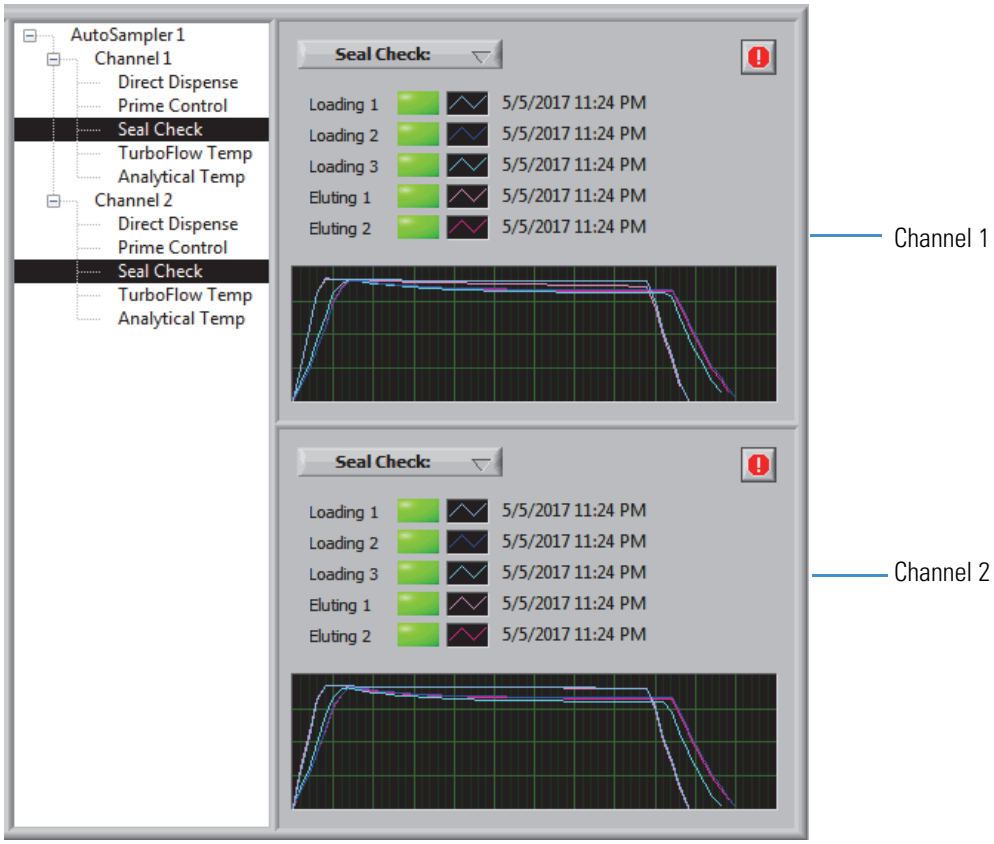
When the test finishes, the pump status color changes to green if the test passes, and to red if the test fails. Additionally, you can point to the pump name to view the test results.

❖ To perform the pump seal check using controls in the Direct Control window

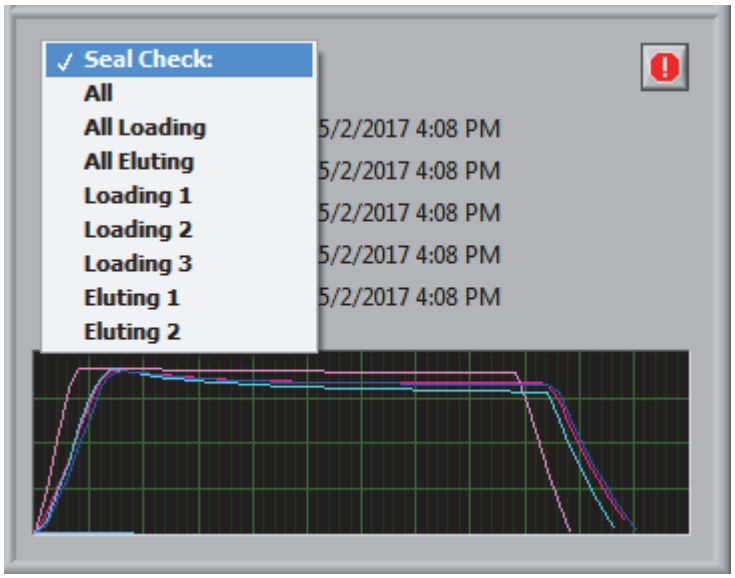
1. Open the Direct Control window.
2. From the middle pane, select **Seal Check**.

   The Seal Check controls for Channel 1 and Channel 2 appear in the right pane (Figure 93).

Figure 93. Seal Check page showing Channel 1 and Channel 2 controls



3. Open the Seal Check list in the right pane.



4. Select the pumps that you want to test.

   The seal check test starts and takes about 90 seconds to complete.

When the test finishes, the pump status color changes to green if the test passes, and to red if the test fails. Additionally, you can point to the pump name to view the test results.

Priming the LC Pumps

If you observe fluctuations in pump pressure, change the solvent bottles, and then prime the pumps. If the instrument has been idle for more than 24 hours, prime the loading and eluting pumps.

