

Waters 1500-Series HPLC Pump and Options Operator's Guide

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|-------------------|---|
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| Telephone and fax | From the USA or Canada, phone 800 252-HPLC, or fax 508 872 1990. For other locations worldwide, phone and fax numbers appear in the Waters Web site. |
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Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, and radiological hazards. You must know the potentially hazardous effects of all substances you work with. Always follow

Good Laboratory Practice, and consult your organization's safety representative for guidance.

Considerations specific to the 1500-Series HPLC pumps and column heater

Radiation hazard

The equipment does not emit any type of hazardous radiation. It emits a minimum amount of electromagnetic radiation that is within the limits of applicable emissions standards (EN61326).

Protective grounding

The pump and column heater requires protective grounding for operation. The three-conductor electrical cord that supplies power also grounds the device. This power cord is approved by a nationally recognized testing laboratory (UL or ETL). It must comprise three, 18-gauge, insulated conductors and be rated for 300 V.

Back siphoning and draining

Check valves in the fluid pump, installed ahead of the column heater, prevent the back-siphoning of fluids.

Drainage systems are installed in this equipment. Drip trays inside the pump and column heater units catch any fluid from leaks or spills. These trays are connected to external drains on the bottom of the units. Tubing connected to this drain routes the fluid into an appropriate waste container.

Hazardous waste

During standard operation, this device does not produce any byproducts or waste. Any waste resulting from a leak or spill is channeled into the drain located on the underside of the device. Tubing connected to this drain directs the flow to an appropriate waste container.

Equipment repair or disposal

Direct questions regarding repair or disposal to Waters. Waters carries out equipment disposal in Europe according to the WEEE directive specific to the

country. Waters also accommodates any special requirements for locations outside of Europe.







Safety advisories

Consult [Appendix A](#) for a comprehensive list of warning and caution advisories.

Operating the Waters 1500-Series HPLC pump and options

When operating these pumps and options, follow standard quality-control (QC) procedures and the guidelines presented in this section.

Applicable symbols

| Symbol | Definition |
|--|--|
|  Waters Corporation 34 Maple Street Milford, MA 01757 U.S.A. | Manufacturer |
|  | Authorized representative of the European Community |
|  | Confirms that a manufactured product complies with all applicable European Community directives |
|  ABN 49 065 444 751 | Australia C-Tick EMC Compliant |
|  | Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements |
|  | Consult instructions for use |

Audience and purpose

This guide is intended for use by individuals who need to install, maintain, and/or troubleshoot the Waters 1500-Series HPLC Pump and options. You should be familiar with HPLC terms, practices, and basic HPLC system operations such as connecting tubing.

Intended use of the Waters 1500-Series HPLC pump and options

Use the Waters 1525, 1525 μ , and 1525EF HPLC pumps and the optional 1500-Series Column Heater and its options to deliver a precisely controlled amount of solvent to a column, maintaining a consistent and reproducible mobile phase composition. The Waters 1500-Series HPLC pump and options are for research use only.

Calibrating

To calibrate LC systems, follow acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for standards should include the entire range of QC samples, typical specimens, and atypical specimens.

When calibrating mass spectrometers, consult the calibration section of the operator's guide for the instrument you are calibrating. In cases where an overview and maintenance guide, not operator's guide, accompanies the instrument, consult the instrument's online Help system for calibration instructions.

Quality-control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. Ensure that QC sample results fall within an acceptable range, and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report these data until you are certain that the instrument performs satisfactorily.

When analyzing samples from a complex matrix such as soil, tissue, serum/plasma, whole blood, and other sources, note that the matrix components can adversely affect LC/MS results, enhancing or suppressing

ionization. To minimize these matrix effects, Waters recommends you adopt the following measures:

- Prior to the instrumental analysis, use appropriate sample pretreatment such as protein precipitation, liquid/liquid extraction (LLE), or solid phase extraction (SPE) to remove matrix interferences.
- Whenever possible, verify method accuracy and precision using matrix-matched calibrators and QC samples.
- Use one or more internal standard compounds, preferably isotopically labeled analytes.

ISM classification

ISM Classification: ISM Group 1 Class B

This classification has been assigned in accordance with CISPR 11 Industrial Scientific and Medical (ISM) instruments requirements. Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment. Class B products are suitable for use in both commercial and residential locations and can be directly connected to a low voltage, power-supply network.

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1

Introduction

This chapter describes key operating principles, pump components, data control configurations, and available options for the 1500-series HPLC pumps.

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HPLC pump operating principles

This section describes these topics:

- Isocratic and gradient LC system operation
- Effects of dissolved oxygen in the mobile phase
- Removing gases from eluents using in-line degassing

Isocratic and gradient LC system operation

Two basic elution modes are used in HPLC: isocratic elution and gradient elution.

In isocratic elution, the mobile phase, either a pure solvent or a mixture, remains the same throughout the run. For LC system operation, a single pump system, such as the 1515 isocratic pump, delivers a controlled amount of solvent into the column to maintain consistent and reproducible mobile phase composition.

In gradient elution, the mobile phase composition changes during the separation. This mode is useful for samples that contain compounds that span a wide range of chromatographic polarity. As the separation proceeds, the elution strength of the mobile phase is increased to elute the more strongly retained sample components.

In the simplest configuration, there are two bottles of solvent and two pumps, the case when using a 1525 binary pump. The speed of each pump is managed by the gradient controller to deliver more or less of each solvent over the course of the separation. The two streams are combined using a mixer to create the actual mobile phase composition that is delivered to the column over time. At the beginning, the mobile phase contains a higher proportion of the weaker solvent (solvent A). Over time, the proportion of the stronger solvent (solvent B) is increased according to a predetermined timetable. Because the mixer is downstream of the pumps, the gradient is created under high pressure.

Other HPLC systems, such as Waters Alliance systems, are designed to mix multiple streams of solvents under low pressure, ahead of a single pump. A gradient proportioning valve selects from the multiple solvent bottles, changing the strength of the mobile phase over time.

Effects of dissolved oxygen on the mobile phase

Dissolved oxygen in the mobile phase can be of special concern. It can under certain circumstances interfere with the detection of analytes by UV/Vis, fluorescence, or electrochemical detectors.¹

Effects on UV/Vis detectors

Oxygen can form UV-absorbing complexes with solvents such as methanol or tetrahydrofuran (THF). These complexes increase the background absorbance, especially at lower wavelengths. This leads to a small decrease in sensitivity of detection. More importantly, however, they cause baseline shifts, or ghost peaks, during gradient separations. Also, a change in the dissolved oxygen level over time, especially when it results from reabsorption of ambient gases following an offline degassing technique, causes baseline drift and irregularity.

Removing dissolved oxygen to a reproducible level greatly enhances the performance of UV/Vis detectors, especially below 254 nm and in gradient systems. It also improves sensitivity in certain fluorescence detection applications.

Effects on fluorescence detectors

For certain analytes at certain wavelengths, oxygen, under certain mobile phase conditions, can quench fluorescence response. Aromatic hydrocarbons, aliphatic aldehydes, and ketones are particularly susceptible to quenching, and decreases in sensitivity of 95% are possible.

Effects on electrochemical detectors

Oxygen can interfere with various electrochemical detection techniques, particularly reductive electrochemistry.

Effects on refractive index detectors

Refractive index detectors are sensitive to changes in solvent density. Removing dissolved gases to a consistent level enhances the performance of refractive index detectors, reducing baseline drift and irregularity.

1. Rollie, Mae E., Gabor Patonay, Isaiah M. Warner, *Ind. Eng. Chem. Res.*, 1987, 26, 1–6.

Using in-line degassing to remove gases from eluents

In-line methods of degassing operate within the chromatographic fluid path. The 1500-series integral vacuum degasser uses this approach. Because degassing occurs close to the pump, this method minimizes reabsorption of ambient gas into the eluent.

The flow rate of eluent through an in-line degasser determines the efficiency of the degassing. At low flow rates, most of the dissolved gas is removed as the eluent passes through the vacuum chambers. At higher flow rates, lesser amounts of gas per unit volume of eluent are removed.

Degassing efficiency

The flow rate of eluent through the degasser determines the efficiency with which the degasser removes gases. As the flow rate increases, the time available to remove dissolved gases from the eluent lessens. The following table shows the relationship between the flow rate of an eluent (water) and the concentration of a gas (oxygen) dissolved in the eluent.

Effect of flow rate on final dissolved gas concentration

| Flow rate (mL/min) | Final oxygen concentration (ppm) |
|--------------------|----------------------------------|
| 1 | ≤1 |
| 2 | ≤1.3 |
| 5 | ≤2.3 |

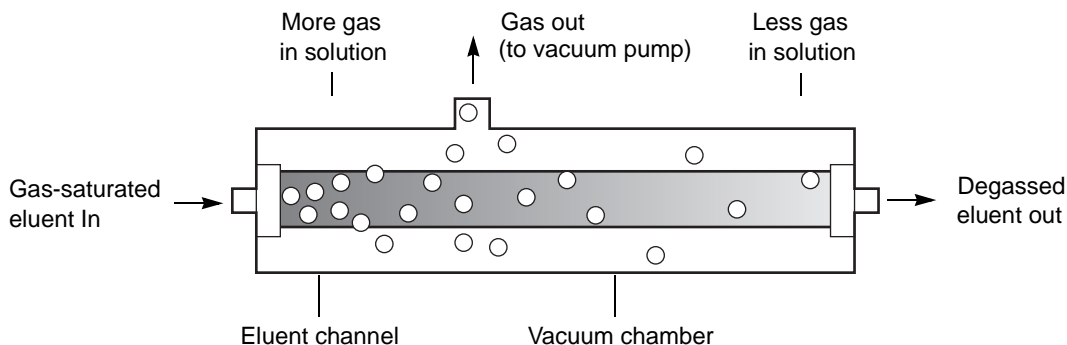
Degasser operating principles

The degasser operates according to Henry's Law, removing dissolved gases from the eluent. Henry's Law states that the mole fraction of a gas dissolved in a liquid is proportional to the partial pressure of that gas in the vapor phase above the liquid. If the partial pressure of a gas on the surface of the liquid is reduced—by evacuation, for example—then a proportional amount of that gas comes out of solution.

The degasser uses a gas-permeable polymer membrane channel to carry the eluent through the vacuum chamber. When the eluent enters the vacuum chamber, the vacuum creates a large differential in gas concentration across the membrane. This differential accelerates the rate at which the dissolved gases diffuse through the polymer membrane into the vacuum chamber. The

gases are then carried away by the vacuum pump. The following figure is a simplified schematic diagram of the vacuum chamber.

Vacuum chamber schematic



The longer the eluent is exposed to the vacuum, the more dissolved gases are removed. Two factors affect the amount of time the eluent is exposed to the vacuum:

- Flow rate – A lower flow rate increases the amount of time the eluent is exposed to the vacuum. [“Degassing efficiency” on page 1-4](#), addresses the effect of different flow rates on the concentration of remaining gas.
- Surface area of degassing membrane – The length of the degassing membrane is fixed in each vacuum chamber.

Overview of the 1500-series HPLC pumps

The 1500-series HPLC pumps combine the most important aspects of eluent delivery for HPLC: high precision, reliability, and smooth eluent flow. All pumps perform their intended functions equally well:

- The 1515 isocratic HPLC pump is designed for precise isocratic analyses, with flow rates up to 10 mL/minute.
- The 1525 binary HPLC pump achieves reproducible, binary gradient delivery, with exceptionally smooth concurrent-stream blending with flow rates of up to 10 mL/minute.

- The 1525EF (Extended Flow) binary HPLC pump is a field-upgrade of the standard 1525 binary pump designed for increased flow rates of up to 22.5 mL/minute.
- The 1525 μ binary HPLC pump is designed for precise, reproducible gradient delivery at low flow rates up to 5 mL/minute.

In an HPLC system, the 1500-series pump is controlled by Waters Breeze™ 2, Empower® 2, or MassLynx® data control software (see [page 1-13](#)).

Tip: For detailed information about specific data control software versions and requirements, refer to the release notes for the Waters 1500-series HPLC pumps.

The microprocessor-controlled stepper motor and noncircular gears of each pump ensures smooth and precise flow regardless of backpressure, flow-rate setting, or eluent compressibility.

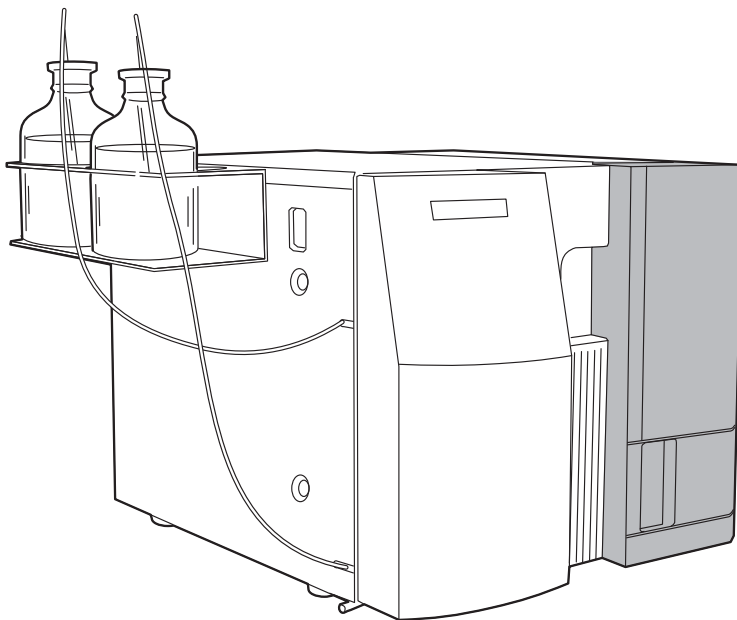
These optional components are available for the 1500-series pump to suit your HPLC applications and site requirements:

- 1500-series column heater – Enables preheating of fluids passing through chromatographic columns.
- Integral vacuum degasser – Provides HPLC pumps with an automatic, continuous method of removing dissolved gasses from mobile phases. The degasser is standard on the 1525 μ pump and is available as an option on the 1515 isocratic pump and 1525 and 1525EF binary pumps.
- Plunger seal wash system – Extends the life of pump seals by lubricating the plunger and flushing away solvent or dried salts forced past the plunger seal from the high-pressure side of each piston chamber.
- Manual injector – For use in place of an autosampler to provide precise manual control of HPLC sample injections.

For more details, see [page 1-15](#).

The following figure shows a 1525 binary pump with an optional column heater.

1525 binary pump with optional column heater

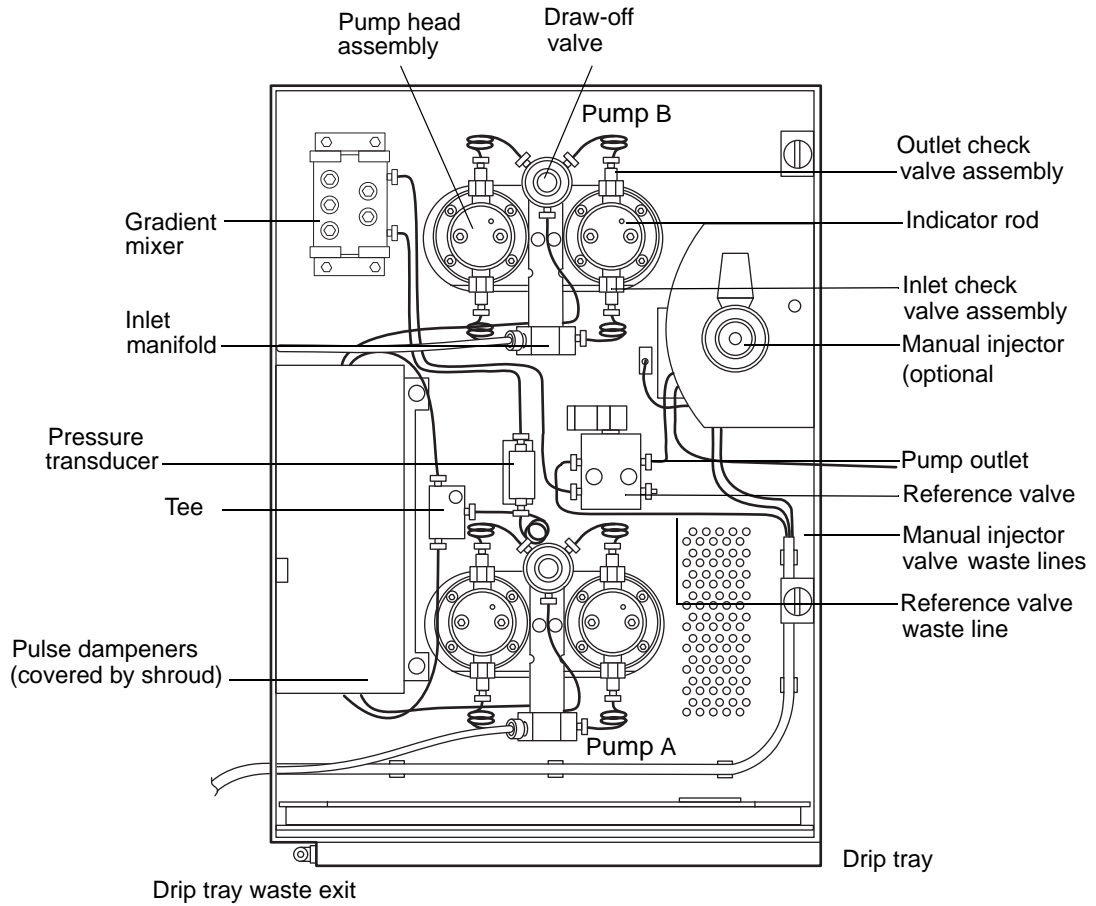


Fluid-handling components

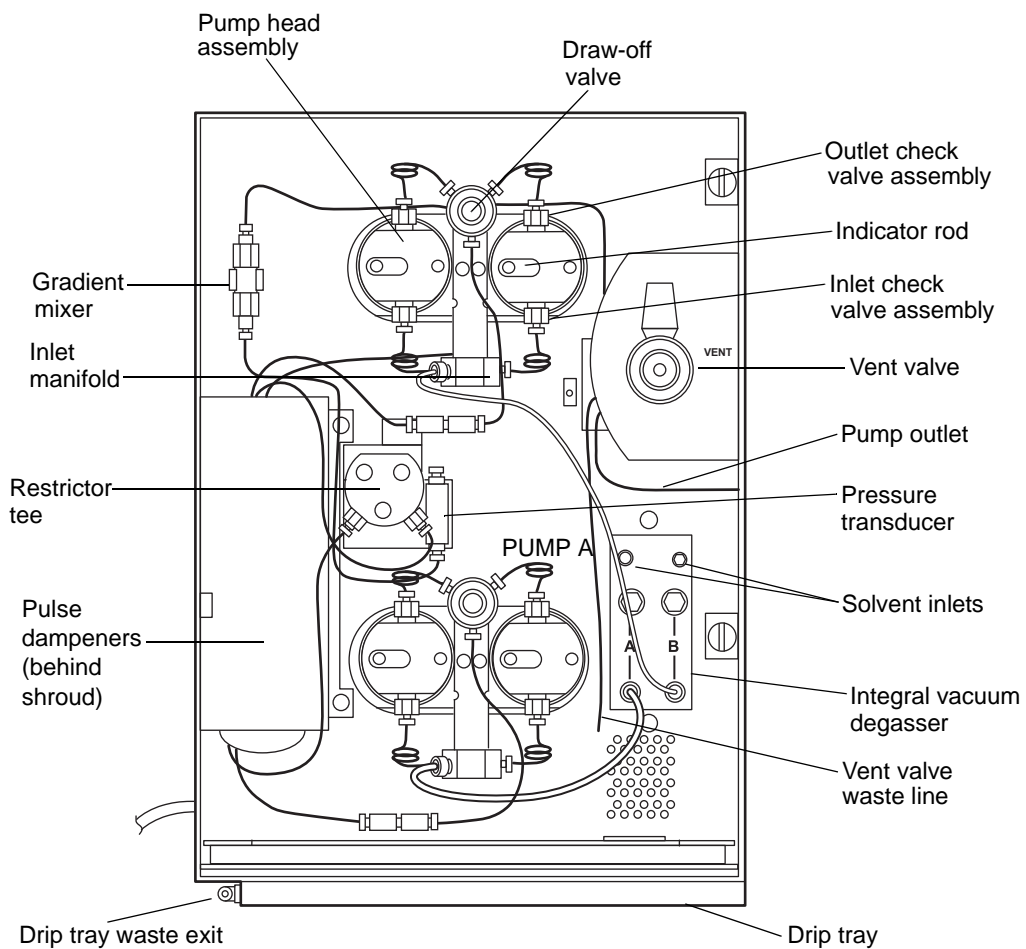
Before you install a 1500-series pump, familiarize yourself with its fluid-handling components. The following figures identify the fluid-handling components of the 1525/1525EF and 1525 μ HPLC pumps.

Tip: The 1515 pump is an isocratic version of the 1525 binary pump. It shares key components with the 1525 binary pump, except for the second pump assembly (Pump B), tee, and gradient mixer (including associated subcomponents).

Fluid-handling components in the 1525/1525EF pumps



Fluid-handling components in the 1525 μ pump



The following table describes the functions of the 1500-series pump's fluid-handling components.

Fluid-handling components

| Component | Description |
|---|---|
| 1500-series column heater (optional) | Maintains elevated column temperature to facilitate method reproducibility. |
| Draw-off valve | Enables attachment of a syringe for drawing eluent through the eluent reservoir line and into the pump for priming. |
| Drip tray | Catches fluid leaks. |
| Drip tray waste exit | Drains accumulated fluids to the waste container. |
| Gradient mixer (optional for 1515 pump) | Increases eluent homogeneity. Also adds volume to the system. |
| Inlet and outlet check valve assemblies | Maintain flow direction and pressure by opening in one direction only. |
| Inlet manifold | Provides the connection for eluent inlet tubing and routes eluent to the inlet check valve on each pump head. |
| Integral vacuum degasser (optional for 1515 and 1525 pumps) | Provides HPLC pumps with an automatic, continuous method of removing dissolved gasses from mobile phases. |
| Manual injector (optional) | Enables a manual sample injection. Generates an inject-start signal to the detector. |
| Manual injector waste line | Routes flow from the manual injector to the waste container. |
| Plunger indicator rods | Show the position of each pump head plunger. |
| Pressure transducer | Senses operating pressure and converts values to electronic signals for monitoring. |
| Pulse dampeners | Dampen operating pressure fluctuations. Located on the left-hand side of the unit, under the mixer and behind the shroud. |
| Pump head assembly | Draws in and delivers eluent. Defines pump capacity. |

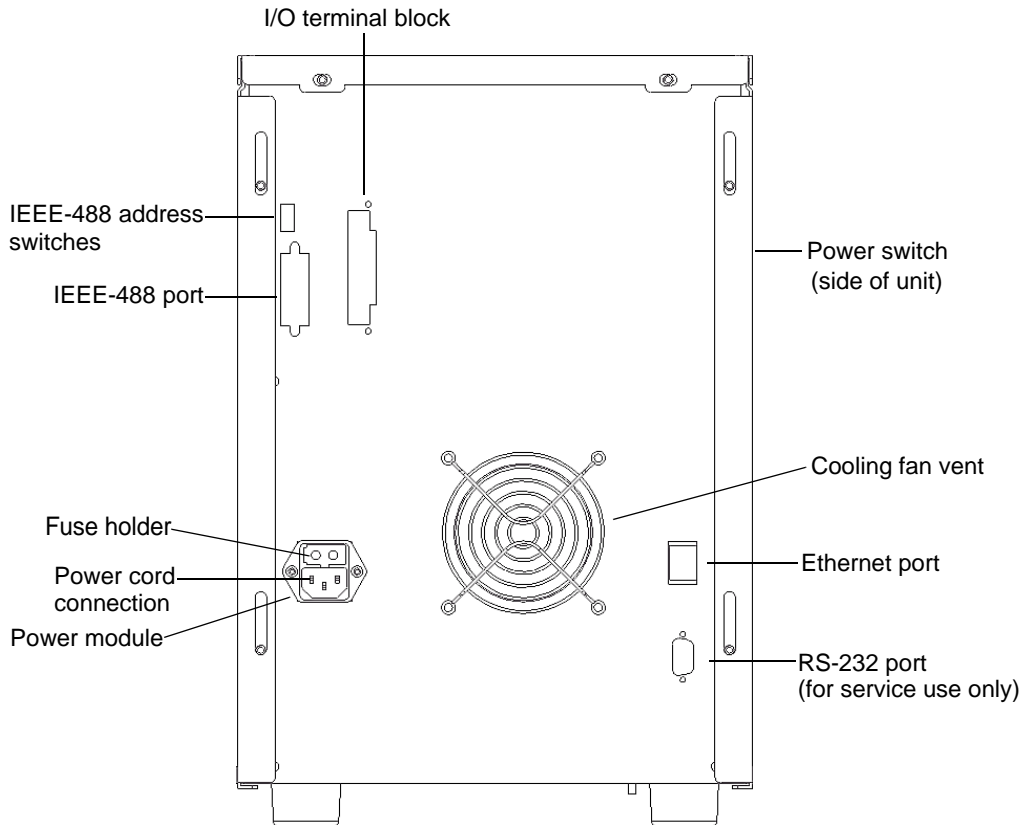
Fluid-handling components (Continued)

| Component | Description |
|---|---|
| Pump outlet | Routes eluent to the injector, column, and detector. |
| Reference valve (or vent valve for 1525 μ) | Directs flow from the pump to waste for purging, or through the injector, the column, and the rest of the system. |
| Reference (or vent) valve waste line | Routes flow from the reference (or vent) valve to the waste container. |
| Restrictor tee (1525 μ pump only) | Blends two solvents (with backflow prevention). |
| Seal wash holes (not visible) | Allow manual flushing of plunger seals. |
| Tee (1525/1525EF pump only) | Blends two solvents. |

Electronic components

Before you install the 1500-series pump, familiarize yourself with its electronic components, as illustrated in the figure below.

Rear-panel electronic components in the 1500-series pump



The following table describes the functions of the 1500-series pump's electronic components.

Electronic components in a 1500-series pump

| Component | Description |
|--------------------|--|
| Cooling fan vent | Exhausts air for cooling internal electronics. |
| I/O terminal block | Provides input and output contact closures for connecting to external devices. |

Electronic components in a 1500-series pump (Continued)

| Component | Description |
|---------------------------|--|
| Power entry module | Provides receptacles for power cord and fuses. |
| Ethernet | Connects the pump to an Ethernet LAN network card or Ethernet switch connected with the data control system. |
| IEEE-488 connector | Connects the pump to a busLAC/E™ or NI GPIB card in the data control system. |
| IEEE-488 address switches | Sets the IEEE-488 address for the pump. |
| Power switch | Powers the pump on and off. |
| Fuse holder | Contains power fuses. |

Pump control

Using Waters data control software, such as Breeze 2 software, you can control and monitor 1500-series HPLC pumps in isocratic or binary applications. For detailed information about specific data control software versions and requirements, refer to the release notes for the Waters 1500-series HPLC pumps.

Use Waters data control software to perform these tasks:

- Set all pump control parameters and operating ranges
- Define binary gradient conditions for a run (binary pumps only)
- Prime and purge the eluent flow path

Under data control, the pump can operate in one of these configurations:

- Where all system components, including a 1500-series pump and optional column heater, communicate with the data system via an Ethernet communications interface
- Where all system components, including a 1500-series pump and optional column heater, communicate with the data system via an IEEE-488 bus interface

You cannot use the pump or column heater's Ethernet port at the same time you are using its IEEE-488 bus interface for communications.

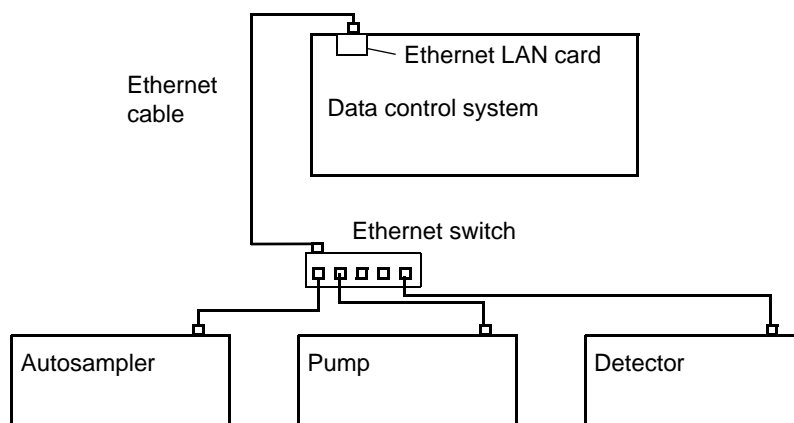
Ethernet configuration

To communicate with the Waters data control system via Ethernet, an Ethernet cable connects the pump with the system's Ethernet network in one of two ways:

- Directly, through the Ethernet LAN card in the data control system
- Through a network switch

For more information, see [page 2-5](#).

