Agilent SD-1 Purification Solution

System User Guide

Agilent Technologies

Notices

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In This Book

This manual provides setup information on following modules:

- Agilent SD-1 Solvent Delivery Module (G9302A/G9303A)
- Agilent 325 UV/VIS Dual WL Detector (G9309A)
- Agilent 410 Autosampler (G9331A/G9332A)
- Agilent 218 Solvent Delivery Module (G9300A/G9301A)
- Agilent 440 Fraction Collector (G9340A)

1 Introduction to the System

This chapter introduces to the Agilent SD-1 Purification Solution system and its components.

2 Site Requirements and Specifications

This chapter provides information on site requirements and specifications of your system.

3 Installation

This chapter gives information about the installation of your Agilent SD-1 Solvent Delivery Module, Agilent 325 UV/VIS Dual WL Detector, Agilent 410 Autosampler and Agilent 440 Fraction Collector.

4 Using, Troubleshooting, Maintenance and Parts

This chapter provides information on how to access further details on the system components.

5 Cables

This chapter provides information on cables used with the instrument.

6 Appendix

This chapter provides addition information on safety, legal and web.

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Contents

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Introduction to the System

This chapter introduces to the Agilent SD-1 Purification Solution system and its components.

Introduction to the System

A complete Agilent SD-1 Purification Solution system includes:

- Agilent SD-1 Isocratic Solvent Delivery Module(s),
- Tubing,
- Mast kit,
- Agilent 325 UV/VIS Dual WL Detector,
- Agilent 410 Autosampler/Agilent 218 Solvent Delivery Module/Agilent SD-1 Isocratic Solvent Delivery Module (for sample injection), and
- Agilent 440 Fraction Collector

As an option the HPLC Control Software Agilent OpenLAB CDS ChemStation Edition is recommended.

The Agilent SD-1 Purification Solution allows to combine the modules listed above with the following Agilent 1200 Infinity Series modules:

- Diode Array Detector (G1315 C/D)
- Multiple Wavelength Detector (G1365 C/D)
- Variable Wavelength Detector (G1314 B/C/E/F)
- Preparative Autosampler (G2260A)
- Dual Loop Autosampler (G2258A)
- Fraction Collector (G1364B)
- Binary Pump (G4220B)
- Manual Injector (G1328C) (combined with one or two Agilent SD-1 Pumps)

NOTE The option to combine several modules requires the Agilent OpenLAB CDS ChemStation software as master controller. The SD-1 Solvent Delivery Module as master controller supports only the modules listed above.

Introduction to the SD-1 Isocratic Solvent Delivery Module

The Agilent SD-1 is an innovative HPLC solvent delivery system engineered with preparative chromatographers in mind. Corrosion-resistant titanium pump heads address the needs of biochemists for compatibility with salt-containing buffers and freedom from unwanted metal ions. Each pump head has a piston washing chamber to prevent deposition of abrasive salt residues behind the high-pressure seal, thereby greatly extending seal life.

Major new technology includes dual independent linear piston drives. Rather than operating both heads from cams attached to a single motor, as in conventional dual piston pumps, the Agilent SD-1 uses two independent stepper motors which connect to pistons via linear screw drives. The motors reciprocate, rather than running only in one direction. Independent drive frees the Agilent SD-1 from the operating constraints of mechanical cam profiles and makes it the first dual piston pump to produce entirely pulse-free flow under all operating conditions, without auxiliary hydromechanical pulse dampers. Totally pulse-free flow favors improved column performance and extended column life by saving expensive preparative columns from the constant pressure-pulse pounding typical of other large piston and diaphragm pumps.

In addition, the Agilent SD-1 meters flow at a constant rate independent of solvent compressibility and without the slight refill-associated flow deficits seen with conventional single-motor dual-piston models. In gradient operation, this produces more precise composition profiles without the time and volume delays introduced by large mixing chambers. Plus, flow rates extend well into the analytical HPLC range, permitting method development on smaller columns without interchanging heads.

Each Agilent SD-1 pump includes a standard serial interface for flow control. A built-in two-channel analog-to-digital converter allows the computer to collect data from HPLC detectors. Contact closure inputs and outputs are provided for system automation. Firmware includes Good Laboratory Practices (GLP) logging features.

Introduction to the Agilent 325 UV/VIS Dual Wavelength Detector

The Agilent 325 UV/VIS Dual WL Detector is integrated into a Liquid Chromatography System. The detector is controlled remotely by OpenLAB through Ethernet communications. In this situation, all functions of the detector are controlled through the Workstation software.

The detector measures the sample absorbance at the user-selected wavelength. The absorbance is displayed. Wavelength absorbance parameters are time programmable.

Features of the Agilent 325 UV/VIS Dual WL Detector:

- Stackable module
- Interchangeable flowcells
- Simple lamp replacement
- Comfortable control (OpenLAB)
- Wide detection range (peaks up to 40 AU/cm with appropriate flowcell)

Introduction to the Agilent 410 Autosampler

The Autosampler is available in two configurations as the *Standard* Autosampler (G9331A) and the Preparative Autosampler (G9332A).

Agilent 410 Autosampler (G9331A)

The Agilent 410 Autosampler has been designed to meet the needs of the modern analytical laboratory. The autosampler has the following features:

- Column temperature control and sample cooling guaranting consistent results
- High resolution syringe control guaranting superior precision for injection and reagent addition
- Fast replacement of the injection valve

Loop injection with Pressure Assisted Sample Aspiration is a proven concept that combines high precision with simplicity and reliability.

Three injection modes can be selected:

- Full loop
- Partial loop filling
- µL Pick-up

Maximum precision, maximum flexibility and zero sample loss can be achieved with these features.

Side-Port Needle

The strong side-port needle combines the optimum point style for septa piercing with a minimum risk of blockage by septum particles.

Column Oven

A column oven is an integral part of the Agilent 410 Autosampler because constant column temperature is important for long term stability of a chromatographic separation and may be required for GLP compliance.

Reagent Addition

Internal Standard addition, sample dilution or derivatization can be programmed in a very simple manner. A single-stage derivatization of a sample in a separate (destination) vial requires no more than 4 program lines. Multi-reagent addition is also possible, two large volume reagent vials are available on the sample tray.

Service Autosampler

Low instrument down time is accomplished by a high Mean Time Between Failure and quick instrument service. Special attention has been paid to these aspects of the concept, as is illustrated by the injection valve. The Agilent 410 Autosampler will alert you when the lifetime of the seal is exceeded or if the switching torque becomes too high. This allows preventive maintenance before injection performance degrades. And if necessary, the entire injection valve can be replaced in seconds with the unique Quick-fit valve mounting mechanism.

Agilent 410 Preparative Autosampler (G9332A)

By just choosing Prep mode in your system settings, you can use the Agilent 410 Autosampler to inject all of your sample into a Preparative LC system or in other areas where large injection volumes are required.

The combination of large sample vials (10 mL), a large sample volume needle and a 2.5 mL syringe enable you to inject large volumes very reproducible with high speeds and only 45 µL of sample loss. The installed large bore valve (0.75 mm) with 10 mL sample loop enables you to inject from microliters to milliliters with the same AutoSampler. Flow rates up to 200 mL per minute are possible when in the Prep mode.

Table 1 Tubing of the Agilent 410 Autosampler prep option

Tubing	Material	Dimensions	Volume
LSV sample needle and tubing	Stainless Steel	70 mm x 0.81 mm o.d. x 0.51 mm i.d.	45 µL
LSV sample needle to high-pressure valve	ETFE	155 mm x 1/16" o.d. x 0.50 mm i.d.	45 µL
Buffer tubing from high-pressure valve to syringe valve	PTFE	2550 mm x 1/16" o.d. x 1 mm i.d.	2000 µL

If the Prep option is factory installed the installation instructions can be skipped.

If the Prep option is bought as a kit, carry out the installation instructions, see "Installing the Agilent 410 Autosampler Prep Option" on page 82 for more information.

Introduction to the Agilent 218 Solvent Delivery Module

For preparative scale HPLC systems the Agilent 218 Pump can be used to quickly and reliably deliver samples to the column, instead of an injection valve or auto sampler. All pumps are controlled by applicable instrument control software on a PC via OpenLAB CDS ChemStation Edition (recommended). The sample injection pump (Agilent 218 Pump) is plumbed into the system with a tee. The column is plumbed after the tee.

When such an injection occurs, the Thru Pump Injector (Agilent SD-1 Pump) automatically begins to ramp up to the correct flow rate. At that instant delivers the sample, and ramps back down to ensure consistent flow rate at all times during the analysis. The Solvent Delivery Module(s) work with the Thru Pump Injector and lower their flow rate during the injection to maintain a constant overall solvent flow rate.

Introduction to the Agilent 440 Fraction Collector

The Agilent 440 Fraction Collector is a random access, single probe fraction collector and can accommodate a variety of racks. It is designed to automate the sample collection process. This fraction collector is designed to meet the diverse requirements of high-throughput laboratories.

Three racks are included with the fraction collector and are made of polypropylene to resist most chemical spills. Up to three racks of many configurations can be placed on the fraction collector. Additional sample racks can be set up in sequence and manually changed during an analysis as each rack's analysis is completed.

The rack closest to the rear of the fraction collector (next to the pillar) is considered rack number one.

2
Site Requirements and Specifications

This chapter provides information on site requirements and specifications of your system.

Site Requirements

Power Considerations

The instrument power supply has wide ranging capability. It accepts any line voltage in the range described in *Physical Specifications*.

WARNING

Hazard of electrical shock or damage of your instrumentation

can result, if the devices are connected to a line voltage higher than specified.

➔ Connect your instrument to the specified line voltage only.

CAUTION

Inaccessible power plug.

In case of emergency it must be possible to disconnect the instrument from the power line at any time.

➔ Make sure the power connector of the instrument can be easily reached and unplugged.

➔ Provide sufficient space behind the power socket of the instrument to unplug the cable.

Power Cords

Different power cords are offered as options with the module. The female end of all power cords is identical. It plugs into the power-input socket at the rear. The male end of each power cord is different and designed to match the wall socket of a particular country or region.

WARNING

Absence of ground connection or use of unspecified power cord

The absence of ground connection or the use of unspecified power cord can lead to electric shock or short circuit.

➔ Never operate your instrumentation from a power outlet that has no ground connection.

➔ Never use a power cord other than the Agilent Technologies power cord designed for your region.

WARNING

Use of unsupplied cables

Using cables not supplied by Agilent Technologies can lead to damage of the electronic components or personal injury.

➔ Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.

WARNING

Unintended use of supplied power cords

Using power cords for unintended purposes can lead to personal injury or damage of electronic equipment.

➔ Never use the power cords that Agilent Technologies supplies with this instrument for any other equipment.

Condensation

CAUTION

Condensation within the module

Condensation will damage the system electronics.

➔ Do not store, ship or use your module under conditions where temperature fluctuations could cause condensation within the module.

➔ If your module was shipped in cold weather, leave it in its box and allow it to warm slowly to room temperature to avoid condensation.

Area selected

WARNING

Explosion, damage and accuracy of the module

➔ Select an area free from drafts, corrosive atmospheres, and vibration.

➔ Select a dust-free, low-humidity environment.

➔ Use air-conditioning for control of the environment.

Bench Space

Make sure that the bench is designed to bear the weight of all modules.

For details on the space needed around the individual modules, refer to the according manuals:

- Agilent SD-1 Isocratic Solvent Delivery Module - User Manual (G9302-90001)
- Agilent 325 UV/VIS Dual Wavelength Detector - User Manual (G9309-90000)
- Agilent 410 Autosampler - User Manual (G9331-90000)
- Agilent 440 Fraction Collector - User Manual (G9340-90000)

Specifications

For details on specifications of the individual modules, please refer to the *Agilent 1200 Infinity Series Specifications Compendium*, or to the corresponding User Manuals.

3
Installation

This chapter gives information about the installation of your Agilent SD-1 Solvent Delivery Module, Agilent 325 UV/VIS Dual WL Detector, Agilent 410 Autosampler and Agilent 440 Fraction Collector.

Delivery

Delivery

For detailed information on parts delivered with the modules, please refer to the according manuals:

- Agilent SD-1 Isocratic Solvent Delivery Module - User Manual (G9302-90001)
- Agilent 325 UV/VIS Dual Wavelength Detector - User Manual (G9309-90000)
- Agilent 410 Autosampler - User Manual (G9331-90000)
- Agilent 440 Fraction Collector - User Manual (G9340-90000)
- Agilent 218 Solvent Delivery Module - User Manual (G9300-90001)
- And to the User Manuals of the Agilent 1200 Infinity Series which allows combining with the modules listed above (see "Introduction to the System" on page 8)

Components of a Complete System

A complete system comprises following modules (as ordered):

Hardware Modules

p/n	Description
G9302A	Agilent SD-1 Isocratic Solvent Delivery Module
G9303A	Agilent SD-1 Add-on Solvent Delivery Module
G9309A	Agilent 325 UV/VIS Dual WL Detector
G9331A	Agilent 410 Autosampler
G9332A	Agilent 410 Preparative Autosampler (OPTIONAL)
G9340A	Agilent 440 Fraction Collector

Software

p/n	Description
M8366-64000	OpenLAB CDS Installation Driver Prep LC
M8367-64000	OpenLAB CDS Installation Upgrade Driver Prep LC
M8500AA	LC driver
M8301AA	OpenLAB CDS ChemStation Edition Workstation A.01.05 or higher

Damaged Packaging

If the delivery packaging shows signs of external damage, please call your Agilent Technologies sales and service office immediately. Inform your service representative that the instrument may have been damaged during shipment.

CAUTION

"Defective on arrival" problems

If there are signs of damage, please do not attempt to install the module. Inspection by Agilent is required to evaluate if the instrument is in good condition or damaged.

➔ Notify your Agilent sales and service office about the damage.

➔ An Agilent service representative will inspect the instrument at your site and initiate appropriate actions.

Check Delivery

Delivery Checklists

Delivery Checklist Pump

The pump is packed in a single carton.

The pressure module ordered with the pump is shipped separately and needs to be installed.

Any pump head ordered with the pump is packed separately.

The following list shows all items delivered in a standard delivery. Your personal list depends on your order, therefore countercheck delivery with your order.

- Agilent SD-1 Solvent Delivery Module
- Power cord
- Solvent inlet assembly
- Compression fitting
- Ferrule
- Pump head kit
- Plumbing kit
- Pressure transducer module
- Mast kit
- Internal mixer

Delivery Checklist Detector

The following list shows all items delivered in a standard delivery. Your personal list depends on your order, therefore countercheck delivery with your order.

- Agilent 325 UV/VIS Dual Wavelength detector
- Assy PWB Sync. Interface 325/335 (optional)
- Cross-over Ethernet cable
- Power cord

NOTE Flow cells are required for the detector, but are ordered separately. See "Hydraulic Connections - Flowcells" on page 64 for suitable flowcells.

Delivery Checklist Autosampler

The autosampler is packed in a single carton.