This procedure flows fluid from the solvent bottle to the pump, and then to waste. The fluids do not reach the columns.

Prime both channels at least six times using Set 1 and at least six times using the Set 2 solvents.

Prime the pumps 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.

Follow these procedures:

❖ To prime the pumps using the channel-preparation procedure

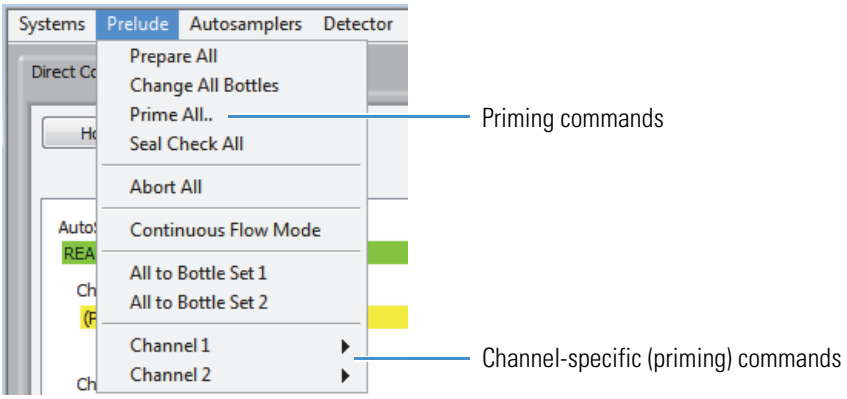
See "Preparing the Channels" on page 21.

❖ To prime the pumps using the controls in the Direct Control window

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.
2. Open the Prelude menu.

   The Prelude menu shows the available priming commands and options.

Figure 94. Prelude menu options



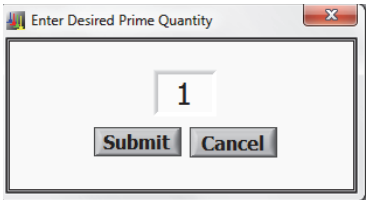
Use one of the following options to initiate the priming operation.

❖ **(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 "Accessing the Direct Control Window" on page 27.
2. Choose **Prelude > Prime All**.

   The Enter Desired Prime Quantity dialog box opens (see Figure ?).

   Figure 95. 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 50.

❖ **(Option 2) To prime individual pumps in the Direct Control window**

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.
2. In the middle pane, select **Prime Control** for any available channel.

   The Direct Control priming options appear to the right (Figure ?).

   Figure 96. 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 50.

❖ (Option 3) To prime both pumps for a specific channel in the Direct Control window

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 27.
2. In the middle pane, select **Prime Control** for any available channel.

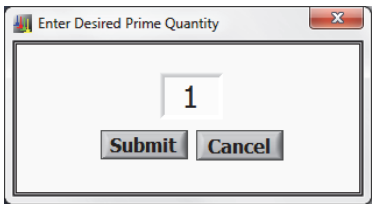
   The Direct Control priming options appear to the right (Figure ?).

   Figure 97. 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 ?).

   Figure 98. 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 50.

❖

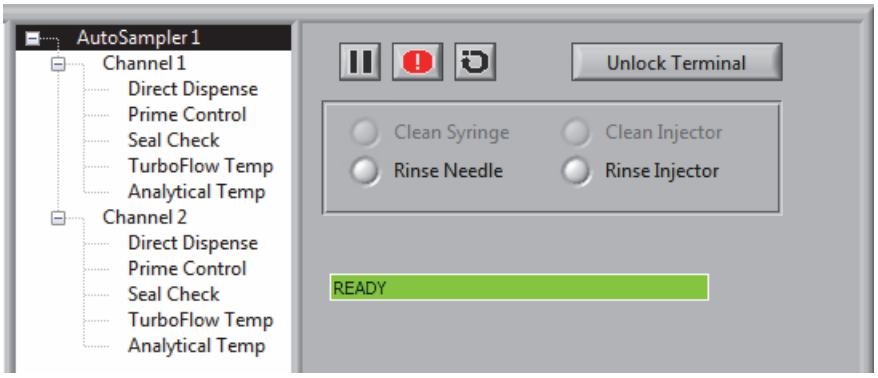
Rinsing the Injector

❖ **To rinse an injector**

1. Open the Direct Control window.
2. Select **Autosampler**.

   The Autosampler options appear on the right.

   Figure 99. Direct Control window showing autosampler options



3. Select the **Rinse Injector** option.

   The Rinse Injector dialog box opens (Figure 100).

   Figure 100. Rinse Injector dialog box

4. In the Wash box, select the wash that you want to use to clean the injector.

5. In the Injector box, select the injector that you want to clean.
6. In the Rinse Time box, enter the number of seconds that you want to wash the injector, and click **OK**.

   Tip Set the rinse time for at least 5 seconds for optimal washing.

   The dialog box closes and the autosampler cleans the injector.

Resetting the XYZ Positions

If you accidentally bump the autosampler arm, reset the XYZ positions.

CAUTION Do not perform this procedure while the autosampler is performing an operation.

❖ **To reset the XYZ position of the autosampler probe**

1. Open the Direct Control window.
2. Select **Autosampler**.

   The Autosampler options appear to the right (see Figure 99 on page 170).
3. Click the **Reset** icon,  .