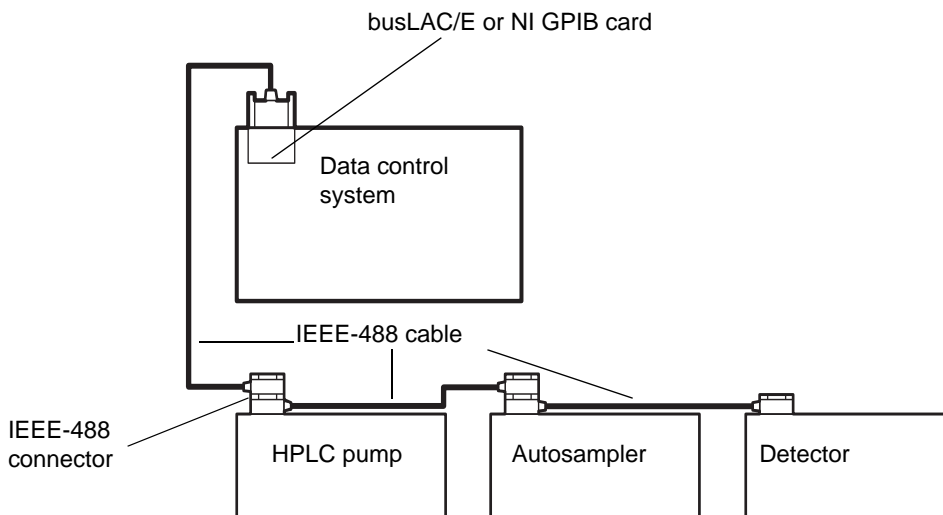
HPLC system configuration using Ethernet communications



IEEE-488 configuration

To communicate with the Waters data control system via IEEE-488, an IEEE-488 cable connects the 1500-series pump to an IEEE-488 controller (a busLAC/E™ card in the data control system for Breeze or Empower control or to a NI GPIB card for MassLynx control). For more information, see [page 2-6](#).

IHPLC system using IEEE-488 connections



Options and accessories

Various options and accessories are available to suit your HPLC pump applications and site requirements.

1500-series column heater

The 1500-series column heater maintains the column at temperatures from 5 °C above ambient (minimum of 20 °C) to 60 °C. It mounts on the side of the pump, for easy access (see the [figure “1525 binary pump with optional column heater” on page 1-7](#)). For installation instructions, see [page 3-2](#).

Integral vacuum degasser

The integral vacuum degasser is a standard feature of the 1525 μ binary pump and an optional feature of the 1515/1525/1525EF HPLC pumps. It provides HPLC systems with an automatic, continuous method of removing dissolved gases from mobile phases. For installation instructions, see [page 3-14](#).

Automated plunger seal wash

The plunger seal wash system is an optional accessory for all 1500-series pumps. The seal wash solvent lubricates the plunger and flushes away any solvent or dried salts forced past the plunger seal from the high-pressure side of each piston chamber. This wash cycle extends the life of the seals. For installation instructions, see [page 3-17](#).

Once the pump is powered-on and seal wash pump is primed, the plunger seal wash system operates automatically (see [page 4-8](#)).

Manual injector

An optional manual injector allows you to manually control sample injections during a run. This technique is useful with preparative or semi-preparative HPLC applications.

Waters offers two different model manual injectors for the 1500-series pumps:

- 1500-series manual injector
- FlexInject manual dual injector

The 1500-series manual injector is supported on all 1500-series pumps except the 1525 μ . It consists of a single injector valve that you can install and use to perform precise manual sample injections.

The FlexInject manual dual injector operates with any fluid-mixing system, including 1500-series pumps. It allows you to inject small or large sample volumes by simply selecting a valve.

The FlexInject injector can operate with flow rates as high as 150 mL/min and mounts on either side of the 1500-series pump for easy access. Its two sample injectors are pre-mounted along with a selector valve, which diverts the flow to small- or large-scale sample preparations. You can connect analytical as well as preparative columns to the respective injector.

For instructions on installing the 1500-series manual injector or the FlexInject manual dual injector, see [page 3-4](#).

Ethernet communications kit

For help with HPLC Ethernet connections, you can order from Waters the Ethernet Switch Communications Kit (part number 700004123). The kit includes an 8-port Ethernet switch, cables, and a mounting bracket, for mounting the switch on the rear panel of the 1500-series pump or separations

module. For additional Ethernet information, see the *Waters Ethernet Instrument Getting Started Guide*.

Gradient mixers

The Waters 1500-series pumps support various configurations of gradient mixers that vary in eluent volume capacity. You can order and install one or more of these mixers in a 1500-series pump. For installation instructions, see [page 3-8](#).

2 Installing the HPLC Pump

This chapter describes how to connect your pump's electrical cables and plumbing.

If a pump's configuration includes options (such as a column heater) or accessories (such as a different eluent mixer), refer to the appropriate sections in [Chapter 3](#) to complete your 1500-series pump installation.

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| Unpacking | 2-3 |
| Connecting power and signal cables | 2-4 |
| Connecting pump inlet and outlet lines | 2-11 |

Site requirements

Install the 1500-series pump at a site that meets the specifications indicated in the following table.

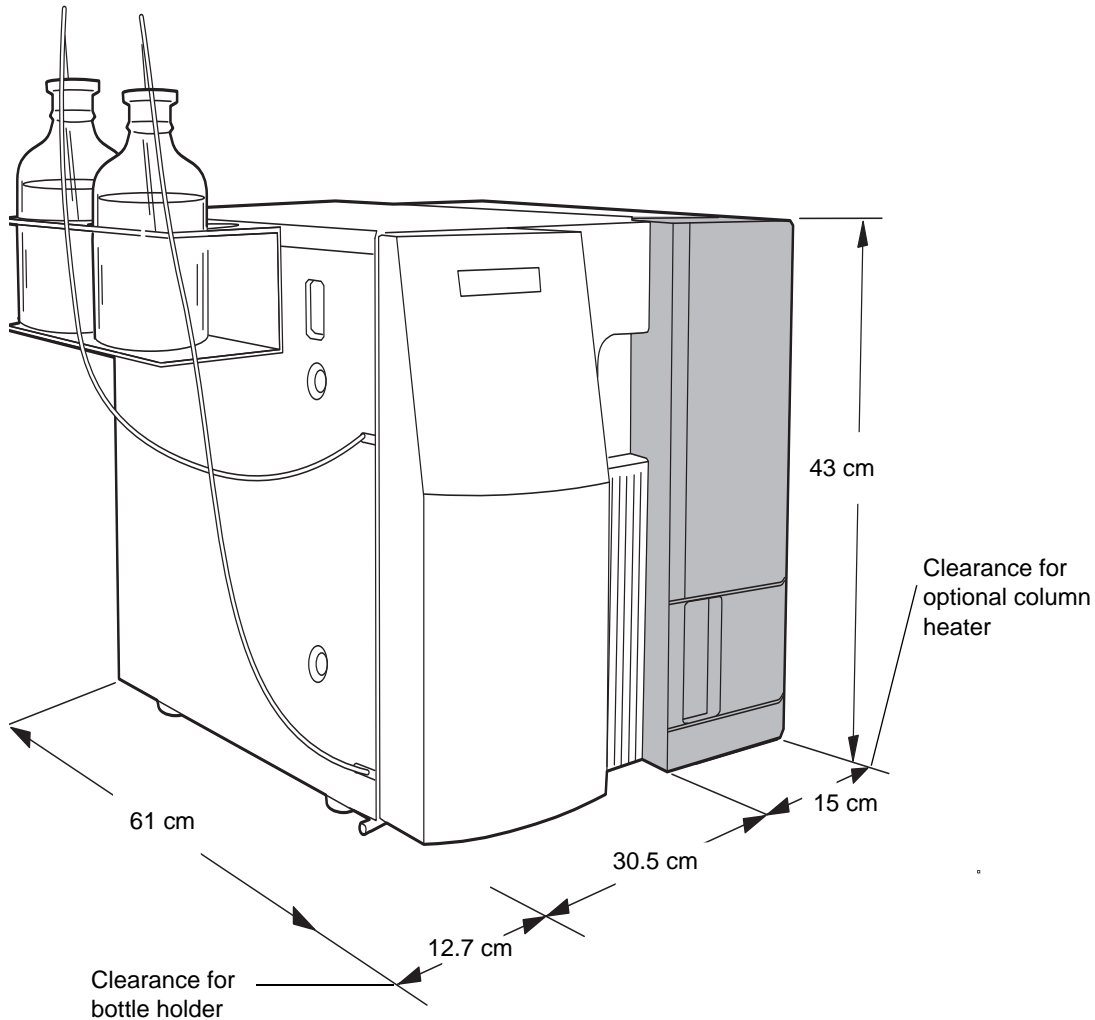
Installation site requirements

| Factor | Requirement |
|-----------------------------|---|
| Temperature | 4 to 40 °C |
| Relative humidity | 20 to 80%, noncondensing |
| Bench space | Width: 43 cm, including bottle holder (58 cm with column heater) Depth: 61 cm Height: 43 cm |
| Vibration | Negligible |
| Clearance | At least 15 cm at rear, for ventilation and cable connections |
| Static electricity | Negligible |
| Input voltage and frequency | Grounded AC, 120/240 VAC, 50/60 Hz, single phase |
| Electromagnetic fields | No nearby source of electromagnetic noise, such as arcing relays or electric motors |
| Power requirement | 200 VA for pump 150 VA for column heater |



Caution: To avoid overheating and to provide clearance for cable connections, ensure there is at least a 15-cm clearance at the rear of the pump.

Dimensions of a 1500-series pump



Unpacking

A Waters 1500-series HPLC pump is shipped in a single carton that contains the following items:

- Waters 1515 Isocratic, 1525 Binary, or 1525 μ HPLC Pump
- Startup kit

- Bottle holder
- Certificate of structural validation

To unpack a 1500-series pump:

1. Open the carton and remove the startup kit and other items from the top of the carton.
2. Using both hands, lift the pump (and its foam packing material) out of the carton.
3. Carefully set the pump down, and then remove the foam packing material from both ends of the pump.
4. Check the contents of the startup kit against the startup kit parts list to confirm that all items are included.
5. Verify that the serial number on the inside-left frame of the pump matches the serial number on the certificate of structural validation.
Tip: Keep the certificate of structural validation with this guide for future reference.
6. Inspect all items for damage, and immediately report any shipping damage to both the shipping company and your Waters representative.

If shipping damage occurred, contact Waters Customer Service. Refer to *Waters Licenses, Warranties, and Support* for complete information on shipping damages and claims.

Connecting power and signal cables

For proper operation, the 1500-series HPLC pump requires these items:

- A grounded, AC power supply with no abrupt voltage fluctuations
- If using Ethernet communications, a connection to a data control system and other Ethernet devices in the HPLC system
- If using IEEE-488 communications, a unique IEEE-488 address for the pump, and an IEEE-488 connection to a data control system and other IEEE-488 devices
- If using an optional manual injector, an inject-start output connection for the detector (see [page 3-5](#))

Required materials

- If using Ethernet communications, an Ethernet cable, and optionally, an Ethernet switch (supplied with the Ethernet Switch Communications Kit, part number 700004123).
- If using IEEE-488 communications, a 2-meter IEEE-488 cable (supplied with the data control system).
- Power cord (startup kit)
- Flat-blade screwdriver – small (required when connecting the inject start signal cable)

Connecting the power supply

The 1500-series pump automatically adjusts for AC input voltage.

To make the power supply connection:

1. Insert the 120 V or 240 V power cord into the power connector on the rear of the pump.
2. Plug the other end of the power cord into a grounded power outlet.

Making ethernet connections

The 1500-series pump, other chromatography equipment in the HPLC system such as the column heater, autosampler, detector, and the data control workstation can interconnect using Ethernet cables and a networking switch (see [page 1-16](#)).

The pump is equipped with a RJ-45 connector for Ethernet port communications. The Ethernet port—a 10/100 Base-T networking interface, is used only for remote control, the case when Empower 2 controls its operation, and for firmware upgrades via the Waters Autoloader utility.

Requirements:

- In an Ethernet configuration, all Waters HPLC system components, including the 1500-series pump and optional column heater, must communicate with the data system via Ethernet communications.
- As with IEEE-488 control, when using an autoinjector, triggering of the inject start signal for the 1500-series pump occurs over the Ethernet

cable, so no external I/O cable is needed. For more information, see [page 3-5](#).

You cannot use the 1500-series pump's Ethernet port at the same time you are using its IEEE-488 bus interface for communications.

To make the ethernet connections:

1. Connect one end of the Ethernet cable to the Ethernet port on the pump's rear panel.
2. Connect the other end of the Ethernet cable to the Ethernet LAN network card in the data control system or an Ethernet switch connected to the data control system. For additional Ethernet configuration information, see the *Waters Ethernet Instrument Getting Started Guide*.

Making IEEE-488 connections

If you are not using Ethernet communications for your HPLC configuration, the 1500-series pump, interconnect other chromatography equipment in the HPLC system such as the column heater, autosampler, detector, and the data control workstation using IEEE-488 cables.

Most chromatography equipment is shipped with a 1-meter cable with dual-receptacle connectors at each end. The Waters data control workstation, such as a Breeze 2 system, comes with a 2-meter cable with a dual-receptacle connector at one end and a single-receptacle connector at the other end.

The IEEE-488 cable transmits digital data between the pump and the IEEE-488 interface card (such as, a busLAC/E card) in the data control system (computer). Observe the IEEE cabling and connection requirements and follow IEEE specifications when adding the pump to the existing IEEE-488 connections.

Requirements:

- In an IEEE-488 configuration, all of the Waters HPLC system components, including the 1500-series pump and optional column heater, must communicate with the data system via IEEE-488 communications.
- When using an autoinjector, triggering of the inject start signal for the 1500-series pump occurs over the IEEE-488 cable, so no external I/O cable is needed. For more information, see [page 3-5](#).

You cannot use the pump's Ethernet port at the same time you are using its IEEE-488 bus interface for communications.

Before you connect the pump, refer to the IEEE-488 guidelines below.

IEEE-488 guidelines

Follow these guidelines when you install and use your system:

- Always keep all devices powered-on while your system is in use.
- While a system is active on the IEEE-488 bus, do not power-on or off any device on the bus.
- The maximum number of devices that can be connected together to form one interface system is 15 (14 instruments plus the busLAC/E or NI GPIB card).
- The maximum total cable length to connect the devices and the busLAC/E or NI GPIB card in one interface system is 2 meters times the number of devices, or 20 meters, whichever is smaller.
- The maximum cable length between two devices is 4 meters.
- The minimum cable length between two devices is 1 meter.

Tip: Use the minimal cable lengths to ensure proper signal transmission. Cable lengths greater than the maximum values, or less than the minimum values, can cause IEEE-488 communication failures.

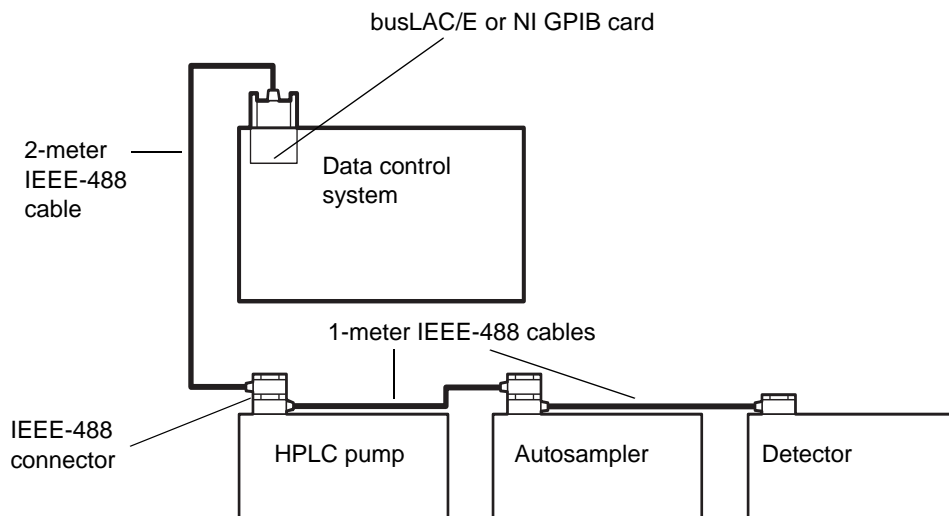
Connecting IEEE-488 devices

The steps in this procedure assume that you have not already connected any of the other HPLC system IEEE-488 devices (the autosampler or detector, for example) to the data control workstation. If you have already connected other devices to the workstation, connect the pump to the existing chain of devices. The order in which you connect IEEE-488 devices is not important.

To make the IEEE-488 connections for the data control system:

1. Connect the single-receptacle end of the 2-meter IEEE-488 cable to the busLAC/E card in the Empower or Breeze 2 workstation, or to the NI GPIB card in the MassLynx workstation.

IHPLC system using IEEE-488 connections



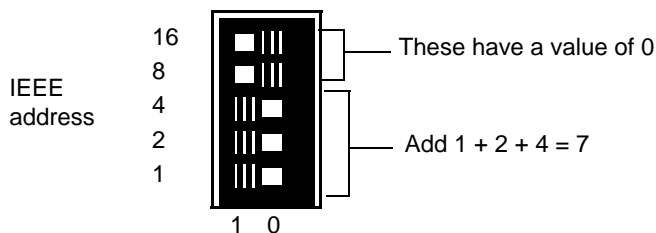
2. Connect the dual-receptacle end of the 2-meter IEEE-488 cable to the IEEE-488 port on one of the IEEE-488 devices (pump, column heater, autosampler, or detector).
3. Connect one end of a 1-meter IEEE-488 cable (with dual-receptacle connectors at each end) to the cable receptacle on the first device. Connect the other end of the cable to the IEEE-488 port on the next device.

4. Repeat [step 3](#) for each additional IEEE-488 device.
5. Ensure all IEEE-488 cable connector screws are finger-tight.

Setting the IEEE-488 address

You must set a unique address for the pump (and for each device connected to the HPLC system on the IEEE-488 bus). Valid IEEE-488 instrument addresses are 2 through 29, and are set using the DIP switches on the rear panel of the pump from 0 to 1 (see the table below). For example, to obtain an address of 7, add the numbers of the switches in the 1 position.

Setting the address switches



To set the IEEE-488 address:

1. Ensure the unit is powered-off.
2. Set the DIP switches on the rear panel of the pump to a unique IEEE-488 address, referring to the following table for DIP switch settings for valid IEEE-488 addresses.

IEEE-488 switch settings

| IEEE-488 address | Switch settings | | | | |
|------------------|-----------------|---|---|---|----|
| | 1 | 2 | 4 | 8 | 16 |
| 2 | 0 | 1 | 0 | 0 | 0 |
| 3 | 1 | 1 | 0 | 0 | 0 |
| 4 | 0 | 0 | 1 | 0 | 0 |
| 5 | 1 | 0 | 1 | 0 | 0 |
| 6 | 0 | 1 | 1 | 0 | 0 |

IEEE-488 switch settings (Continued)

| IEEE-488 address | Switch settings | | | | |
|------------------|-----------------|---|---|---|----|
| | 1 | 2 | 4 | 8 | 16 |
| 7 | 1 | 1 | 1 | 0 | 0 |
| 8 | 0 | 0 | 0 | 1 | 0 |
| 9 | 1 | 0 | 0 | 1 | 0 |
| 10 | 0 | 1 | 0 | 1 | 0 |
| 11 | 1 | 1 | 0 | 1 | 0 |
| 12 | 0 | 0 | 1 | 1 | 0 |
| 13 | 1 | 0 | 1 | 1 | 0 |
| 14 | 0 | 1 | 1 | 1 | 0 |
| 15 | 1 | 1 | 1 | 1 | 0 |
| 16 | 0 | 0 | 0 | 0 | 1 |
| 17 | 1 | 0 | 0 | 0 | 1 |
| 18 | 0 | 1 | 0 | 0 | 1 |
| 19 | 1 | 1 | 0 | 0 | 1 |
| 20 | 0 | 0 | 1 | 0 | 1 |
| 21 | 1 | 0 | 1 | 0 | 1 |
| 22 | 0 | 1 | 1 | 0 | 1 |
| 23 | 1 | 1 | 1 | 0 | 1 |
| 24 | 0 | 0 | 0 | 1 | 1 |
| 25 | 1 | 0 | 0 | 1 | 1 |
| 26 | 0 | 1 | 0 | 1 | 1 |
| 27 | 1 | 1 | 0 | 1 | 1 |
| 28 | 0 | 0 | 1 | 1 | 1 |
| 29 | 1 | 0 | 1 | 1 | 1 |

Connecting pump inlet and outlet lines

This section describes how to make these connections and attachments to the 1500-series pump:

- Eluent supply connections
- Outlet connection
- Fluid waste lines

Connecting the eluent supply

Follow the instructions in this section to connect the pump inlet to an eluent reservoir.

Tip: When using an integral vacuum degasser, refer to [page 3-15](#) for instructions on connecting the degasser to the eluent reservoir and pump inlet.

Required materials

- Tefzel[®] ferrule and compression screw (startup kit) – one set per pump
- 1/8-inch OD ETFE tubing (part number WAT270714 in startup kit)
- ETFE tubing (startup kit) (part number WAT024036 in startup kit)
- Reservoir containing filtered, degassed eluent – one per pump
- Bottle holder
- Bottle caps (1 L) (part number WAT062479 in startup kit)
- Stainless steel solvent filter (startup kit) – one per pump (part number WAT025531 in startup kit)
- Plastic tubing cutter (not included) (part number WAT031795) or razor blade
- Inlet tubing labels (not included with the 1515 pump) (part numbers WAT087186[A] and WAT087187[B])

Installing the bottle holder

To install the bottle holder:

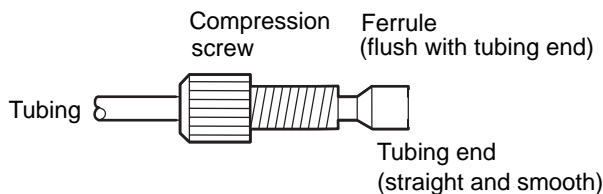
1. Position the rack along the pump's left-hand side.
The two cutouts at the bottom inside of the rack rest must on their corresponding positioning screws on the pump.
2. Holding the rack flush against the surface of the pump, finger tighten the captive screw to secure the rack.

Connecting to the pump inlet

To connect the eluent tubing to the pump inlet:

1. Measure the length of 1/8-inch ETFE tubing required to connect an eluent reservoir (mounted in the bottle holder) to an inlet manifold on the pump.
When using high-viscosity eluents, you can possibly need to increase reservoir height, 46 to 61 cm, above the pump's inlet manifold.
2. Insert the ETFE tubing into the 1/8-inch diameter hole of the tubing cutter, making sure that the tubing that extends from the metal side of the cutter is the correct length (as determined in [step 1](#)).
3. Insert the razor blade into the cutter, and press down to cut the tubing. Ensure the cut end is straight and free from burrs.
4. Slide a compression screw over one end of the tubing, followed by a ferrule with its tapered end facing away from the tubing end (its wide end flush with the tubing end), as shown in the figure below.

Reverse ferrule and compression screw assembly



5. Firmly seat the tubing end into the inlet manifold on the pump, and then finger-tighten the compression screw.



Caution: To avoid damaging the ferrule, do not overtighten the compression screw.

6. If you have a binary pump, repeat [step 1](#) through [step 5](#) for the second pump assembly.

Preparing solvent reservoirs

General recommendations:

- When using the instrument for general chromatography (that is, reverse and/or normal phase and gel permeation), use high-quality lab glassware made of borosilicate glass for all reservoirs (solvent, seal wash, and needle wash).
- When using techniques such as ion chromatography, where glass containers can contribute ionic contamination (sodium and/or chloride ions), use laboratory-grade polypropylene or polyethylene containers as reservoirs.
- When using the instrument in combination with a mass spectrometer, refer to recommendations published in the most recent version of “Controlling Contamination in UPLC/MS and HPLC/MS Systems” on the Waters Web site.
- Choose 1-L solvent reservoirs that provide a snug fit for the reservoir caps supplied in the startup kit. The solvent reservoir caps help control solvent evaporation. Three feather-edged holes in each cap make a gas tight seal around the solvent and vent tubes.

Place the reservoirs in the solvent bottle tray, and set the tray above the solvent management system components.

Connecting to an eluent reservoir

Tip: To avoid having eluent leak from a pump outlet, position each eluent reservoir at a level below its corresponding pump inlet until the outlet is connected to the system.

To connect the inlet tubing to an eluent reservoir:

1. If you have a binary pump, slide the inlet tubing label onto appropriate tube.
2. Insert the free end of the 1/8-inch ETFE inlet tubing into the cap of an eluent reservoir.
3. Slide one of the pieces of tubing (part number WAT024036) approximately 2 cm over the end of the 1/8-inch ETFE tubing.
4. Insert the stainless tubing fitting on the solvent filter into the open end of this tubing.
5. Install the cap onto the eluent reservoir, and push the tubing through the cap until the filter reaches the bottom of the reservoir.
6. If you have a binary pump, repeat [step 1](#) through [step 5](#) for the second pump assembly.

Tip: If you are connecting the pump to a Waters 717plus or 2707 autosampler, you can use one position of the bottle holder to hold a bottle that contains the needle wash solvent.

Connecting the pump outlet



Caution: To avoid having eluent leak from a pump outlet, position each eluent reservoir below its corresponding pump inlet until the outlet is connected to the system.

Follow the instructions in this section to connect the pump outlet to the injector (or next component in the flow path). Making pump outlet connections involves these tasks:

- Cutting the tubing
- Attaching a compression fitting to each end of the tubing
- Connecting each end of the tubing

Required materials

- Two stainless steel ferrules and standard compression screws (part number WAT025604) (startup kit)
- 1/16-inch OD stainless steel tubing (startup kit)

- Circular tubing cutter (part number WAT022384) or knife-edge file
- Needle-nose pliers (two pairs if cutting tubing with a knife-edge file)
- 5/16-inch open-end wrench (part number WAT022527 (startup kit))

Cutting the tubing to length

To cut the pump outlet tubing you will need:

1. Measure the length of stainless steel tubing required to connect the pump outlet to the injector or other instrument.
2. Use a knife-edge file to scribe the circumference of the tubing at the desired break.

Tip: Whenever possible, use a circular tubing cutter instead of the knife-edge file to obtain a clean, square cut.

3. Grasp the tubing on both sides of the scribe mark with cloth-covered pliers (to prevent marring of the tube surface) and gently work the tube back and forth until it snaps.

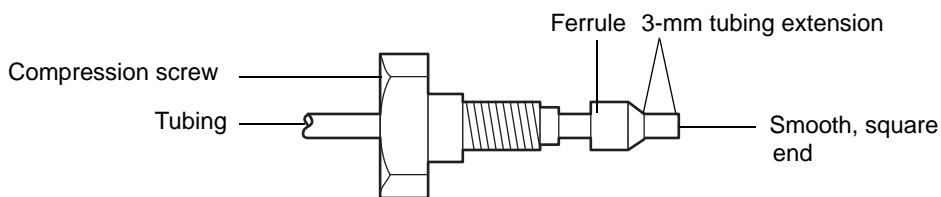
Requirement: Ensure that the break is square and free of burrs.

Attaching the compression fittings to the tubing

To attach the fittings:

1. Slide a compression screw onto one end of the tubing, and then slide a ferrule onto the tubing, with the large end of the taper toward the screw, as shown below.

Standard ferrule and compression screw assembly



2. While firmly pressing the tubing into the pump outlet or other system component, finger-tighten the compression screw.

3. Use the 5/16-inch wrench to tighten the screw another quarter turn. This seats the ferrule against the tubing.
4. Unscrew the assembled fitting, and verify that the length of tubing extending beyond the ferrule is 3 mm.
5. Repeat [step 1](#) to [step 4](#) for the other end of the stainless steel tubing.

Performing the connections

To connect the outlet tubing to the injector or other device in your HPLC configuration:

1. While pressing one end of the tubing assembly into the pump outlet fitting, finger-tighten the compression screw, and then use the 5/16-inch wrench to tighten the screw another 1/8-turn.
Tip: Leave the instrument end of the outlet tubing disconnected until you have primed the pump as described in [Chapter 4](#).
2. After you primed the pump, press the free end of the tubing assembly into the injector or next device in your HPLC system, and finger-tighten the compression screw.
3. Use the 5/16-inch wrench to tighten the screw another 1/8-turn.

Connecting fluid waste lines

Follow the instructions in this section to connect the fluid-waste lines. The instructions describe how to do the following:

- Connect the reference valve (or vent valve for 1525 μ) and optional manual injector waste lines
- Connect the drip tray waste line

Required materials

- Tubing (part number WAT076775 in startup kit)
- Teflon tubing (part number WAT050693 in startup kit)
- Convoluted ETFE tubing (part number WAT241095 in startup kit)
- Plastic tubing cutter (not included) (part number WAT031795) or razor blade

Connecting pump waste tubing

To connect the tubing:

1. Obtain a piece of convoluted tubing and stretch it apart.
2. Feed a piece of smooth Teflon[®] tubing through the convoluted tubing until ends of both pieces are flush with one another.

Tip: This dual tubing prevents the waste line from developing kinks.