The following list shows all items delivered in a standard delivery. Your personal list depends on your order, therefore countercheck delivery with your order.

- Agilent 410 Autosampler (G9331A/G9332A)
- Power cord
- Agilent 410 Reservoir Rack
- Standard Tray Assy (G9332A)
- Agilent and PrepStar Mast kit
- Prime purge valve bracket
- Prime purge valve stainless steel

Delivery Checklist Fraction Collector

The following list shows all items delivered in a standard delivery. Your personal list depends on your order, therefore countercheck delivery with your order.

- Agilent 440-LC Fraction Collector
- Assy USB RS-232 serial adaptor (optional)
- Rack
- Funnel Rack Kit (optional)
- Power cord

Delivery Checklist Capillary Kits

Your order contains one of the following Capillary Kits:

- Binary Pump Stainless Steel Tubing Kit, 1/16 x 0.02 i.d.
- Binary Pump Stainless Steel Tubing Kit, 1/16 x 0.03 i.d.
- Binary Pump Stainless Steel Tubing Kit, 1/16 x 0.04 i.d.
- Binary Pump Stainless Steel Tubing Kit, 1/8 x 0.08 i.d.
- Isocratic Pump Stainless Steel Tubing Kit, 1/16 x 0.02 i.d.
- Isocratic Pump Stainless Steel Tubing Kit, 1/16 x 0.03 i.d.
- Isocratic Pump Stainless Steel Tubing Kit, 1/16 x 0.04 i.d.
- Isocratic Pump Stainless Steel Tubing Kit, 1/8 x 0.08 i.d.

In addition, a Technical Note with installation instructions is included.

Delivery Checklist User Documentation CD

Part of a standard delivery is also the Agilent Purification & Preparative LC - User Documentation CD (G9300-64500).

Unpacking and Inspection

Unpacking and Inspection

1. Check carefully to make sure you received all the items listed on the packing list.
2. Carefully unpack all the containers and inspect the contents for damage as soon as possible.
3. Save the packing containers; they will be useful if you have to file a claim for damage, or in the case of future transit.

Unpacking the Detector

Prerequisites Ensure there is enough room on the bench for the detector.

WARNING

Heavy weight

The Agilent 325 UV/VIS Dual Wavelength Detector weighs in excess of 15 kg (33 lb).

➔ Carry the instrument at least with 2 people.

➔ Avoid back strain or injury by following all precautions for lifting heavy objects.

➔ Ensure that the load is as close to your body as possible.

➔ Ensure that you can cope with the weight of your load.

1 Carefully unpack the unit from the shipping carton and place it on the bench.

2 Make sure to check carefully for all miscellaneous components that might be contained in the inner compartments.

NOTE The detector is a sensitive instrument and should always be handled with the degree of care appropriate for laboratory instrumentation.

HINT Keep the shipping carton, as it provides excellent protection if you have to transport or store the detector in the future.

Unpacking the Autosampler

CAUTION Risk of damaging the autosampler.

➔ Do not lift the Agilent 410 Autosampler by the front cover.

1 Lift the Agilent 410 Autosampler as shown in Figure 1 on page 34 with both hands under the instrument or with one hand under the front and the other hand grasping the rear top of the Agilent 410 Autosampler.

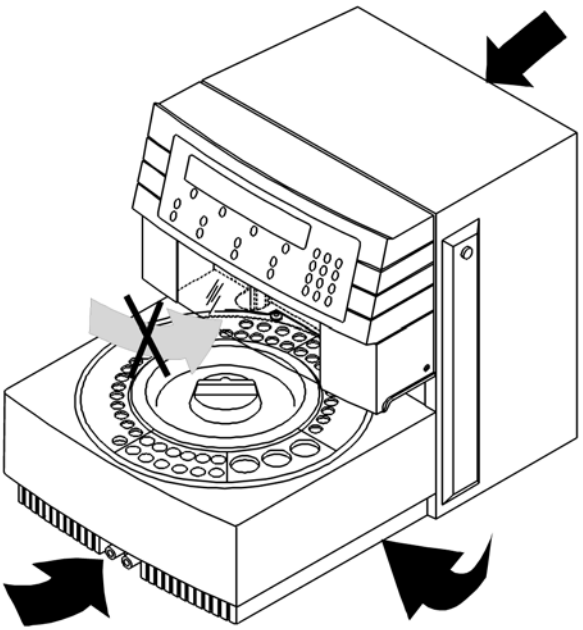


Figure 1 Agilent 410 Autosampler lifting instructions

Unpacking the Solvent Delivery System

WARNING

Danger to hands and feet

The instrument is heavy.

➔ Always use a fork lift or other suitable lifting device when moving the instrument.

CAUTION

Overheating of the pump

Objects interfering with airflow to the pump

➔ Maintain at least 15 cm (6 inches) clear space next to the fan.

Unpacking the Fraction Collector

Prerequisites Ensure there is enough room on the bench for the fraction collector.

WARNING

Heavy weight

The Agilent 440 Fraction Collector weighs in excess of 18 kg.

➔ Carry the instrument at least with 2 people.

➔ Avoid back strain or injury by following all precautions for lifting heavy objects.

➔ Ensure that the load is as close to your body as possible.

➔ Ensure that you can cope with the weight of your load.

1 Carefully unpack the unit from the shipping carton and place it on the bench.

2 Make sure to check carefully for all miscellaneous components that might be contained in the inner compartments.

NOTE The fraction collector is a sensitive instrument and should always be handled with the degree of care appropriate for laboratory instrumentation.

HINT Keep the shipping carton, as it provides excellent protection if you have to transport or store the detector in the future.

Optimizing the Stack Configuration

This section contains information on how to stack your modules, depending on the preparative method.

For optimal performance for the given examples respect the following rules:

- The orientation is also suggested if you are only installing the Agilent SD-1 Pump.
- For a two modules systems, the modules can be stacked one on the other.
- On the side of the stacked modules a mast can be attached to hold the mixer, drain valve, inject valve and column hanger.
- For three modules systems, stack two of the modules and place the third on the bench.
- If an autosampler is present, it should be placed on top of the detector.
- Position the master pump as highest pump in the stack.

  This position allows easy access to keypad and display to control the HPLC-system via pump instead of software.

Isocratic System

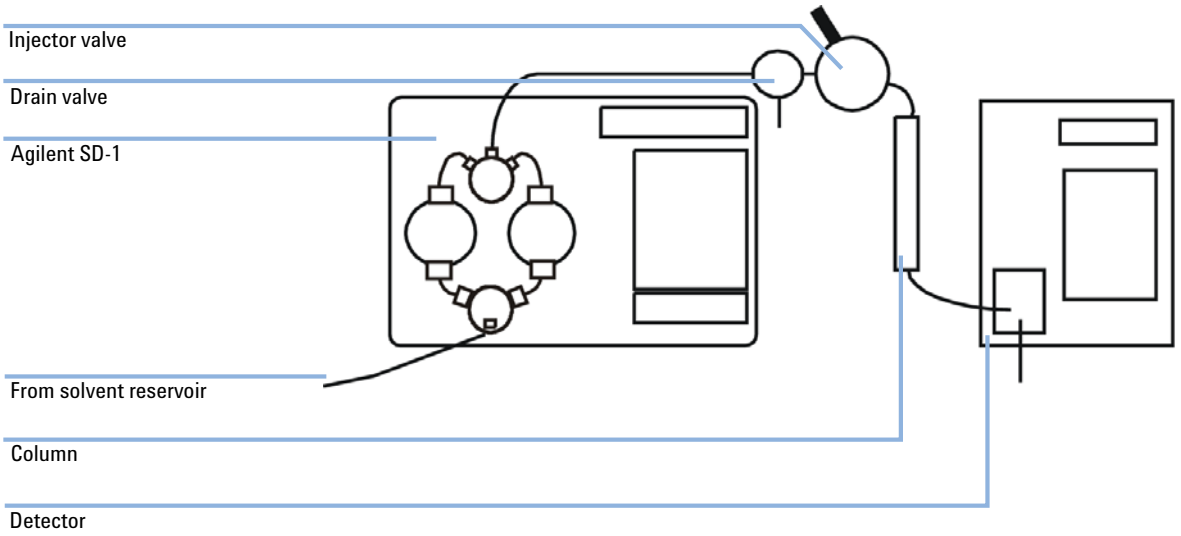


Figure 2 Isocratic HPLC system

The high pressure outlet from the Agilent SD-1 connects to the system drain valve, to the injector valve, the column and to the detector flow cell. The outlet from the detector can be directed to waste or collected.

Gradient System

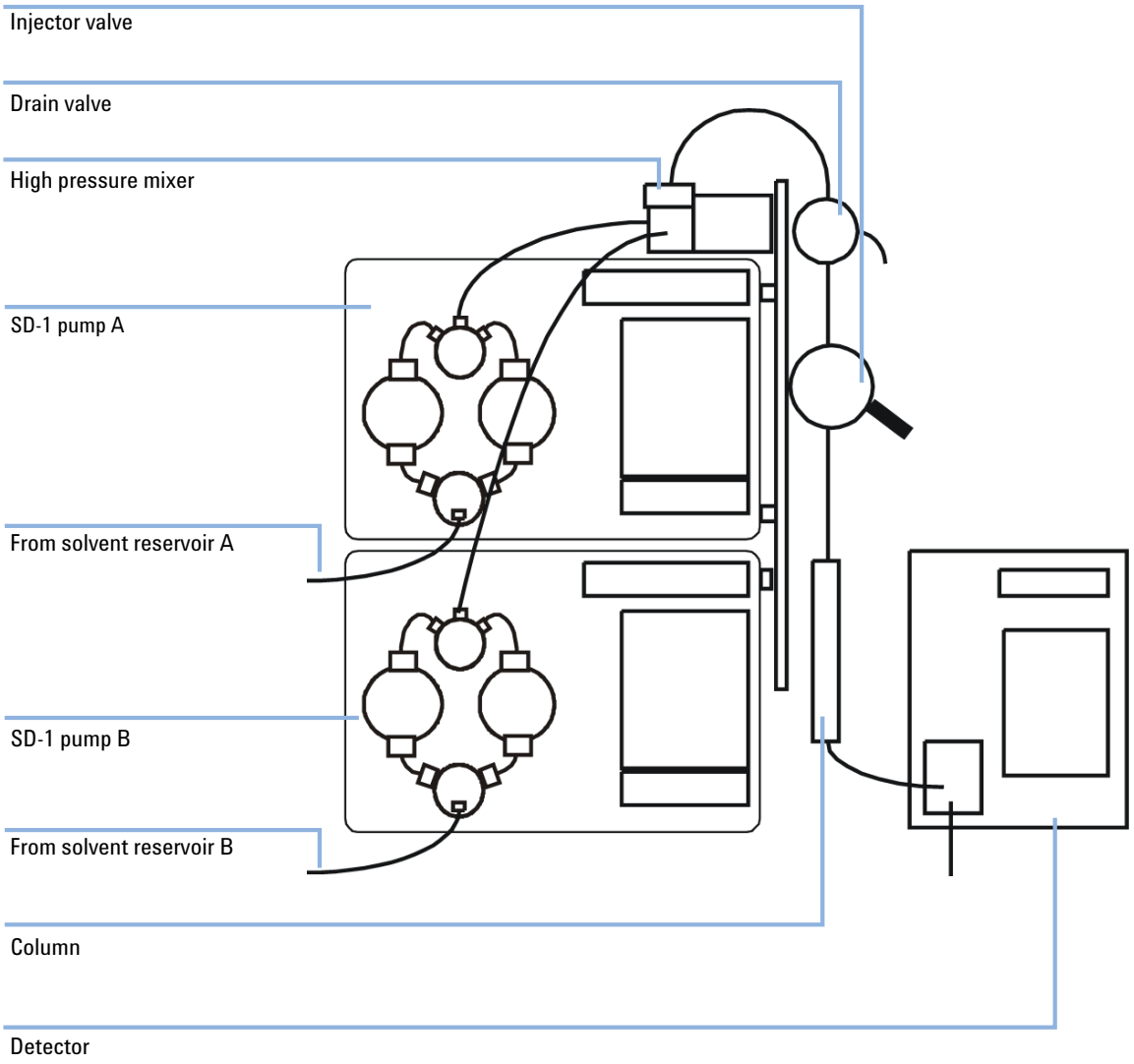


Figure 3 Binary gradient HPLC system

The two Agilent SD-1 pumps can be used to pump two solvents in a gradient, under front panel control. A binary gradient HPLC system can also be controlled by a applicable instrument control software on a computer. The software controls the flow rates for both Agilent SD-1 pumps.

Plumbing is the same for both these systems. The high pressure outlets from both Agilent SD-1 pumps are connected to a high pressure mixer, to ensure that both solvents are mixed thoroughly. The system drain valve is plumbed after the mixer. The injector valve is plumbed after the drain valve, then the column, then the detector flow cell. The outlet from the detector can be directed to waste or collected.