   The instrument resets positions, injectors, and the syringe. The autosampler then goes 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 from chemical and biohazard conditions.

CAUTION Observe the appropriate biohazard and chemical safety precautions defined by your laboratory while performing the decontamination procedure.

❖ To decontaminate the Prelude instrument

1. Wipe down the table and external surfaces of the Prelude 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 167.
4. Schedule five samples to run using any TurboFlow method. Install a sample blank in the autosampler, and start the run.
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 xviii. Complete the decontamination form before shipping the instrument.

Operating Hazards

These topics describe the operation hazards and the location of hazard labels on the instrument.

Contents

Before Operating the Instrument

Observe the precautions listed in this topic 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 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 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 safety training for hazardous chemicals, when handling solvents, changing tube lines, or both. Consult the pertinent material safety data sheets (MSDSs) 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.

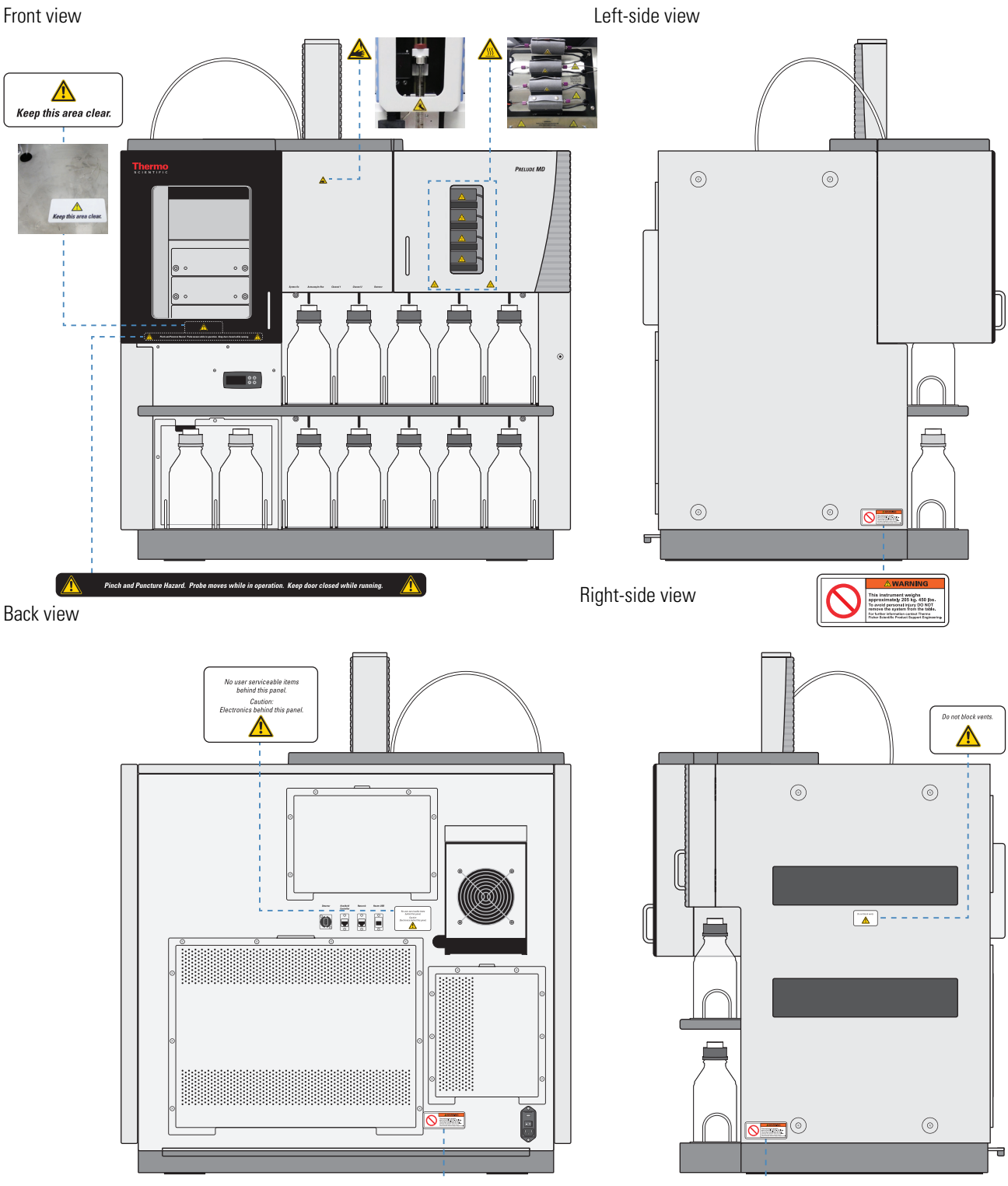
CAUTION Do not use a damaged or expired TurboFlow or HPLC column on the system. Run a preview batch at regular intervals to evaluate the quality of the TurboFlow and HPLC columns.

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.

Caution-Specific Labels on the Instrument

Figure 101 shows the placement of caution labels on the instrument.