3. Mount this piece of dual tubing parallel with the drip tray (see the [figure “Fluid-handling components in the 1525/1525EF pumps” on page 1-8](#) or the [figure “Fluid-handling components in the 1525 \$\mu\$ pump” on page 1-9](#)), and secure it in the provided spring clips.
4. Insert the reference valve (or vent valve) waste line and the optional manual injector waste lines together in the top end of the dual tubing.
5. Insert the other end of the dual tubing in a properly situated waste container.

To connect the drip tray to the waste line:

1. Attach tubing to the drip tray waste exit (see the [figure “Fluid-handling components in the 1525/1525EF pumps” on page 1-8](#) or [“Fluid-handling components in the 1525 \$\mu\$ pump” on page 1-9](#)).
2. Insert the other end of the tubing in a waste container.

3

Installing Options and Accessories

This chapter describes how to install the various options and accessories for 1500-series HPLC pumps.

For the current list of 1500-series pump options and accessories, as well as ordering information and prerequisites, visit the Waters Web site or contact Waters.

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| Topic | Page |
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| Installing the 1500-series column heater | 3-2 |
| Installing a manual injector | 3-4 |
| Installing different eluent mixers | 3-8 |
| Installing the integral vacuum degasser | 3-14 |
| Installing the plunger seal wash system | 3-17 |

Installing the 1500-series column heater

You can mount the column heater on the right-hand side of 1500-series pumps using three screws. Connectors on the rear panel of the column heater provide power to the device and enable its control.

Installation considerations

- The column heater weighs approximately 5.9 kg. No sub-assemblies are associated with the device, but it is used in conjunction with other devices and instruments. Consider the additional weight requirements for any components used in addition to the column heater, and provide sufficient support for the total system weight at the installation location.
- To facilitate ventilation and cooling, allow at least 15-cm of clearance at the rear of the column heater unit.
- Ensure that users have unobstructed access to the column heater compartment.
- The column heater does not contain any user-serviceable parts. Do not use tools to access the components inside the device.



Caution:

- All circuits connected to the column heater must be protected for Class I operation.
- To facilitate disconnecting the power source, do not install the column heater in a location that prevents access to the power switch or electrical cord connections.

To install the column heater:

1. Remove the three screws from the pump's right-hand side panel.
2. Insert the screws through the standoffs (included with the Column Heater Startup Kit) so that the flat side of the standoffs are flush with the right-hand side panel.
3. Mount the column heater on the three screws on the right-hand side-panel.
4. Connect one end of the power cable to the power connector at the rear of the column heater and the other end to the power source outlet.

5. Connect one end of the signal cable (Ethernet or IEEE-488) to the appropriate connector at the rear of the column heater, and connect the other end of the cable to the Ethernet switch or IEEE-488 bus.

If you are using IEEE-488 communications, verify that the column heater has a unique IEEE-488 address in your HPLC system (see [page 2-9](#)).

6. Attach waste tubing to the drain port on the underside of the device drip tray.

Tip: The column heater has an internal drip tray to catch any leaks that can occur during normal operation, or when changing columns.

7. Direct the waste tubing into an appropriate waste container.

To relocate the column heater after initial installation:

1. Note the current location of all electrical and tubing connections.
2. Power-off the system, and disconnect all electrical cables.
3. Disconnect any tubing that directs flow through the column heater.



Warning: To prevent injury from splashing solvent, wear eye protection and powder-free, nonlatex gloves when handling tubing that can contain solvents. If possible, drain tubing before handling it.

4. Verify that the drip tray is empty, and then disconnect the drip tray hose from the underside of the device.
5. Carefully move the device to the new location.



Caution: To avoid damage during shipment, transport the device in its original shipping container with the custom foam insert. Special shipping considerations are printed on the shipping container. Store the device according to the guidelines given in [Appendix B](#).

Operating the column heater



Warning: To avoid chemical exposure risk, always observe Good Laboratory Practices when you use this equipment and when you work with solvents and solutions. Know the chemical and physical properties of the solvents and test solutions you use. See the material safety data sheet for each solvent and test solution in use.



Warning: To avoid the possibility of electric shock, do not remove any covers from the device. The column heater unit does not contain any user-serviceable parts.

There are no operating controls located on the column heater unit. You control the temperature of the heater via data control software installed on the HPLC system's workstation. The workstation communicates with the column heater through the IEEE-488 or Ethernet signal cable. (For detailed information about specific software versions and requirements, refer to the release notes for the Waters 1500-series column heater.)

You can clean the device with mild detergent and a damp cloth.

For additional information, see *Controlling Contamination in UltraPerformance LC/MS and HPLC/MS Systems* on the Waters Web site.

Installing a manual injector

If you are installing a 1500-series manual injector or FlexInject dual manual injector, refer to the installation instructions and startup list included with the injector.

Requirements:

- If you are installing a manual injector, you must connect the pump outlet and injector waste lines according to the instructions on [page 2-14](#) and [page 2-16](#).
- When using a manual injector, you must configure an inject start signal for the detector (see [page 3-5](#)).

Connecting to the column or column heater

To connect the manual injector to the column or column heater:

Tip: Ensure you have already installed the manual injector as described in the instructions included with the manual injector:

1. Prime the pump, purging the injector (see the instructions appropriate for your data control system in [Chapter 4](#)).
2. Connect the free end of the manual injector outlet tubing to the column or column heater.

Connecting the inject start signal (for manual injector)

For 1500-series pumps with an optional 1500-series manual injector or FlexInject dual manual injector, you make an inject-start output signal connection from the I/O terminal block, on the pump, to the appropriate inputs on the detector.

This section describes the connections for Waters 2489 and 2414 Detectors. For a description of signal connections to other model detectors, refer to the detector's operator's guide.

Required materials

- Two-wire cable from the manual injector
- Screwdriver, small, flat-blade

To make the inject start signal connection from the pump to the detector:

1. Remove the terminal block from the rear of the pump.
2. Insert the red stripped wire into position 1 of the terminal block, then tighten the screw to secure the wire.
3. Insert the black stripped wire into position 2 of the terminal block, then tighten the screw to secure the wire.
4. Firmly push the terminal into the back of the pump.
5. Remove the terminal block from connector A on the back of the detector (see the [figure “Inject start connection to a 2489 detector”](#) on [page 3-7](#) and the [figure “Inject-start connection to a 2414 detector”](#) on [page 3-8](#), depending on which model detector you have).

6. Insert the red stripped wire into position 1 of the detector's terminal block, as indicated in the table below (Inject Start + on the 2489 or Chart Mark + on the 2414), and then tighten the screw to secure the wire.

I/O terminal block connections

| Function | Pin number | Direction |
|----------------|------------|-----------------|
| Inject Start + | 1 | Output or Input |
| Inject Start – | 2 | |
| Stop Flow + | 3 | Input |
| Stop Flow – | 4 | |
| Event Relay 1A | 5 | Output |
| Event Relay 1B | 6 | |
| Event Relay 2A | 7 | Output |
| Event Relay 2B | 8 | |
| Event Relay 3A | 9 | Output |
| Event Relay 3B | 10 | |
| Event Relay 4A | 11 | Output |
| Event Relay 4B | 12 | |

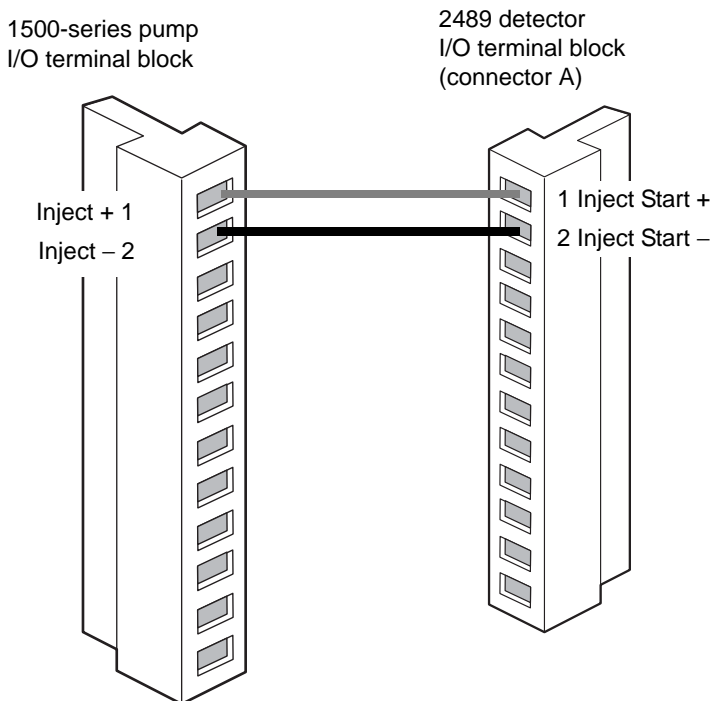
7. Insert the black stripped wire into position 2 of the terminal block (Inject Start – on the 2489 or Chart Mark – on the 2414), and then tighten the screw to secure the wire.

Tip: To connect the inject-start pump signal to other detector inputs (for example, to the Autozero inputs), refer to the appropriate detector operator's guide for details.

8. Firmly push the terminal block into the back of the detector

The following figure shows the inject-start connection from the I/O terminal block on the pump to connector A on a 2489 detector.

Inject start connection to a 2489 detector



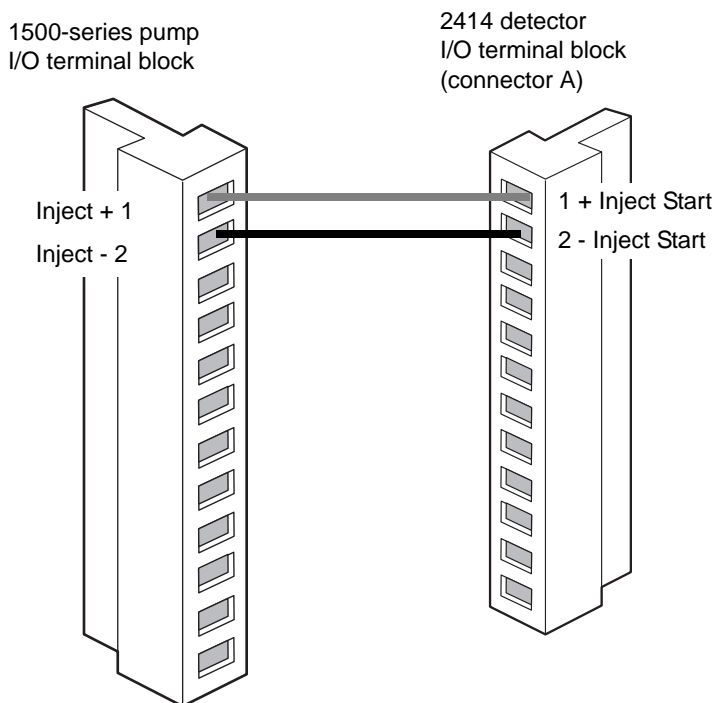
Requirements:

- Set the manual injector in the LOAD position when you start the data control software. The busLAC/E card can fail to boot if the injector is left in the INJECT position.
- When you use the manual injector in conjunction with compositional gradients, turn the injector back to the LOAD position before the end of the run. Leaving the injector in the INJECT position automatically triggers the gradient table for subsequent injections, but data acquisition does not start.

For instructions on operating the FlexInject Dual Manual Injector with a detector, refer to the *Waters FlexInject Dual Manual Injector Module Installation Guide*.

The following figure shows the inject-start connection from the I/O terminal block on the pump to connector A on a 2414 detector.

Inject-start connection to a 2414 detector



If you are using the 2414 detector, with the manual injector, the instrument's acquisition does not start until you turn the injector back to the LOAD position. When injecting, make sure you keep the injector in the INJECT position for a minimum of two loop volumes before turning back to the LOAD position to start acquiring data.

Tip: For instructions on operating the FlexInject Dual Manual Injector with a detector, refer to the *Waters FlexInject Dual Manual Injector Module Installation Guide*.

Installing different eluent mixers

You can use various eluent mixer configurations in the 1500-series pumps. This section describes these configurations, including how to install a new mixer, or replace an existing mixer, in your pump.

The pump has four pairs of mounting holes on the upper left-hand side of the chassis face that are used for installing one or more mixers. These hole pairs are designated 1 to 4, top to bottom (see below).

Position of mixer mounting hole pairs on pump chassis face

○ — 1 — ○

○ — 2 — ○

○ — 3 — ○

○ — 4 — ○

1500-series mixer options

| Pump model | Standard mixer configuration | Option 1 | Option 2 | Option 3 |
|------------|------------------------------|--------------|-----------------------|-----------------------|
| 1515 | No mixer | Single GM150 | Dual GM150s | Large-volume gradient |
| 1525 | Single GM150 | Dual GM150s | Large-volume gradient | N/A |
| 1525 μ | 50 μ l | Single GM150 | N/A | N/A |

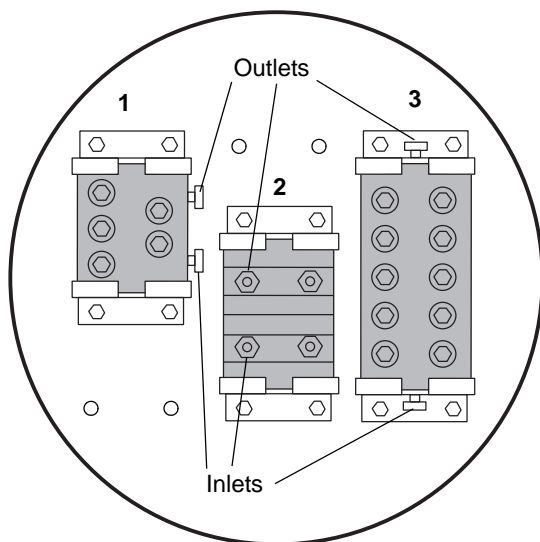
1515 isocratic HPLC pump

Most isocratic separations do not need a mixer, so the 1515 pump does not include one. If you need mixing capability, you can obtain any of the three configurations shown in the table above.

1525 binary HPLC pump

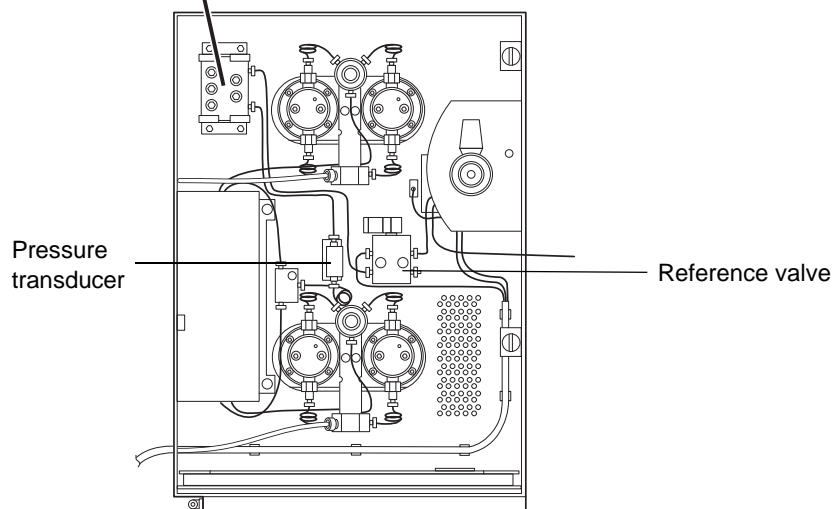
The 1525 pump is shipped with one standard GM150 gradient mixer (part number WAT055847) mounted on the upper left-hand side of the chassis face (shown as configuration 1 in the figure, below). If you require a second GM150 mixer, see configuration 2. If you want to replace the standard GM150 mixer with a large-volume gradient mixer, see configuration 3.

1525 pump showing different mixer configurations (labeled 1, 2, and 3)



Configuration 1 – Single GM150 mixer
Configuration 2 – Dual GM150 mixers*
Configuration 3 – Large-volume mixer

*Oriented horizontally top to bottom.



Installing a single GM150 gradient mixer

Tip: This mixer is standard on the 1525 pump.

To install the single GM150 gradient mixer on the 1515 pump:

1. Acquire a mixer (part number WAT055847).
2. Remove the mounting screws and mixer clamps from the upper-left corner of the chassis face.
3. Orient the mixer between the clamps as shown in configuration 1 of the [figure “1525 pump showing different mixer configurations \(labeled 1, 2, and 3\)” on page 3-10](#) using hole pairs 1 and 3.
4. Firmly tighten the mounting screws.
5. Cut an appropriate length of 0.009-inch diameter tubing (part number WAT026973 in the startup kit), and attach one end to the pressure transducer outlet and the other end to the mixer inlet using a compression screw and ferrule on each end.
6. Cut another length of 0.009-inch diameter tubing. Connect one end to the mixer outlet and the other end to the reference valve inlet using a compression screw and ferrule on each end.

To install the single GM150 gradient mixer on the 1525 μ pump:

1. Acquire a mixer (part number WAT055847) and the mixer mounting bracket and (4) mounting screws (startup kit).
2. Loosen the compression screw, and disconnect the tubing that connects the pressure transducer outlet to the 50 μ l mixer inlet.
3. Loosen the compression screw, and disconnect the tubing that connects the 50 μ l mixer outlet to the system/vent valve, and set the mixer aside.
4. Orient the new mixer between the clamps, as shown in configuration 1 of the [figure “1525 pump showing different mixer configurations \(labeled 1, 2, and 3\)” on page 3-10](#), using hole pairs 1 and 3.
5. Firmly tighten the mounting screws.
6. Reconnect the pressure transducer outlet tubing to the mixer inlet tightening the compression screw.
7. Reconnect the mixer outlet tubing to the system/vent valve inlet tightening the compression screw.

Installing dual GM150 gradient mixers

To install the dual GM150 gradient mixers in the 1515 pump:

1. Acquire two mixers (part number WAT055847).
2. Remove the mounting screws and the mixer clamps from their positions on the upper left-hand corner of the chassis face.
3. Put the mixers inside the mounting clamps, as shown in configuration 2 of the [figure “1525 pump showing different mixer configurations \(labeled 1, 2, and 3\)” on page 3-10](#), using hole pairs 2 and 4.
Tip: To position the two new mixers inside the mounting clamps, orient the mixers horizontally, top to bottom, so that their inlets and outlets face forward.
4. Firmly tighten the mounting screws.
5. Cut an appropriate length of 0.009-inch diameter tubing (part number WAT026973 in the startup kit) and attach one end to the pressure transducer outlet and the other end to the bottom mixer inlet using a compression screw and ferrule on each end.
6. Cut one 9.25-cm minimum length of 0.009-inch diameter tubing (part number WAT026973 in the startup kit), bend it to form a U-tube, and place a compression screw and ferrule on each end.
7. Connect the outlet of the bottom mixer to the inlet of the top mixer with the U-tube.
8. Cut another length of 0.009-inch diameter tubing. Connect one end to the top mixer outlet and the other end to the reference valve inlet using a compression screw and ferrule on each end.

To install the dual GM150 gradient mixers in the 1525 pump:

1. Acquire one additional mixer (part number WAT055847).
2. Loosen the compression screws on the inlet and outlet tubing of the mixer.
3. Move the tubing enough to allow repositioning of the mounting clamps.
4. Loosen the compression screw on the outlet tubing.
5. Remove the mounting screws, the mixer, and mounting clamps.

6. Put both mixers inside the mounting clamps, as shown in configuration 2 of the [figure “1525 pump showing different mixer configurations \(labeled 1, 2, and 3\)”](#) on page 3-10, using hole pairs 2 and 4.
7. Firmly tighten the mounting screws.
8. Reattach the pressure transducer outlet tubing to the inlet of the bottom mixer.
9. Connect the outlet of the bottom mixer to the inlet of the top mixer with the U-tube (part number 430000405, startup kit).
10. Reconnect the outlet of the top mixer to the reference valve inlet tubing.

Installing a large-volume gradient mixer

To prepare a 1515 pump:

1. Acquire a large-volume gradient mixer (part number WAT051518).
2. If the 1515 pump was previously configured for a GM150 mixer, perform these actions:
 - a. Remove the tubing that connects the pressure transducer outlet to the GM150 mixer inlet, and set it aside.

Tip: You can use the tubing as the inlet tubing to the large-volume gradient mixer. However, the existing tubing that connects the GM150 mixer outlet to the reference valve is too short to be used with the large-volume mixer.
 - b. Remove the tubing that connects the GM150 mixer outlet to the reference valve, and set it aside.
3. Remove the mounting screws, GM150 mixer (if installed) and the mixer clamps from their positions on the upper-left corner of the chassis face.

To prepare a 1525 pump:

1. Acquire a large-volume gradient mixer (part number WAT051518).
2. Remove the mounting screws, the single standard GM150 gradient mixer, and mounting clamps.

Tip: If you intend to use the GM150 mixer in the future, label the mixer outlet tube and store it with the GM150 mixer.

To install the large-volume gradient mixer in a 1525 or 1515 pump:

1. Put the mixer inside the mounting clamps, as shown in configuration 3 of the [figure “1525 pump showing different mixer configurations \(labeled 1, 2, and 3\)”](#) on page 3-10, using hole pairs 1 and 4.
2. Firmly tighten the mounting screws.
3. Cut an appropriate length of 0.009-inch diameter tubing (part number WAT026973, startup kit) and attach one end to the pressure transducer outlet and the other end to the mixer inlet using a compression screw and ferrule on each end.
4. Cut another length of 0.009-inch diameter tubing. Connect one end to the mixer outlet and the other end to the reference valve inlet using a compression screw and ferrule on each end.

Installing the integral vacuum degasser

The degasser is a standard feature of the 1525 μ pump and an optional feature of the 1515 isocratic and 1525/1525EF binary pumps. It provides HPLC systems with an automatic, continuous method of removing dissolved gases from mobile phases (for operating principles, see [page 1-4](#)).

The degasser’s vacuum system provides vacuum at a preset level to the connected vacuum chambers. The vacuum system consists of the vacuum pump, vacuum sensor, and vacuum chambers.

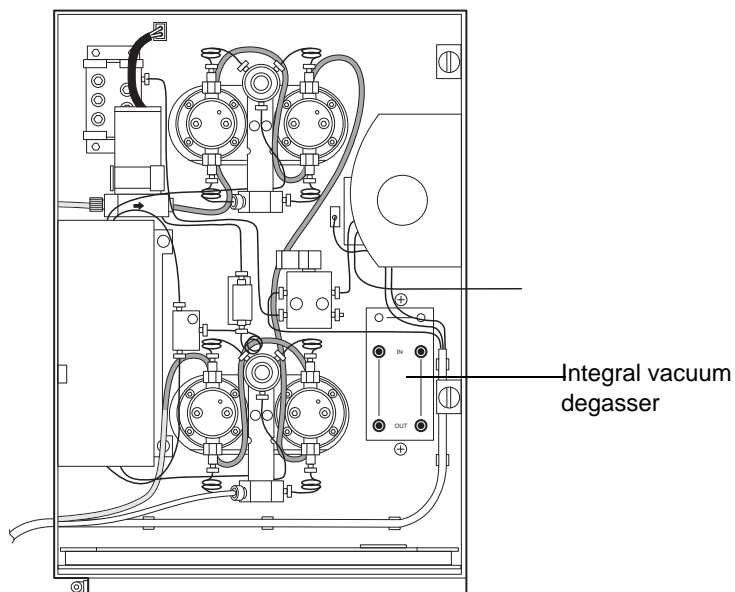
The control system consists of the control board used to monitor and control the vacuum pump, and the two-color front panel LED (see [page 4-6](#)).

The degasser removes dissolved gasses from the eluent as it passes through a tubular membrane. The membrane, enclosed in a vacuum chamber, is in the eluent flow path between the reservoir and the pump inlet. The eluent enters and exits through inlet and outlet fittings on the vacuum chamber. These fittings are labeled on—and accessible from—the pump’s front panel.

The degasser installation procedure involves removing the 1500-series pump’s top and side panels to incorporate the degasser assembly, along with its wiring and plumbing (see the installation instructions included with the Integral Vacuum Degasser Kit (P/N 205000253)).

Tip: The degasser installation is typically performed by a Waters service engineer. The instructions in this section describe only the external plumbing connections for connecting the degasser inlet and outlet tubing and vent line.

1525 binary pump with integral vacuum degasser



Required materials

- A tubing cutter that suits the tubing type: razor knife or razor blade, or a file with a cutting edge
- Tubing, 1/8-inch, OD, thick-walled Tefzel (startup kit) or 1/16-inch OD stainless steel
- Four ferrules and compression screws (startup kit) for each channel
- Tubing, 0.149-inch, OD, PTFE¹ (startup kit)

Connecting tubing to the degasser inlet and outlet

The connectors on the front panel of the degasser are 1/4-28 flat-bottom fittings. The startup kit contains the compression screws and ferrules needed to connect tubing to these fittings.

1. Polytetrafluoroethylene

Requirements:

- You connect the eluent supply tubing from the eluent reservoirs to the degasser's pump A and pump B (In) inlet fittings, instead of directly to the inlet connectors on the pump manifolds (see [page 2-11](#)).
- You connect the pump inlet tubing between the degasser's pump A and pump B (Out) outlet and the pump inlet connectors on the corresponding pump manifolds (see [page 2-12](#)).
- If you are installing the degasser on a 1515 isocratic pump, you use only one degasser vacuum chamber (A). In this case, fill the degasser's unused vacuum chamber (B) with eluent, and use pin plugs to cap the unused chamber's inlet and outlet fittings to prevent leaks.

Tip: To minimize degasser equilibration time when using only one pump, fill the degas tubing in the unused vacuum chamber with a fluid such as water or eluent. Install caps on the chamber's inlet and outlet fittings to prevent leaks.

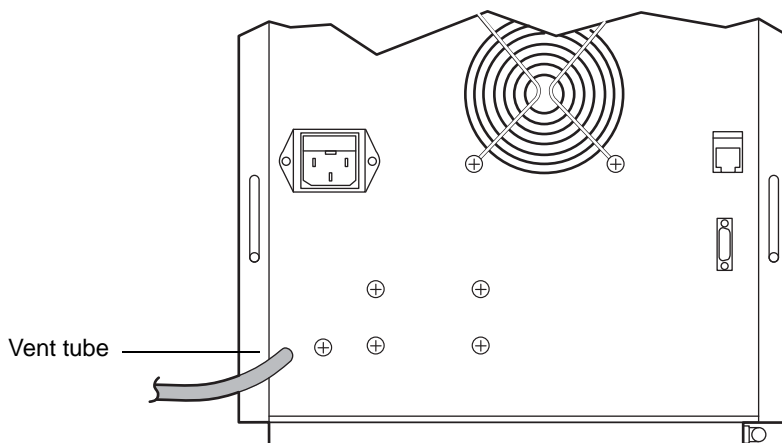
Installing the degasser vent line



Warning: To avoid exposure to eluent vapors, connect the outlet vent on the pump's rear panel to a suitable fume hood. Consult your local building and health codes for specific requirements regarding the venting of eluent vapors.

Besides removing dissolved gases from the eluents, the degasser can vaporize some eluent components. The vapors can then condense, forming droplets in the exhaust system. The degasser exhausts these gases and droplets through a vent line that exits the instrument at the rear panel, as shown in the figure, below.

Integral vacuum degasser vent tubing



To install the vent line, place the unattached end of the vent tubing in a waste container. The container catches any incidental leaks or condensates from the degasser.

Using the degasser

Electrical power is supplied to the degasser whenever you power-on the pump. With the pump's switch set to On, the degasser operates automatically while you perform HPLC runs. There are no controls to adjust as it removes gases from the eluents. For additional information, see [page 4-2](#).

Installing the plunger seal wash system

The plunger seal wash system is an optional accessory for all of the 1500-series pumps. The seal wash solvent lubricates the plunger and flushes away any solvent or dried salts forced past the plunger seal from the high-pressure side of each piston chamber. This wash cycle extends the life of the seals.

Once you install the seal wash option, use it at all times during instrument operation to protect plungers and seals.

Required materials

- Plunger seal wash system kit. Use the appropriate kit for your model pump:
 - 1515 – part number 205000251
 - 1525 – part number 205000251
 - 1525 μ – part number 205000250
 - 1525EF – part number 205000252
- Open-end wrench, 5/16-inch (2)
- Open-end wrench, 1/2-inch
- T-handle hex wrench, 5/32-inch
- T-handle hex wrench, 9/64-inch
- TORX[®] wrench, T15
- HPLC-grade methanol
- Tweezers or tape
- Razor knife or tubing cutter

Preparing the instrument

To prepare the pump:

1. Purge the pump with methanol (see [Chapter 4](#)). If methanol is not miscible with your mobile phase, use an intermediate mobile phase.
2. Insert the priming syringe into the Luer[®] fitting at the center of the draw-off valve handle, and then rotate the handle counterclockwise, about 1/2-turn, to open the valve.
3. Remove the solvent inlet lines from the reservoirs.
4. Use the syringe to withdraw all the methanol from the instrument.