Auto-Preparative System

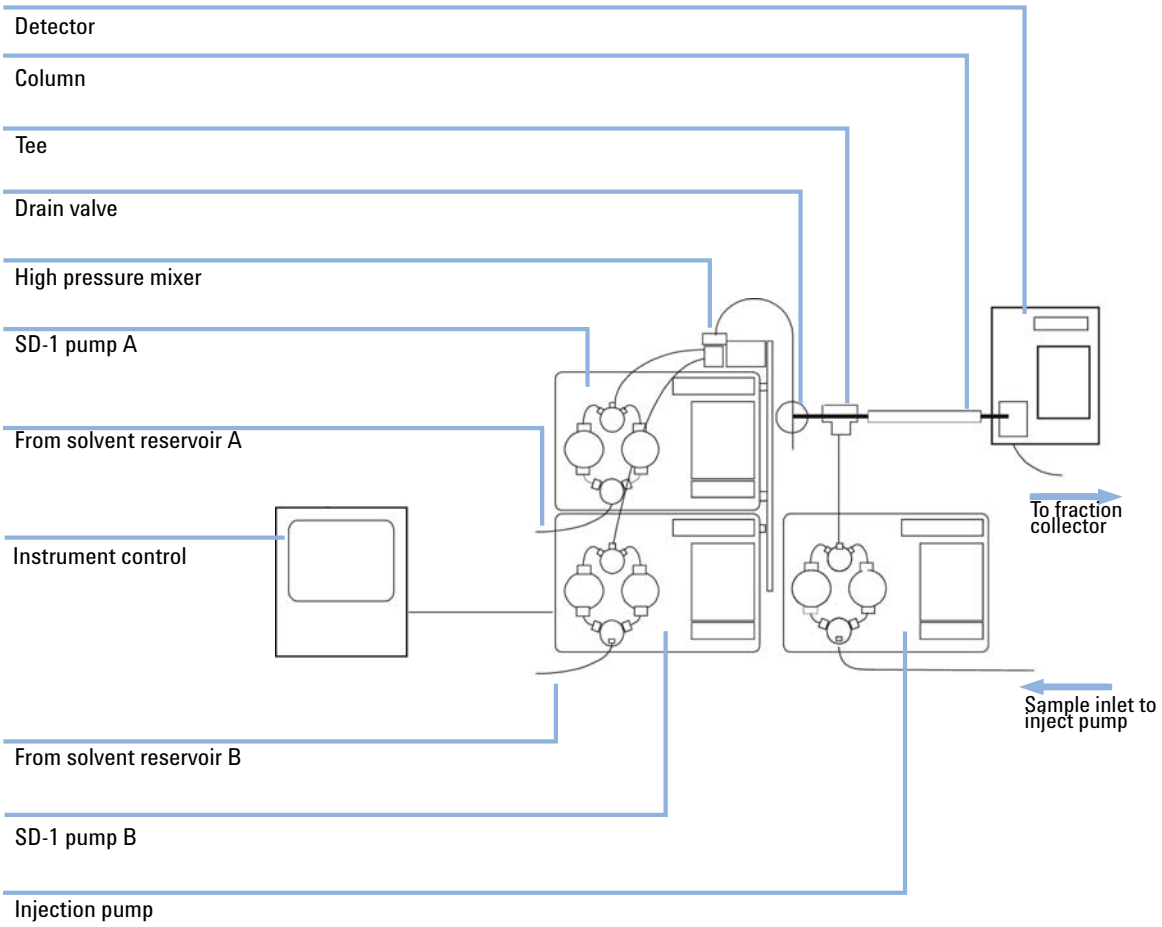


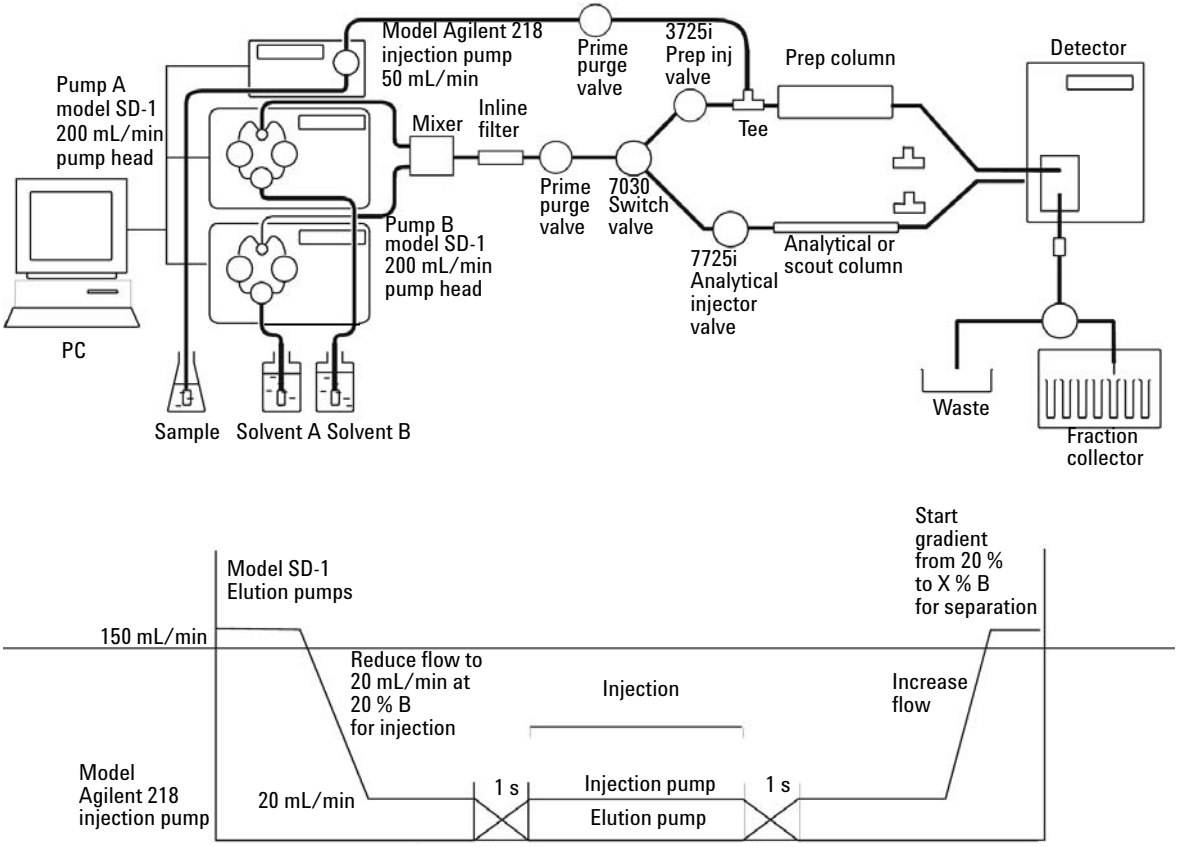
Figure 4 Auto-Preparative HPLC System

Instead of an injection valve this special binary gradient HPLC system uses a third pump for sample injection. All pumps are controlled by applicable instrument control software on a PC.

The high pressure outlets from both Agilent SD-1 solvent pumps are connected to a high pressure mixer. The system drain valve is plumbed after the mixer. The sample inject pump is plumbed into the system with a tee. The column is plumbed after the tee, then the detector. The outlet from the detector flow cell would normally be connected to a fraction collector.

Scale-up the HPLC System

Scale-up the HPLC System



The diagram shows the SD-1 based system for preparative scale-up. The system is controlled by a PC using instrument control software. The two elution pumps are Agilent SD-1's with 200 mL/min pump heads fitted. Downstream from the elution pumps is a high-pressure mixer, filter, drain valve and a switching valve. The switching valve selects between the analytical components (injection valve, column) and the preparative components (preparative injection valve, preparative column). In this example, both a preparative injection valve, and an injection pump are shown - this provides more flexibility. The injection pump is an Agilent 218 with a 50 mL/min head.

The injection pump's outlet is sent to a tee upstream of the preparative column. The eluent from either column flows to the detector flow cell then to collection.

Processing the Scale-up

The HPLC system has all the characteristics necessary to effectively handle both analytical and preparative HPLC with small particle columns.

As with all method development, one needs to develop analytical methods which meet purity requirements. For example, there may be a specific contaminant which must be completely removed while other peptides must be lower than some specific level, and specific buffers or salts may be prohibited. Once purity requirements are met with the analytical method, the method is scaled-up to maximum load on the analytical column. Again, knowing purity requirements is very beneficial, since sample displacement with an overloaded column can often provide adequate resolution with greatly increased loading capacity compared to a separation with optimal efficiency. Once the analytical method is perfected, all that is needed is to increase the flow rate and loading by the ratio of the cross-sectional areas. Maintaining the same particle size, linear velocity and proportional load will give the same results with a prep column as with an analytical column.

The next step is to develop a strategy for scale-up from analytical to preparative purifications of protein samples. There are three basic steps to scale-up:

1 Develop the analytical separation method to meet purity requirements. Determine the flow rate increase on your preparative column (F2) with the following formula.

$$F_2 = \sqrt{\frac{(F_1)^2 \times (ID_2)^2 \times L_2}{(ID_1)^2 \times L_1}}$$

F1	Flow rate on your analytical column
F2	Flow rate on your preparative column
ID1	Inner diameter of your analytical column
ID2	Inner diameter of your preparative column
L1	Length of your analytical column
L2	Length of your preparative column

OR

Determine the linear scale-up factor (LSF) and then multiply your analytical flow rate and volume to determine your preparative scale flow rate and volume.

$$LSF = \frac{(ID_2)^2 \times L_2}{(ID_1)^2 \times L_1}$$

LSF	Linear scale-up factor
ID1	Inner diameter of your analytical column
ID2	Inner diameter of your preparative column
L1	Length of your analytical column
L2	Length of your preparative column

2 Scale the method to maximum load on analytical column.

3 Scale flow rate and loading for preparative column with same particle size and column length.

Installing the Solvent Delivery Module

Electrical Setup

Electrical Setup

1 Setup electrical connections.

2 Check that the ON/OFF power switch is off (in the O position).

3 Connect the power cord to the back panel of the module and plug it into a grounded power socket.

NOTE A good ground connection is necessary to ensure safety for users and proper communications.

4 Turn on the power switch.

NOTE If the pump does not start check following items:

1 Proper connection of the power cord

2 Power at the wall receptacle

3 Functionality of the main power fuse (F1)

Connecting the External Contacts Panel

The Agilent SD-1 Pump has a number of analog and digital connections on the rear panel. These can be used to digitize data from an analog detector, start and stop other devices through contact closures and to receive contact closures to start and stop the pump.

The external contacts are not used with the standard systems offered by Agilent. The standard systems using the serial interface connections for communication and is organized by the OpenLAB software.

The external contacts panel can be removed for convenience when attaching the connections.

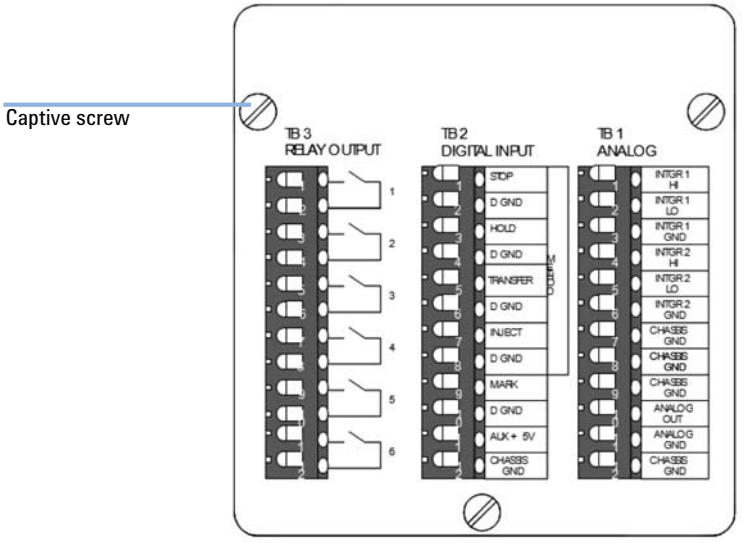


Figure 5 External contacts panel (panel turned 90° for clarity)

1 Remove the panel by loosening the three captive screws holding it in place and pulling the handle away from the pump.

WARNING

Incorrect voltage to the instrument

Damage to your instrumentation can result if the devices are applied to a voltage higher than specified.

➔ Connect the terminal block 2 (digital input) of your instrument only to a voltage from –0.5 – 5.5 VDC.

➔ Connect the terminal block 3 (relay output) of your instrument only to a voltage of 24 VDC and a current of 1 A.

➔ If any module you are switching requires higher voltage or current than the specified maximum ratings for the terminal block 3, use an auxiliary power relay to isolate the module from the instrument.

2 Attach the connections for each terminal block (see Table 2 on page 47, Table 3 on page 48 and "Terminal Block 3 (TB-3) Relay Output" on page 48).

3 Replace the panel by carefully aligning the main connector and firmly pushing it in place.

4 Tighten the captive screws finger-tight.

Terminal Block 1 (TB1) Analog

Table 2 Terminal Block 1 (TB-1) Analog

Name	Connection
INTGR 1 HI	Connect the “high” signal line from the channel A detector. Range from –0.5 – 2.5 V.
INTGR 1 LO	Connect the “low” signal line from the channel A detector.
INTGR 1 GND	Connect the “ground” line (if present) from the channel A detector.
INTGR 2 HI	Connect the “high” signal line from the channel B detector. Range from –0.5 – 2.5 V.
INTGR 2 LO	Connect the “low” signal line from the channel B detector.
INTGR 2 GND	Connect the “ground” line (if present) from the channel B detector.
CHASSIS GND	Connected internally to case ground.
CHASSIS GND	Connected internally to case ground.
CHASSIS GND	Connected internally to case ground.
ANALOG OUT	The “analog out” connections are bi-functional.
ANALOG GND 1	For wavelength control using a P325 UV/VIS Dual Wavelength Detector, connect this output to the “External Wavelength” input of the detector. For wavelength control a 1 V analog output from the pump equates to 100 nm from the detector. Alternatively, analog out can be used to output the pressure signal in mV, when the contacts are not used for wavelength control and when the **ANALOG PRESSURE MONITOR** menu item in the SD-1 pressure menu is selected to **ON**. For Analog Out 1 V = 1000 psi, (68 bar or 6.9 MPa). **NOTE** The pressure signal output is cancelled when using these outputs for wavelength control.
CHASSIS GND	Connected internally to case ground.

Terminal Block 2 (TB-2) Digital Input

Table 3 Terminal Block 2 (TB-2) Digital Input

Name	Connection
STOP D GND	Contact closure sends a "stop pump" signal to the Agilent SD-1 which will send it to the data system software controlling the instrument. If there is no "shutdown" method specified, this input stops the pump immediately and informs the instrument control software that the pump has stopped. If there is a shutdown method specified control will branch to that method, either immediately, or after the current method pass, depending on the preference set.
HOLD	Contact closure sends a "hold flow rate" signal to the instrument control software. Contact closure duplicates the **HOLD** key on the front panel.
TRANSFER	Contact closure sends a "transfer method" signal to the instrument control software. If there is no "link" method specified, the method will stop looping after the current method pass. If there is a link method specified, the method will stop looping and transfer to the link method at the end of the current method pass.
INJECT D GND	Connect these inputs to the position sensing switch of the injector or to the "injection complete" output from the automatic sample injector. The contact should close when the valve is in the "inject" position, or when the autosampler completes injection. Contact closure cancels "inject wait" programmed in the pump.
MARK D GND	Inputs can accept all contact closures relays, open collector transistors, and open collector integrated circuits. When there is no event, the input must be greater than 4.75 V; with a contact closure, the input must be equal to or less than 1.2 V with a duration of 0.1 s or longer.
AUX + 5V	Provides 5 V logic voltage with a 47 Ω resistor in series. Low current loads (less than 20 mA) such as sensitive relays, LEDs, and simple logic circuits can be run off this output. To maintain output greater than 4.75 V, the load must be less than 5 mA.
CHASSIS GND	Connected internally to case ground.

Terminal Block 3 (TB-3) Relay Output

The "relay out" connectors are for switching power to user-defined external devices. Each pair of output connectors is connected to a relay inside the Agilent SD-1.

NOTE To use the relay outputs to operate any inductive-load devices please contact your Agilent Technologies sales and service office.

Connecting Cables to the Injection Pump

Start and stop of the injection pump is controlled by a contact closure which is set up in the external event table in the PC configuration.

1 Connect a cable between the inject and ground of the injection pump to a contact closure on the SS-420 board.

2 Connect a cable between stop and ground of the injection pump to another contact closure on the SS-420 board.

3 Set up the trigger of these contact closures at the appropriate time in the external event table.

The injection pump must not be on the bus or it will limit the maximum flow rate to the maximum allowable system flow rate of the injection pump.