Figure 101. Placement of caution labels on the instrument



Residual Product Risks

This topic lists residual product risks that are associated with the Prelude MD instrument. Residual product risks are those risks that remain after all efforts have been made to mitigate or eliminate them.

IMPORTANT Be sure that you read and understand the following residual product risks that are associated with the instrument.

- 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. For more information, see "Assigning a Pressure Profile" on page 127.

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Maintenance Procedures

These topics describe the procedures that will help maintain your system's operation quality.

Contents

Maintenance Schedule

Table 43 provides the schedule for performing system maintenance procedures.

Table 43. Maintenance schedule

Frequency	Procedure
Daily	Accessing the Direct Control Window
	Tracking the Number of Injections
	Recording Pump Pressures
	Preparing the Aqueous Mobile Phases
	Priming the Dynamic Load Wash (DLW)
	Rinsing the Needle
Weekly	Preparing the Cleaning Solution
	Preparing the Autosampler Wash Solutions
	Changing the Solvent Bottles
	Preparing the Solvent Mobile Phases
Every 500 injections	Replacing the TurboFlow Column
Every 2000 injections	Replacing the Analytical Column
Every 10 000 injections	Preventive maintenance performed by a qualified service engineer

Accessing the Direct Control Window

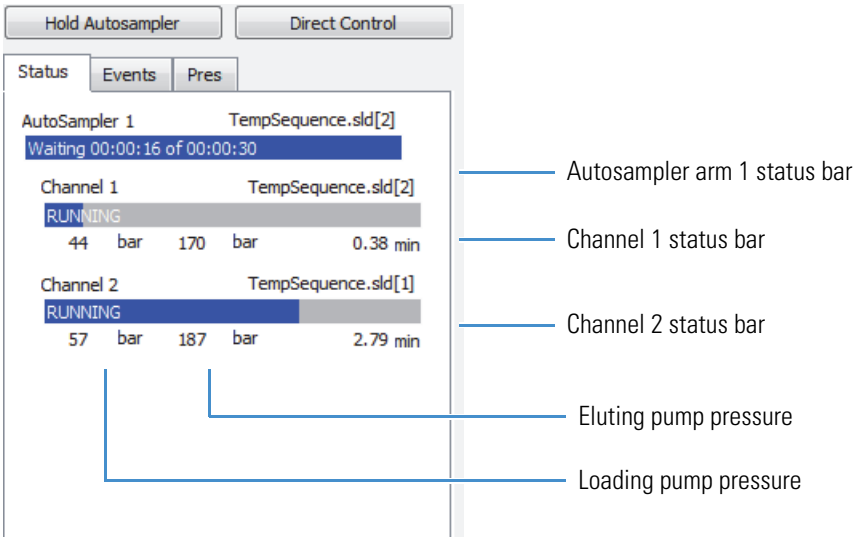
Access the Direct Control window to perform many of the procedures described in this topic.

❖ **To access the Direct Control window**

1. Open the status window using the detector control application.

   The Status information appears.

Figure 102. Prelude Status Area



2. Click **Direct Control**.

   The Direct Control window opens.

Tracking the Number of Injections

The Maintenance dialog box helps you optimize instrument performance by tracking the number of injections made by each probe or system.

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

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

   The Maintenance dialog box opens.

Figure 103. Maintenance dialog box

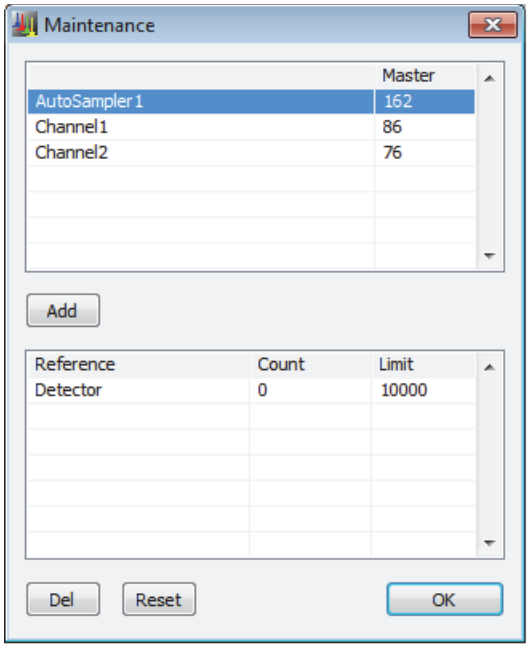


Table 44 describes the Maintenance dialog box options.

Table 44. Maintenance dialog box options

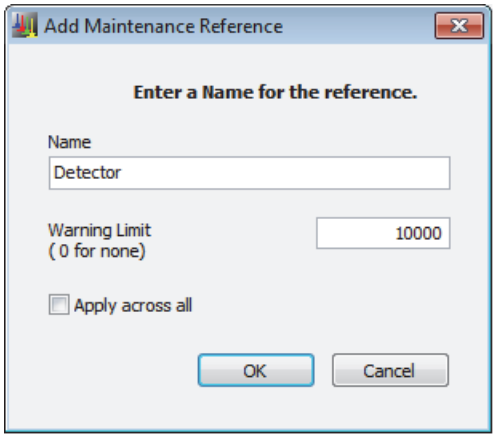
Column	Description
Master	The total, cumulative number of injections for each component.
Reference	A user-defined interval of injections for tracking the number of injections for a system component. For example, define a reference named "TurboFlow column," and install a new column. At the start of the reference, the count shows 0. Reset the count to zero each time you change the TurboFlow 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.