Removing pump head components

To remove pump head components for all 1500-series pumps:

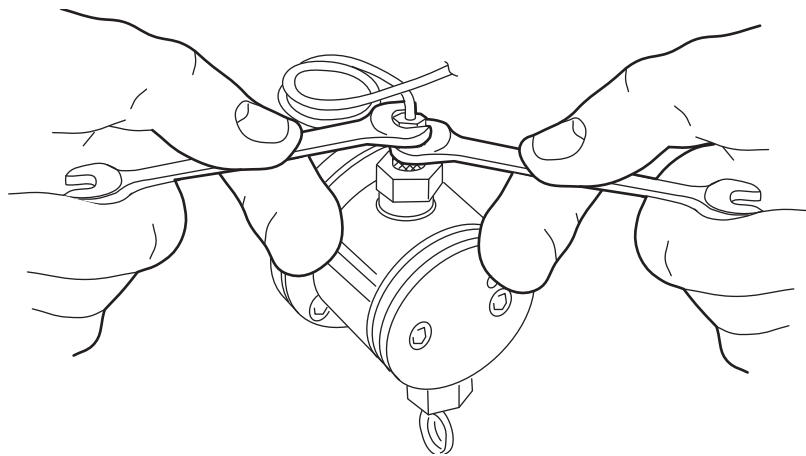
Tip: When installing the seal wash system, work on one pump head support at a time. Complete the procedure for one pump head before removing another.

1. In your data control software, set the flow to 0.3 mL/min.
2. Operate the pump until the indicator rod fully retracts into the pump head to be removed, and then stop the pump.

Tip: Doing so ensures that the relatively heavy pump head will not rest on the plunger as you remove the head.


3. Loosen and remove the inlet and outlet tubing assemblies from the pump head:
 - For 1515/1525 pump, use a 5/16-inch open-end wrench to hold the knurled portion of the check valve housings in place while loosening the inlet and outlet tubing assemblies with another 5/16-inch open-end wrench (see the figure below).
 - For the 1525EF and 1525 μ pump, use the 1/2-inch open-end wrench to hold the check valve housings in place while loosening the inlet and outlet tubing assemblies with the 5/16-inch open-end wrench.

Loosening fittings (1515/1525 pumps)

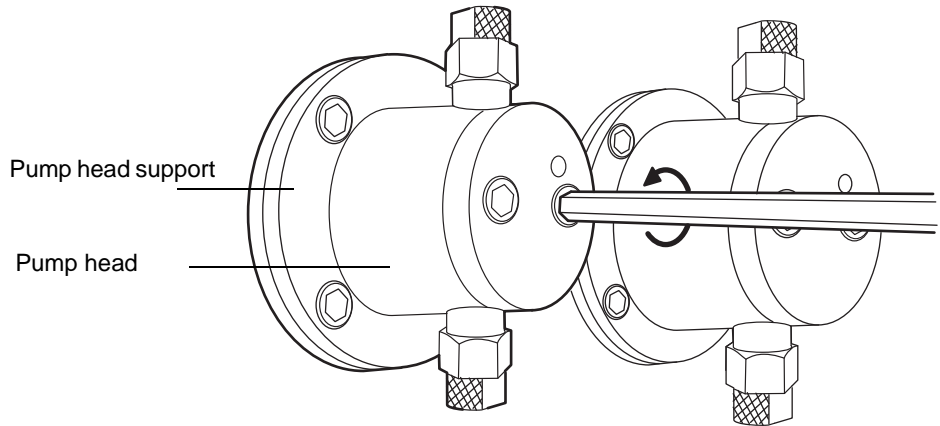


4. While holding the pump head in place, use the 5/32-inch T-handle hex wrench to remove the two pump head mounting screws. Alternately

loosen the screws 1/2-turn at a time for the first two turns (see the figure, below).

 **Caution:** To avoid breaking the plunger, pull the pump head off straight toward you.

Removing head bolts (1515, 1525 and 1525EF pumps)



5. Carefully remove the head from its support.
6. Proceed as follows:
 - If you are installing the seal wash kit in a 1525 μ model, stop here and follow the instructions in [page 3-27](#).
 - If you are installing the seal wash kit in a 1515, 1525, or 1525EF model, continue with [step 7](#) below.
7. While holding the head support in place, use the 9/64-inch T-handle hex wrench to remove the four head support mounting screws.
8. Carefully slide the pump head support straight off.
9. Complete the procedure below “Installing head support components (1515/1525 pump only)”, or [page 3-25](#), as appropriate for your instrument.

Installing head support components (1515/1525 pump only)

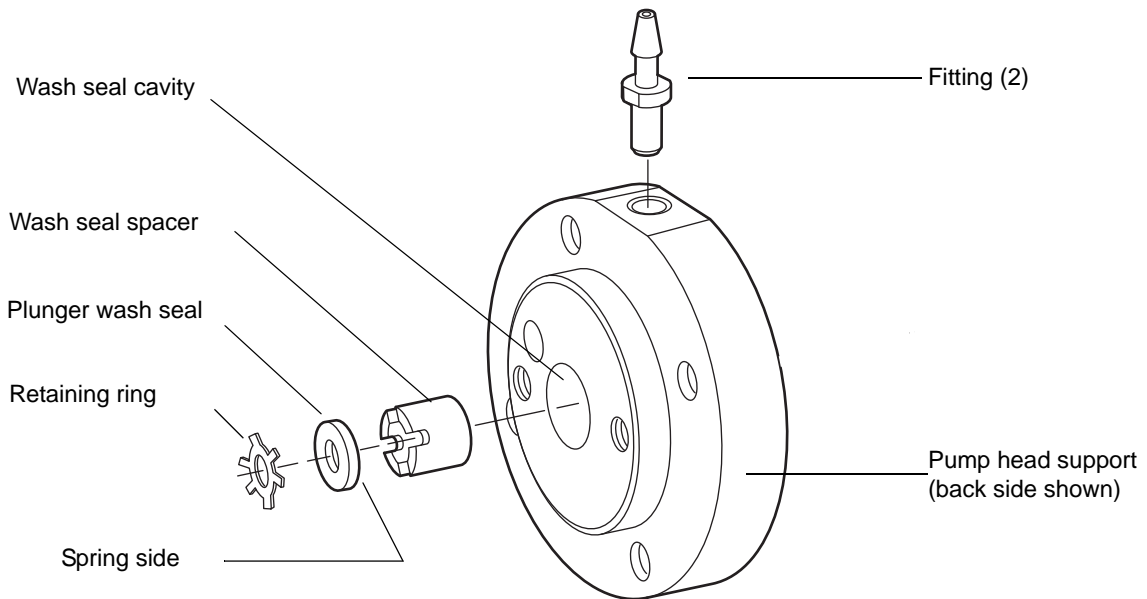
Replace the high pressure seal while the head is removed. See [page 5-6](#) for more information.

Perform these steps only if you have the 1515 or 1525 pump:

1. Lay the head support on a hard surface, back side up.

Tip: You can identify the back side of the pump head support by its larger wash seal cavity. The following figure shows the back side of the pump head support.

Pump head support components (1515/1525 pump)

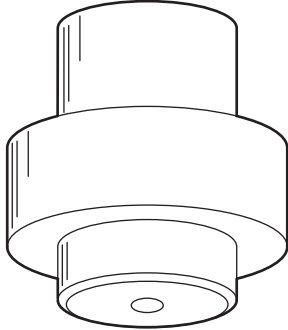


2. Insert the wash seal spacer into the wash seal cavity in the center of the head support.

Tip: Because the spacer fits tightly, start its edge into the cavity at a slight angle and then rotate it into the cavity while pressing down.

3. Using the wash seal installation tool, press the wash seal spacer until it bottoms in the wash seal cavity.

Wash seal installation tool



4. Rotate the wash seal spacer with a screwdriver until the slots line up with the holes in the edge of the head support.
Doing so creates a fluid path for the seal wash solvent.
5. Inspect the fluid path for obstructions by holding the head support up to a light source and looking through the holes in the edge of the head support.
The fluid path must be unobstructed. If it is not, blow through the hole, or use a thin wire to clear any obstructions.
6. Lay the head support on a hard surface, back side up.
7. Insert the plunger wash seal (spring side down) into the wash seal cavity in the center of the head support.
8. Use the wash seal installation tool to bottom the plunger wash seal against the wash seal spacer.
9. Slide the retaining ring onto the thin end of the wash fitting installation tool.
The raised edges of the retaining ring must point downward onto the wash fitting installation tool.

Wash fitting installation tool



10. Hold the wash fitting installation tool upright, and carefully lower the head support onto the tool so that the retaining ring fits into the center of the wash seal cavity and the thin end of the tool slides through the hole in the plunger wash seal.
11. Hold the wash fitting installation tool and the head support together, and lay the head support on a hard surface, back side up.
12. Press down on the wash fitting installation tool to start the retaining ring into the wash seal cavity.
13. Carefully remove the wash fitting installation tool, and then use the wash seal installation tool to bottom the retaining ring against the wash seal.



Caution: To avoid damaging the plunger, ensure that the retaining ring is seated against the wash seal.

14. Insert a tube fitting, barbed end out, into each hole on the edge of the head support.
15. Slide the tubular end of the wash fitting installation tool over the fitting and press down until the fitting flange is firmly seated against the flat area on the head support edge (see the [figure “Pump head support components \(1515/1525 pump\)”](#) on page 3-21).

16. From the back side of the head support, slide the plunger indicator rod through the hole in the head support.
17. Lubricate the plunger rod and seals with methanol.
18. Align the head support on the pump housing face.

The back side of the head support must contact the pump housing face. When viewed from the front of the pump, the indicator rod must be oriented to the upper right of the head support.



Caution: To avoid damaging the support head, support it securely at all times while installing and tightening the screws.

19. Insert the four 9/64-inch hex screws, and alternately tighten them with a T-handle wrench.
20. Ensure the indicator rod slides freely.
21. Lubricate the pump head seal with methanol.
22. Align the pump head carefully over the plunger and indicator rod, and then slide the head onto the head support.
23. Insert the two 5/32-inch hex screws, and alternately tighten them with a T-handle wrench.
24. Ensure the gap between the pump head and the head support is uniformly spaced all around.
25. Pull out and release the indicator rod:
 - If the rod does not snap back easily, the head is misaligned. Loosen the pump head, and then repeat [step 21](#) through [step 23](#), above.
 - If the rod snaps back easily, continue with [step 25](#), below.
26. Reconnect the inlet and outlet tubing assemblies to the pump head.

Tip: Use the 1/2-inch open-end wrench to hold the check valve housings in place while tightening the tubings with the 5/16-inch open-end wrench.
27. Repeat the process for the remaining pump head supports, and then complete the steps in [page 3-29](#).

Installing head support components (1525EF pump only)

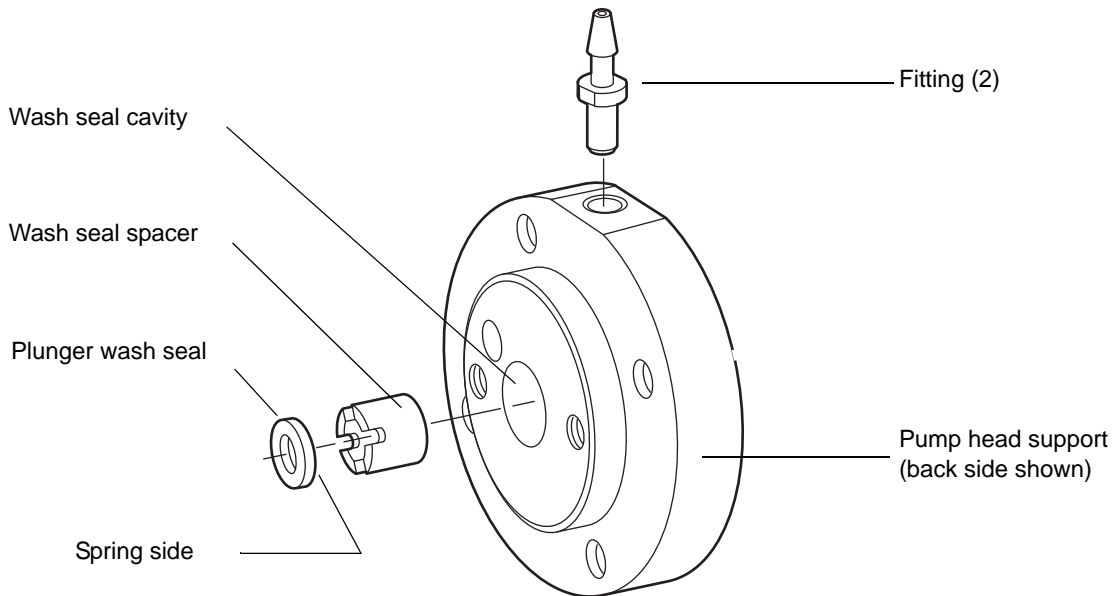
Replace the high pressure seal while the head is disassembled. See [page 5-6](#) for more information.

Perform these steps only if you have the 1525EF model:

1. Lay the head support on a hard surface, back side up.

Tip: The back side of the pump head support is identified by the larger wash seal cavity. The following figure shows the back side of the pump head support.

1525EF pump head support components

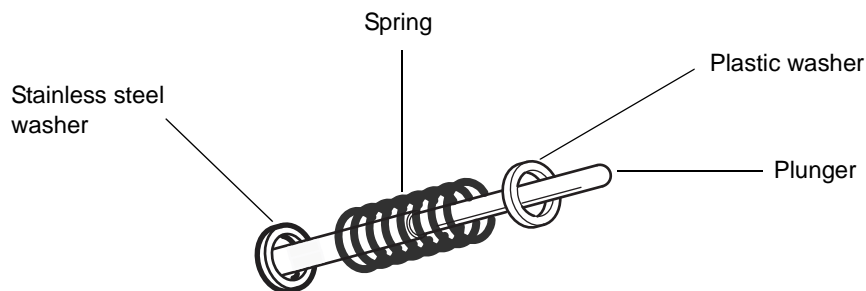


2. Insert the wash seal spacer into the wash seal cavity at the center of the head support.
3. Using the wash seal installation tool, press the wash seal spacer until it bottoms in the wash seal cavity.
4. Rotate the wash seal spacer with a screwdriver until its slots line up with the holes in the edge of the head support.

Doing so creates a fluid path for the seal wash solvent.

5. Inspect the fluid path for obstructions by holding the head support up to a light source and looking through the holes in the edge of the head support.
The fluid path must be unobstructed. If it is not, blow through the hole, or use a thin wire to clear any obstructions.
6. Lay the head support on a hard surface, back side up.
7. Insert the plunger wash seal (spring side down) into the wash seal cavity.
8. Use the wash seal installation tool (see the [figure “Wash seal installation tool” on page 3-22](#)) to bottom the plunger wash seal against the wash seal spacer.
9. Insert a tube fitting, barbed end out, into each hole on the edge of the head support.
10. Slide the tubular end of the wash fitting installation tool over the fitting and press down until the fitting flange is firmly seated against the flat area on the head support edge.
11. Slide the stainless steel washer, spring, and plastic washer included in the kit carefully onto the pump plunger.

Installing the 1525EF washers and spring



12. Slide the plunger indicator rod from the back side of the head support through the hole in the head support.
13. Lubricate the plunger and seals with methanol.
14. Align the head support on the pump housing face.

The back side of the head support must contact the pump housing face. When viewed from the front of the pump, the indicator rod must be oriented to the upper right of the head support.



Caution: To avoid damaging the support head, support it securely at all times while installing and tightening the screws.

15. Insert the four 9/64-inch hex screws and alternately tighten them with a T-handle wrench.
16. Ensure the indicator rod slides freely.
17. Lubricate the pump head seal with methanol.
18. Align the pump head carefully over the plunger and indicator rod, and then slide the head onto the head support.
19. Insert the two 5/32-inch hex screws, and alternately tighten them with a T-handle wrench.
20. Ensure the gap between the pump head and the head support is uniformly spaced all around.
21. Pull out and release the indicator rod:
 - If the rod does not snap back easily, the head is misaligned. Loosen the pump head, and then repeat [step 17](#) through [step 19](#).
 - If the rod snaps back easily, continue with [step 21](#).
22. Reconnect the inlet and outlet tubing assemblies to the pump head.
23. Use the 1/2-inch open-end wrench to hold the check valve housings in place while tightening the tubings with the 5/16-inch open-end wrench.
24. Repeat the process for the remaining pump head supports, and then complete the steps in [“Installing the solenoid” on page 3-29](#).

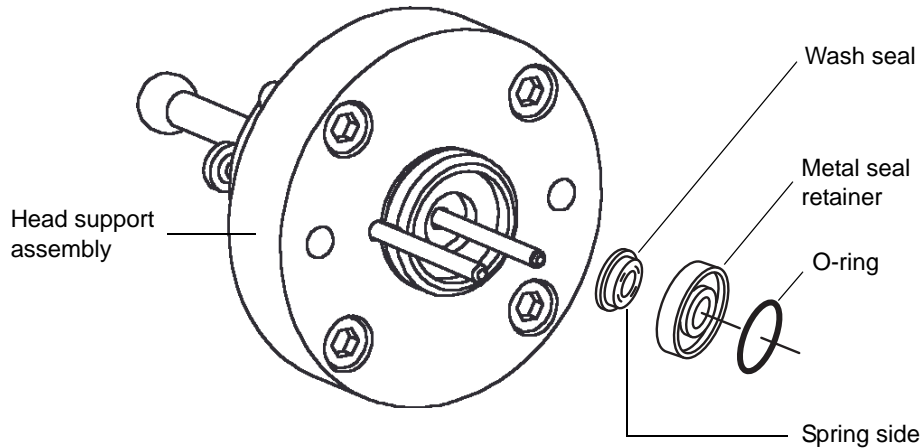
Installing head components (1525 μ pump only)

Replace the high pressure seal while the head is disassembled. See [page 5-6](#) for more information.

Perform these steps only for the 1525 μ pump:

1. Use a piece of tape or tweezers to remove the metal seal retainer from the head support.

1525 μ head support components



2. Press the seal retainer O-ring into the front of the seal retainer.
Tip: After starting the O-ring into the groove, turn over the retainer and O-ring, and press them onto a flat surface to seat the O-ring into the O-ring groove.
3. Press the wash seal onto the opposite side of the seal retainer so that its spring faces the seal retainer (see the figure, above).
4. Carefully slide the retainer assembly over the plunger and into the head support.
5. Ensure the indicator rod slides freely.
6. If the pump head has a plastic cap behind the top check valve housing, use the 5/16-inch open-end wrench to remove the cap.
7. Carefully start the barbed fittings into the threaded holes at the top and bottom of the pump head. Tighten the fittings so that they are finger tight.
8. Lubricate the plunger, plunger seal, and high-pressure seal with methanol.



Caution: To avoid damaging the support head, support it securely at all times while installing and tightening the screws.

9. Align the pump head carefully over the plunger and indicator rod, and then slide the head onto the head support.
10. Insert the two 5/32-inch hex screws, and alternately tighten them with a T-handle wrench.
11. Ensure the gap between the pump head and the head support is uniformly spaced all around.
12. Reconnect the inlet and outlet tubing assemblies to the pump head. Use the 1/2-inch open-end wrench to hold the check valve housings in place while tightening the tubings with the 5/16-inch open-end wrench.
13. Repeat the process for the remaining pump heads, and then complete the steps in “Installing the solenoid”, below.

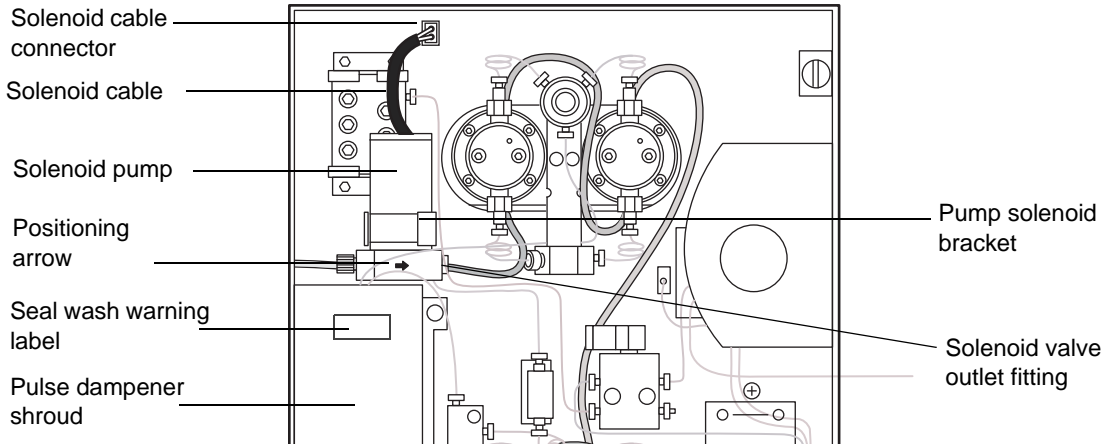
Installing the solenoid

Perform these steps for all 1500-series pumps:

1. Install the solenoid bracket on the pump chassis with the two TORX head screws provided with the kit.
Tip: The solenoid bracket mounting holes are located slightly below and to the left of the upper-left pump head.
2. Align the solenoid so that the arrow on its base faces forward and points to the right.

3. Push the metal body of the solenoid into the bracket until it snaps into position.

Correct solenoid positioning



4. Ensure that the solenoid bracket does not contact the label on the solenoid. If it does, adjust the solenoid position.
5. Carefully start the barbed fitting into the threaded hole on the right side of the solenoid base, and tighten the fitting finger-tight.
6. Plug the solenoid cable into the solenoid cable connector.
The solenoid cable must be plugged in at all times to ensure that the seal wash system is active whenever the instrument is operating.
7. Affix the warning sticker to the pulse dampener shroud.
8. For all models, complete [“Installing seal wash tubing”](#) on page 3-31.

Installing seal wash tubing

Perform these steps for all 1500-series pumps:

1. Cut the PharMed[®] tubing, provided with the kit, into the lengths described in the following table.

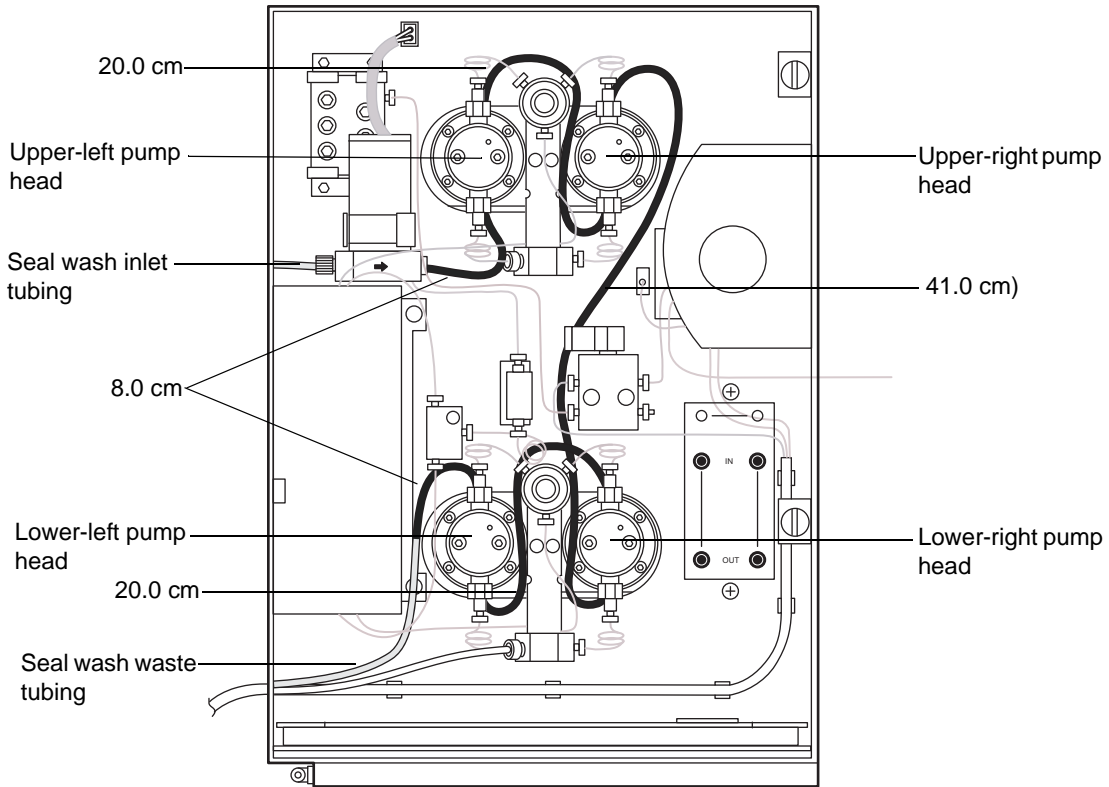
PharMed[®] tubing lengths

| Length | Number of pieces |
|---------|------------------|
| 8.0 cm | 2 |
| 20.0 cm | 2 |
| 41.0 cm | 1 |

2. Connect one end of a 8-cm piece of tubing to the solenoid valve outlet fitting (see the figure, above). Connect the other end to the bottom fitting on the upper-left pump head.
3. Connect one end of a 20.0-cm piece of tubing to the top fitting on the upper-left pump head. Connect the other end of the tube to the bottom fitting on the upper-right pump head.
4. Connect one end of the 41.0-cm piece of tubing to the top fitting on the upper-right pump head. Connect the other end of the tube to the bottom fitting on the lower-right pump head.
5. Connect one end of the remaining 20.0-cm piece of tubing to the top fitting on the lower-right pump head. Connect the other end of the tube to the bottom fitting on the lower-left pump head.
6. Connect one end of the remaining 8-cm piece of tubing to the top fitting on the lower-left pump head. Slide the other end over the 1/8-inch OD ETFE (clear plastic) tubing from the kit.

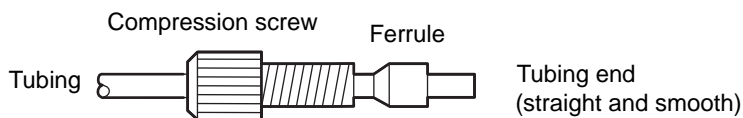
Tip: The ETFE tubing must extend at least 0.5 cm inside the PharMed tubing.

Seal wash tubing routing



7. Route the ETFE tubing to a suitable waste receptacle, and cut the tubing to length.
8. Slide one end of the remaining portion of the ETFE tubing into the inlet slot on the left side of the instrument.
9. Slide a compression screw over the end of the tubing, followed by a ferrule with its tapered end facing away from the tubing end and its wide end flush with the tubing end, as shown in the figure, below.

Reverse ferrule and compression screw assembly



10. Firmly seat the tubing end into the inlet on the left-hand side of the solenoid pump, and then hold the tubing in place while finger tightening the compression screw.



Caution: To avoid damaging the ferrule, do not overtighten the compression screw.

11. Attach a sinker to the free end of the inlet tubing, and then insert the tubing into the seal wash solvent reservoir.
12. Prime the pump and check for leaks.

Using the seal wash system

The seal wash solvent lubricates the plunger. It also flushes away solvent or precipitated salts forced past the plunger seal from the high-pressure side of the solvent piston chamber.



Caution: Once you install the seal wash option, use it at all times during instrument operation to protect plungers and seals.

4 Preparing for Operation

This chapter describes how to prepare the 1500-series pump for making chromatographic runs via Waters data control software.

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Startup and initial preparation

Before you use your data control software to ready the pump for making chromatographic runs, familiarize yourself with these procedures and recommendations:

- Powering-on the pump
- Recommendations for preparing the pump for operation
- Dry priming the pump
- Operating the pump with the integral vacuum degasser
- Operating the pump with the plunger seal wash system
- Operating within the pump's maximum flow rate

Powering-on the pump

Requirements:

- Power-on the pump and other chromatographic system devices before you power-on the system workstation, and startup the data control application.
- Ensure the pump is installed according to the instructions in [Chapter 2](#).
- Ensure the options and accessories included with your pump are installed according to the instructions in [Chapter 3](#).

To power-on the pump, locate the power switch on the left-hand side panel and set it to the 1 (ON) position.

Pump preparation recommendations

The procedures to prepare your pump for operation depend on the status of your chromatography system, as indicated in the following table. Use this table as a quick reference guide for determining when to perform certain preparation procedures.

Pump preparation recommendations

| Status | Prime | Purge | Equilibrate |
|-------------------|-------|-------|-------------|
| Initial operation | ✓ | ✓ | ✓ |

Pump preparation recommendations (Continued)

| Status | Prime | Purge | Equilibrate |
|------------------------------------|-------|-------|-------------|
| Changing eluent | ✓ | ✓ | ✓ |
| Adding fresh eluent | | ✓ | ✓ |
| Changing columns | | | ✓ |
| System has been idle for some time | ✓ | ✓ | ✓ |

Priming, purging, and equilibrating procedures are fully described later in this chapter.

Eluent guidelines

When you prepare a 1500-series pump for operation, follow these guidelines:

- Use methanol to prime a new pump, or one that has been stored for an extended period.
- Ensure that the solvents are totally miscible at all compositional combinations to prevent precipitation, check valve fouling, and other related problems.
- Use an intermediate solvent when you change between eluents that are not miscible.
- Use only HPLC-grade eluents, to obtain accurate, reproducible chromatography.
- To prevent air from being drawn into the pump, keep the eluent level in the reservoir at least four inches (10 cm) higher than the inlet manifold. Do not place eluent reservoirs on top of a 1500-series pump.
- Use only filtered, degassed eluents.
- After use with a buffer, purge the pump with HPLC-grade water.
- When possible, use eluents of similar viscosity when you run a gradient.
- Consider column chemistry. If the column can be damaged by an intermediate or new eluent, remove it from the system, and substitute a union. Refer to the care and use guide for your column.