Serial Interface Connections for Remote Control

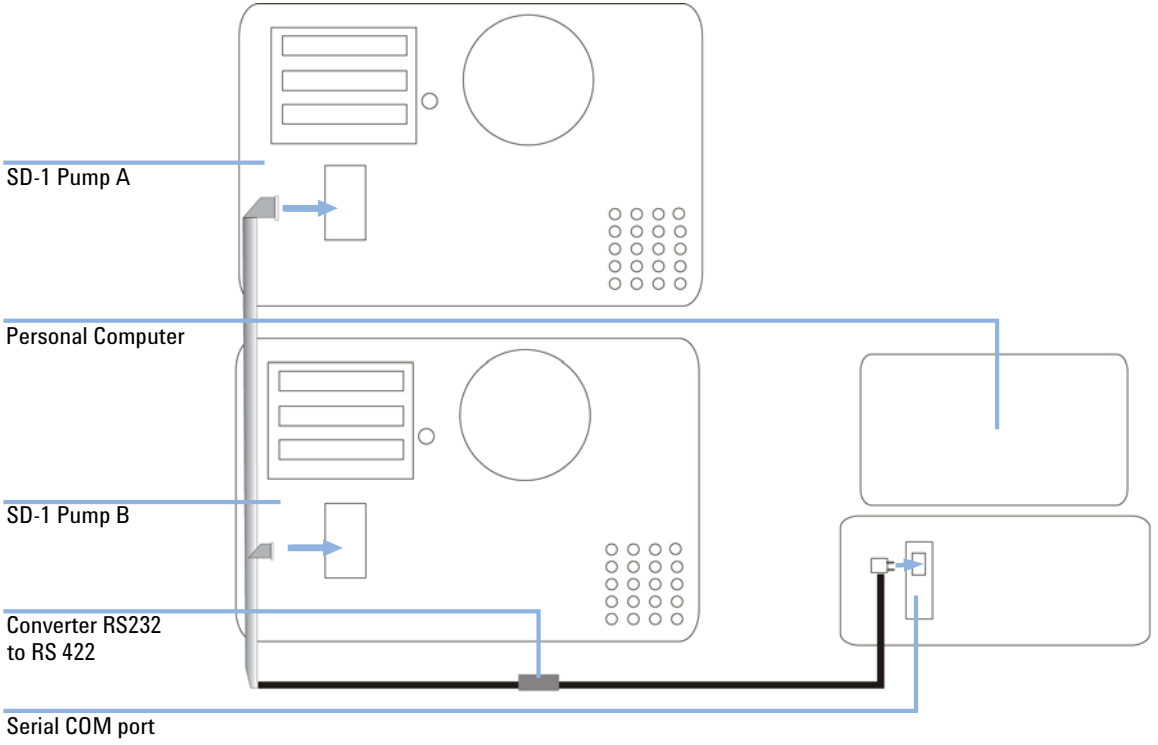


Figure 6 Connections for instrument control software (IBM/PC compatible)

1. Plug the serial interface cable into serial COM port 1 on the back of the PC.
2. Connect the serial interface cable with the RS-232 to RS-422 converter.

   The four 9-pin female connectors on the serial interface cable are all equivalent.
3. Connect one of the connectors to the serial interface port on each SD-1 pump being used.

   Extra connectors may be left unconnected.
4. If applicable, plug the AC connector on the serial interface cable into the AC power supply.
5. Configure the pump IDs (see Agilent SD-1 Isocratic Solvent Delivery Module - User Manual (G9302-90001)).

Plumbing Connections 200 to 800 mL Pump Heads

Low Pressure Inlet Tubing

Tools required **Description**

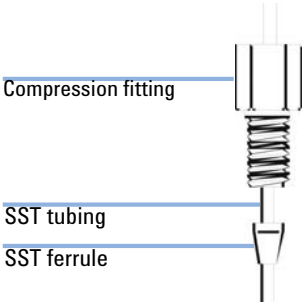
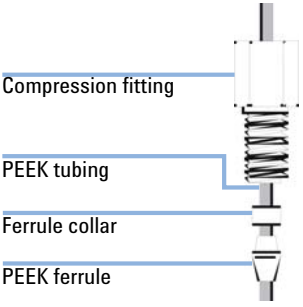
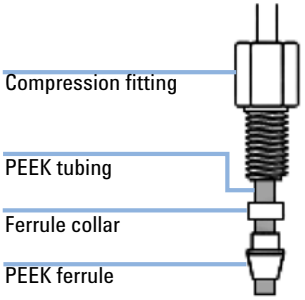
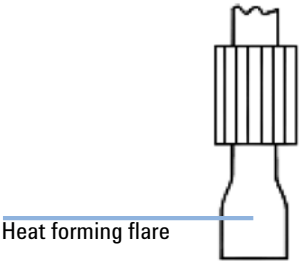
Wrench, open-ended, ½ inch

1. Remove the solvent inlet assembly from the accessories package.
2. Immerse the inlet filter into clean, HPLC-grade water.
3. Remove the plug from the low pressure inlet tee and connect the inlet fitting to the inlet tee.
   a. Store the plug in a safe place.
4. Thread the fitting into the inlet tee and finger-tighten.
5. Tighten the fitting in the inlet tee with the ½ inch open-ended wrench and tighten ¼-turn past finger-tight.

High Pressure Outlet Tubing

Refer to the following figure to find the outlet connections for your SD-1 pump. The appropriate compression fitting for your SD-1 pump is in the accessories package.

Table 4 Connections for the outlet check valve

Type of pump head/tubing	Ferrule/Tubing (Titanium/SST)	Ferrule/Tubing (Titanium/PEEK)	Ferrule/Tubing (PFA, PVDF/FEP)
200 mL/min 0.159 cm (1/16 inch)	 over 172 bar[1]	 up to 172 bar	
500/800 mL/min 0.318 cm (1/8 inch)		 up to 172 bar	
3.2 L/min 0.635 cm (1/4 inch)			

[1] Unit conversion: 172 bar = 2500 psi = 17.2 MPa

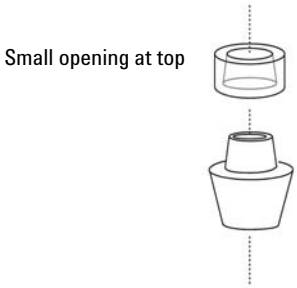


Figure 7 Detail of PEEK ferrule and ferrule collar

Tools required

Description

Wrench, open-ended, 5/16 inch

1 Remove the plug from the outlet tee port.
   a Store the plug in a safe place.
2 Assemble the ferrule (and ferrule collar for PEEK tubing) and compression fitting onto the outlet tubing (see Table 4 on page 52).
3 Assemble the ferrule (and ferrule collar for PEEK tubing) and compression fitting onto the outlet tubing as shown.
   a For PEEK ferrules, place the ferrule collar onto the shoulder of the ferrule, large opening toward the ferrule.

      The collar will seat properly as you tighten the fitting.
4 Holding the compression fitting, ferrule and collar in place on the tubing, push the end of the tubing as far as possible into the port on the outlet tee.
5 Carefully thread the compression fitting into the outlet port on the tee.
6 Tighten the compression fitting finger-tight.
7 Tighten the fitting in the outlet tee with the 5/16 inch open-ended wrench and tighten ¼-turn past finger-tight.
8 Immerse the other end of the outlet tubing into the solvent in the reservoir, and tape the outlet tubing firmly to the neck of the solvent reservoir.
9 As an alternative you can finish connecting the instrument through the drain valve to the rest of the HPLC system and prime the instrument with the drain valve open to waste (see "Priming the Pump Heads" on page 58).

Plumbing Connections 3.2 L Pump Head

Inlet and outlet plumbing use flared tubing compatible with PFA or PVDF fittings.

CAUTION

Damage to the fittings

Use of tools such as wrenches or pliers to tighten the fittings may damage the fitting or cause the tubing to fail under pressure.

➔ The fittings, with the exception of the check valves, should only require hand tightening.

1 Remove the white shipping plug from the inlet valve.

   a Store the plug in a safe place.

2 Attach the inlet and outlet tube assemblies (see Figure 8 on page 55).

a Keep the inlet tube to the solvent reservoir short.

A short tube prevents inlet pressure oscillations.

b Flush the tubing and reservoirs with air or filtered solvent.

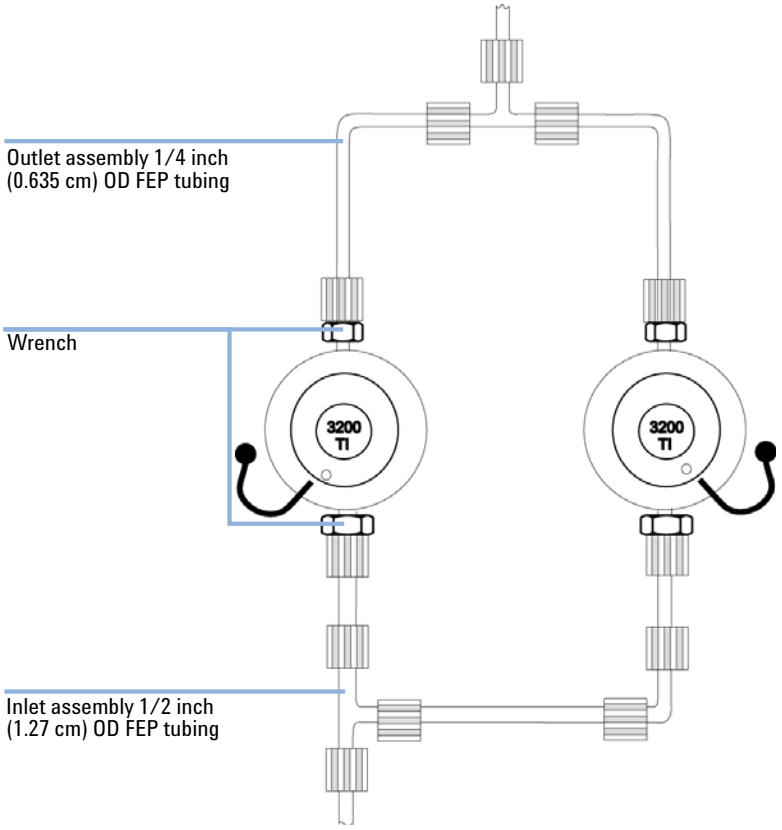


Figure 8 Connections for 3.2 L pump heads

3 Fill the system with filtered solvent.

Pump Head Installation

When

The pumps are shipped without the pump head installed. You will have to install the pump head before beginning to run. You can also change pump heads at any time.

Tools required

Description

Wrench, open-ended, 5/16 inch

Wrench, open-ended, ½ inch

Wrench, 9/64 inch hexagonal, T-handle

Wrench, 5/32 inch hexagonal, T-handle

Wrench, 3/16 inch hexagonal, T-handle

1 To open the **SETUP** menu, press the ~ key.

2 Press the **DOWN ARROW** key to access the setup functions.

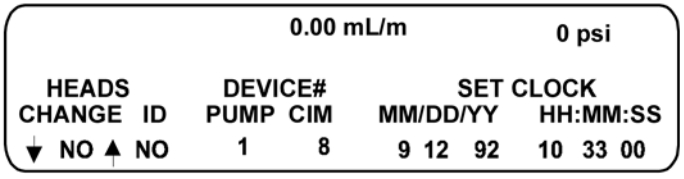


Figure 9 SETUP display

3 Place the cursor on **HEADS CHANGE** and press the **DOWN ARROW** key to scroll to **YES**.

4 Press **ENTER**.

5 Take the pump head set assembly and hold both heads in position, with the piston cups inside the open piston collets.

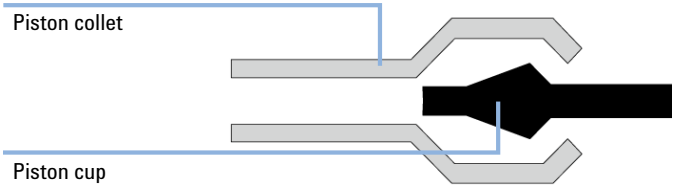


Figure 10 Piston cup and piston collet

6 To begin the pump head engagement sequence, press **ENTER**.

The collets will close over the piston cups and when fully closed will draw the pump heads into place. When both pump heads are in place the following message will appear: **Replace and tighten screws on both heads. Plumb connections. Press ENTER when completed.**

7 Tighten the mounting screws in each pump head with a 3/16 inch wrench.

8 Tighten the mounting screws in the inlet tee with a 5/32 inch wrench.

9 Tighten the mounting screws in the outlet tee with a 9/64 inch wrench.

10 Connect the inlet tubing (tee to inlet check valves).

11 Press **ENTER** to confirm the mounting screws and plumbing are connected.

The following message will appear: **Plug in BOTH connectors. Press ENTER when completed.**

12 Connect the pressure sensor cables.

13 Press **ENTER** to complete the head replacement procedure.

The following display will read (with appropriate values for the actual head size):

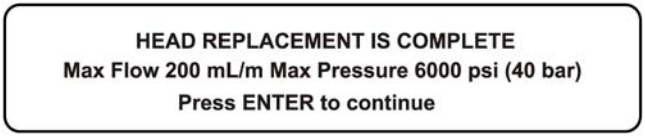


Figure 11 Pump head replacement

Priming the Pump Heads

All pump heads are self-priming.

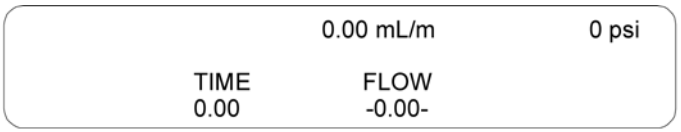
Preparations

- The detector is bypassed.
- If a prime purge valve is connected, open the prime purge valve to relieve the pressure.

1 Tighten the outlet tee fitting ¼-turn past finger-tight.

2 To switch on the pump power, press the power switch.

After a few moments the **Flow** display is displayed:



3 If the **Flow** field is not flashing, press the **RIGHT ARROW** key to get the cursor to the flow field.

4 To prime the 200 mL/min pump head, type in 150 and confirm with **ENTER**.

OR

To prime the 500 mL/min pump head, type in 400 and confirm with **ENTER**.

OR

To prime the 800 mL/min pump head, type in 600 and confirm with **ENTER**.

OR

To prime the 3.2 L/min pump head, type in 2000 and confirm with **ENTER**.

5 Start the pump by pressing **ENTER**.

The pump will start to run and solvent will be drawn into the solvent inlet line. After the inlet line is full, let the pump continue to run for a few moments until the pressure display is steady.

6 Press **STOP** to stop the pump.

The SD-1 pump is now primed.

7 Switch off the SD-1 by pressing the power switch.

Flushing the HPLC System

Before pumping solvent through an HPLC system, it is important to flush all tubing to prevent impurities or particulates from the new tubing (such as dust, cutting oils, metal swarf, etc.) from clogging the column frit or contaminating the column.

WARNING

Chemical Hazard

➔ Danger of burns. To avoid injury to personnel or damage to equipment, always follow appropriate safety guidelines when using chemicals and always wear appropriate safety equipment and clothing.