❖ 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.

Figure 104. 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, and then enter a name that reflects the component you are tracking, such as "**Detector**".
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 using the component, leave the box value set to **0**.
5. If you want the setting to apply 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**.

   The window closes.

❖ 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.

Preparing the Aqueous Mobile Phases

Prepare aqueous mobile phases according to these guidelines:

- Prepare fresh aqueous mobile phases daily in clean bottles. Do not refill or top off standing bottles.
- Make aqueous mobile phases in quantities that will be used on a daily basis.
- Do not use a thermoplastic sealing film, such as Parafilm, as a mobile phase reservoir cover. Use an appropriate bottle cap that accommodates the solvent lines. If caps are not available, use aluminum foil to secure the solvent lines in the bottle and protect the solvent from dust. Make sure that the mobile phase line reaches the bottom of its intended 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 appear 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, replace the bottles. Replace the solvent filters and purge the lines fully with new, clean, LC/MS-grade mobile phase.

Recording 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. See "Monitoring the Pump Pressure" on page 30.

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 xviii.

Priming the Dynamic Load Wash (DLW)

Prime the DLW according to your laboratory's maintenance schedule by using the Rinse Needle option in the Direct Control window.

❖ To prime the DLW

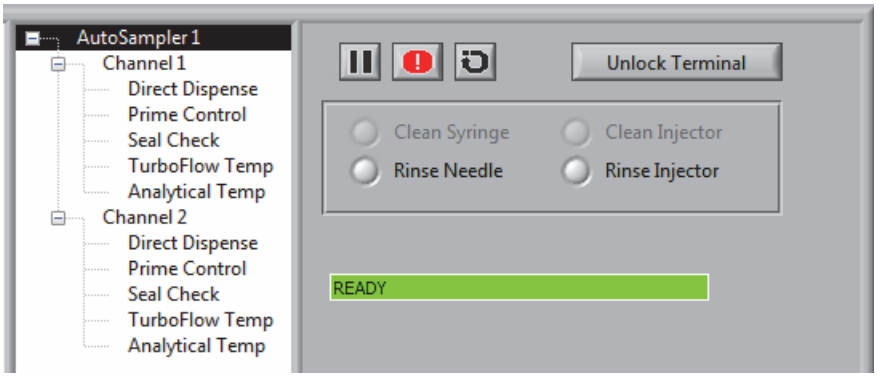
1. Perform the Rinsing the Needle procedure using Wash 1 for at least 5 seconds. See "Rinsing the Needle."
2. Perform the Rinsing the Needle procedure using Wash 2 for at least 5 seconds. See "Rinsing the Needle."

Rinsing the Needle

❖ **To rinse the needle**

1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 180.
2. Click **Autosampler**. The Autosampler options appear. See Figure 105.

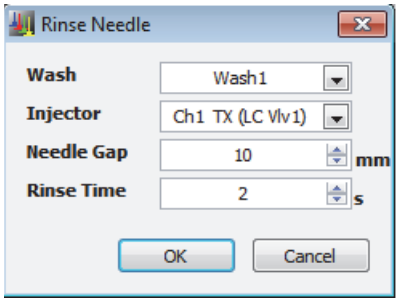
   Figure 105. Direct Control window showing autosampler options



3. Select the **Rinse Needle** option.

   The Rinse Needle dialog box opens.

   Figure 106. Rinse Needle dialog box



4. In the Wash box, select the wash solution that you want to use to rinse the needle.
5. In the Injector box, select the injector where you want to rinse the needle.
6. In the Needle Gap box, leave the value at the default setting, unless a service engineer instructs you to change it.
7. In the Rinse Time box, type the number of seconds that you want to rinse the needle, and click **OK**.

   The autosampler rinses the needle.

Preparing the Cleaning Solution

Prepare a 45:45:10 acetonitrile/isopropanol/acetone solution in a clean, 1-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

Use this solution to fill your cleaning solution reservoir. The solution is stable for 30 days at room temperature.

Preparing the Autosampler Wash Solutions

❖ **To prepare the autosampler wash solution**

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 DLW. See "Priming the Dynamic Load Wash (DLW)" on page 184.