- Dedicate an eluent supply tube and filter assembly for each eluent. If doing so is not possible, purge the tube and filter of any remaining eluent before you use a new eluent.
- To prevent cross contamination of eluents, dedicate a set of glassware for preparing, storing and supplying each eluent. Once a container has been used for an eluent, do not use it for any other eluent unless it has been thoroughly cleaned.



Caution: To avoid chemical hazards, observe safe laboratory practices when you handle eluents. Refer to the Material Safety Data Sheets for the eluents you use. For more information on eluent properties, see [Appendix C](#).

Dry priming the pump

Dry priming is necessary during initial pump startup or when the entire eluent line is dry.

Tip: For binary pumps, you can possibly need to dry prime one or both pump assemblies.

If your pump configuration includes a plunger seal wash system, you can possibly need to prime the seal wash pump as well (see [page 4-9](#)).

Required material

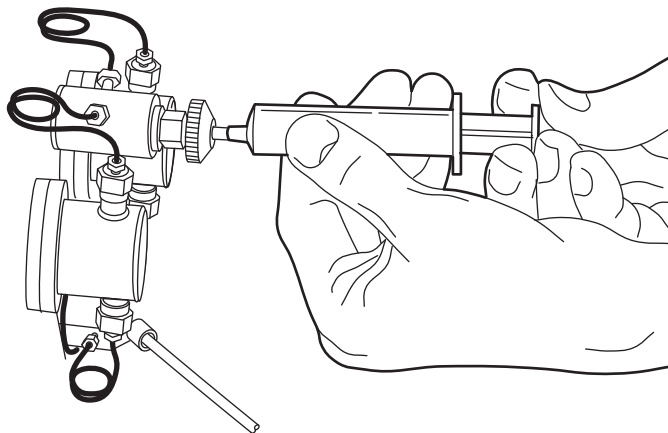
Priming syringe (P/N WAT010337 in startup kit)

To dry prime the pump:

1. Fully depress the priming syringe plunger to remove all air from the syringe.

2. Insert the syringe into the Luer fitting at the center of the draw-off valve handle.

Priming the pump



3. Rotate the draw-off valve handle counterclockwise, 1/2-turn, to open the valve.
4. Withdraw several milliliters of eluent with the syringe, and then close the valve handle.
5. Expel the contents of the syringe into a suitable waste container.
6. For binary pumps, repeat [step 1](#) through [step 5](#) to dry prime the second pump assembly if necessary.



Caution: To prevent eluent flow through the pump heads due to gravity pressure, never leave the pump unattended with the draw-off valve in the open position.

Operating with the integral vacuum degasser

If your HPLC pump includes the integral vacuum degasser, electrical power is supplied to the degasser when you power-on the pump. With the pump's switch set to On, the degasser operates automatically while you perform HPLC runs. There are no controls to adjust as it removes gases from the eluents.

During normal operation, the degasser stops operating if the pump does not deliver flow for 30 minutes. During the startup sequence, the degasser stops operating if the pump does not deliver flow for 3 minutes.

Before you start the pump, make sure these tasks are completed:

- Degasser eluent tubing is connected to the eluent reservoirs and pump inlet manifold
- The degasser vent line is connected to a suitable waste container

The degasser's degassing membrane can withstand a maximum pressure of 10 psi (70 kPa).



Caution: To avoid damaging the degasser, do not apply more than 10 psi (70 kPa) to the eluent reservoirs.

Controlling the degasser

The degasser is enabled by default. It operates briefly, following power-up, and continuously during pump flow.

These events take place when the degasser is enabled:

- The LED on the 1500-series pump front panel illuminates.
- The vacuum pump begins its evacuation.

Normal operation begins when the vacuum pump reaches its vacuum target and changes from high to low RPM.

If the degasser fails, you can continue pump operation without the degasser by setting the degasser toggle switch (located at the top of the degasser unit) to Off.

Tip: The degasser is intended for use at all times during pump flow. Set the toggle switch to Off only if the degasser fails.

These events take place when you disable the degasser by setting the degasser toggle switch to Off:

- Vacuum pump operation stops.
- The LED on the pump front panel flashes yellow (0.5 seconds on and 2 seconds off).

The degasser remains in the disabled mode until you set the switch to On. Data control software cannot override the disabled mode.

Degasser operating statuses

The LED on the 1500-series pump front panel indicates the status of the degasser as described in the table, below.

LED indications

| LED state | Description |
|--|--|
| Unlit | Pump is powered-off. |
| Steady yellow | Degasser is operating with pump at high RPM, vacuum level above 60 mmHgA/1.16 psiA. (This is typically a brief, transitional state during initial evacuation.) |
| Steady green | Degasser is operating with pump at low RPM, vacuum level below 60 mmHgA/1.16 psiA, normal operation. |
| Green flash, 0.5 seconds on and 0.5 seconds off | Degasser is operating but vacuum level is unstable. This condition can also indicate a sudden change in degasser work load. |
| Yellow flash, 0.5 seconds on and 2 seconds off | Degasser is not operating. Degasser toggle switch is set to Off. Operation should resume after setting the switch to On. |
| Yellow flash, 2 seconds on and 0.5 seconds off | Degasser is not operating. Vacuum signal is out of usable range, indicating an electronic or pressure sensor failure. |
| Yellow flash, 0.5 seconds on and 0.5 seconds off | Degasser is not operating. Target vacuum level was not reached within 10 minutes, indicating a vacuum leak. |
| Alternating yellow and green flash | Degasser is not operating. Target vacuum level was reached but then rose above upper limit, indicating a vacuum failure. |

Compatibility with solvents

The degasser vacuum chamber consists of a tubular membrane that is made of a proprietary, specially engineered, fluorocarbon polymer for compatibility with a wide spectrum of liquids, including all mobile phases commonly encountered in HPLC. See [page B-7](#) and [Appendix C](#) for more information about solvent compatibility.

Operating with the plunger seal wash system

If your HPLC pump includes the plunger seal wash system, during operation, plunger seal wash solvent flows from a reservoir to the solenoid wash pump, which pumps the solvent through the fluid path.

- In the 1515 isocratic, 1525 binary, and 1525EF models, the solvent flows through the pump head supports.
- In the 1525 μ model, the solvent flows through the pump heads.

When the solvent has passed through all four pump heads or pump head supports, it flows to waste. The plunger seal wash solvent is not recycled.

Powering-on the instrument triggers the seal wash pump's priming sequence, which lasts for one minute. The seal wash pump then shifts to its normal operating mode, intermittently pumping seal wash solvent according to the duty cycles in the following table.

Seal wash pump duty cycles

| Mode | Operation |
|---------|---|
| Priming | Solenoid energized for 0.5 seconds, solenoid off for 0.5 seconds, repeating. |
| Normal | Solenoid energized for 0.25 seconds, solenoid off for 59.75 seconds, repeating. |

Once you stop using the instrument, seal washing continues for 30 minutes after the mobile phase stops flowing.

Tip: The seal wash pump returns to the priming sequence each time the instrument is powered-on. However, the seal wash pump requires priming only for first-time use or when the line is dry (see [page 4-9](#)).

The following table shows the amount of seal wash solvent consumed during normal operation.

Seal wash solvent consumption

| Model | Seal wash solvent consumption |
|-------------|-------------------------------|
| 1525 binary | 12 mL/hr |
| 1525 μ | 11 mL/hr |
| 1525EF | 13.5 mL/hr |

The seal wash solvent lubricates the plunger. It also flushes away solvent or precipitated salts forced past the plunger seal from the high-pressure side of the solvent piston chamber.

Tips:

- Once you install the seal wash option, use it at all times during instrument operation to protect plungers and seals.
- For reversed-phase HPLC applications, use an aqueous plunger seal wash solution with enough organic content to inhibit bacterial growth. For example, use a 4:1 water methanol or water acetonitrile solution, depending on your application.
- For all GPC separations, use a 1:1 water methanol seal wash solution. If required, use an intermediate solution to prevent immiscibility or precipitation problems when you switch between GPC and reversed-phase analytical solvents.



Caution: To avoid contamination issues, never recycle seal wash.

Priming the plunger seal wash pump

You must prime the plunger seal wash pump for first-time use or when the entire line is dry.

Ensure the plunger seal wash supply line (labeled “Seal Wash In”) is in the plunger seal wash solvent bottle and that the plunger seal wash waste line (clear) is in an appropriate waste container.

To prime the seal wash pump:

1. Fill the plunger seal wash solvent bottle with an appropriate solvent.
2. Place the plunger seal wash supply line in the plunger seal wash solvent bottle.
3. Connect the tubing adapter to the syringe provided with the kit.
4. Disconnect the seal wash outlet tube from the waste tubing.
5. Attach the syringe to the seal wash outlet tube, and pull out the syringe plunger to create vacuum in the system.
6. Power-on the pump.

The seal wash solenoid will make a rapid clicking sound as you draw solvent through the seal wash system.

7. When solvent begins to flow into the syringe, remove the syringe from the seal wash outlet tube.
8. Reconnect the seal wash outlet tube to the waste tubing.

Tip: If the seal wash system runs dry during operation, shut down and restart the instrument to return the seal wash pump to the priming mode. Then perform this procedure to prime the pump.

Maximum flow rates for 1500-series pumps

To prevent the pump from stalling, do not exceed the pump's maximum flow rate.

When specifying flow rates via Waters data control software, do not exceed the maximum flow rate that is specified for the pump.

- For the 1515 or 1525 pump (combined flow from Pumps A and B), do not exceed 10.00 mL/minute
- For the 1525EF (combined flow from Pumps A and B), do not exceed 22.5 mL/minute
- For the 1525 μ pump (combined flow from Pumps A and B), do not exceed 5.00 mL/minute

Tip: The data control software can possibly allow you to enter values that exceed the pump's maximum flow rate.

Preparing for Breeze 2 operation

Use this section to ready the 1500-series pump for performing runs under Breeze 2 software control. It describes simple techniques for accomplishing these tasks:

- Priming and purging the pump
- Purging the system
- Equilibrating the system

For additional information and instructions on setting up the HPLC system and creating instrument methods, see the Breeze 2 online Help.

For software-release-specific details, see the release notes for the Waters 1500-series pumps and the Waters Breeze 2 software.

Power-on the pump and other chromatographic system devices before you power-on the Breeze 2 system workstation, and start the Breeze 2 application (see [page 4-2](#)).

Priming and purging the pump via Breeze 2 control

You must prime and purge the pump to ensure its proper operation (see [page 4-2](#)).

Before priming and purging the pump

- If the pump is dry, dry prime it before proceeding (see [page 4-4](#)).
- Ensure the eluent flow is directed to waste—bypassing any connected components downstream of the pump—by turning the pump’s reference valve knob 1/2-turn to the right (for the 1525 μ pump, position the vent valve knob to Vent).



To prime and purge the pump:

1. At the Breeze 2 workstation, log in to Breeze 2.
2. In the Select Project and System dialog box, verify that your chromatographic system, with a 1500-series pump, is configured and online.

If you did not yet create a chromatographic system with the 1500-series pump, do so now.

- Ensure the pump is properly configured in the Node Properties, which allows you to scan for available instruments (including the pump), and set the pump-head size in the Instruments tab (for 1525 μ and 1525EF only).
- Bring the system online.

For detailed instructions, refer to the Breeze 2 online Help.

3. In the Run Samples window, click the Start Flow icon , and enter appropriate values for the pump's flow rate and gradient settings.
Tip: To quickly prime and purge just the pump,
 - for the 1515 pump, set the rate to 2.0 mL/min.
 - for the 1525 pump, set the rate to 4.0 mL/min and gradient to 50% A, 50% B.
 - for the 1525 μ pump, set the rate to 2.0 mL/min and gradient to 50% A, 50% B.
 - for the 1525EF pump, set the rate to 6.0 mL/min and gradient to 50% A, 50% B.
4. Select Immediate Flow Change and click OK.
5. Allow the pump to run for approximately one minute, and then verify that the eluent flow exiting to waste is normal and consistent for the specified flow rate.
6. Click the Stop Flow icon  in the Run Samples window to stop the flow.

Purging the flow path via Breeze 2 control

Purging ensures that all eluent in the flow path is replaced with new eluent before you equilibrate the system and run samples (see [page 4-2](#)).

Tip: When bringing new instruments online or troubleshooting a solvent problem, you can restrict the flow path to only those sections of the system that require purging by disconnecting the flow path at an inlet or outlet junction, such as the injector outlet or column outlet, and routing the flow to waste.

Before purging

- Make sure the eluent is filtered and degassed.
- Prime and purge the pump with fresh eluent as described in the section above.
- Ensure the pump's reference valve knob is turned to the left, directing the eluent flow to the system (for the 1525 μ pump, position the vent valve knob to the system).


When you change between two eluents that are not totally miscible, perform the steps in this procedure using an intermediate eluent before purging with the final eluent. See [Appendix C](#) for more information.

Purging



Caution: To avoid damaging the column, ensure any changes to conditions that affect the column, such as flow or pressure, are gradual.

To purge the flow path:

1. Determine flow rate and gradient settings appropriate for your column and application, and then follow [step 1](#) through [step 4](#) on [page 4-11](#).
2. Allow the pump to run for a few minutes to complete the purge.
Tip: The amount of running time required to purge the flow path depends on total system volume, eluent miscibility, flow rate, and so on.
3. Verify that the flow exiting to waste is normal and consistent for the specified flow rate, and then click the Stop Flow icon  in the Run Samples window to stop the flow.

For information about creating an instrument method to prime and purge the pump, refer to the Breeze 2 online Help.

Equilibrating the system via Breeze 2 control

Equilibrate the system to achieve a final state of system readiness for running samples (see [page 4-2](#)).

Before equilibrating

- Make sure the eluent is filtered and degassed.
- Allow the eluent and the system to reach proper temperatures before pump operation.
- Prime the pump, as described in the section above.
- Ensure the pump's reference valve knob is turned to the left, directing the eluent flow to the system (for the 1525 μ pump, position the vent valve knob to the system).

To equilibrate the system:

1. Determine flow rate and gradient settings appropriate for your column and application, and then follow [step 1](#) through [step 4](#) on [page 4-11](#).

2. Allow the system to equilibrate for several minutes.

Tip: The amount of running time required for equilibration depends on total system volume, eluent miscibility, flow rate, and so on.

3. Monitor the system pressure to verify it is normal and stable.
4. Inspect the eluent flow exiting the system to verify it is normal and consistent for the specified flow rate.

Tip: To monitor the detector baseline during system equilibration, in the Acquisition bar, click the Equilibrate System/Monitor Baseline icon



. Select an instrument method for the pump and click Equilibrate/Monitor. For information about creating an instrument method used for equilibration, refer to the Breeze 2 online Help.

5. Click the Stop Flow icon  in the Run Samples window to stop the flow.

Preparing for Empower 2 operation

Use this section to ready the 1500-series pump for performing runs under Empower 2 software control. It describes simple techniques for accomplishing these tasks:

- Priming and purging the pump
- Purging the system
- Equilibrating the system

For additional information and instructions on setting up the HPLC system and creating methods, see the Empower 2 online Help.

For software-release-specific details, see the release notes for the Waters 1500-series pumps and the Waters Empower 2 software.

Power-on the pump and other chromatographic system devices before you power-on the Empower 2 system workstation, and start the Empower 2 application (see [page 4-2](#)).

Priming and purging the pump via Empower 2 control

You must prime and purge the pump to ensure its proper operation (see [page 4-2](#)).

Before priming and purging the pump

- If the pump is dry, dry prime it before proceeding (see [page 4-4](#)).
- Ensure the eluent flow is directed to waste—bypassing any connected components downstream of the pump—by turning the pump's reference valve knob 1/2-turn to the right (for the 1525 μ pump, position the vent valve knob to Vent).


To prime and purge the pump:

1. At the Empower 2 workstation, log in to Empower 2.
2. In the Navigation pane, click Configure System to display the Configuration Manager window.
3. Select Systems, and verify that your chromatographic system with a 1500-series pump is configured and online.


If you did not yet create a chromatographic system with the 1500-series pump, do so now.

- Ensure the pump is properly configured in the Empower Node Properties, which allows you to scan for available instruments (including the pump), and set the pump-head size in the Instruments tab (for 1525 μ and 1525EF only).
- Bring the system online.

For detailed instructions, refer to the Empower 2 online Help.

4. In the Navigation pane, click Run Samples.
5. Select the system and click OK.
6. In the Run Samples window, click the Start Flow icon , and enter appropriate values for the pump's flow rate and gradient settings.

Tip: To quickly prime and purge just the pump,

- for the 1515 pump, set the rate to 2.0 mL/min.
 - for the 1525 pump, set the rate to 4.0 mL/min and gradient to 50% A, 50% B.
 - for the 1525 μ pump, set the rate to 2.0 mL/min and gradient to 50% A, 50% B.
 - for the 1525EF pump, set the rate to 6.0 mL/min and gradient to 50% A, 50% B.
7. Select Immediate Flow Change and click OK.
 8. Allow the pump to run for approximately one minute, and then verify that the eluent flow exiting to waste is normal and consistent for the specified flow rate.
 9. Click the Stop Flow icon  in the Run Samples window to stop the flow.

Purging the flow path via Empower 2 control

Purging ensures that all eluent in the flow path is replaced with new eluent before you equilibrate the system and run samples (see [page 4-2](#)).

Tip: When bringing new instruments online or troubleshooting a solvent problem, you can restrict the flow path to only those sections of the system that require purging by disconnecting the flow path at an inlet or outlet junction, such as the injector outlet or column outlet, and routing the flow to waste.

Before purging

- Make sure the eluent is filtered and degassed.
- Prime and purge the pump with fresh eluent, as described in the section above.
- Ensure the pump's reference valve knob is turned to the left, directing the eluent flow to the system (for the 1525 μ pump, position the vent valve knob to the system).


When you change between two eluents that are not totally miscible, perform the steps in this procedure with an intermediate eluent before purging with the final eluent. See [Appendix C](#) for more information.

Purging



Caution: To avoid damaging the column, ensure any changes to conditions that affect the column, such as flow or pressure, are gradual.

To purge the flow path:

1. Determine flow rate and gradient settings appropriate for your column and application, and then follow [step 1](#) through [step 7](#) on [page 4-15](#).
2. Allow the pump to run for a few minutes to complete the purge.
Tip: The amount of running time required to purge the flow path depends on total system volume, eluent miscibility, flow rate, and so on.
3. Verify that the flow exiting to waste is normal and consistent for the specified flow rate, and then click the Stop Flow icon  in the Run Samples window to stop the flow.

For information about creating and using a purge method, refer to the Empower 2 online Help.

Equilibrating the system via Empower 2 control

Equilibrate the system to achieve a final state of system readiness for running samples (see [page 4-2](#)).

Before equilibrating

- Make sure the eluent is filtered and degassed.
- Allow the eluent and the system to reach proper temperatures before pump operation.
- Prime the pump, as described in the section above.
- Ensure the pump's reference valve is turned clockwise to the closed position, directing the eluent flow to the system (for the 1525 μ pump, position the vent valve knob to the system).

To equilibrate the system:

1. Determine flow rate and gradient settings appropriate for your column and application, and then follow [step 1](#) through [step 7](#) on [page 4-15](#).
2. Allow the system to equilibrate for several minutes.

Tip: The amount of running time required for equilibration depends on total system volume, eluent miscibility, flow rate, and so on.

3. Monitor the system pressure to verify it is normal and stable. Inspect the eluent flow exiting the system to verify it is normal and consistent for the specified flow rate.

Tip: To monitor the detector baseline during system equilibration, select an instrument method for the pump and click Monitor. For information about creating an instrument method used for equilibration, refer to the Empower 2 online Help.

4. Click the Stop Flow icon  in the Run Samples window to stop the flow.

Preparing for MassLynx operation

Use this section to ready the 1500-series pump for performing runs under MassLynx software control. It describes simple techniques for accomplishing these tasks:

- Priming and purging the pump
- Purging the system
- Equilibrating the system

For additional information and instructions on setting up the HPLC system and creating inlet methods, see the MassLynx online Help.

For software-release-specific details, see the release notes for the Waters 1500-series pumps and the Waters MassLynx software.

Power-on the pump and other chromatographic system devices before you power-on the MassLynx system workstation, and start the MassLynx application (see [page 4-2](#)).

Priming and purging the pump via MassLynx control

You must prime and purge the pump to ensure its proper operation (see [page 4-2](#)).

Before priming and purging the pump

- If the pump is dry, dry prime it before proceeding (see [page 4-4](#)).
- Ensure the eluent flow is directed to waste—bypassing any connected components downstream of the pump—by turning the pump's reference valve knob 1/2-turn to the right (for the 1525 μ pump, position the vent valve knob to Vent).

To prime and purge the pump:

1. At the MassLynx workstation, log on to MassLynx.
2. From the MassLynx Main window, click Status.
3. Click the Inlet Method button to open the Inlet Editor window.
4. Click the Inlet icon to go to the pump method.

If you did not yet create an inlet method with the 1500-series pump under Waters Pump Control, do so now. Ensure the pump is properly configured in the Waters Pump Control Configuration, which allows you to scan for available instruments (including the pump), and set the pump-head size (for 1525 μ and 1525EF only). For detailed instructions, refer to the MassLynx online Help.

5. Enter appropriate values for the pump's flow rate and gradient settings and click OK.

Tip: To quickly prime and purge just the pump,

- for the 1515 pump, set the rate to 2.0 mL/min.
- for the 1525 pump, set the rate to 4.0 mL/min and gradient to 50% A, 50% B.
- for the 1525 μ pump, set the rate to 2.0 mL/min and gradient to 50% A, 50% B.
- for the 1525EF pump, set the rate to 6.0 mL/min and gradient to 50% A, 50% B.

6. Click the Load Method icon  to download the method to the system.

Doing so initiates the pump and starts flow.

7. Allow the pump to run for approximately one minute, and then verify that the eluent flow exiting to waste is normal and consistent for the selected flow rate.

8. Click the Start/Stop Pumping icon  in the Inlet Method window to stop the flow.

Purging the flow path via MassLynx control

Purging ensures that all eluent in the flow path is replaced with new eluent before you equilibrate the system and run samples (see [page 4-2](#)).

Tip: When bringing new instruments online or troubleshooting a solvent problem, you can restrict the flow path to only those sections of the system that require purging by disconnecting the flow path at an inlet or outlet junction, such as the injector outlet or column outlet, and routing the flow to waste.

Before purging

- Make sure the eluent is filtered and degassed.
- Prime and purge the pump with fresh eluent, as described in the section above.
- Ensure the pump's reference valve knob is turned to the left, directing the eluent flow to the system (for the 1525 μ pump, position the vent valve knob to the system).


When you change between two eluents that are not totally miscible, perform the steps in this procedure with an intermediate eluent before purging with the final eluent. See [Appendix C](#) for more information.

Purging



Caution: To avoid damaging the column, ensure any changes to conditions that affect the column, such as flow or pressure, are gradual.

To purge the flow path:

1. Determine flow rate and gradient settings appropriate for your column and application, and then follow [step 1](#) through [step 6](#) on [page 4-19](#).
2. Allow the pump to run for a few minutes to complete the purge.
Tip: The amount of running time required to purge the flow path depends on total system volume, eluent miscibility, flow rate, and so on.
3. Verify that the flow exiting to waste is normal and consistent for the specified flow rate, and then click the Start/Stop Pumping icon  in the Inlet Method window to stop the flow.

For more information about selecting an purge method, refer to the MassLynx online Help.


Equilibrating the system via MassLynx control

Equilibrate the system to achieve a final state of system readiness for running samples (see [page 4-2](#)).

Before equilibrating

- Make sure the eluent is filtered and degassed.
- Allow the eluent and the system to reach proper temperatures before pump operation.
- Prime the pump, as described in the section above.
- Ensure the pump's reference valve knob is turned to the left, directing the eluent flow to the system (for the 1525 μ pump, position the vent valve knob to Vent).

To equilibrate the system:

1. Determine flow rate and gradient settings appropriate for your column and application, and then follow [step 1](#) through [step 6](#) on [page 4-19](#).
2. Allow the system to equilibrate for several minutes.
Tip: The amount of running time required to equilibrate the system depends on total system volume, eluent miscibility, flow rate, and so on.
3. Monitor the system pressure and detector baseline to verify they are normal, and inspect the eluent flow exiting the system to verify it is normal and consistent for the specified flow rate.
4. Click the Start/Stop Pumping icon  in the Inlet Method window to stop the flow.

For information about creating and using an equilibration method, refer to the MassLynx online Help.

Powering off the pump

To power-off the pump:

1. If you have been using a buffer, purge it from the pump and other HPLC system components with HPLC-grade water.
2. If the pump will be idle for more than one day, purge with a methanol/water solution to prevent the growth of microorganisms.
3. If the pump is running, use a shutdown method to ramp the flow to zero.



Caution: Since abrupt flow changes can damage columns, it is advisable to ramp flow to 0 using an appropriate shutdown method. Refer to your data control software's online Help for details.

4. Turn the power switch to the O (OFF) position.

5

Maintaining the HPLC Pump

This chapter provides important safety and handling considerations for the Waters 1500-series pump, describes how to run diagnostic tests and set pump calibration parameters, and explains how to replace pump components.

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Maintenance considerations

Safety and handling

When you perform maintenance procedures on your pump, keep the following safety considerations in mind.



Warning:

- To prevent injury, always observe Good Laboratory Practices when you handle eluents, change tubing, or operate the 1500-series HPLC pump. Know the physical and chemical properties of the eluents. Refer to the Material Safety Data Sheets for the eluents in use.
- To avoid the possible eye injury or cuts, handle the plunger with care. Wear safety glasses and use the plunger insertion tool. Be aware that the pieces of a broken plunger are very sharp.
- To avoid possible electric shock, do not remove the cover. The interior of the pump contains no user-serviceable parts.

Proper operating procedures

Follow the operating procedures and guidelines on [page 4-2](#).

Spare parts

For a comprehensive catalog of spare parts, see the Waters Quality Parts Locator on the Waters Web site's Services & Support page.

Contacting Waters Technical Service

If you encounter any problems replacing parts in the 1500-series pump, contact Waters Technical Service.

Performing pump diagnostic tests

The following diagnostic tests can help you track system performance and prevent or identify potential problems before they interfere with operation:

- Retention time stability test
- Ramp-and-decay test

Retention time stability test

Observing retention time stability during system performance monitoring tests is useful for determining the performance of your HPLC system and its components, including the pump. Erratic or changing retention times could be a result of dirty or malfunctioning check valves, worn plungers or plunger seals, air bubbles in the lines, incorrectly set flow rate, leaks, or other pump-related problems. Be aware that other factors, such as system and column equilibration, column age, operating temperature, and so on, can also affect retention time stability. See your system suitability documentation for more information.

Ramp-and-decay test

Use the ramp-and-decay test to monitor check valve performance.

Required materials

- Three compression plugs (part number WAT025566, startup kit)
- Tissues
- Open-end wrench, 5/16-inch (startup kit)
- HPLC-grade methanol

Monitor check valve performance

To perform the ramp-and-decay test:

1. Disconnect the pump outlet tube to the system at the reference valve (or vent valve for the 1525 μ), and install a compression plug in its place.
2. Purge Pump A with 100% methanol (see [Chapter 4](#)).

Requirements:

- If your eluent is not miscible with methanol, use an intermediate eluent.
 - Move the eluent reservoir below the pump head level to prevent gravity flow of the eluent.
3. For binary pumps, disconnect Pump B from the tee by removing the outlet tube of the pulse dampener; install a compression plug in its place.
 4. Disconnect the left-hand pump head outlet tube from the draw-off valve and install a compression plug in its place.
 5. Place absorbent tissues under the disconnected pump head outlet tube and the disconnected pulse dampener tube to catch drips.
 6. Ensure that the draw-off valves are closed and the eluent directed to waste by turning the reference valve 1/2-turn to the right (for 1525 μ , position the vent valve to vent).
 7. Use the data control software to set the following pump parameter values:
 - Flow rate:
 - For 1515 isocratic and 1525 binary pump – 0.3 mL/min
 - For 1525EF pump – 0.5 mL/min
 - For 1525 μ pump – 0.2 mL/min
 - High pressure limit:
 - For 1515 isocratic, 1525 binary pump and 1525 μ pump – 41,370 kPa (401 bar, 6000 psi)
 - For 1525EF pump – 34,475 kPa (334 bar, 5000 psi)
 8. With the 100% methanol reservoir still connected to the pump inlet, initiate the pump flow. Monitor pump pressure:
 - If the check valves are operating properly, pressure rises with each plunger stroke on the connected head and then holds steady as the plunger recedes.
 - If there is a bad inlet check valve, pressure can possibly stop at a certain point, or not rise at all.