1 Disconnect the tubing fitting at the column inlet and place the fitting into a waste container. It is vital that the tubing is thoroughly flushed before flow is introduced to the column.

2 To set the **FLOW** to 15 mL/min on each Agilent SD-1 pump, press 15 and confirm with **ENTER**.

3 Press **RUN** to start each pump.

4 Pump solvent through the tubing for several minutes.

The tubing is thoroughly flushed before flow is introduced to the column.

Installing the Detector

Location of the Detector Module

Place the detector conveniently near your HPLC system. The modular design of the detector enables you to locate it anywhere within the limitations imposed by the length of the power cord, fluid lines and signal cables. In order to keep liquid dead volume as low as possible and to minimize peak broadening in the lines, the distance between the column outlet and the flowcell inlet should be kept to a minimum.

For best performance, the detector should be located on a clean, sturdy, vibration free bench in an area free of:

- Heat sources (such as direct sunlight or a heater vent)
- Drafts (such as an open doorway, window, or air conditioner vent)
- Smoke or UV-absorbing vapor
- Corrosive or dusty atmosphere
- Potential liquid spills

Provide approximately 4 inches of space behind the unit so that the cooling fan intake is not impeded, and to allow easy access to the rear panel services (see “Power Connection and Rear Panel Services” on page 61).

Power Connection and Rear Panel Services

Power Connection and Rear Panel Services

WARNING

Electrical shock

Risk of stroke and other personal injury.

➔ Turn off the power to the detector before making power and signal cable connections.

The detector can communicate with other modules and devices in the entire HPLC system. All power and signal connections are made on the rear panel of the detector (see Figure 12 on page 61). The connectors on the rear panel support communication configurations to a wide range of Agilent modules and non-Agilent devices.

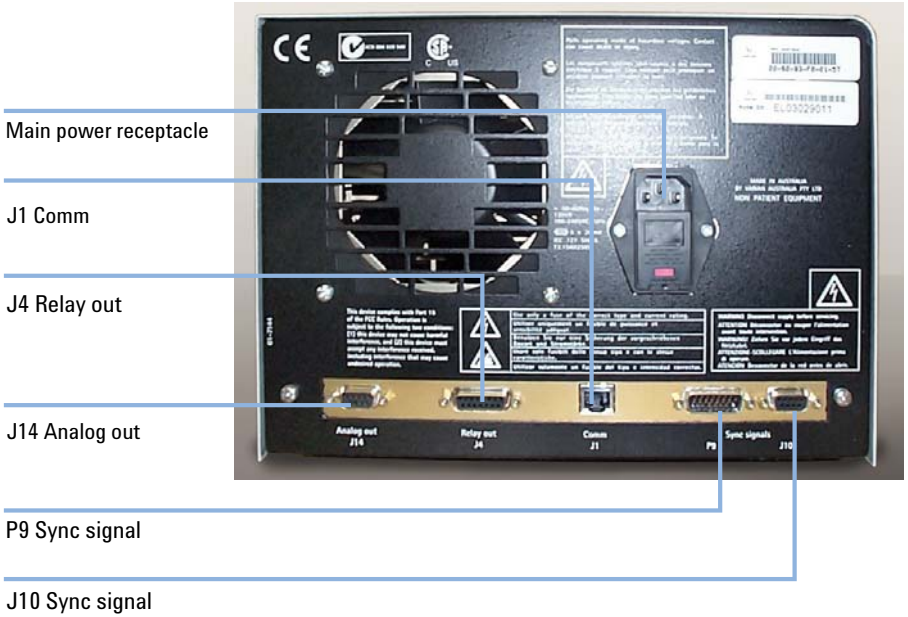


Figure 12 Agilent 325 Detector rear panel services

The following table summarizes the function of each connection:

Table 5 Rear panel services functions

Connection	Function
Main power receptacle	3-pin receptacle with 2 fuses
J14 Analog out	9-pin female "D" shell connector used for two channels (A and B) of analog output
J4 Relay out	15-pin female "D" shell connector used for time programmed contact closures
J1 Comm	RJ-45 type connector used to interface the Agilent 325 to a desktop PC
P9 Sync signal	15-pin male "D" shell connector used with synchronization signal cable
J10 Sync signal	9-pin female "D" shell connector used with synchronization signal cable

For more information about the connections see chapter Cables.

AC Power

The Agilent 325 UV/VIS Dual Wavelength Detector may be connected to any voltage in the range 100 – 240 VAC ±10 %, 50 /60 Hz ±1 Hz, single phase, without modification or the need to change fuses.

Before connecting power to the detector, ensure that the power switch on the front of the instrument is OFF (the rocker switch *O* is pressed). The power switch is a rocker switch that connects from the front of the detector directly to the power receptacle on the rear panel. Plug one end of the power cord into the power receptacle on the rear panel and the other end into your AC power source.

WARNING

Absence of ground connection or use of unspecified power cord

The absence of ground connection or the use of unspecified power cord can lead to electric shock or short circuit.

➔ Never operate your instrumentation from a power outlet that has no ground connection.

➔ Never use a power cord other than the Agilent Technologies power cord designed for your region.

All devices in the liquid chromatography system should be connected to the same power source, using a properly grounded (3rd wire to earth) multiple outlet power strip.

NOTE

Do not turn on the AC power yet. All required external devices and hydraulics must first be connected.

Avoiding Harmful Interferences to Radio or Television Reception

Operation is subject to the following two conditions:

- This device may not cause harmful interference.
- This device must accept any interference received, including interference that may cause undesired operation.

If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try one or more of the following measures:

1. Relocate the radio or antenna.
2. Move the device away from the radio or television.
3. Plug the device into a different electrical outlet, so that the device and the radio or television are on separate electrical circuits.
4. Make sure that all peripheral devices are also certified.
5. Make sure that appropriate cables are used to connect the device to peripheral equipment.

6 Consult your equipment dealer, Agilent Technologies, or an experienced technician for assistance.

7 Changes or modifications not expressly approved by Agilent Technologies could void the user's authority to operate the equipment.

Removing the Front Panel

1 If installed, remove the door from the Agilent 325 Detector (see "Removing the Door" on page 71 for more information).

2 Remove the panel on the front right side of the detector by unscrewing the captive screw in the top left corner of the panel.

Figure 13 Removing the front panel

Hydraulic Connections - Flowcells

Hydraulic connections are located at the front of the Agilent 325 Detector.

The only line installed by the user where dead volume and low holdup are critical is the line from the column exit to the flowcell inlet port. This line should be as short as possible.

The Agilent 325 Detector can be fitted with any one of four flowcells (ordered separately). Each one has an inlet and outlet connection and quartz optics cell

window. Your chosen flowcell type is packed internally in the detector. The four flowcell types are outlined in Table 6 on page 65.

Table 6 Compatible flowcells

Flowcell type	Flowcell p/n	Pathlength[1]	Column ID	Flow rate	Maximum pressure
Analytical	210181800	9 mm x 0 mm	2 – 8 mm	0.0001 – 10 mL/min	69 bar (1000 psi)
Preparative[2]	210181900	9 mm x 1 mm	4 – 76 mm	1 – 500 mL/min	69 bar (1000 psi
Scale Up[2]	210224200	4 mm x 0.25 mm	4 – 76 mm	10 – 200 mL/min	69 bar (1000 psi
Super Prep[2]	210182000	4 mm x 0.15 mm	8 – 152 mm	30 – 1200 mL/min	69 bar (1000 psi
Micro-analytical	210182100	4 mm x 0 mm	1 – 4 mm	0 – 20 mL/min	69 bar (1000 psi

1 A pathlength of a mm x b mm means that the sample light path has a pathlength of a mm, and the reference light path has a pathlength of b mm. A reference light path of zero means there is no fluid in the cell - air acts as the reference.

2 Extended range cell

NOTE It is important for the extended range cells to run at least at the minimum flow rate.

Installing a Flowcell

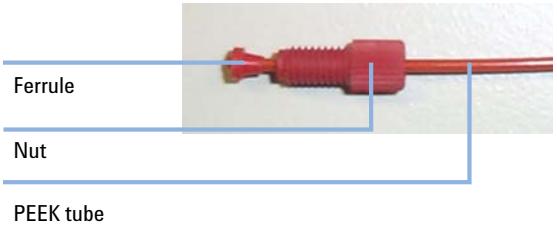
The Agilent 325 UV/VIS Dual Wavelength Detector is not shipped with a flowcell installed. You will need to install the flowcell that you purchased with the detector. Each flowcell comes with a set of recommended nuts and ferrules that may be fitted to tubing.

1/16" tubing is used on all flowcells. However for the 4 mm x 0.15 mm super prep. flowcell it is recommended to use 1/8" tubing at higher flow rates. In this case you can add the Adaptor 1/8" - 1/16" (1610126800). This will require the 1/8" tubing and the 1/8" flowcell fittings.

Tubing connections are PEEK type, except for the Super Prep flowcell which uses ETFE tubing 0.125 mm x 0.062 mm.

Parts required	#	p/n	Description
	1	210181800	Flowcell 9 mm x 0 mm, inert (analytical)
OR	1	210181900	Flowcell 9 mm x 1 mm, inert (prep.)
OR	1	210224200	Flowcell 4 mm x 0.25 mm, inert (scale up)
OR	1	210182100	Flowcell 4 mm x 0 mm, inert (micro-analytical)
	1	9910128300	Flow cell replacement fittings
	1	210182000	Flowcell 4 mm x 0.15 mm,inert (super prep.)
	1	1610126900	Fitting 1/8" tube nut flat bottom
	1	1610126400	Fitting 1/8" tube ferrule, Pack of 10
	1	1610126800	Adaptor 1/8" - 1/16"

1 Fit the nuts and ferrules on the PEEK tubes.

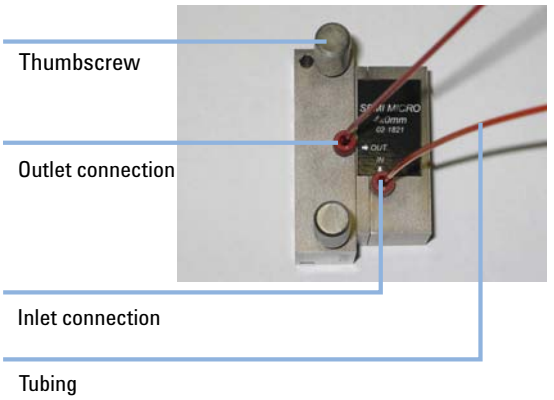


2 Screw the two tubes into the inlet and outlet connection of the flowcell. Each flowcell has an inlet and outlet connection.

NOTE

Be careful not to cross thread the connector into the flowcell body.

Do not over tighten, as this will damage the threads.



NOTE

The position of the inlet and outlet connections varies for different flowcells.

3 Remove the front panel (see "Removing the Front Panel" on page 64 for more information).

4 Carefully position the flowcell so that the two thumbscrews are positioned in the threaded holes in the flowcell compartment.

a Push at the center of the flowcell to ensure it is positioned squarely in its housing and that it is not tilted in any way.

NOTE

It is critical to the performance of the detector that the flowcell is inserted correctly. The internal optical components of the flowcell are an integral part of the detector's optical system. If the flowcell is not fitted correctly, it will have an adverse effect on detector performance.

Next Steps:

5 Secure the flowcell by tightening the thumbscrews with your fingers. Alternate tightening the thumbscrews until they are snug.

6 Replace the front panel.

NOTE

For optimum performance, the detector should be operated with the front panel in place. This is because the foam on the inside of the panel stops breezes, which may cause instability and noise, from reaching the flowcell.

NOTE

The flowcell should be removed with the connecting tubing fitted. These must be removed outside the detector compartment.

7 Perform a lamp calibration (see chapter maintenance in Agilent 325 UV/VIS Dual Wavelength Detector - User Manual (G9309-90000)).

Detector Outlet Back Pressure Restrictor

The Back pressure restrictor (110743300) should be added to all flow cells, unless a fraction collector is installed after the detector. The back pressure restrictor assembly that was supplied with your detector should be threaded into the outlet line from the flowcell. The back pressure restrictor applies approximately 2.76 bar (40 psi) back pressure on the flowcell. This prevents outgassing and bubbles from forming or being trapped in the flowcell, which can cause an unstable baseline.

Note the arrow stamped on the restrictor body. This arrow must point away from the flowcell outlet port, and toward the waste receiver. The threaded plastic fittings should be finger tightened only enough to prevent leaks.

About 122 cm (48") of 1.6 mm (1/16") tubing is supplied at both the inlet and outlet of the restrictor. The inlet tubing is provided with a 1.6 mm (1/16") plastic tubing fitting for connecting to the flowcell outlet port. Either the outlet tubing can be directed to the waste container, or the tubing removed, and the restrictor itself dropped to the bottom of the waste bottle.

The restrictor pressure setting is not adjustable. If the restrictor fails or becomes plugged, replace the existing cartridge with a new 2.76 bar (40 psi) replacement.

Installing the Door

The module door may be attached to the front of the Agilent 325 Detector to cover the tubing connections to the flowcell.

NOTE If the Agilent 325 Detector is the top module in the stack, the door cap should be installed before installing the door on the module.

Installing the Door Cap

If the Agilent 325 Detector is not at the top of the stack, do not install the cap and proceed to the instructions describing door installation.

1 Stand the door upside down on a flat surface (i.e., with the two ribs towards the bottom).

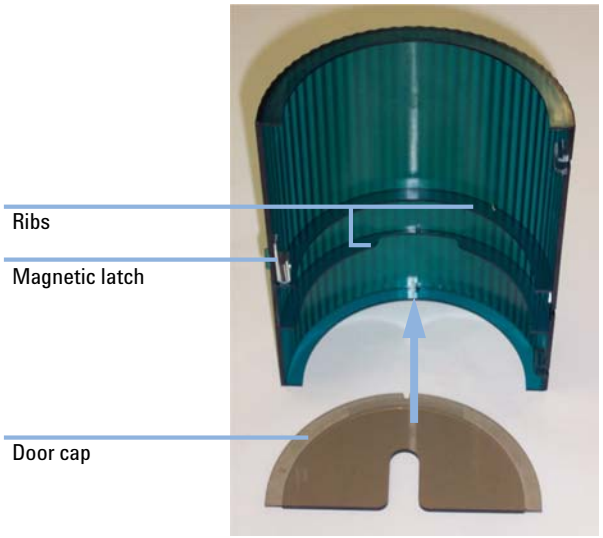


Figure 14 Installing the door cap

2 Take the cap and remove the protective paper exposing the adhesive that will attach the cap to the door.

3 Insert the cap into the door and press the adhesive onto the inside of the door lip. Be sure to keep the door edges and cap edges flush.

Installing the Door

1 Insert the top hinge pin into the top hinge.

2 Gently press down on the top of the door and slide the lower hinge pin into the lower hinge. The door should now pivot on the pins and close. The magnetic door latch should stick to the instrument.