Changing the Solvent Bottles

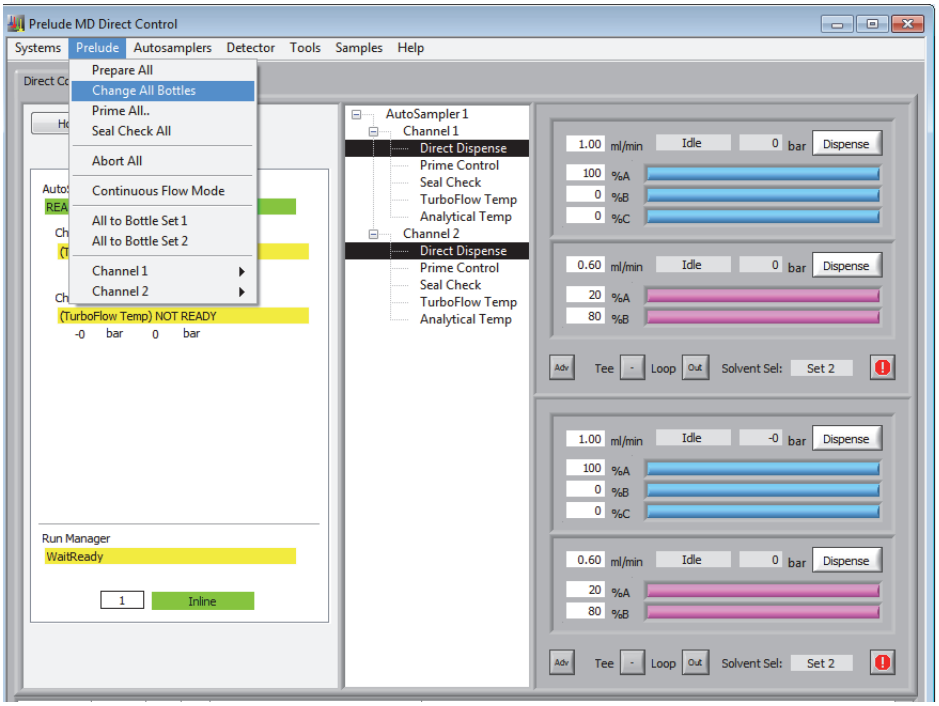
This topic describes how to replace the solvent bottles.

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

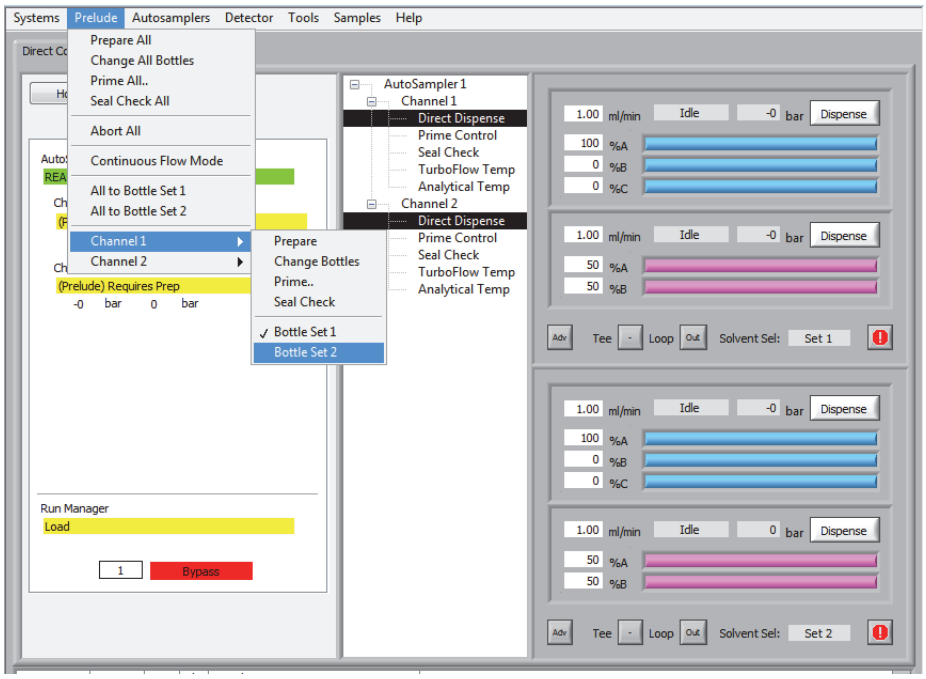
1. Open the Direct Control window. See "Accessing the Direct Control Window" on page 180.
2. Do one of the following:
   - If you refilled the bottles and you want all of the channels to draw fresh solvent without changing bottle selections, choose **Prelude > Change All Bottles** from the main menu (see Figure 107). This runs the predefined Change Bottles macro consisting of a number of prime cycles and the optional seal check.

     Figure 107. 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 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 108), 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 108. 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.

Preparing the Solvent Mobile Phases

Prepare solvent mobile phases according to the following guidelines:

- Prepare fresh solvent mobile phases weekly in clean bottles. Do not refill or top off standing bottles.
- Use LC/MS-grade solvents or higher. Fisher Scientific offers mobile phase blends for your convenience. Refer to the *Prelude 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.

- 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.

Replacing the TurboFlow Column

Replace the TurboFlow column every 500 injections. Use the Maintenance dialog box to track injection numbers. See "Tracking the Number of Injections" on page 181.

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 columns until you are ready to install it onto the system.