- If there is a bad outlet check valve, pressure can possibly increase, and then immediately decrease as the plunger recedes.
 - If the pressure does not rise to the high-pressure limit, try repriming the pump or increasing the flow rate parameter setting.
9. Allow the pump to reach the high-pressure limit. The pump should stop flow automatically.
 10. After two minutes, record the pressure (P1). One minute later, record the pressure again (P2).
Calculate head pressure decay with the formula $(P1 - P2)/P1$. Verify that head pressure decay is 0.15 or less. If the pressure decay for the pump head is greater than 0.15, you can possibly have one or more faulty check valves. Remove and clean or replace the inlet and outlet check valves (see [page 5-14](#)).
 11. Relieve head pressure by slowly turning the reference valve knob 1/2 turn to the left (for the 1525 μ , position the vent valve to the system).
 12. Disconnect the right-hand pump head outlet tubing from the draw-off valve.
 13. Transfer the compression plug from the left-hand port of the draw-off valve to the right-hand one.
 14. Reconnect the left-hand pump head outlet tubing to the left-hand port of the draw-off valve.
 15. Repeat [step 5](#) through [step 12](#) to test the left-hand pump head.
 16. Remove the compression plugs from the draw-off valve and the tee. Reconnect the right pump head outlet tubing. Reconnect the tubing from the pulse dampener.
 17. For binary-type pumps, repeat [step 3](#) through [step 14](#) on Pump B, in [step 3](#), disconnecting Pump A from the tee.
 18. Remove the compression plug from the reference valve (or vent valve) and reconnect the outlet tube to the system.
 19. When you finish, purge the pump with eluent (see [Chapter 4](#)). Direct the eluent to system by turning the reference valve to the left (for the 1525 μ , position the vent valve to the system).

Replacing and cleaning plunger seals and plungers

This section describes these procedures:

- Removing and installing plunger seals for replacement
- Removing and installing plungers for cleaning and replacement

The plungers in a 1500-series pump are ultrasMOOTH, chemically inert sapphire rods. Salt crystals that precipitate from the eluent can form on the plunger and cause wear on the plunger seals and on the plunger itself. The result is a slow leak and a very slight cyclic pressure fluctuation, with a possible increase in retention time.

For continued high-performance operation of the pump, perform the following maintenance tasks:

- Replace the plunger seals and bearings twice yearly, or as needed
- Replace the plunger twice yearly, or as needed
- Clean and inspect the plunger every six months (or sooner if using abrasive eluents)

Preparing for plunger seal replacement

Required materials

- Open-end wrench, 5/16-inch (startup kit)
- Allen wrench, 5/32-inch (startup kit)
- Adjustable wrench (startup kit)
- Priming syringe (startup kit)
- Fitting plug (startup kit)
- Replacement seal kit, plunger seal insertion tool, and seal extraction tool (startup kit)
- HPLC-grade methanol

Removing the pump head

To gain access to a plunger seal, you must first remove the pump head.

Before starting this procedure, move the eluent reservoir below the pump head level to prevent gravity flow of the eluent.

To remove a pump head:

1. Purge the pump with methanol (see [Chapter 4](#)).

Requirements:

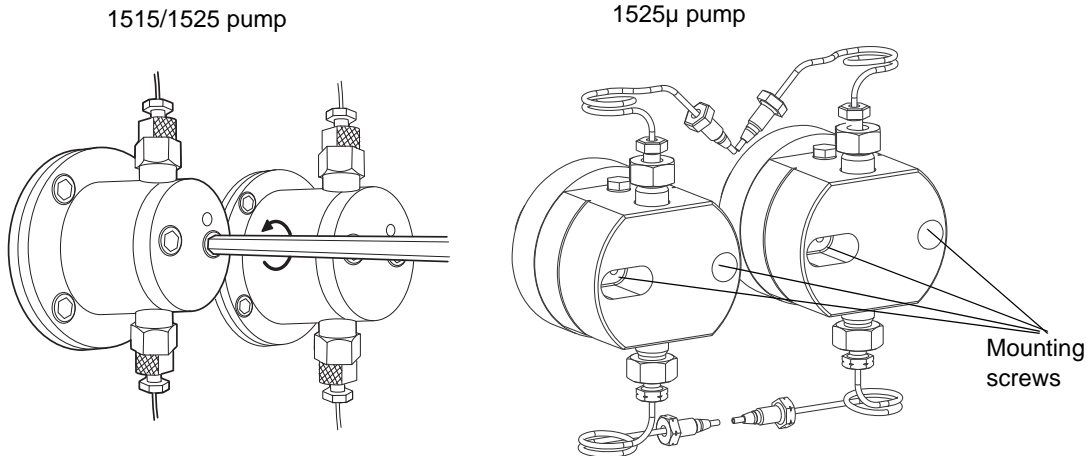
- If your eluent is not miscible with methanol, use an intermediate eluent.
 - Move the eluent reservoir below the pump head level to prevent gravity flow of the eluent.
2. Insert the priming syringe into the Luer fitting at the center of the draw-off valve handle, and then turn the handle counterclockwise about 1/2-turn to open the valve.
 3. Use the syringe to withdraw all methanol.
 4. Use the data control software to set the flow rate to 0.3 mL/min and to initiate pump flow.
 5. When the indicator rod fully retracts into the pump head, use the software to stop the flow, turning off the pump.

Tip: This action ensures that the weight of the pump head does not rest on the plunger while you remove the head.

6. While using the adjustable wrench to hold the check valve housings in place, use the 5/16-inch open-end wrench to remove the inlet and outlet tubing from the check valves on the pump.
7. Holding the pump head against the pump, use the 5/32-inch Allen wrench to remove the two, pump-head-assembly mounting screws.

Tip: Loosen the screws 1/2-turn at a time for the first two turns.

Removing the pump head mounting screws



8. Carefully slide the pump head assembly off the pump.

Removing and replacing the plunger seal

To remove and replace the plunger seal:

1. For the 1525 μ pump only, remove the plunger bearing from the pump head.
2. For all 1500-series pumps, use the seal insertion tool or other nonmetallic tool to pry the seal out of the pump head.
Tip: Twist the plastic screw end of the tool clockwise into the seal, and then pull the seal to remove it from the pump head.
3. Place the new plunger seal onto the tip of the seal insertion tool.
Tip: Make sure the spring in the seal is facing away from the end of the tool (you must be able to see the seal spring at the tip of the tool).
4. Moisten the seal with methanol.
5. Use the seal insertion tool to firmly seat the seal in the pump head.
6. Place the small end of the block part of the seal insertion tool into the pump head.
7. Slide the tip of the tool (with the seal on it) through the block and into the pump head.

8. Press the tool firmly into the pump head, and then remove it.
9. For the 1525 μ pump only, refit the plunger bearing on top of the seal.
Make sure the grooves in the bearing are facing outward and are visible as you look at the pump head.
10. Remoisten the seal and plunger with methanol.
11. Carefully slide the pump head assembly into position over the plunger.



Caution: To avoid breaking the plunger, hold the pump head securely against the pump.

12. Holding the pump head securely against the pump, reinstall the two pump head mounting screws, alternately tightening the screws using half turns to evenly align the assembly.
13. Use the data control software to set the flow rate to 0.3 mL/min and initiate pump flow.
14. Verify that the indicator rod moves freely, and then use the software to stop the flow, turning off the pump.
15. Use the adjustable wrench to hold the check valve housings in place while reconnecting the inlet and outlet tubing to the pump head.
16. Tighten the tubing using the 5/16-inch open-end wrench.
17. Prime the pump as described in [Chapter 4](#). If you notice leaks, verify pump head and plunger seal installation.

Cleaning and replacing the plungers



Warning: To avoid eye injury or lacerations, handle the plunger with care. Wear safety glasses and use the plunger insertion tool. Pieces of a broken plunger are very sharp.

The plungers are sapphire rods that require careful handling. Although cleaning the plungers is not difficult, it is important to follow these instructions carefully to avoid damaging the plungers. Assemble all materials and read the procedure thoroughly before you begin.

Required materials

- Open-end wrench, 5/16-inch (startup kit)
- Allen wrench, 5/32-inch (startup kit)
- Allen wrench, 9/64-inch (startup kit)
- Adjustable wrench (startup kit)
- Snap-ring pliers (for 1515/1525 pumps only) (part number WAT025263)
- Plunger insertion tool and seal extraction tool (startup kit)
- Sonicator
- HPLC-grade water
- HPLC-grade methanol
- Replacement plunger
- Replacement plunger seals

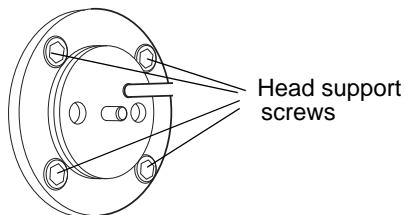
Removing the plunger

To remove the plunger from the pump:

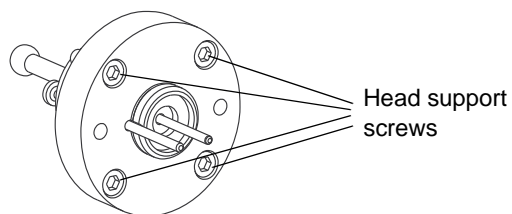
1. Remove the pump head as described on [page 5-6](#).
2. Remove the four head-support screws with the 9/64-inch Allen wrench, and then carefully slide the head support assembly and the indicator rod off the pump.

Exposed head support assembly

1515/1525 head support assembly



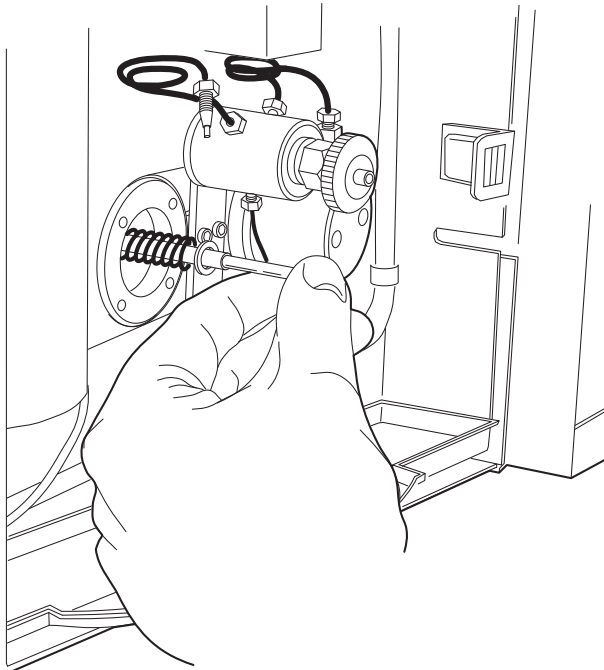
1525 μ head support assembly



3. Set the head support assembly on the benchtop.
4. Use the data control software to set the flow rate to 0.3 mL/min and initiate pump flow.

5. When the indicator rod is fully extended from the pump head, use the software to stop the flow, turning off the pump.
6. Carefully remove the plunger:
 - For a 1525 μ pump, slide the plunger assembly out of the back of the support assembly. Set it aside for cleaning.
 - For 1515/1525 pumps, use the snap-ring pliers to remove the snap-ring that holds the plunger in place (see the figure, below). Carefully remove the plunger assembly and set it aside for cleaning.

Removing the plunger assembly (1515/1525 pumps)

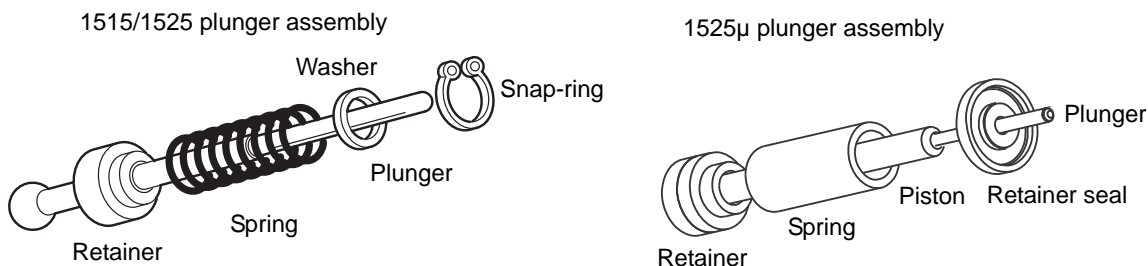


Cleaning the plunger

To clean the plunger:

1. Remove the plunger from the plunger assembly.
2. Clean the plunger by sonicating it in 50:50 methanol/water for a few minutes.

Assembled plunger



Inspecting the plunger

After cleaning the plunger, inspect it for damage by holding it under a bright white light and looking down its length for nicks and scratches. It is easier to see scratches under a bright light than to feel them with your fingers.

- If the plunger is not scratched or otherwise damaged, reassemble it with new seals. Continue with [“Replacing the Plunger” on page 5-12](#).
- If the plunger is damaged, replace both the plunger and the seals. Continue with [“Replacing the Plunger” on page 5-12](#).

Replacing the Plunger

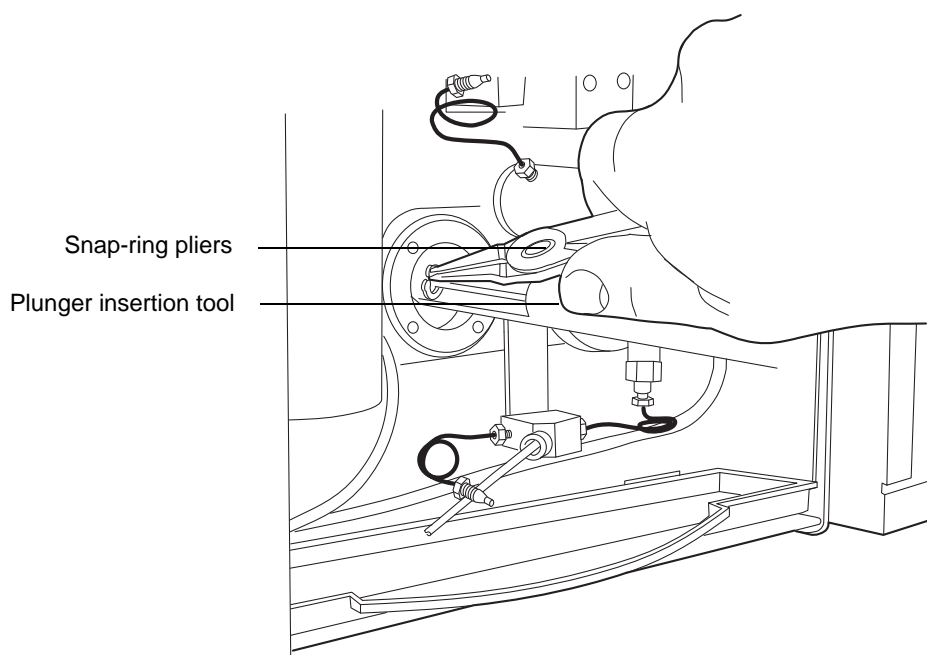
To replace the plunger:

1. Reassemble the components of the plunger assembly as shown in the figure above.
2. Lubricate the ball and seat with high-pressure grease.
Make sure the brass seat is located at the bottom of the piston.

3. Reinstall the plunger assembly
 - For 1525 μ pump, reinstall the plunger assembly into the head support assembly.
 - For 1515/1525 pumps, use the plunger insertion tool to push the plunger assembly into the pump piston as far as it will go, and then use the snap-ring pliers to insert the snap ring.

Make sure the snap ring is fully seated in the groove before you release the pliers.

Inserting the plunger (1515/1525 pumps)



4. Use the data control software to set the flow rate to 1.0 mL/min and initiate pump flow.
5. When the indicator rod is fully retracted into the pump head, use the software to stop the flow, turning off the pump.
6. With the indicator rod hole oriented to the upper right (to the upper left for 1525 μ), reinstall the head support assembly. Alternately tighten the four screws, taking care not to overtighten.
7. Moisten the plunger seal and plunger with methanol.

- Carefully slide the pump head over the plunger and onto the pump.



Caution: To avoid breaking the plunger, hold the pump head securely against the pump.

- Holding the pump head securely against the pump, alternately tighten the two screws.
- Check for even head alignment by observing the gap between the pump head and the pump head support assembly.
- Use the data control software to set the flow rate to 1.0 mL/min and initiate pump flow.
- Verify the pump head alignment.
 - For 1525 μ pump, observe the movement of the indicator rod while the pump is running.
 - For 1515/1525 pumps, pull out and release the indicator rod.
 - If the rod does not move smoothly (or snap back easily), the head is misaligned. Stop the pump, loosen the pump head, and then repeat [step 8](#) through [step 11](#).
 - If the rod moves smoothly (snaps back easily), use the software to stop the flow, turning off the pump. Continue with [step 13](#).
- Using the adjustable wrench to hold the check valve housings in place, reconnect the inlet and outlet tubing assemblies to the pump head. Tighten the tubings using the 5/16-inch open-end wrench.
- Prime the pump as described in [Chapter 4](#). If you notice leaks, verify pump head and plunger seal installation (see [page 5-6](#)).

Replacing check valves

This section describes how to replace the pump's inlet and outlet check valves for the 1515/1525 pumps, 1525EF pump, and the 1525 μ pump.

Recommendations:

- Replace the inlet and outlet check valves every 6 months, or more frequently if required by your application.
- Keep a spare set of new check valves on hand.

Requirements:

- Replace each check valve and check valve housing as a single unit. Do not separate the check valve from the housing.
- Before replacing a check valve, move the eluent reservoir to a level below the pump head to prevent the gravity flow of eluent.

Required materials

- Open-end wrench, 5/16-inch (startup kit)
- Adjustable wrench, 1/2-inch (startup kit)
- Priming syringe (startup kit)
- Replacement check valves (assembled with housings)
- HPLC-grade methanol
- Forceps

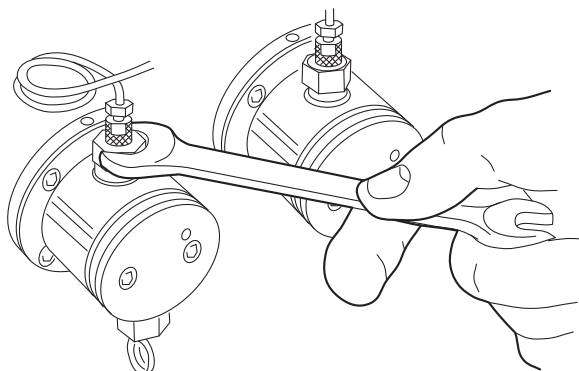
Replacing 1515/1525 check valves

The 1515 isocratic and 1525 binary pumps employ a check valve cartridge for both the inlet and outlet check valves.

To replace a check valve:

1. Purge the pump with methanol (see [Chapter 4](#)). If methanol is not miscible with your current eluent, use an intermediate eluent.
2. Use the open-end wrench to remove the check valve housing from the inlet or outlet side of the pump head.

Disconnecting the outlet tubing (1515/1525 pump)

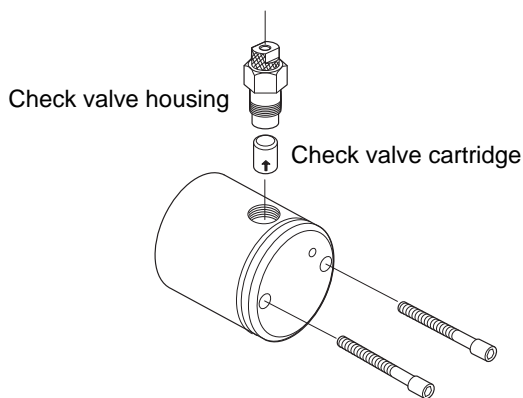


3. Using forceps, remove the cartridge from the housing and replace it with the new cartridge.

Requirements:

- The arrow printed on the check valve cartridge indicates the direction that liquid can flow. For both inlet and outlet check valve assemblies, the arrow must point upward when installed in the pump head.
- To help keep the cartridge seated within the housing, moisten it with methanol during installation.

Outlet check valve assembly (1515/1525 pumps)



4. With the check valve held upright to prevent the cartridge from falling out, hand-tighten the check valve into the pump head. Tighten the check valve another 1/2-turn with the open-end wrench.
5. Reconnect the eluent supply line to the inlet manifold on the pump.
6. Prime the pump as described in [Chapter 4](#). Check for leaks.

Replacing 1525EF check valves

The 1525EF pump employs ball and socket check valve assemblies that are installed in the pump inlet and outlet housings.

Replacing the 1525EF inlet check valve

To replace the inlet check valve:

1. Purge the pump with methanol (see [Chapter 4](#)). If methanol is not miscible with your current eluent, use an intermediate eluent.
2. While holding the inlet check valve housing with the adjustable wrench, use the 5/16-inch open-end wrench to loosen the compression screw and remove the tubing assembly from the check valve housing (see the figure, below).
3. Use the adjustable wrench to remove the entire check valve assembly, including the housing, from the pump head.
4. Replace the entire old check valve housing with a new one. Finger-tighten the new check valve housing onto the pump head. Tighten the check valve with the adjustable wrench.
5. Reinstall the tubing assembly. Holding the housing with the adjustable wrench, tighten the compression screw with the 5/16-inch open-end wrench.
6. Repeat [step 2](#) through [step 5](#) to replace the other inlet check valves on the pump.
7. Prime the pump as described in [Chapter 4](#). Check for leaks.

Replacing the 1525EF outlet check valve

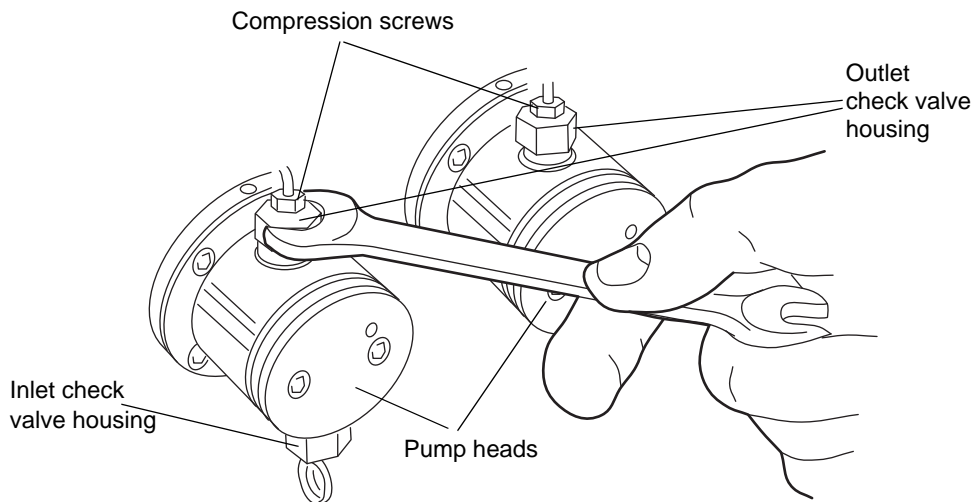
To replace the outlet check valve:

1. Purge the pump with methanol (see [Chapter 4](#)). If methanol is not miscible with your current eluent, use an intermediate eluent.
2. Use the data control software to run the pump until the indicator rod fully retracts into the pump head, and then stop the flow, turning off the pump.

This ensures that the weight of the pump head does not rest on the plunger when you remove the pump head.

3. Use the adjustable wrench to hold the housings in place. Use the 5/16-inch open-end wrench to loosen the compression screws and remove the inlet and outlet tubing assemblies from the check valve housings.

Disconnecting the outlet tubing (1525EF pump)



4. Use the adjustable wrench to loosen the outlet check valve housing.
5. While holding the pump head in place, use the 5/32-inch Allen wrench to remove the two pump head assembly mounting screws. Loosen the screws 1/2-turn at a time for the first two turns.

- Carefully slide the pump head assembly off the pump (see [page 5-6](#)).



Caution: Pull the pump head off straight to avoid breaking the plunger.

- Hold the pump head upside-down in one hand with the outlet check valve housing facing downward. Remove the entire outlet check valve housing and replace it with a new one.
- Finger-tighten the new outlet check valve housing into the pump head.
- Turn the pump head over and carefully slide the pump head over the plunger and onto the pump.



Caution: Ensure that the pump head is straight and the indicator rod is properly aligned with the pump head to avoid damaging the plunger.

- Holding the pump head securely against the pump, reinstall the two screws, alternately tightening the screws to secure the pump head to the pump. Do not overtighten.
- Tighten the outlet check valve housing with the adjustable wrench.
- Reinstall the outlet tubing and inlet tubing assemblies.
Tip: Use the 5/16-inch open-end wrench to tighten the compression screw while holding the housing with the adjustable wrench.
- Prime the pump as described in [Chapter 4](#). Check for leaks.

Replacing 1525 μ check valves

This section describes how to replace inlet, outlet, and restrictor tee check valves for the 1525 μ pump. These check valves employ a cartridge assembly that you install in the check valve housing.

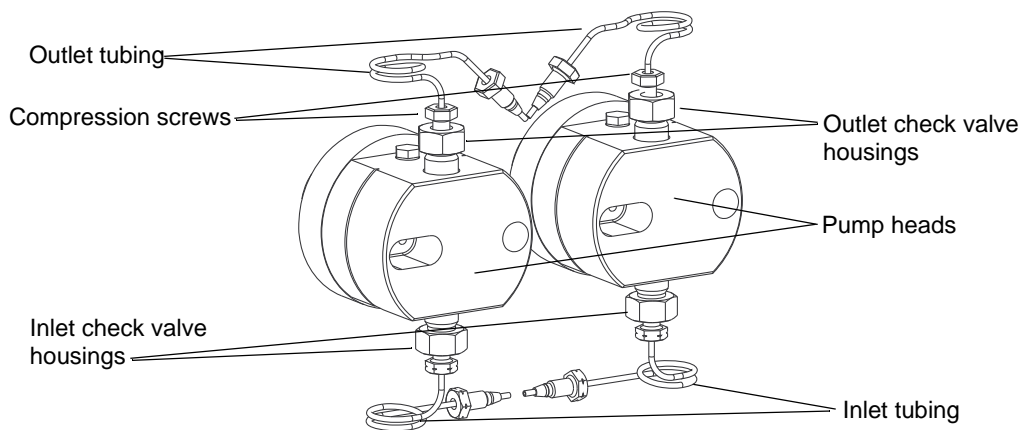
Replacing the 1525 μ inlet check valve

To replace the inlet check valve:

- Purge the pump with methanol (see [Chapter 4](#)). If methanol is not miscible with your current eluent, use an intermediate eluent.

2. While holding the check valve housing with the adjustable wrench, use the 5/16-inch open-end wrench to loosen the compression screw, and remove the tubing assembly from the inlet check valve housing.
3. Use the adjustable wrench to remove the inlet check valve housing from the pump head.

Disconnecting the inlet and outlet tubing (1525 μ pump)



4. Remove the cartridge from the housing and replace it with the new cartridge.
The arrow printed on the check valve cartridge indicates the direction that liquid can flow. For all check valve assemblies, to ensure proper operation, the arrow must point upward when installed on the pump.
Tip: To help keep the cartridge seated within the housing, moisten it with methanol during installation.
5. With the housing held upright to prevent the cartridge from falling out, finger-tighten the housing into the pump head. Tighten the check valve another 1/4-turn with the adjustable wrench.
6. Reinstall the inlet tubing assembly.
Tip: Use the 5/16-inch open-end wrench to tighten the compression screw while holding the housing secure with the adjustable wrench.
7. Repeat [step 2](#) through [step 6](#) to replace the other inlet check valves as necessary.
8. Prime the pump as described in [Chapter 4](#). Check for leaks.

Replacing the 1525 μ outlet check valve

Before you replace the outlet check valve, move the eluent reservoir to a level below the pump head to prevent the gravity flow of eluent.

To replace the outlet check valve:

1. Purge the pump with methanol (see [Chapter 4](#)). If methanol is not miscible with your current eluent, use an intermediate eluent.
2. Use the data control software to run the pump until the indicator rod fully retracts into the pump head, and then stop the flow, turning off the pump.

This step ensures that the weight of the pump head does not rest on the plunger when you remove the pump head.

3. Use the adjustable wrench to hold the housings in place. Use the 5/16-inch open-end wrench to loosen the compression screws and remove the inlet and outlet tubing assemblies from the check valve housings.
4. Use the adjustable wrench to loosen the outlet check valve housing.
5. While holding the pump head securely against the pump, use the 5/32-inch Allen wrench to remove the two pump head assembly mounting screws. Loosen the screws 1/2-turn at a time for the first two turns.
6. Carefully slide the pump head assembly off the pump (see the instructions on [page 5-6](#)).



Caution: Pull the pump head off straight to avoid breaking the plunger.

7. Hold the pump head upside-down in one hand with the outlet check valve housing facing downward, and then remove the housing. Remove the cartridge from the housing and replace it with the new cartridge.

The arrow printed on the check valve cartridge indicates the direction that liquid can flow. For all check valve assemblies, to ensure proper operation, the arrow must point upward when installed on the pump.

Tip: To help keep the cartridge seated within the housing, moisten it with methanol during installation.

8. With the outlet check valve housing held upright to prevent the cartridge from falling out, finger-tighten the housing into the pump head.
9. Turn the pump head over and carefully slide it over the plunger and onto the pump.



Caution: To avoid damaging the plunger, ensure that the pump head is straight and the indicator rod is properly aligned with the pump head.

10. Holding the pump head securely against the pump, reinstall the two mounting screws. Alternately tighten the screws to secure the pump head to the pump. Do not overtighten.
11. Tighten the check valve housing 1/4-turn with the adjustable wrench.
12. Reinstall the outlet tubing assembly.
Tip: Use the 5/16-inch open-end wrench to tighten the compression screw while holding the housing with the adjustable wrench.
13. Reinstall the inlet tubing assembly.
Tip: Use the 5/16-inch open-end wrench to tighten the compression screw while holding the housing with the adjustable wrench.
14. Prime the pump as described in [Chapter 4](#). Check for leaks.