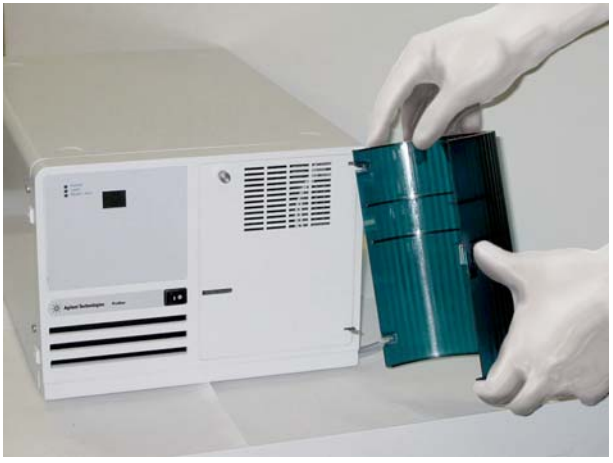


Figure 15 Installing the Agilent 325 Detector door

Removing the Door

1 Gently push down on the door and slide the lower hinge pin out of the lower hinge.

2 Lift and slide out the top of the door.

Installing the Autosampler

Location of the Autosampler Module

The best place to install your Agilent 410 Autosampler is at the top of the module stack. This stack would normally be; solvent delivery module on the bench, detector on top of the SDM, and finally the AutoSampler. From the right-hand side you have the shortest connection to the injection valve of the Agilent 410 Autosampler to the column.

Overview of the Module

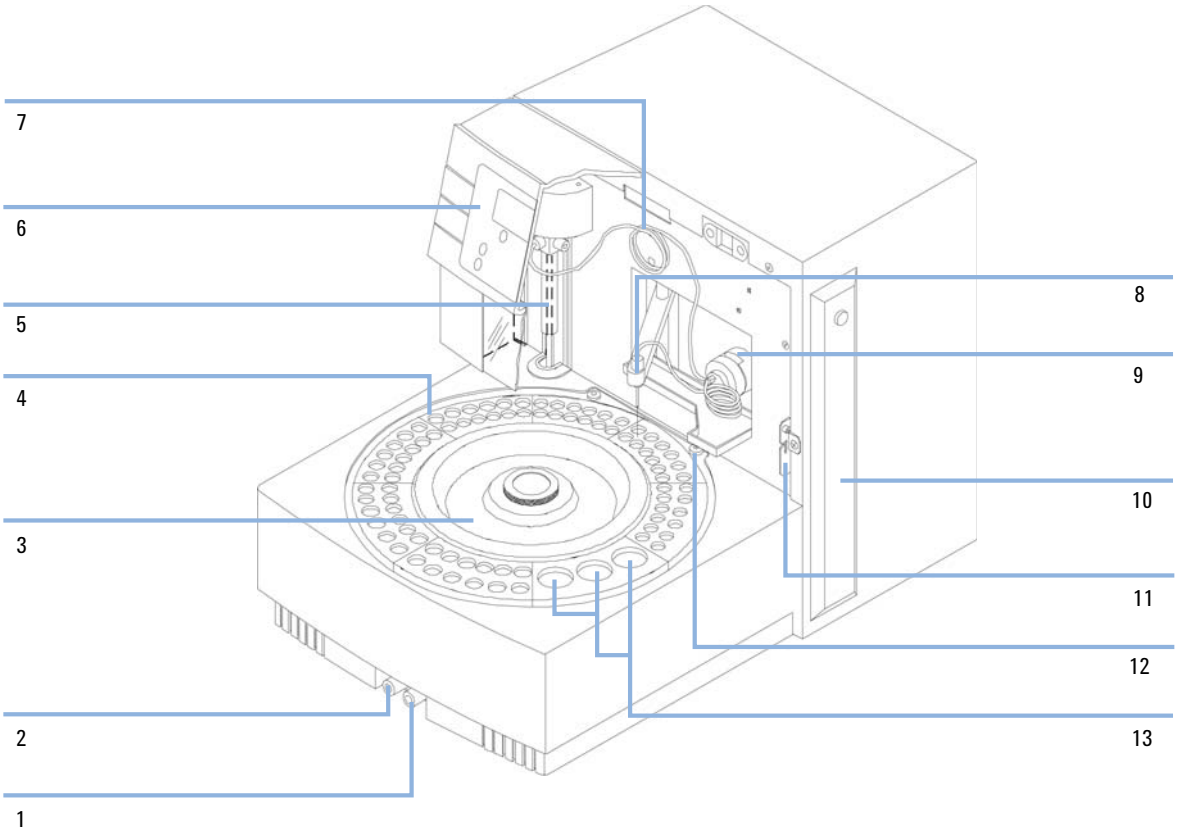


Figure 16 Autosampler overview

1	Vial wash drain
2	Condensed water and leakage drain
3	Tray fixation knob
4	Sample tray
5	Syringe dispenser

6	Keyboard
7	Buffer tubing
8	Needle arm
9	Injection valve
10	Oven compartment
11	Tubing guide
12	Wash position
13	Position for transport solvent and reagent vials

The rear view of the autosampler is shown in Figure 17 on page 75.

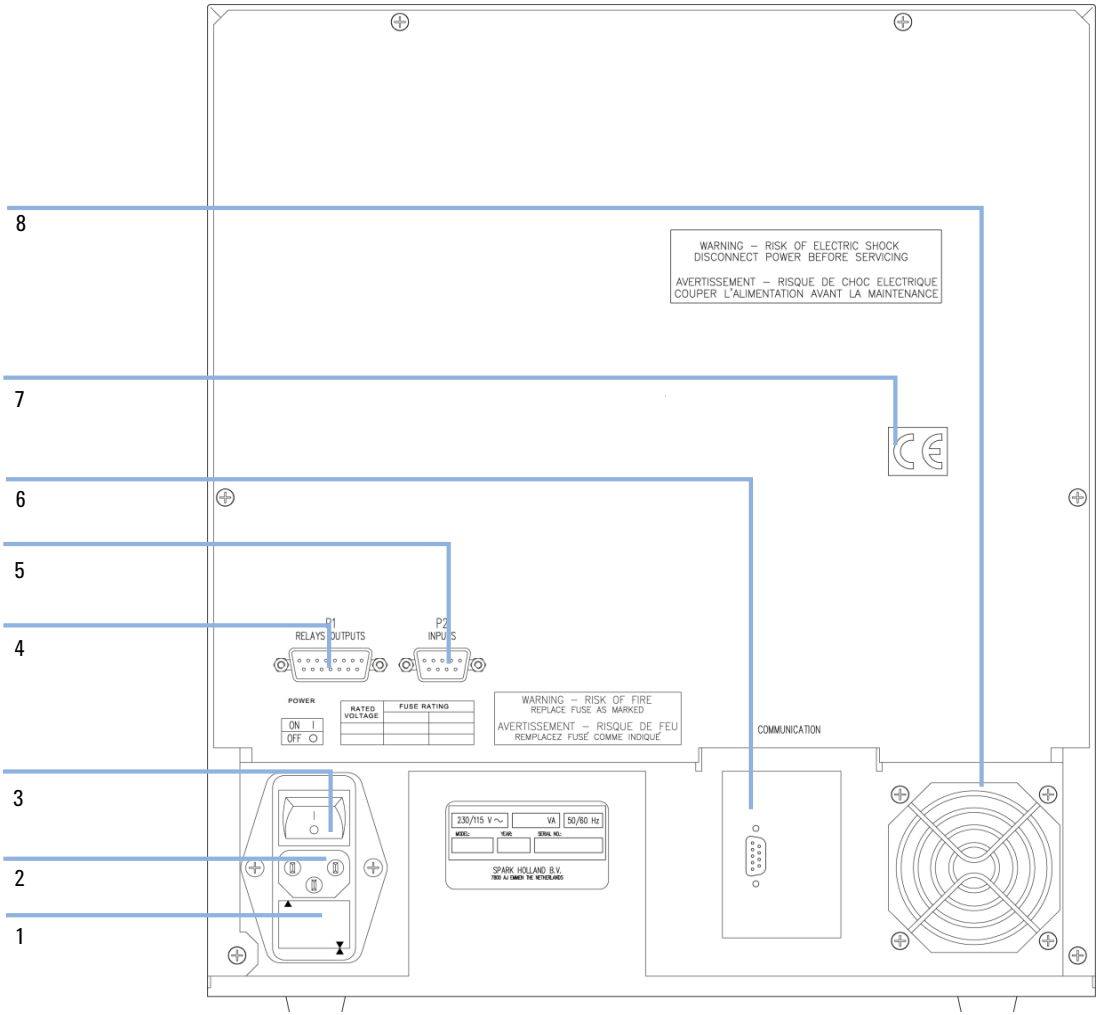


Figure 17 Rear View of the autosampler

1	Fuses and voltage selector
2	Main input
3	Main switch
4	P1-I/O connector

5	P2-I/O connector
6	RS-232 communication interface connector
7	CE-mark
8	Fan (only if optional Peltier tray cooling option is installed)

Installing the Sample Tray

1 Place the sample tray in the autosampler and rotate it until the tray drops into the slot, only one position is possible.

2 Turn the tray fixation knob clock-wise to fixate the tray (see "Overview of the Module" on page 73 for an overview).

Connecting the Waste Tubings

The following Waste Tubings must be connected to the autosampler:

- General waste tubing
- Syring waste tubing
- Water and leakage drain

Connecting the General Waste Tubing

1 Connect the drain tubing to the right-hand drain hose connector of the autosampler and put it in a bottle which is placed on the floor.

All the liquid dispensed while the autosampler is in the wash position is removed through this drain

Connecting the Syringe Waste Tubing

1 Place the syringe waste tubing into a small bottle next to the autosampler. If no injection volumes are programmed that are larger than the buffer tubing can contain, the syringe waste will only be wash solvent.

Connecting the Water and Leakage Drain

All solvents that result from a leak in the system and condensed water are drained through the left hand hose connector.

CAUTION

Risk of damaging the autosampler

Drain and waste tubing are twisted thereby obstructing the flow path.

➔ Be sure that the drain and waste tubing are not twisted.

1 Connect the hose connector to a waste container on the floor.

Starting the Autosampler

Tools required	Description
	Distilled Water
	Isopropanol
	Helium
OR	Ultrasonic bath

Preparations

- Allow the Agilent 410 Autosampler to reach ambient temperature for at least one hour.
- Connect the waste tubings (see "Connecting the Waste Tubings" on page 76 for more information).

WARNING

Electrical shock

Risk of stroke and other personal injury due to reduced safety protection or unwanted fusing.

➔ Ensure that the code on the fuse cap matchs the information next to the fuse holders.

WARNING

Risk of fire and damaging the module

➔ For 115 VAC ±15 %, use two 5 A T-fuses (slow-blow).

➔ For 230 VAC ±15 %, use two 2.5 A T-fuses (slow-blow).

➔ All fuses must be UL listed and CSA certified, or IEC 127 type.

NOTE

Keep the keyboard front cover closed during operation.

1 Remove the safety screw on the right-hand side of the front cover.
2 Check fuses and voltage settings on the rear of the instrument.
3 Connect the power cable.
4 Turn the power switch to ON.
The Ready screen appears displaying the firmware revision number.

Rinsing the System with Wash Solvent

Tools required	Description
	Distilled Water
	Isopropanol
	Helium
OR	Ultrasonic bath

CAUTION

Damage to the system

Crystals from salts or buffer solutions may block or damage the system.

➔ Never use salts or buffer solutions as wash solvents.

➔ Only use water/organic solvents.

1. Place a clean bottle for the wash solvent at the left-hand side of the autosampler
2. Use a mixture of distilled water and isopropanol (80 / 20 v/v %) or the mobile phase as wash solvent.
3. Degas the wash solvent with Helium or an ultrasonic bath.
4. Put the wash solvent tubing in the filled was solvent bottle.
5. Press **MAINTENANCE** to go the autosampler maintenance functions.
6. Fill the tubing using the autosampler soft-function keys **SYR END** and **SYR HOME**.
7. With **SYR END** a syringe volume of wash solvent is aspirated from the wash solvent bottle and the wash solvent tubing is filled. With **SYR HOME** the syringe contents will be dispensed to the syringe waste.
8. Repeat this action until the wash solvent tubing and the syringe are completely filled and no air bubbles appear in the syringe.
9. Press **Escape** to leave the maintenance screen.

10 After the wash solvent tubing and the syringe are filled, press **WASH** to perform a standard wash routine. Repeat the wash routine 2 or 3 times. All tubing connected to the syringe valve will be rinsed with wash solvent.

NOTE The autosampler will give the best results if all air is removed from the syringe.

Connecting to the Autosampler

To ensure reproducible injections the following connections to your HPLC system should be made:

1 Connect the HPLC pump to port 1 of the injection valve.

2 Connect the HPLC column to port 6 of the injection valve.

3 Check for leakage and let the system equilibate for at least 5 min.

NOTE The instrument is flushed with Isopropanol. Make sure that the mobile phase of your HPLC system is miscible with Isopropanol. If your mobile phase is not miscible with Isopropanol, then start with an intermediate solvent that is a transition to the mobile phase. To avoid possible contamination of the column, it is good practice to not have the column installed during this operation.

NOTE It is essential that the contents of the sample loop are injected as a back flush onto the column, therefore do not exchange column and pump connections at the injection valve.

Filling and Sealing the Vials

1 Fill the standard vials, as well as the conical vials by means of a narrow–end pipette to allow air to escape when filling the vial.

NOTE Do not fill vials completely to the top. In that case sample will be forced into the air needle, risking cross-contamination of samples and fouling of the needle pair.

2 Check that the seal is airtight due to maintain a pressure on the vial for air bubble prevention and to prevent evaporation of volatile samples.

3 Check seal after crimping, if the cap can be turned easily, the seal is not airtight (re-adjust hand crimper).

NOTE When using uncapped vials, the performance of the AutoSampler may not meet the specifications (precision). Do not re-use a sample vial without replacing its cap or septum.

Loading the Sample Tray

1 Place the vial in the tray and link them to methods in the Series. For details, see Agilent 410 Autosampler - User Manual (G9331-90000), Chapter *Using*, section *Programming the Run Sequence or Series*.

2 If the autosampler ist not running, manually rotate the tray to gain access to all vial positions.

Installing the Agilent 410 Autosampler Prep Option

1. Replace the standard injection valve with the special Agilent 410 Prep valve.
2. Replace standard sample needle, air needle and buffer tubing with the ones supplied in Prep Upgrade Option, Includes Needle, Syringe, Injection Valve and Large Volume Sample Tray (393590791).
3. Re-connect all tubing to the injection valve.
4. Replace standard Syringe with the 2500 µL syringe.
5. Install the 24 vials (LSV) tray.
6. Choose Prep Mode in system.