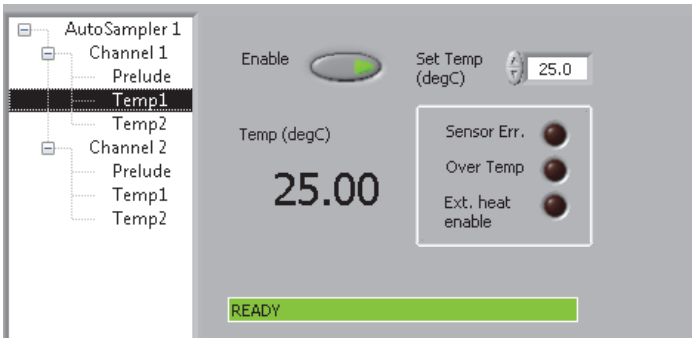
CAUTION Column heaters can become extremely hot and become unsafe to handle. Allow the column and tubing to cool to below 50 degrees Celsius before handling the column, tubing, and other system components that are near the heater.

CAUTION If you use solvents that emit hazardous vapors, take appropriate chemical and hazardous vapor precautions when you remove the TurboFlow or 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 TurboFlow column

1. Turn off the column heater by doing the following, and allow the column heater to cool to room temperature.
   a. Open the Direct Control window.
   b. Click a column heater that appears under the appropriate channel name.

Figure 109. Direct Control window showing column heater control



c. To disable the column heater, click the **Enable** button until the bright green icon becomes dark green.

d. To disable the second column heater, repeat step b through step c.

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

3. Replace the column by doing the following:

> **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 and the grip from the column.

> **IMPORTANT** The silver grip and graphite-tipped ferrule must remain with the tubing.

c. Remove the tubing from the other side of the column in the same way, 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. Finger tighten the silver grip.

g. Finger tighten 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. Start the pumps and monitor the pressure until it reaches your typical operating pressure.

5. Dispense fluid through the column. See "To dispense solvent through the system" on page 44.

Replacing the Analytical Column

Replace the analytical column every 2000 injections. Use the Maintenance dialog box to track injection numbers. See "Tracking the Number of Injections" on page 181.

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.

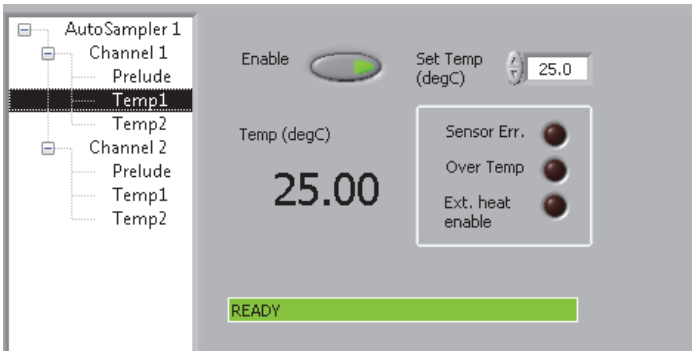
CAUTION Column heaters can become extremely hot and become unsafe to handle. Allow the column and tubing to cool to below 50 degrees Celsius before handling the column, tubing, and other system components that are near the heater.

CAUTION If you use solvents that emit hazardous vapors, take appropriate chemical and hazardous vapor precautions when you remove the TurboFlow or 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 by doing the following, and allow the column heater to cool to room temperature:
   a. Open the Direct Control window.
   b. Click a column heater for the appropriate channel.

      Figure 110. Direct Control window showing column heater control

   c. Click the **Enable** button until the bright green icon becomes dark green.
   d. Repeat step b through step c for the second column heater if applicable.

2. When the column heater has cooled, unwrap the column heater from the column.
3. Replace the column by doing the following:

   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.

      IMPORTANT The silver grip and graphite-tipped ferrule must remain with the tubing.

   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. Start the pumps and monitor the pressure until it reaches your typical operating pressure.
5. Dispense fluid through the column. See "To dispense solvent through the system" on page 44.

Consumables

This topic contains part numbers and descriptions of consumable parts for the Prelude MD instrument. To ensure proper results in servicing the instrument, order only the parts that are listed or their equivalent. See Table 45.

Table 45. Consumable part numbers and descriptions

Part number	Description
00301-01-00032	Corning Square Solvent Bottle, 1L
00301-01-00036	Solvent Inlet Filter, 1/8-inch
00109-02-00041	PEEK Tubing, 0.005 ID x 0.062 OD x 1.5m
00109-00314	One Piece Fingertight PEEK Fitting, 10-32
00109-02-00044	Graphite Ferrule, 1/16-inch, FLEXCHROM, 5-PK

A

Columns

This appendix describes the TurboFlow columns for use on the Prelude MD instrument.

Contents

TurboFlow Column Descriptions

TurboFlow columns come in a wide range of chemistries for diverse applications. This broad range of chemistries provides you with separation flexibility to accommodate variations in the compounds' structure, polarity, and solubility. Table 46 and Table 47 describe the chemistries available with TurboFlow columns. Table 48 describes the column characteristics for each TurboFlow column chemistry.

Select the most appropriate column by determining the overall polarity of the analyte, the mobile phase needed to solubilize it, and the sample matrix from which it must be extracted.

Table 46. Silica-based TurboFlow columns (Sheet 1 of 2)

Column	Description
C18	Most retentive of the alkyl-bonded phases for nonpolar solutes. You can also use the C18 packing for moderately polar solutes.
C18-P	Has lower carbon load than C18 and without end capping. Applicable to the analysis of polar and nonpolar solutes.
C8	Less retentive than C18 packings but with similar reversed-phase selectivity. Useful for recovering solutes that would be excessively retained by a C18 packing.
Phenyl	Reversed-phase material with unique selectivity. Especially useful for aromatic compounds. Less retentive than C18 packings.