Replacing the 1525 μ restrictor tee check valves

To replace the restrictor tee check valves, follow the procedure on [page 5-19](#).

Replacing a draw-off valve

Replace a draw-off valve if you notice that the valve leaks even after you tighten the knob.

Required materials

- Open-end wrench, 5/16-inch (startup kit)
- Allen wrench, 7/64-inch (startup kit)
- Adjustable wrench, 1/2-inch (startup kit)

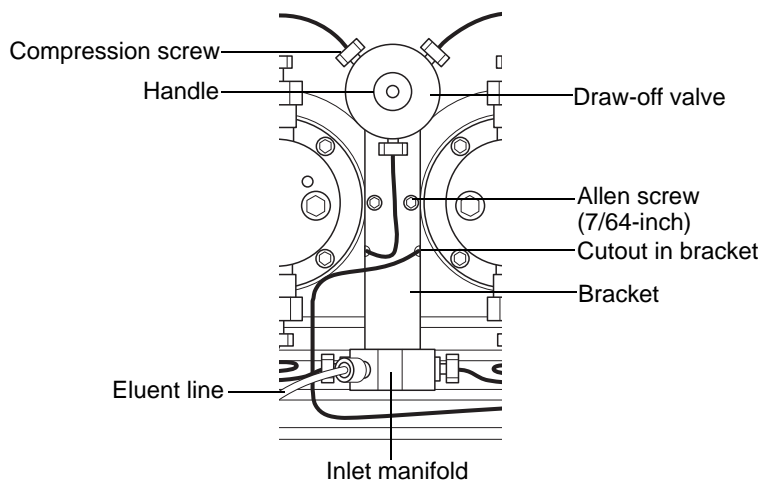
- Priming syringe (startup kit)
- Phillips screwdriver
- Replacement draw-off valve
- HPLC-grade methanol

Removing the draw-off valve

To remove the draw-off valve:

1. Purge the pump with methanol (see [Chapter 4](#)). If methanol is not miscible with your current eluent, use an intermediate eluent.
Before you continue with this procedure, lower the eluent reservoir to halt gravity flow of eluent. Disconnect the eluent supply line from the inlet manifold on the pump.
2. Insert the priming syringe into the Luer fitting at the center of the draw-off valve handle, and then rotate the handle counterclockwise about 1/2-turn to open the valve.
3. Use the syringe to withdraw all the methanol.
4. Use the 5/16-inch open-end wrench to disconnect the three stainless steel compression screws from the draw-off valve.

Draw-off valve/inlet manifold assembly




5. Use the 5/16-inch open-end wrench to disconnect the two stainless steel compression screws from the inlet manifold. (These screws connect the inlet manifold to the inlet check valves.)
6. Use the 7/64-inch Allen wrench to remove the two Allen screws that hold the draw-off valve assembly and bracket to the pump. Carefully remove the assembly.
7. Remove the two Phillips screws that hold the draw-off valve to the bracket.

Installing a draw-off valve

To install the draw-off valve:

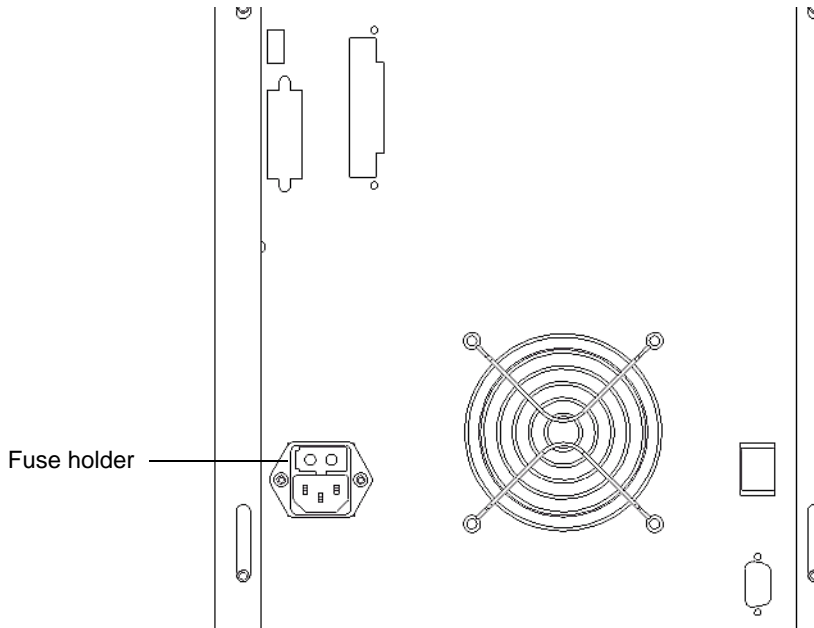
1. Attach the new draw-off valve to the bracket using the two Phillips screws.
Tip: Ensure the new valve is oriented on the bracket as shown in the figure above.
2. Reposition the draw-off valve/inlet manifold assembly onto the pump, being careful not to pinch the stainless tubing behind the bracket.
When correctly positioned, the stainless tubing extends through the cutouts on the sides of the bracket.
3. Secure the bracket to the pump with the two Allen screws.
4. Reconnect the inlet tubing to the inlet manifold.
5. Reconnect the outlet tubing to the new draw-off valve.
6. Reposition the eluent reservoir, and then prime the pump (see [Chapter 4](#)). Check for leaks.

Replacing fuses

 **Warning:** To avoid possible electrical shock, power-off and unplug the instrument before checking the fuses. For continued protection against fire hazard, replace fuses only with those of the same type and rating.

The fuse holder is located on the pump's rear panel. The 1500-series pump is shipped with two factory-installed 3.15 A fuses.

Rear panel fuse holder



Identifying faulty fuses

Suspect a faulty fuse if either of these conditions occur:

- The pump fails to power-on.
- The fan does not operate.

Replacing the rear panel fuses

Required materials

- Screwdriver, flat-blade, small
- Replacement fuses

To replace a blown or faulty fuse:

- Power-off the pump, and then remove the power cord from its connector.
 - Use the small flat-blade screwdriver to remove the fuse holder located just above the power cord connector.
1. Remove and discard the old fuses.
 2. Insert the new fuses into the fuse holder.
 3. Insert the fuse holder into the receptacle and gently push until it locks into position.

6 Troubleshooting

This chapter describes how to troubleshoot pump problems based on error messages and malfunctions, identify and correct noises, and identify pump-related chromatographic problems.



Warning: To prevent injury, always observe Good Laboratory Practices when you troubleshoot the 1500-series pump

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| Identifying and correcting noises | 6-8 |
| Identifying chromatographic problems | 6-9 |

Troubleshooting pump problems

The following table is a guide to troubleshooting pump problems. It lists pump error messages and pump-related symptoms and their possible causes, and suggests corrective actions.

Determining pump malfunctions

| Symptom | Possible cause | Corrective action |
|---------------|---|---|
| High pressure | Pump flow rate set too high | Set to correct flow rate. |
| | High pressure limit set too low | Set high pressure limit to the correct value. |
| | Blocked column | Clean the column according to the column care and use manual. |
| | Blocked mixer | Backflush or disassemble the mixer. |
| | Viscous eluents in use | Verify eluent viscosity (the observed pressure may be normal for the column/eluent blend). If necessary, change to a less viscous eluent. |
| | Defective pressure transducer | Contact Waters Technical Service. |
| | Blocked outlet tubing or fluid path (in pump outlet, detector, column, or injector) | Locate the source of blockage. Clean or replace tubing according to the appropriate operator's manual. |
| | Ambient temperature has changed | Stabilize the operating temperature. |

Determining pump malfunctions (Continued)

| Symptom | Possible cause | Corrective action |
|----------------------------|--|---|
| Low pressure | Pump flow rate set too low | Set to the correct flow rate. |
| | Low pressure limit set too high | Set low pressure limit to correct value. |
| | Eluent reservoir empty | Fill the reservoir. |
| | Air in eluent lines | Prime the pump (see Chapter 4). |
| | Incorrect eluent used | Change to the correct eluent. |
| | Visible leaks | Carefully tighten any loose fittings. |
| | Ambient temperature has changed | Stabilize the operating temperature. |
| Pump does not run | Pump not connected to power source | Ensure power cable is properly connected to power source and pump. |
| | No power at outlet | Confirm the outlet is serviceable by connecting to another electrical unit known to be working. If that unit does not work, relocate the pump to a functioning electrical outlet. |
| | Blown fuse | Replace the fuses. |
| Pump not delivering eluent | Draw-off valve open or leaking | Close the draw-off valve. If eluent still leaks, replace the valve (see page 5-22). |
| | Pump low-pressure limit set higher than operating pressure | Set to the correct low-pressure limit. |
| | Flow rate set to zero | Set the desired pump flow rate. |
| | Pressure transducer out of adjustment or defective | Contact Waters Technical Service. |

Determining pump malfunctions (Continued)

| Symptom | Possible cause | Corrective action |
|--|---|--|
| Pump not delivering eluent (continued) | Pump not primed | Prime the pump (see Chapter 4). |
| | Immiscible eluents in pump head | Purge pump with appropriate eluents. Verify miscibility of eluents being used and change to more miscible eluents. |
| | Dirty or malfunctioning inlet or outlet check valve | Try to isolate the problem. Varying pressure/flow usually indicates an outlet check valve problem. Try loosening and then retightening the check valve to dislodge any stuck particles. Often, a sticking check valve is unstuck by letting the pump run for a few minutes at pressure. If there is no flow from one head, replace the head's inlet check valve. (see page 5-14). |
| | Damaged plunger seal (indicated by eluent leaking from behind the pump head or by salt crystal build-up around the back of the pump head) | Confirm that both pump heads can maintain pressure as outlined on page 5-3 . If a head is leaking, replace the plunger seal (see page 5-6). |

Determining pump malfunctions (Continued)

| Symptom | Possible cause | Corrective action |
|--|---|---|
| Pump not delivering eluent (continued) | Pump cavitation due to one of the following: Eluent reservoirs positioned at or below the height of the pump | Perform the following corrective action: Raise eluent reservoirs above the pump. |
| | Loose, bent, or blocked inlet tubing | Inspect tubing. Tighten, straighten, or replace tubing. |
| | Improperly degassed eluent | Verify degasser operation. |
| | Dirty eluent reservoir inlet filter | Replace filter. |
| Pump not delivering eluent (continued) | Volatile eluents in pump head | Prime pump (see Chapter 4). |
| | Tubing ID too small | Use correct tubing. |
| | Ruptured high-pressure noise filter | Contact Waters Technical Service. |
| Pump not delivering eluent (continued) | Defective pump motor | Contact Waters Technical Service. |
| Pump not delivering eluent (continued) | Defective circuit board | Contact Waters Technical Service. |

Determining pump malfunctions (Continued)

| Symptom | Possible cause | Corrective action |
|-----------------------------------|-----------------------------------|--|
| Leak from pump head | Worn pump plunger seals | Replace defective plunger seals (see page 5-6). |
| | Worn plunger | Replace the plunger (see page 5-6). |
| | Loose pump head | Tighten the two pump head screws. Ensure that both screws are tightened equally, otherwise seal wear may result. Do not overtighten. |
| | Loose inlet or outlet check valve | Tighten the loose check valve(s). Do not overtighten. |
| | | Inspect fittings and ferrules for wear. Replace if necessary. |
| Leak from draw-off valve | Draw-off valve open or broken | Close the draw-off valve. If the leak continues, replace the valve (see page 5-22). |
| Erratic flow rate/pump pulsations | Pump not primed | Prime the pump. If you are using a volatile eluent (such as hexane), prime the pump with a miscible, less volatile eluent such as THF or methanol. Ensure that the column is disconnected to avoid disrupting equilibrium. |
| | Reservoir low or out of eluent | Refill reservoir (filter and degas eluent). |
| | Air bubble in pump head | Prime pump to remove bubble. Ensure there are no air bubbles in the inlet lines. Degas eluents. |

Determining pump malfunctions (Continued)

| Symptom | Possible cause | Corrective action |
|---|--|--|
| Erratic flow rate/pump pulsations (continued) | Dirty or malfunctioning check valve(s) | Try to isolate the problem. Varying pressure/flow usually indicates an outlet check valve problem. Try loosening, and then retightening the check valve to dislodge any stuck particles. Often, a sticking check valve is resolved by letting the pump run for a few minutes at pressure. If there is no flow from one head, replace the head's inlet check valve. (see page 5-14). |
| | Inlet filter or inlet lines blocked | Inspect lines for blockages. Replace the inlet filter frit. |
| | Pump plunger seal leaking (under pump head) | Replace pump plunger seal (see page 5-6). |
| | Worn pump plunger | Replace the plunger (see page 5-6). |
| | Immiscible eluents in pump head | Refer to the Corrective Action under the "Pump not delivering eluent" symptom listed above. |
| | Pump cavitation | Refer to the Corrective Action(s) under the "Pump not delivering eluent" symptom listed above. |
| | Pump electronics failure | Contact Waters Technical Service. |
| High system pressure due to pump | Pump Flow parameter set too high | Set the correct flow parameter value. |
| | Pressure transducer out of adjustment or defective | Contact Waters Technical Service. |

Identifying and correcting noises

The following table is a guide to troubleshooting and correcting noises in the Waters 1500-series pump.

Identifying noises

| Symptom | Possible cause | Corrective action |
|--------------------|------------------------------|---|
| Click or loud snap | Binding plunger seal | If click does not stop and cannot be isolated to one pump head, replace the plunger seals, one at a time (see page 5-6). |
| | Worn indicator rod spring | Replace the spring. |
| Squeak | Plunger seals dry | Wet plunger with appropriate eluent through pump head access holes. |
| | Binding plunger seal | Replace plunger seal assembly (see page 5-6). |
| | Improper plunger seal | Install correct pump plunger seal (see page 5-6). |
| | Binding piston indicator rod | Replace indicator rod. |

Identifying chromatographic problems

The following table is a guide to troubleshooting and correcting chromatographic problems.

Correcting chromatographic problems

| Symptom | Possible cause | Corrective action |
|---------------------------|------------------------------|--|
| Erratic retention times | Air bubble in pump head | Degas all eluents and prime pump (see Chapter 4). |
| | Malfunctioning check valves | Replace pump check valves (see page 5-14). |
| | Leaking pump seals | Replace pump seals (see page 5-6). |
| | Leaks around fittings | Tighten fittings |
| | Separation chemistry | Check eluent and column. |
| | Clogged eluent filters | Replace filters. |
| Increased retention times | Incorrect flow rate | Change flow rate. |
| | Incorrect eluent composition | Change eluent composition. |
| | Column heater not turned on | Turn column heater on. |
| | Incorrect eluent | Use correct eluent. |
| | Column contaminated | Clean or replace column. |
| | Incorrect column | Use correct column. |
| | Air bubble in pump head | Degas all eluents and prime pump (see Chapter 4). |
| | Clogged eluent filter | Replace filter. |

Correcting chromatographic problems (Continued)

| Symptom | Possible cause | Corrective action |
|-------------------------|--|---|
| Reduced retention times | Incorrect flow rate | Change flow rate. |
| | Incorrect eluent composition | Change composition. |
| | High column temperature | Reduce column temperature. |
| | Incorrect eluent | Use correct eluent. |
| | Column contaminated | Clean or replace column. |
| | Incorrect column | Use correct column. |
| | Eluent not properly degassed | Degas eluent. |
| Reproducibility errors | Eluent not properly degassed | Degas eluent. |
| | Incorrect chemistry/integration | Verify chemistry/integration. |
| Baseline drift, rapid | Column not equilibrated | Equilibrate column. |
| | Eluent contaminated | Use fresh eluent. |
| | Eluent not properly degassed (rapid or slow drift) | Degas eluent. |
| | Flow fluctuations (rapid or slow drift) | Fix pump problems, replace pump seals and check valves. |
| Baseline drift, slow | Eluent contaminated | Use fresh eluent. |
| | Ambient temperature fluctuations | Stabilize operating environment temperature enough to allow full equilibration. |

Correcting chromatographic problems (Continued)

| Symptom | Possible cause | Corrective action |
|---|---|--|
| Baseline noise cycling, short-term (30 to 60 sec) | Fluctuating flow rate | Degas eluent. Try repriming the pump(s). If necessary, check flow accuracy of the pump(s). |
| | Inadequate eluent blending | Install a mixer as shown on page 3-8 . |
| | Power source (short- or long-term cycling) | Disconnect other instruments on the power line; try a different wall outlet; have line voltage checked; use power conditioner. |
| | Radio frequency noise (short- or long-term cycling) | Eliminate interference. |
| Baseline noise cycling, long-term (for 1 hour) | Ambient temperature fluctuations | Stabilize ambient temperature. |
| | Integrator or recorder faulty | Inspect integrator or recorder for excessive baseline noise. |

Correcting chromatographic problems (Continued)

| Symptom | Possible cause | Corrective action |
|-----------------------------|---|--|
| Baseline noise, random | Eluents not properly degassed | Degas eluents. |
| | Flow erratic, pump not primed | Prime the pump (see Chapter 4). |
| | | Purge the pump to dislodge any air bubbles, inspect for failing seals. |
| | Eluents contaminated | Use fresh eluent. |
| | Column contaminated | Clean/replace column. |
| | System improperly grounded | Plug into outlet on different electrical circuit. |
| | | Use power conditioner. |
| | Recorder voltage incorrect | Set recorder to correct voltage. |
| Radio frequency noise | Eliminate interference. | |
| Straight baseline, no peaks | No pump flow | Set pump flow rate. |
| | Leak in eluent path | Repair the leak. |
| Sensitivity loss | Degraded, contaminated, or improperly prepared sample | Use fresh sample. |
| | Column contaminated | Clean or replace column. |
| | Loss of column efficiency | Clean or replace column. |
| | Change in eluent composition | Correct eluent pH or ionic composition. |
| | Incorrect flow rate | Change flow rate. |

A Safety Advisories

Waters instruments display hazard symbols designed to alert you to the hidden dangers of operating and maintaining the instruments. Their corresponding user guides also include the hazard symbols, with accompanying text statements describing the hazards and telling you how to avoid them. This appendix presents all the safety symbols and statements that apply to the entire line of Waters products.

Contents

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| Caution symbol | A-5 |
| Warnings that apply to all Waters instruments | A-6 |
| Electrical and handling symbols | A-12 |

Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with an instrument's use or misuse. Heed all warnings when you install, repair, and operate Waters instruments. Waters assumes no liability for the failure of those who install, repair, or operate its instruments to comply with any safety precaution.

Task-specific hazard warnings

The following warning symbols alert you to risks that can arise when you operate or maintain an instrument or instrument component. Such risks include burn injuries, electric shocks, ultraviolet radiation exposures, and others.

When the following symbols appear in a manual's narratives or procedures, their accompanying text identifies the specific risk and explains how to avoid it.



Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)



Warning: (Risk of burn injury from contacting hot surfaces.)



Warning: (Risk of electric shock.)



Warning: (Risk of fire.)



Warning: (Risk of sharp-point puncture injury.)



Warning: (Risk of hand crush injury.)



Warning: (Risk of exposure to ultraviolet radiation.)



Warning: (Risk of contacting corrosive substances.)



Warning: (Risk of exposure to a toxic substance.)



Warning: (Risk of personal exposure to laser radiation.)



Warning: (Risk of exposure to biological agents that can pose a serious health threat.)



Warning: (Risk of tipping.)



Warning: (Risk of explosion.)



Warning: (Risk of eye injury.)

Specific warnings

The following warnings can appear in the user manuals of particular instruments and on labels affixed to them or their component parts.

Burst warning

This warning applies to Waters instruments fitted with nonmetallic tubing.



Warning: Pressurized nonmetallic, or polymer, tubing can burst.

Observe these precautions when working around such tubing:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to incompatible compounds like tetrahydrofuran (THF) and nitric or sulfuric acids.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, which significantly reduces the pressure at which the tubing can rupture.

Mass spectrometer flammable solvents warning

This warning applies to instruments operated with flammable solvents.



Warning: Where significant quantities of flammable solvents are involved, a continuous flow of nitrogen into the ion source is required to prevent possible ignition in that enclosed space.

Ensure that the nitrogen supply pressure never falls below 690 kPa (6.9 bar, 100 psi) during an analysis in which flammable solvents are used. Also ensure a gas-fail connection is connected to the LC system so that the LC solvent flow stops if the nitrogen supply fails.

Mass spectrometer shock hazard

This warning applies to all Waters mass spectrometers.



Warning: To avoid electric shock, do not remove the mass spectrometer's protective panels. The components they cover are not user-serviceable.

This warning applies to certain instruments when they are in Operate mode.



Warning: High voltages can be present at certain external surfaces of the mass spectrometer when the instrument is in Operate mode. To avoid non-lethal electric shock, make sure the instrument is in Standby mode before touching areas marked with this high voltage warning symbol.

Biohazard warning

This warning applies to Waters instruments that can be used to process material that might contain biohazards: substances that contain biological agents capable of producing harmful effects in humans.



Warning: Waters instruments and software can be used to analyze or process potentially infectious human-sourced products, inactivated microorganisms, and other biological materials. To avoid infection with these agents, assume that all biological fluids are infectious, observe Good Laboratory Practices, and consult your organization's biohazard safety representative regarding their proper use and handling. Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Chemical hazard warning

This warning applies to Waters instruments that can process corrosive, toxic, flammable, or other types of hazardous material.



Warning: Waters instruments can be used to analyze or process potentially hazardous substances. To avoid injury with any of these materials, familiarize yourself with the materials and their hazards, observe Good Laboratory Practices (GLP), and consult your organization's safety representative regarding proper use and handling. Guidelines are provided in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals*.

Caution symbol

The caution symbol signifies that an instrument's use or misuse can damage the instrument or compromise a sample's integrity. The following symbol and its associated statement are typical of the kind that alert you to the risk of damaging the instrument or sample.



Caution: To avoid damage, do not use abrasives or solvents to clean the instrument's case.

Warnings that apply to all Waters instruments

When operating this device, follow standard quality control procedures and the equipment guidelines in this section.



Attention: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

Important: Toute modification sur cette unité n'ayant pas été expressément approuvée par l'autorité responsable de la conformité à la réglementation peut annuler le droit de l'utilisateur à exploiter l'équipement.

Achtung: Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.

Avvertenza: qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.

Atencion: cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.

注意: 未經有關法規認證部門允許對本設備進行的改變或修改,可能會使使用者喪失操作該設備的權利。

注意: 未經有關法規認證部門明確允許對本設備進行的改變或改裝,可能會使使用者喪失操作該設備的合法性。

주의: 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.

注意: 規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザーとしての承認が無効になる可能性があります。



Warning: Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames.
- Do not use tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.

Attention: Manipulez les tubes en polymère sous pression avec précaution:

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Eteignez toute flamme se trouvant à proximité de l'instrument.
- Evitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Evitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.

Vorsicht: Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:

- In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.
- Alle offenen Flammen in der Nähe löschen.
- Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.
- Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.
- Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.



Attenzione: fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnere tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.

Advertencia: se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Si hubiera alguna llama las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.

Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.

警告：當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。

要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。



警告: 当有压力的情况下使用管线时, 小心注意以下几点:

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚砜会导致非金属管线膨胀, 大大降低管线的耐压能力。

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸설폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.

警告: 圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン(THF)や高濃度の硝酸または硫酸などを流さないでください。
- 塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があります、その場合、チューブは極めて低い圧力で破裂します。



Warning: The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Attention: L'utilisateur doit être informé que si le matériel est utilisé d'une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d'être défectueuses.

Vorsicht: Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwendung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.

Attenzione: si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.

Advertencia: el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.

警告: 使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被削弱。

警告: 使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被削弱。

경고: 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.

警告: ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。



Warning: To protect against fire, replace fuses with those of the type and rating printed on panels adjacent to instrument fuse covers.



Attention: pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués sur le panneau à proximité du couvercle de la boîte à fusible de l'instrument.



Vorsicht: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert auf den Tafeln neben den Sicherungsabdeckungen des Geräts gedruckt sind.



Attenzione: per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate sui pannelli adiacenti alla copertura fusibili dello strumento.



Advertencia: Para evitar incendios, sustituir los fusibles por aquellos del tipo y características impresos en los paneles adyacentes a las cubiertas de los fusibles del instrumento.



警告: 為了避免火災，更換保險絲時，請使用與儀器保險絲蓋旁面板上所印刷之相同類型與規格的保險絲。



警告: 为了避免火灾，应更换与仪器保险丝盖旁边面板上印刷的类型和规格相同的保险丝。



경고: 화재의 위험을 막으려면 기기 퓨즈 커버에 가까운 패널에 인쇄된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.







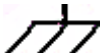
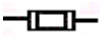



警告: 火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

Electrical and handling symbols





Electrical symbols

These can appear in instrument user manuals and on the instrument's front or rear panels.

| | |
|---|--|
|  | Electrical power on |
|  | Electrical power off |
|  | Standby |
|  | Direct current |
|  | Alternating current |
|  | Protective conductor terminal |
|  | Frame, or chassis, terminal |
|  | Fuse |
|  | Recycle symbol: Do not dispose in municipal waste. |

Handling symbols

These handling symbols and their associated text can appear on labels affixed to the outer packaging of Waters instrument and component shipments.

| | |
|---|---------------|
|  | Keep upright! |
|  | Keep dry! |
|  | Fragile! |
|  | Use no hooks! |

B Specifications

This appendix provides the following types of specifications for the Waters® 1500-series pumps:

- Physical
- Environmental
- Electrical
- Performance
- Degasser
- Instrument control and communication

Physical specifications for 1500-series pump

| Item | Specification |
|-------------------------|---|
| Height | 43 cm |
| Depth | 61.0 cm |
| Width | 30.5 cm {without bottle holder} |
| Weight (1525) | 27.2 kg |
| Weight (1515) | 20.4 kg |
| Wetted Surface Material | 316 stainless steel, sapphire, reinforced fluorocarbon polymer seals (1525 μ pump), UHMWPE seals (1525EF pump), carbon-reinforced Tefzel® |

Physical specifications for 1500-series column heater

| Item | Specification |
|--------|---------------|
| Height | 43 cm |
| Depth | 35.5 cm |
| Width | 15.2 cm |
| Weight | 5.9 kg |


Environmental specifications

| Item | Specification |
|-----------------------|--|
| Operating Temperature | 4 to 40 °C |
| Humidity | 20 to 80%, noncondensing |
| Audible Noise | <70 dBA at operator position |
| Solvent Compatibility | Solvents consistent with materials of construction. Salts and buffers can reduce seal life, especially at pressures in excess of 20,684.3 kPa (206.8 bar, 3000 psi). |

Electrical specifications

| Item | Specification |
|------------------------------------|---------------|
| Protection class ^a | Class I |
| Overvoltage category ^b | Category II |
| Pollution degree ^c | Degree 2 |
| Moisture protection ^d | Normal (IPX0) |
| Power requirements (pump) | 200 VA |
| Power requirements (column heater) | 150 VA |

Electrical specifications (Continued)

| Item | Specification |
|--|------------------------|
|  Line voltage | 120/140 VAC |
| Frequency | 50/60 Hz, single phase |
| Fuses | 3.15 A, 250 V, two |

- a. Protection Class I – The insulating scheme used in the instrument to protect you from electrical shock. Class I identifies a single level of insulation between live parts (wires) and exposed conductive parts (metal panels), in which the exposed conductive parts are connected to a grounding system. In turn, this grounding system is connected to the third pin (ground pin) on the electrical power cord plug.
- b. Overvoltage Category II – Pertains to instruments that receive their electrical power from a local level such as an electrical wall outlet.
- c. Pollution Degree 2 – A measure of pollution on electrical circuits, which may produce a reduction of dielectric strength or surface resistivity. Degree 2 refers to normally only nonconductive pollution. Occasionally, however, a temporary conductivity caused by condensation must be expected.
- d. Moisture Protection – Normal (IPXO) – IPXO means that there is no Ingress Protection against any type of dripping or sprayed water. The X is a placeholder to identify protection against dust, if applicable.