Installing the Fraction Collector

Overview of the Module

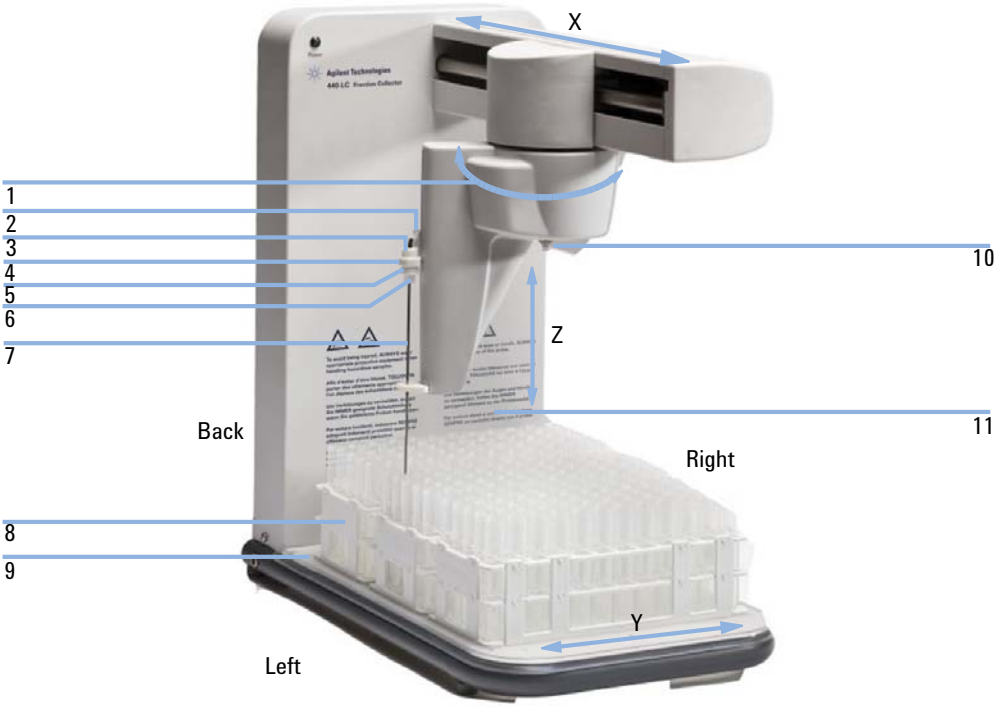


Figure 18 Fraction collector components

1	Theta axis
2	Z axis slide
3	Top knurled mount nut on the probe assembly
4	Probe mounting block
5	Lower knurled mount nut on the probe assembly

6	Nut holding the metal probe
7	Probe
8	Rack
9	Rack location mat
10	Split retaining ring
11	Fraction collector pillar (control column)

Installation Overview

Basic installation involves:

- Assembling the fraction collector
- Connecting the fraction collector to the HPLC system
- Installing Agilent OpenLAB CDS ChemStation PrepLC Drivers
- Determining the delay volume and probe depth

NOTE All external DIP switches should always be in the *OFF* position.

Assembling the Fraction Collector

Carefully follow the instructions provided below to assemble your instrument.

Assembling the Agilent 440 Fraction Collector includes installing the:

1. Spill tray
2. Rack location mat
3. Probe and tubing
4. Sample racks

NOTE Position the fraction collector on the side of the HPLC system closest to the detector output to help decrease the delay volume.

Installing the Spill Tray

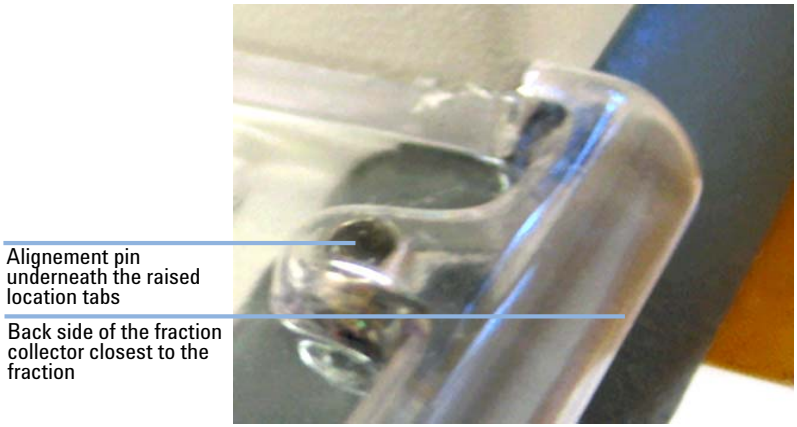


Figure 19 Spill tray alignment pin and mat for the rack location raised locating tabs

Preparations Position the fraction collector closest to the detector output. This decreases the delay volume.

NOTE The spill tray will catch most solvent spills. Always clean up spills immediately.

1. Locate the alignment pins protruding above the base support tube.
2. Lower the tray so that the two alignment pins closest to the fraction collector pillar fit into the slots in the spill tray.
3. Lower the spill tray until the front edge rests on the front of the base support tube.
4. Check that the spill tray is firmly pushed down on all alignment pins.

NOTE The spill tray must be firmly in position so that it does not move.

Installing the Rack Location Mat

The rack location mat is installed on top of the spill tray.

Preparations Spill tray installed

NOTE There are cut-outs around the rack location mat. The cut-outs slot over the raised locating tabs that are molded into the spill tray to ensure proper alignment (see Figure 20 on page 90).

1 Position the rack location mat over the spill tray and press the mat onto the locating tabs.

2 Check that the rack location mat fits firmly in place by trying to move it side to side. There should not be any movement. If the rack location mat is loose, refit it.

Installing the Probe and Tubing

The sample probe comes preassembled with 0.050 cm (0.020 in) ID tubing. There are three additional coils of tubing included in the probe kit.

- The longer piece of tubing with a nut and ferrule is used for the drain tube on the divert valve.
- The shorter tubing with a nut and ferrule is used to connect fraction collector divert valve to the sample output from the HPLC system.
- The third piece of tubing is 0.025 cm (0.010 in) ID tubing and can be used for low delay volumes for applications with flow rates of 2 mL/min or less.

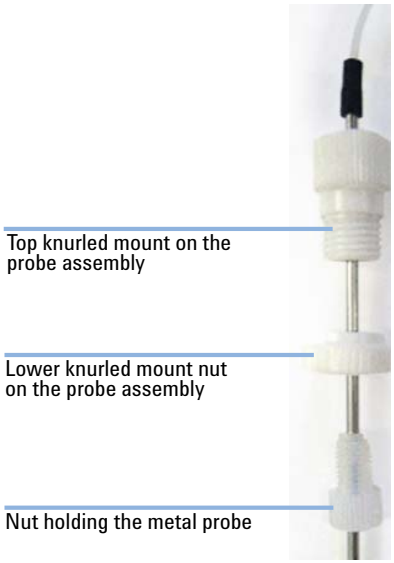
For details on installing the tubing, refer to Agilent 440 Fraction Collector - User Manual (G9340-90000).

1 Turn off the fraction collector.

2 Slide the Z-axis slide to the top of the probe carriage.

3 Manually rotate the probe arm so that it can be easily accessed.

4 Disassemble the probe kit by unscrewing the nut holding the metal probe from the bottom of the lower knurled mount nut and then the lower knurled mount nut from the top knurled mount nut.



5 Insert the probe into the probe mounting block (see Figure 18 on page 83).

6 Push the tubing down into the probe so that at least 0.5 cm tubing protrudes from the end of the probe.

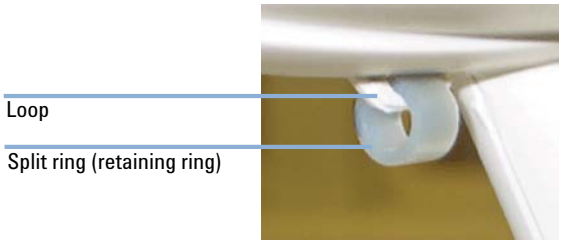
7 Secure the lower knurled mount nut to the top knurled mount nut.

8 Secure the nut holding the metal probe into the bottom of the lower knurled mount nut.

9 Clip the tubing into the tube restraint which is the small hook at the top of the Z-axis slider. Allow for a small length in the tubing to prevent kinking the tubing.

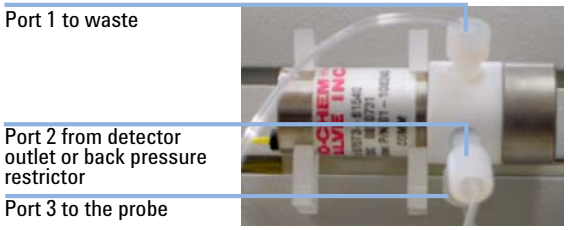
10 Run the tubing along the back side of the probe arm housing.

11 Attach the split ring (also called the retaining ring) as shown below. The retaining ring is split to attach it through the loop on the underside of the probe arm housing.



12 Feed the tubing through the retaining loop attached to the underside of the housing.

13 Screw the tubing fitting into the bottom (port 3) of the valve.



NOTE

It is important to ensure that the length of the tubing from the probe to the valve allows the probe arm to move freely in all axes. If it is too tight, it will restrict movement and may cause movement failure. If it is too loose, the probe arm may become tangled in the tubing as it moves. Tubing length between the valve and the detector depends on fraction collector placement. When choosing the length of tubing ensure it is long enough to allow free movement of the arm but not too long to delay sample delivery into the tubes. If the tubing is too long between the detector output and the fraction collector probe end, your samples may not correspond correctly to the fraction collector markers on your chromatogram. The delay volume calculation will compensate for this effect.

14 Move the probe arm to the front-right side of the fraction collector and rotate the probe arm fully to the right to check that the tubing is completely free to move.

15 Manually position the probe arm in the middle along the X axis.

16 Rotate the probe arm through its full extent to check that the tubing is free to allow full movement in all directions.

NOTE If the length is not correct, remove and then replace the tubing, install the fittings and tubing onto the fraction collector. Repeat step 14 on page 88 - step 16 on page 88 to test the tubing length

17 Adjust the length of the sample line from the HPLC system. In port 2 (see Figure on page 88) screw in the fitting with ETFE tubing. Connect the other end of this tubing to the HPLC system's detector outlet or to the back pressure restrictor if one is present.

NOTE This tubing should be as short as possible.

18 In port 1 (see Figure on page 88) screw in the fitting with PTFE tubing. Put the other end of the tubing into your waste container.

Assembling the Sample Racks

CAUTION Loss of sample

If a tube sits at an angle in the rack, the probe may hit the side of the tube as it enters or leaves the tube. This can affect the alignment of the probe and/or damage the tube.

➔ Ensure that the tubes sit vertically within the sample rack.

1 Follow the manufacturer's instructions to assemble the sample racks.

2 If needed: Insert the overlay by placing the overlay over the top of the rack.

Installing the Sample Racks

1 *Standard racks:*

- Fit the pegs on the rack into the holes on the rack location mat. The rack closest to the pillar of the fraction collector is considered rack number one.

OR

Autosampler rack (Type 128 or Type 200):

- Slide the extended portion on the bottom of the rack into the long slit on the rack location mat.

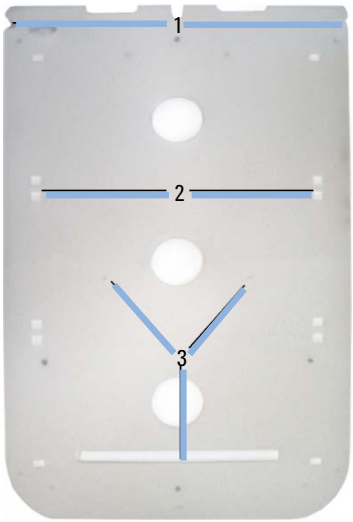


Figure 20 Rack location mat

1	Cut-outs on the rack location mat
2	Holes for regular tube racks
3	Holes for the autosampler rack

Rack Orientation

1 Place the rack in the correct orientation to get your samples in the desired order.

The following image shows an example of where the first sample is delivered to and the orientation of the regular racks provided with the fraction collector.

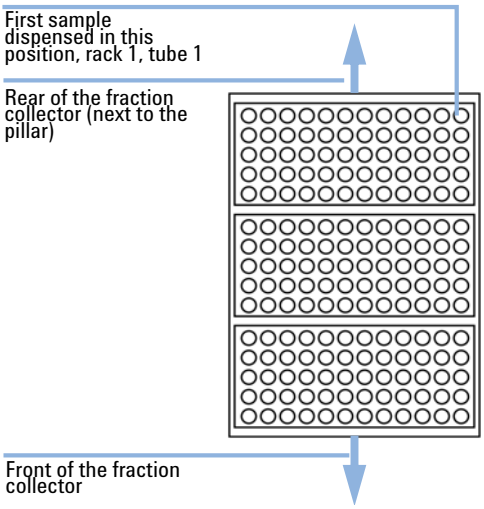


Figure 21 Tube positions on the fraction collector

Connecting the Power to the Fraction Collector

Connecting the Power to the Fraction Collector

The connection panel of the Agilent 440 Fraction Collector (G9340A) is located on the pillar (also called a control column). The panel contains an I/O port, indicators, DIP switches, the power socket and switch, and a RS 232 port for communication between the Agilent 440 Fraction Collector (G9340A) and the system (or computer) that is running instrument control software.

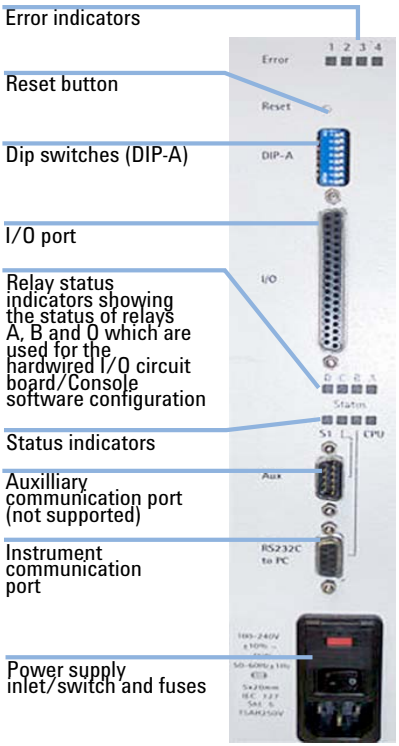


Figure 22 Connection panel

Prerequisites Three power cables are supplied with the module. Select the correct one for your location.

WARNING

Electrical shock

Electrical power for the module must be provided through a three wire outlet with ground connection. The outlet must be rated for at least 75 VA.

➔ Ensure that power receptacles are earth-grounded at the grounding pin.

NOTE

Ensure that the probe arm's movement is not interfered with during operation/initialization.

1 Ensure that the power switch is turned off (0).

2 Plug one end of the power cable into the module (both the switch and the power socket are located on the connection panel on the pillar) and the other into the mains power outlet.

NOTE

In some countries, it may be necessary to fit a suitable three pin power plug to the cord. A three pin earthed power outlet must be used.

Ensure the module is always connected to the mains supply protective earth.