Table 46. Silica-based TurboFlow columns (Sheet 2 of 2)

Column	Description
Fluoro	Fluorinated alkyl stationary phase provides a unique selectivity compared to other reversed phases.
C2	Recommended for the extraction of extremely nonpolar constituents that would be difficult to elute from C8 or C18 packings.

Table 47. Polymer-based TurboFlow columns

Column	Description
Cyclone	Recommended for the determination of a wide range of analytes in complex matrices. Silanophilic compounds can perform significantly better on Cyclone than on silica-bonded phases.
Cyclone-P	Recommended for the determination of a wide range of analytes in complex matrices. Cyclone-P is more polar than Cyclone and provides different selectivity.
Cyclone MCX (Mixed Cation Exchange TurboFlow Column)	A mixed-mode material with both strong cation exchange and reversed-phase binding capacity. Both neutral and positively charged molecules can be captured by Cyclone MCX columns. Since the stationary phase is negatively charged across the entire operating pH range, a combination of increasing pH and increasing solvent strength might be required for eluting weak bases.
Cyclone MAX (Mixed Anion Exchange TurboFlow Column)	A mixed mode material with both strong anion exchange and reversed-phase binding capacity. Both neutral and negatively charged molecules can be captured by Cyclone MAX columns. Since the stationary phase is positively charged across the entire operating pH range, a combination of decreasing pH and increasing solvent strength might be required for eluting weak acids.
Cyclone MCX-2	A mixed-mode material with both strong anion exchange and reversed-phase binding capacity. Compared to the Cyclone MCX, the Cyclone MCX-2 column has lower ion exchange capacity. Both neutral and positively charged molecules can be captured on the Cyclone MCX-2 columns. Since the stationary phase has a negative charge across the entire operating pH range, a combination of decreasing pH and solvent strength might be required for eluting weak bases.

Table 48. TurboFlow column characteristics

Column	Chemistry	Hydro- phobicity	Operating pH	Typical applications
C18	C18 bonded silica	Very high	2–9	Pharmaceuticals, fatty acids
C18-P	C18 bonded silica	High	2–9	Polar pharmaceuticals, metabolites
C8	C8 bonded silica	High	2–9	Pharmaceuticals, fatty acids
Phenyl	Phenyl bonded silica	Moderate	2–9	Aromatic compounds
Fluoro	Alkyl fluoro bonded silica	Slight	2–9	Highly lipophilic solutes, perfluorinated compounds
C2	C2 bonded silica	Slight	2–9	Extremely nonpolar, multifunctional pharmaceuticals
Cyclone	Styrene-divinylbenzene copolymer bead	Very high	1–13	Nonpolar pharmaceuticals
Cyclone-P	Styrene-divinylbenzene copolymer bead with polar modification	High with polar modification	1–13	Nonpolar and moderately polar pharmaceuticals, steroids
Cyclone MCX	Styrene-divinylbenzene copolymer with sulfonic acid modification	Moderately high	1–13	Polar, weakly basic drugs and metabolites; drugs of abuse
Cyclone MCX-2	Styrene-divinylbenzene copolymer with sulfonic acid modification	Moderately high	1–13	Polar, weakly basic drugs and metabolites. Cyclone MCX-2 has lower ion exchange capacity than Cyclone MCX.
Cyclone MAX	Styrene-divinylbenzene copolymer with quaternary ammonium	Moderately high	1–13	Polar, weakly acidic drug compounds and metabolites; antibiotics

TurboFlow Column Part Numbers

Table 49 lists the TurboFlow columns and their part numbers. Columns are 0.5 × 50 mm.

Table 49. TurboFlow column part numbers

Column	Part number
TurboFlow XL C18	CH-953280
TurboFlow XL C18-P	CH-953281
TurboFlow XL C8	CH-953282
TurboFlow XL Fluoro	CH-953283
TurboFlow XL Phenyl	CH-953284
TurboFlow XL C2	CH-953285
TurboFlow Cyclone	CH-953288
TurboFlow Cyclone-P	CH-953289
TurboFlow Cyclone MAX	CH-953286
TurboFlow Cyclone MCX	CH-953287
TurboFlow Cyclone MCX-2	CH-953457
TurboFlow Phenyl[a]	CH-952820

[a] Phenyl columns work reliably at pressures up to 250 bar.

Analytical Column Description

Thermo Scientific Accucore™ HPLC columns contain solid core particles, which are engineered to a diameter of 2.6 mm and a very narrow particle size distribution. Accucore HPLC columns allow high speed, high resolution separations. They are available in a variety of sizes and types, including Accucore PFP, Accucore aQ, Accucore C18, Accucore C8, and Accucore RP-MS, to name a few. Accucore UHPLC columns are also available.

Manufacturer Addresses

Manufacturer

Thermo Finnigan LLC
355 River Oaks Parkway
San Jose, CA 95134
U.S.A.

European Authorized Representative

Thermo Fisher Scientific (Bremen) GmbH
Hanna-Kunath-Straße 11
28199 Bremen
Germany

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