Performance specifications for the 1515 and 1525 pumps

| Item | Specification |
|--|--|
| Programmable Flow Rate Range 100- μ L heads | 0.00 to 10.00 mL/min, in 0.01-mL/min increments |
| Maximum Operating Pressure | 41,368 kPa (414 bar, 6000 psi); programmable upper and lower limits |
| Pressure Ripple (one pump) | $\leq 2.0\%$ at 1 mL/min, degassed methanol, at 6894.75 kPa (68.9 bar, 1000 psi) backpressure |
| Flow Precision | $\leq 0.1\%$ RSD or 2 seconds SD, six replicates based on retention time or volumetric measures at 1 mL/min, 1000 to 2000 psi backpressure, PQ test (each pump individually) |
| Flow Accuracy | $\pm 1.0\%$ of setting at 1 mL/min, or 30 μ L/min, whichever is larger, using degassed methanol at 6894.75 kPa (68.9 bar, 1000 psi) to 13,789.51 kPa (137.9 bar, 2000 psi) backpressure (each pump individually) |

Performance specifications for the 1515 and 1525 pumps (Continued)

| Item | Specification |
|---------------------------|--|
| Gradient Accuracy (1525) | ±0.5% of setting at 1 mL/min and 6894.75 kPa (68.9 bar, 1000 psi) backpressure (Methanol: methanol with 5.6 mg/L propyl paraben) with one GM-150 mixer |
| Gradient Precision (1525) | <0.5% RSD at 1 mL/min and 6894.75 kPa (68.9 bar, 1000 psi) backpressure (Methanol: methanol with 5.6 mg/L propyl paraben) with one GM-150 mixer |

Performance specifications for the 1525 μ pump

| Item | Specification |
|---|---|
| Programmable flow rate, range 50- μ L heads | 0.00 to 5.00 mL/min, in 0.01-mL/min increments |
| Maximum operating pressure | 41,368 kPa (414 bar, 6000 psi) with 50- μ L heads |
| Pressure ripple (one pump) | <2.0% at 0.5 mL/min, degassed methanol, at 6894.75 kPa (68.9 bar, 1000 psi) to 13,789.51 kPa (137.9 bar, 2000 psi) backpressure |
| Flow precision | 2 seconds SD, six replicates based on retention time or volumetric measures at 0.2 mL/min, 6894.75 kPa (68.9 bar, 1000 psi) to 13,789.51 kPa (137.9 bar, 2000 psi) backpressure, PQ test (each pump individually) |
| Flow accuracy | 2% of setting at 0.1 mL/min using degassed methanol at 6894.75 kPa (68.9 bar, 1000 psi) to 13,789.51 kPa (137.9 bar, 2000 psi) backpressure (each pump individually) |
| Gradient accuracy | <1% (typical) between 10 to 90%, and 1000 psi backpressure (methanol: methanol with propylparaben and one 50- μ L mixer) |

Performance specifications for the 1525 μ pump (Continued)

| Item | Specification |
|-----------------------|---|
| Gradient precision | <0.5% RSD (typical) between 10 to 90%, 0.1 mL/min, and 6894.75 kPa (68.9 bar, 1000 psi) backpressure (methanol: water with octanophenone and one 50- μ L mixer) |
| Gradient delay volume | <30 μ L (without mixer, no manual injector and no column heater) <100 μ L (with one 50- μ L mixer, no manual injector, and no column heater) |

Performance specifications for the 1525EF pump

| Item | Specification |
|--|--|
| Programmable flow rate, range 225- μ L heads | 0.00 to 22.5 mL/min, in 0.01-mL/min increments |
| Maximum operating pressure | 34,474 kPa (345 bar, 5000 psi) with 225- μ L heads; programmable upper and lower limits |
| Pressure ripple (one pump) | \leq 3.0% at 2.25 mL/min, degassed methanol, at 6894.75 kPa (68.9 bar, 1000 psi) backpressure |
| Flow precision | \leq 0.1% RSD or 2 seconds SD, six replicates based on retention time or volumetric measures at 1 mL/min, 6894.75 kPa (68.9 bar, 1000 psi) to 13,789.51 kPa (137.9 bar, 2000 psi) backpressure, PQ test (each pump individually) |
| Flow accuracy | \pm 1.0% of setting at 1 mL/min, or 30 μ L/min, whichever is larger, using degassed methanol at 6894.75 kPa (68.9 bar, 1000 psi) to 13,789.51 kPa (137.9 bar, 2000 psi) backpressure (each pump individually) |
| Gradient accuracy | < \pm 1.0% between 10 to 90%, 1 mL/min, and 6894.75 kPa (68.9 bar, 1000 psi) backpressure (methanol: methanol with 5.6 mg/L propylparaben > 05 AUFS @257 nm and one GM150 mixer) |

Performance specifications for the 1525EF pump (Continued)

| Item | Specification |
|-----------------------|--|
| Gradient precision | <0.5% RSD between 10 to 90%, 1 mL/min, and 6894.75 kPa (68.9 bar, 1000 psi) backpressure (methanol: methanol with 5.6 mg/L propylparaben > 05 AUFS @257 nm and one GM150 mixer) |
| Gradient ripple | <1.5% normalized to full scale between 10 to 90%, 1 mL/min, and 6894.75 kPa (68.9 bar, 1000 psi) backpressure (methanol: methanol with 5.6 mg/L propylparaben > 05 AUFS @257 nm and one GM150 mixer) |
| Gradient delay volume | <100 μ L (without mixer, no manual injector and no column heater) < 600 μ L (with one GM150 mixer, no manual injector, and no column heater) |

Integral vacuum degasser operational specifications

| Item | Specification |
|------------------------------------|---|
| Chemical resistance | Unaffected by full range of organic solvents and aqueous solutions of acids, bases, salts, and surfactants |
| pH range | 0 to 14 |
| Gas removal efficiency | Varies with flow rate (see the table, above) |
| Typical operating flow range | 200 μ L/min to 10 mL/min (1525 model) 200 μ L/min to 5 mL/min (1525 μ model) 200 μ L/min to 22.5 mL/min (1525EF models) |
| Equilibration time | <1 hour |
| Pressure drop (across one channel) | <0.55 kPa (0.0055 bar, 0.08 psi) at 1 mL/min., Milli-Q [®] water, STP |
| Wetted surfaces | PPS ^a , Teflon AF ^b , Tefzel ^c |
| Inputs | DC power, QSPI serial data |

Integral vacuum degasser operational specifications (Continued)

| Item | Specification |
|--|---|
| Output | QSPI serial data |
| Eluent connections | 1/4-28 reversed ferrule fittings; 2 inlet and 2 outlet fittings |
| Vacuum chambers | One chamber/eluent, two channels standard <0.5 mL internal volume/channel |
| Tubular membrane | 1/vacuum chamber, Teflon AF |
| Vapor exhaust | 1/8-inch inside diameter ID × 1.8 meter tubing |
| Vacuum source | Built-in, 2-head diaphragm pump, solvent resistant |
| Vacuum sensor | Detects vacuum from 0 to 15.6 psiA |
| Maximum pressure on inlet and outlets | 70 kPa (0.7 bar, 10 psi) |

- a. High-density polyethylene
- b. polytetrafluoroethylene
- c. ethylenetetrafluoroethylene

The following table lists Teflon[®] AF chemical compatibility test results from Waters.

Tip: Additional materials in the flowpath are PPS and PTFE.

Teflon AF chemical compatibility test results

| Compatible ^a | Incompatible ^b |
|-------------------------|---------------------------|
| Acetonitrile | Fluorinert [®] |
| Chloroform | Fomblin [®] |
| Dimethyl acetamide | Freon [®] |
| Dimethyl formamide | Galden [®] |
| Dimethylsulfoxide | HFIP |
| Ethanol | Perfluorinated solvents |
| Ethylacetate | |

Teflon AF chemical compatibility test results (Continued)

| | |
|---------------------|--|
| Hexane | |
| Isopropanol | |
| Methanol | |
| Methylene chloride | |
| N-methylpyrrolidone | |
| Tetrahydrofuran | |
| Toluene | |
| Trichlorobenzene | |
| Water | |

- a. Compatible solvents are not so soluble that they change the appearance or weight of Teflon AF after prolonged exposure at 23 °C.
- b. Incompatible solvents immediately and irreversibly damage Teflon AF.

Instrument control and communication specifications

| Item | Specification |
|-------------------------|--|
| Inject | Voltage Range: 0 to 30 V Current: 200 mA |
| Stop Flow Input | 0 to 30 V |
| Contact Closure Outputs | Voltage Range: \pm 30 V Current: 500 mA |
| External Control | Ethernet or IEEE-488 |

C Solvent Considerations

Contents

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| Solvent compatibility | C-3 |
| Solvent miscibility | C-6 |
| Buffered solvents | C-8 |
| Head height | C-9 |
| Solvent viscosity | C-9 |
| Mobile phase solvent degassing | C-9 |
| Wavelength selection | C-12 |

Introduction

Clean solvents

Clean solvents provide these benefits:

- Reproducible results
- Operation with minimal instrument maintenance

A dirty solvent can cause these issues:

- Baseline noise and drift
- Blockage of the solvent filters with particulate matter

Solvent quality

Use HPLC-grade solvents to ensure the best possible results. Filter solvents through 0.45- μm filters before use. Solvents distilled in glass generally maintain their purity from lot to lot; use them to ensure the best possible results.

Preparation checklist

The following solvent preparation guidelines help to ensure stable baselines and good resolution:

- Filter solvents with a 0.45- μm filter.
- Degas the solvent.
- Stir the solvent.
- Keep in a place free from drafts and shock.

Water

Use water only from a high-quality water purification system. If the water system does not provide filtered water, filter it through a 0.45- μm membrane filter before use.

Buffers

When you use buffers, dissolve salts first, adjust the pH, then filter to remove insoluble material.

Tetrahydrofuran (THF)

When using unstabilized THF, ensure that your solvent is fresh. Previously opened bottles of THF contain peroxide contaminants, which cause baseline drift.



Warning: THF contaminants (peroxides) are potentially explosive if concentrated or taken to dryness.

Solvent compatibility

A Waters 1500-series HPLC pump is constructed of stainless steel (316) components that, with some minor restrictions, can be used with all solvents. This section lists the solvents that have and have not been approved for use with a 1500-series HPLC pump.

Solvents to avoid

You can use any solvent with a 1500-series HPLC pump. However, long-term static exposure to halide salts (for example, fluoride, bromide, chloride, and iodide) cause pitting and corrosion of stainless steel parts. When using these salts, flush your system thoroughly with water if the pump will be idle for more than two days.

Solvents to use

Materials of construction used in a 1500-series HPLC pump do not react with most acids, bases, salts, and organic solvents.

The solvents listed in the tables on [page C-4](#) through [page C-5](#) are approved for use with the 1500-series HPLC pump. These include salts, acids and bases in concentrations up to 1 M (unless otherwise noted), and organic solvents in concentrations of up to 100% (unless otherwise noted). Higher concentrations can be used in many instances.

Seek information on the use of a specific solvent or concentration not listed in this manual directly from Waters.

Aqueous buffers for use with the 1500-series HPLC pump

| Aqueous buffers | | | |
|--------------------|---------------|----------------|--|
| Acetate | K_2SO_4 | Na_2S | Perfluorobutyric acid |
| Al_2SO_4 | $K_3Fe(CN)_6$ | Na_2CO_3 | NH_4Cl |
| $Ca(OCl)_2$ | $K_4Fe(CN)_6$ | Na_2SO_4 | Phosphate |
| $CaCl_2$ | KBr | NaCl | Tartrate |
| Citrate | KCl | Sodium acetate | Trilithium citrate |
| H_2O_2 up to 10% | $KHCO_3$ | NaH_2BO_3 | Tris |
| HIBA | $KMnO_4$ | $NaHCO_3$ | 4-(2-pyridylazo) resorcinol mono-sodium salt monohydrate |
| K_2CO_3 | KNO_3 | $NaHSO_4$ | |
| $K_2Cr_2O_3$ | $LiClO_4$ | $NaNO_3$ | |
| K_2S | $Na_2B_4O_7$ | NaOCl | |

Acids for use with the 1500-series HPLC pump

| Acids | | |
|----------------------|--------------------------------|---------------------------------------|
| Acetic acid, glacial | Hydrochloric acid | Perchloric acid |
| Benzoic acid | Lactic acid | Phosphoric acid |
| Chromic acid | Methanesulphonic acid | Pyridine-2,6-dicarboxylic acid |
| Citric acid | Nitric acid, up to 37.5% (6 N) | Sulfuric acid, up to 0.20 M |
| Formic acid | Octanesulphonic acid | Trifluoroacetic acid (TFA), up to 10% |
| Glyceric acid | Oxalic acid | |

Bases for use with the 1500-series HPLC pump

| Bases | |
|---------------------|--|
| Ba(OH) ₂ | NaOH, up to 10 M |
| KOH | NH ₄ OH, up to 3 M |
| LiOH | Tetramethylammonium hydroxide pentahydrate |

Organic solvents for use with the 1500-series HPLC pump

| Organic solvents | | | |
|----------------------|---------------------|----------------------|----------------------------------|
| 4-cyanophenol | Chloroform | Ethylene glycol | Methylene chloride |
| Acetone | Cyclohexane | Formaldehyde | <i>n</i> -Propanol |
| Acetonitrile | Cyclohexanone | Heptane | Phenol |
| Amyl acetate | Dibutyl phthalate | Hexane | Tetrahydrofuran (THF) |
| Benzaldehyde | Dimethyl formamide | iso-Octane | Toluene |
| Benzene | Dimethyl sulfoxide | iso-Propanol | Waters PIC [®] Reagents |
| Benzyl alcohol | Ethanol | Lysine hydrochloride | Xylene |
| Butanol | Ethyl acetate | Methanol | |
| Carbon tetrachloride | Ethylene dichloride | Methyl ethyl ketone | |

Solvent miscibility

Before you change solvents, refer to the table below to determine the miscibility of the solvents to be used. When you change solvents, be aware that:

- You can make changes involving two miscible solvents directly. Changes involving two solvents that are not totally miscible (for example, from chloroform to water), require an intermediate solvent (such as methanol).
- Temperature affects solvent miscibility. If you are running a high-temperature application, consider the effect of the higher temperature on solvent solubility.
- Buffers dissolved in water may precipitate when mixed with organic solvents.

When you switch from a strong buffer to an organic solvent, flush the buffer out of the system with distilled water before you add the organic solvent.

Solvent miscibility

| Polarity index | Solvent | Viscosity CP, 20 °C | Boiling point °C (@1 atm) | Miscibility number (M) | λ Cutoff (nm) |
|----------------|--------------------|---------------------|---------------------------|------------------------|-----------------------|
| -0.3 | N-decane | 0.92 | 174.1 | 29 | — |
| -0.4 | Iso-octane | 0.50 | 99.2 | 29 | 210 |
| 0.0 | N-hexane | 0.313 | 68.7 | 29 | — |
| 0.0 | Cyclohexane | 0.98 | 80.7 | 28 | 210 |
| 1.7 | Butyl ether | 0.70 | 142.2 | 26 | — |
| 1.8 | Triethylamine | 0.38 | 89.5 | 26 | — |
| 2.2 | Isopropyl ether | 0.33 | 68.3 | — | 220 |
| 2.3 | Toluene | 0.59 | 100.6 | 23 | 285 |
| 2.4 | <i>P</i> -xylene | 0.70 | 138.0 | 24 | 290 |
| 3.0 | Benzene | 0.65 | 80.1 | 21 | 280 |
| 3.3 | Benzyl ether | 5.33 | 288.3 | — | — |
| 3.4 | Methylene chloride | 0.44 | 39.8 | 20 | 245 |

Solvent miscibility (Continued)

| Polarity index | Solvent | Viscosity CP, 20 °C | Boiling point °C (@1 atm) | Miscibility number (M) | λ Cutoff (nm) |
|----------------|---------------------|---------------------|---------------------------|------------------------|-----------------------|
| 3.7 | Ethylene chloride | 0.79 | 83.5 | 20 | — |
| 3.9 | Butyl alcohol | 3.00 | 117.7 | — | — |
| 3.9 | Butanol | 3.01 | 177.7 | 15 | — |
| 4.2 | Tetrahydrofuran | 0.55 | 66.0 | 17 | 220 |
| 4.3 | Ethyl acetate | 0.47 | 77.1 | 19 | 260 |
| 4.3 | 1-propanol | 2.30 | 97.2 | 15 | 210 |
| 4.3 | 2-propanol | 2.35 | 117.7 | 15 | — |
| 4.4 | Methyl acetate | 0.45 | 56.3 | 15, 17 | 260 |
| 4.5 | Methyl ethyl ketone | 0.43 | 80.0 | 17 | 330 |
| 4.5 | Cyclohexanone | 2.24 | 155.7 | 28 | 210 |
| 4.5 | Nitrobenzene | 2.03 | 210.8 | 14, 20 | — |
| 4.6 | Benzonitrile | 1.22 | 191.1 | 15, 19 | — |
| 4.8 | Dioxane | 1.54 | 101.3 | 17 | 220 |
| 5.2 | Ethanol | 1.20 | 78.3 | 14 | 210 |
| 5.3 | Pyridine | 0.94 | 115.3 | 16 | 305 |
| 5.3 | Nitroethane | 0.68 | 114.0 | — | — |
| 5.4 | Acetone | 0.32 | 56.3 | 15, 17 | 330 |
| 5.5 | Benzyl alcohol | 5.80 | 205.5 | 13 | — |
| 5.7 | Methoxyethanol | 1.72 | 124.6 | 13 | — |
| 6.2 | Acetonitrile | 0.37 | 81.6 | 11, 17 | 210 |
| 6.2 | Acetic acid | 1.26 | 117.9 | 14 | — |
| 6.4 | Dimethylformamide | 0.90 | 153.0 | 12 | — |
| 6.5 | Dimethylsulfoxide | 2.24 | 189.0 | 9 | — |
| 6.6 | Methanol | 0.60 | 64.7 | 12 | 210 |
| 7.3 | Formamide | 3.76 | 210.5 | 3 | — |
| 9.0 | Water | 1.00 | 100.0 | — | — |

How to use miscibility numbers (M-numbers)

Use miscibility numbers (M-numbers) to predict the miscibility of a liquid with a standard solvent (see the table above).

To predict the miscibility of two liquids, subtract the smaller M-number value from the larger M-number value.

- If the difference between the two M-numbers is 15 or less, the two liquids are miscible in all proportions at 15 °C.
- A difference of 16 indicates a critical solution temperature between 25 and 75 °C, with 50 °C as the optimal temperature.
- If the difference is 17 or greater, the liquids are immiscible or their critical solution temperature is above 75 °C.

Some solvents prove immiscible with solvents at both ends of the lipophilicity scale. These solvents receive a dual M-number:

- The first number, always lower than 16, indicates the degree of miscibility with highly lipophilic solvents.
- The second number applies to the opposite end of the scale. A large difference between these two numbers indicates a limited range of miscibility.

For example, some fluorocarbons are immiscible with all the standard solvents and have M-numbers of 0 and 32. Two liquids with dual M-numbers are usually miscible with each other.

A liquid is classified in the M-number system by testing for miscibility with a sequence of standard solvents. A correction term of 15 units is then either added or subtracted from the cutoff point for miscibility.

Buffered solvents

When using a buffer, use a good quality reagent and filter it through a 0.45- μ m filter.

Do not leave the buffer stored in the system after use. Flush all fluidic pathways with HPLC-quality water before shutting down the system and leave water in the system (flush with 90 percent HPLC-quality water:10 percent methanol for shutdowns scheduled to be more than one day). Flush using a minimum of 15 mL.

Head height

Position the solvent reservoirs in the bottle holder supplied with the 1500-series HPLC pump (with adequate spill protection).

Solvent viscosity

Generally, viscosity is not important when you are operating with a single solvent or under low pressure. However when you are running a gradient, the viscosity changes that occur as the solvents are mixed in different proportions can result in pressure changes during the run. For example, a 1:1 mixture of water and methanol produces twice the pressure of either water or methanol alone.

If the extent to which the pressure changes affect the analysis is not known, monitor the pressure during the run.

Mobile phase solvent degassing

Mobile phase difficulties account for the majority of all liquid chromatographic problems. Using degassed solvents is important, especially at wavelengths below 220 nm. Degassing provides:

- Stable baselines and enhanced sensitivity
- Reproducible retention times for eluting peaks
- Reproducible injection volumes for quantitation
- Stable pump operation

This section presents information on the solubility of gases, solvent degassing methods, and solvent degassing considerations.

Gas solubility

Only a finite amount of gas can be dissolved in a given volume of liquid. This amount depends on:

- The chemical affinity of the gas for the liquid
- The temperature of the liquid
- The pressure applied to the liquid

Changes in the composition, temperature, or pressure of the mobile phase can lead to outgassing.

Effects of intermolecular forces

Nonpolar gases (N_2 , O_2 , CO_2 , He) are more soluble in nonpolar solvents than in polar solvents. Generally, a gas is most soluble in a solvent whose intermolecular attractive forces are similar to those in the gas (“like dissolves like”).

Effects of temperature

Temperature affects the solubility of gases. If the heat of solution is exothermic, the solubility of the gas decreases when you heat the solvent. If the heat of solution is endothermic, the solubility increases when you heat the solvent. For example, the solubility of He in H_2O decreases with an increase in temperature, but the solubility of He in benzene increases with an increase in temperature.

Effects of partial pressure

The mass of gas dissolved in a given volume of solvent is proportional to the partial pressure of the gas in the vapor phase of the solvent. If you decrease the partial pressure of the gas, the amount of that gas in solution also decreases.

Eluent degassing methods

You can degas eluents using any of the following methods:

- In-line vacuum degassing
- Heating
- Vacuum sonication

Vacuum degassing

An in-line vacuum degasser operates on the principle of Henry’s Law to remove dissolved gases from the solvent. According to Henry’s Law, the mole fraction of a gas dissolved in a liquid is proportional to the partial pressure of that gas in the vapor phase above the liquid. If the partial pressure of a gas on the surface of the liquid is reduced, for example, by evacuation, then a proportional amount of that gas comes out of solution.

Note: Vacuum degassing can change the composition of mixed solvents.

In-line degassing

In-line degassing removes gases from the eluent as it passes through a gas-permeable membrane enclosed in a vacuum chamber. The vacuum in the chamber accelerates the rate at which the dissolved gas diffuses through the gas-permeable membrane. This method provides an automatic, continuous method of removing dissolved gasses, and allows for quick eluent changeover. Waters makes two in-line degassers: part numbers WAT079700 (2 Eluent) and WAT079800 (4 Eluent). Contact Waters for details.

The longer a solvent is exposed to a vacuum, the more dissolved gases are removed. Two factors affect the amount of time the solvent is exposed to the vacuum:

- Flow rate – At low flow rates, most of the dissolved gas is removed as the solvent passes through the vacuum chamber. At higher flow rates, lesser amounts of gas per unit volume of solvent are removed.
- Surface area of the degassing membrane – The length of the degassing membrane is fixed in each vacuum chamber. To increase the length of membrane, you can connect two or more vacuum chambers in series.

Heating

Heating to remove dissolved gas is usually not effective unless you boil the eluent, and doing so is not practical or safe for mixed, flammable, or volatile eluents. However, raising the temperature even slightly raises the partial pressure of the eluent, and thereby reduces the rate of resolubilization of a gas.

Vacuum sonication

Sonication in combination with a vacuum degasses eluents very quickly. This technique does not change the composition of mixed eluents appreciably.



Caution: Apply vacuum only to suitable vessels. The brown gallon bottles in which eluent is shipped are not designed for vacuum degassing. There is a high risk of implosion if these bottles are used for vacuum degassing.

Conclusions

With any of the above techniques, the eluent reequilibrates to air saturation in 12 to 24 hours, depending on the eluent.

Degassing by vacuum or sonication or both is often performed for improved pump performance in multipump gradient applications.

Wavelength selection

The tables in this section provide UV cutoff values for:

- Common solvents
- Common mixed mobile phases
- Chromophores

UV cutoffs for common solvents

The following table shows the UV cutoff (the wavelength at which the absorbance of the solvent is equal to 1 AU) for some common chromatographic solvents. Operating at a wavelength near or below the cutoff increases baseline noise due to the absorbance of the solvent.

UV cutoff wavelengths for common chromatographic solvents

| Solvent | UV cutoff (nm) | Solvent | UV cutoff (nm) |
|----------------------|----------------|------------------------|----------------|
| 1-Nitropropane | 380 | Ethylene glycol | 210 |
| 2-Butoxyethanol | 220 | Iso-octane | 215 |
| Acetone | 330 | Isopropanol | 205 |
| Acetonitrile | 190 | Isopropyl chloride | 225 |
| Amyl alcohol | 210 | Isopropyl ether | 220 |
| Amyl chloride | 225 | Methanol | 205 |
| Benzene | 280 | Methyl acetate | 260 |
| Carbon disulfide | 380 | Methyl ethyl ketone | 330 |
| Carbon tetrachloride | 265 | Methyl isobutyl ketone | 334 |
| Chloroform | 245 | Methylene chloride | 233 |

UV cutoff wavelengths for common chromatographic solvents (Continued)

| Solvent | UV cutoff (nm) | Solvent | UV cutoff (nm) |
|---------------------|----------------|---------------------------|----------------|
| Cyclohexane | 200 | <i>n</i> -Pentane | 190 |
| Cyclopentane | 200 | <i>n</i> -Propanol | 210 |
| Diethyl amine | 275 | <i>n</i> -Propyl chloride | 225 |
| Dioxane | 215 | Nitromethane | 380 |
| Ethanol | 210 | Petroleum ether | 210 |
| Ethyl acetate | 256 | Pyridine | 330 |
| Ethyl ether | 220 | Tetrahydrofuran | 230 |
| Ethyl sulfide | 290 | Toluene | 285 |
| Ethylene dichloride | 230 | Xylene | 290 |

Mixed mobile phases

The following table provides approximate wavelength cutoffs for some other solvents, buffers, detergents, and mobile phases. The solvent concentrations represented are those most commonly used. If you want to use a different concentration, you can determine approximate absorbance using Beer's Law, since absorbance is proportional to concentration.

Wavelength cutoffs for different mobile phases

| Mobile phase | UV cutoff (nm) | Mobile phase | UV cutoff (nm) |
|-----------------------------|----------------|----------------------------|----------------|
| Acetic acid, 1% | 230 | Sodium chloride, 1 M | 207 |
| Ammonium acetate, 10 mM | 205 | Sodium citrate, 10 mM | 225 |
| Ammonium bicarbonate, 10 mM | 190 | Sodium dodecyl sulfate | 190 |
| BRIJ 35, 0.1% | 190 | Sodium formate, 10 mM | 200 |
| CHAPS, 0.1% | 215 | Triethyl amine, 1% | 235 |
| Diammonium phosphate, 50 mM | 205 | Trifluoroacetic acid, 0.1% | 190 |

Wavelength cutoffs for different mobile phases (Continued)

| Mobile phase | UV cutoff (nm) | Mobile phase | UV cutoff (nm) |
|---|----------------|--|----------------|
| EDTA, disodium, 1 mM | 190 | TRIS HCl, 20 mM, pH 7.0, pH 8.0 | 202, 212 |
| HEPES, 10 mM, pH 7.6 | 225 | Triton-X™ 100, 0.1% | 240 |
| Hydrochloric acid, 0.1% | 190 | Waters PIC Reagent A, 1 vial/liter | 200 |
| MES, 10 mM, pH 6.0 | 215 | Waters PIC Reagent B-6, 1 vial/liter | 225 |
| Potassium phosphate, monobasic, 10 mM dibasic, 10 mM | 190 190 | Waters PIC Reagent B-6, low UV, 1 vial/liter | 190 |
| Sodium acetate, 10 mM | 205 | Waters PIC Reagent D-4, 1 vial/liter | 190 |

Refractive indices of common solvents

The following table lists the refractive indices for some common chromatographic solvents. Use this table to verify that the solvent you intend to use for your analysis has a refractive index (RI) significantly different from the RIs of the sample components.

Refractive indices for common chromatographic solvents

| Solvent | RI | Solvent | RI |
|------------------------------|--------|-----------------------|-------|
| Fluoroalkanes | 1.25 | Tetrahydrofuran (THF) | 1.408 |
| Hexafluoroisopropanol (HFIP) | 1.2752 | Amyl alcohol | 1.410 |
| Methanol | 1.329 | Diisobutylene | 1.411 |
| Water | 1.33 | <i>n</i> -Decane | 1.412 |
| Acetonitrile | 1.344 | Amyl chloride | 1.413 |
| Ethyl ether | 1.353 | Dioxane | 1.422 |
| <i>n</i> -Pentane | 1.358 | Ethyl bromide | 1.424 |
| Acetone | 1.359 | Methylene chloride | 1.424 |

Refractive indices for common chromatographic solvents (Continued)

| Solvent | RI | Solvent | RI |
|---------------------------|-------|---------------------------------------|-------|
| Ethanol | 1.361 | Cyclohexane | 1.427 |
| Methyl acetate | 1.362 | Ethylene glycol | 1.427 |
| Isopropyl ether | 1.368 | <i>N,N</i> -Dimethyl formamide (DMF) | 1.428 |
| Ethyl acetate | 1.370 | <i>N,N</i> -Dimethyl acetamide (DMAC) | 1.438 |
| 1-Pentene | 1.371 | Ethyl sulfide | 1.442 |
| Acetic acid | 1.372 | Chloroform | 1.443 |
| Isopropyl chloride | 1.378 | Ethylene dichloride | 1.445 |
| Isopropanol | 1.38 | Carbon tetrachloride | 1.466 |
| <i>n</i> -Propanol | 1.38 | Dimethyl sulfoxide (DMSO) | 1.477 |
| Methylethylketone | 1.381 | Toluene | 1.496 |
| Diethyl amine | 1.387 | Xylene | ~1.50 |
| <i>n</i> -Propyl chloride | 1.389 | Benzene | 1.501 |
| Methylisobutylketone | 1.394 | Pyridine | 1.510 |
| Nitromethane | 1.394 | Chlorobenzene | 1.525 |
| 1-Nitropropane | 1.400 | <i>o</i> -Chlorophenol | 1.547 |
| Isooctane | 1.404 | Aniline | 1.586 |
| Cyclopentane | 1.406 | Carbon disulfide | 1.626 |

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