3 Turn on the module.

The module will go through initialization tests and set the probe position. If the initialization process is not successful, refer to chapter Troubleshooting and Diagnostics in Agilent 440 Fraction Collector - User Manual (G9340-90000).

If the fraction collector does not start up, check each fuse as described in chapter maintenance in Agilent 440 Fraction Collector - User Manual (G9340-90000).

During the initialization sequence:

1 The probe rises to the full extreme of the Z axis.

2 The probe travels to the full extremes of the X axis and rotates to the full extremes of the Theta axis.

3 Then the probe is positioned at the front-left of the fraction collector.

Instrument Communication Port

The instrument communication port, see Figure 22 on page 92, is used to connect the Agilent 440 Fraction Collector (G9340A) to the computer or MIB controlling the instrument.

Auxiliary Communication Port

The auxiliary communications port provided on the Agilent 440 Fraction Collector (G9340A) is not used with current Agilent software or hardware.

Connecting the Fraction Collector to the HPLC System

For details on setting up the fraction collector to the HPLC System, see "Setup the System with OpenLAB CDS ChemStation Edition - Control Panel" on page 102.

Determining the Delay Time

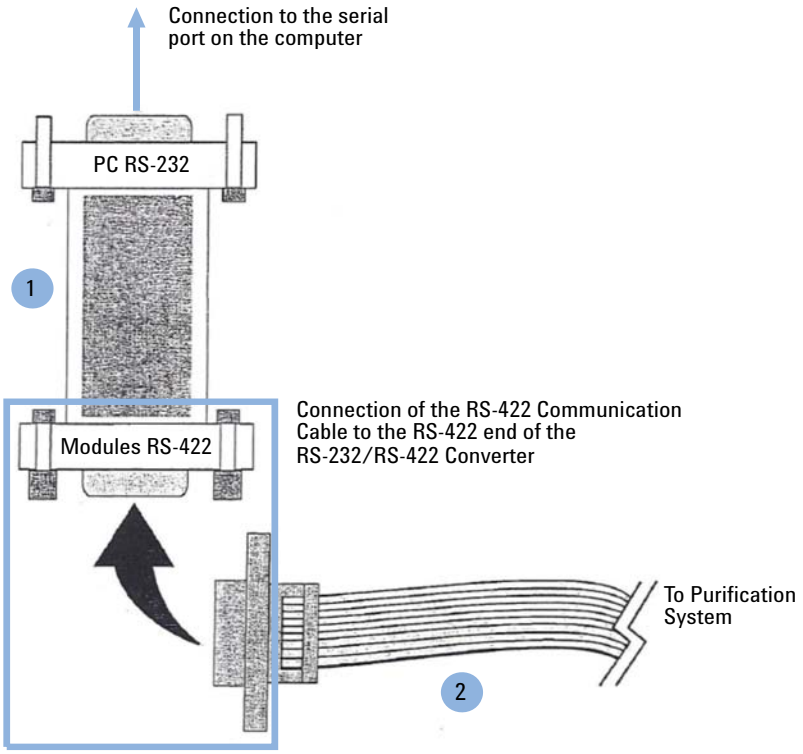
1 For information refer to the online help of the Agilent 440 Fraction Collector.

Determining the Correct Probe Depth

1 For information refer to the online help of the Agilent 440 Fraction Collector.

Installing the RS-422/485 Communication Kit

1 Connect the Serial extension cable (R000815107) to the end of RS-422 connector on the Converter RS-232 to RS-422 (393597601).



1	Converter RS-232 to RS-422 (393597601)
2	Serial extension cable (R000815107)

2 Connect the 9-pin "D"-shell connector on the RS-232 end of the Converter RS-232 to RS-422 (393597601) to a serial communication port on the computer.

NOTE The screws on the RS-232 end of the RS-232/RS-422 Converter should be screwed into the nuts on the computer 9-pin "D"-shell serial port connector.

Installing the Stream Splitter

Agilent Stream Splitters are designed for preparative HPLC applications with flow rates up to 1200 mL/min to split stream between preparative HPLC detector and an analytical detector. If modules in the flow pass are not capable of the pressure, the stream splitter must be installed. PCG426000001 is designed for flow rates up to 200 mL/min. It uses 1/16 inch connection fittings (PTFE tubing). PCG426000002 is designed for higher flow rates up to 1200 mL/min. It uses 1/8 inch and 1/16 inch connection fittings (PTFE tubing), depending on the detector being used. Some tubing's and fitting are supplied to connect with both preparative and analytical flow cells as required.

1. Install the stream splitter as close as possible to the outlet of the UV detector, the inlet of the ELSD and the fraction collector.
2. Cut the appropriate lengths of the chosen diameter tubing.
3. Install outlet from the UV detector to the IN port on the stream splitter.
4. Connect the OUT port on the stream splitter to the fraction collector.
5. Connect To Detector port in the back of stream splitter to the ELSD detector.

Setup Hardware

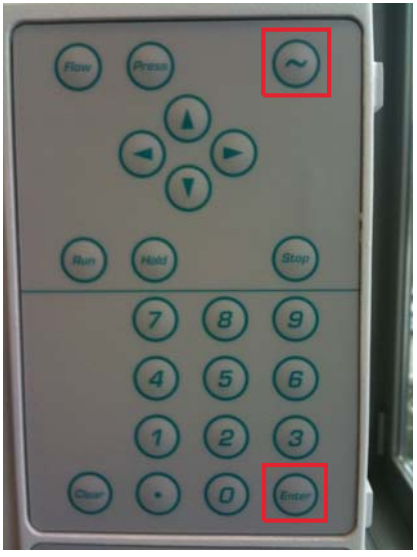
Setup the Hardware

This is done on the modules regardless of whether you are using HPLC control software or using one of the pumps as a master controller including following modules:

- Different pumps,
- 325 UV/VIS Dual WL Detector,
- 440 Fraction Collector, and
- 410 Autosampler.

Setup the Pump

1. Turn the module on.
2. Press ~.



3 Press **Enter**.

4 With the left arrow key select:

- Headsize is defined as NO
- **ID of the Pump:3** (select a different ID for every devices)

NOTE

(Available choices are: 0 – 63 , **MC** (master controller) or – – (no ID).

For OpenLAB, an ID between 1 – 63 must be used.

- **CIM: 5**

5 Press **Enter**.

6 Reboot the module.

Setup the Autosampler

Preparation for general and tray settings:

1 Press **Serial** on the autosampler keyboard.

To set up the general system settings:

1 Turn the module on.

2 Press **SYSTEM**.

3 Press **GENERAL**

4 Confirm or change each of the settings that appear on the display:

- Volume of installed loop (0 – 5000 µL)
- Volume of tubing "needle↔valve" 0 – 999 µL
- Syringe volume (250 /1000)
- Syringe speed (**LOW**/**NORMAL**/**HIGH**)
- Skip missing vials (**YES**/**NO**)
- Air segment (**YES**/**NO**)
- Headspace pressure (**YES**/**NO**)

To set up the tray settings:

1 Turn the module on.

2 Press **SYSTEM**.

3 Press **Tray**

4 Confirm each of the tray settings that appear on the display:

- Tray type (84 +3 /9 /24)
- Vial type (STANDARD/2.5 mL)

5 Reboot the module.

Setup the Detector

The detector is set up at the factory with a BOOTP (or DHCP) IP address. If need be, this can be changed by a trained service engineer who will own a copy of the diagnostics software or by setting up Agilent BootP Service to provide the detector with the right IP details.

Setup the Fraction Collector

The Fraction Collector is ready to be set up in OpenLAB and does not need to have the hardware set up.

Setup the Software

Install Agilent OpenLAB CDS ChemStation PrepLC Drivers

Parts required	p/n	Description
	M8301AA	OpenLAB CDS ChemStation Edition Workstation A.01.05 or higher
	M8500AA	M8500AA LC driver
	M8366-64000	OpenLAB CDS Installation Driver Prep LC
	M8367-64000	OpenLAB CDS Installation Upgrade Driver Prep LC

Hardware required Computer with *OpenLAB CDS ChemStation Edition* installed. For details refer to the *OpenLAB WorkStation Installation Guide*.

1 Copy AgilentDriversPrepLCChemstationSet_up.msi from CD to your computer.

2 Doubleclick on AgilentDriversPrepLCChemstationSet_up.msi and follow the steps in the setup wizard.

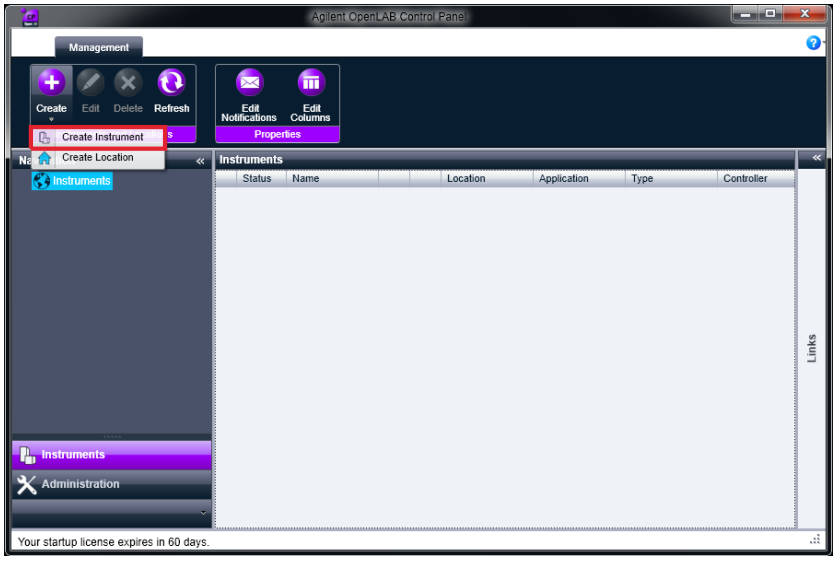
Setup the System with OpenLAB CDS ChemStation Edition - Control Panel

Preparations

- Latest driver package installed, see OpenLAB CDS Installation Driver Prep LC (M8366-64000)
- Modules are wired as described in "Cable Connections" on page 125
- All modules are setup as described in "Setup the Hardware" on page 98
- All modules are switched on

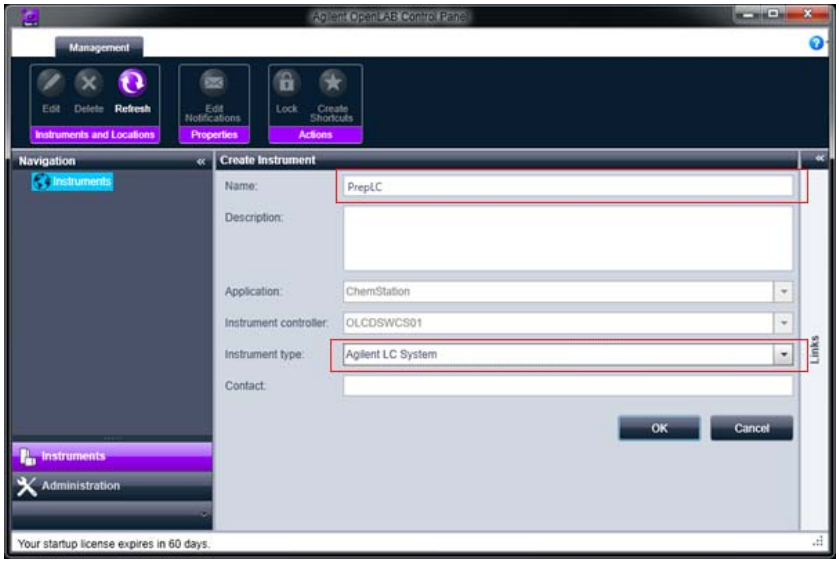
OpenLAB configuration

1 Open the **Agilent OpenLAB Control Panel** and select **Create > Create Instrument**.

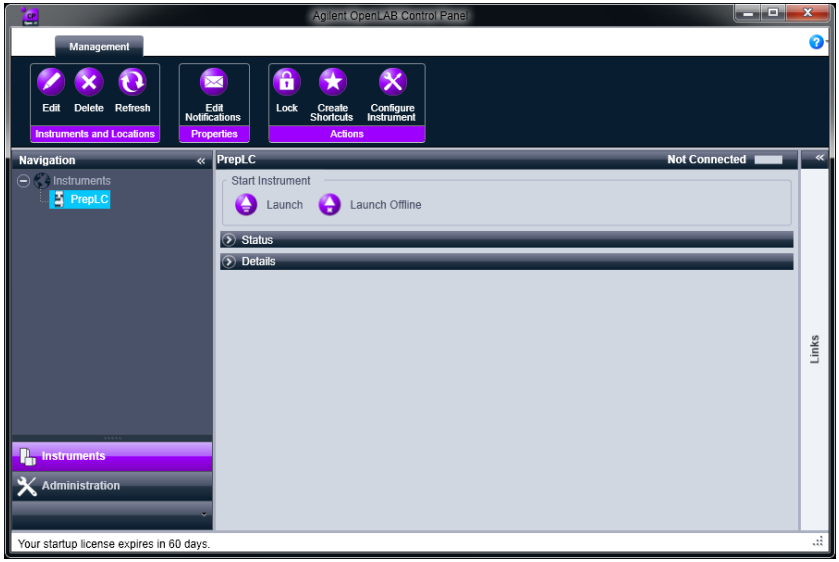


2 Define the **Name** (Example **PrepLC**).

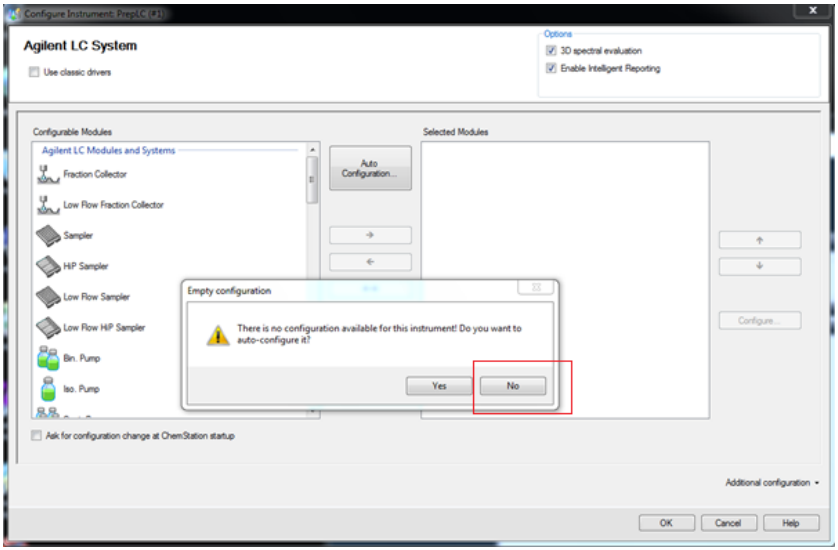
3 Select **Agilent LC System** from the **Instrument type** drop-down list.



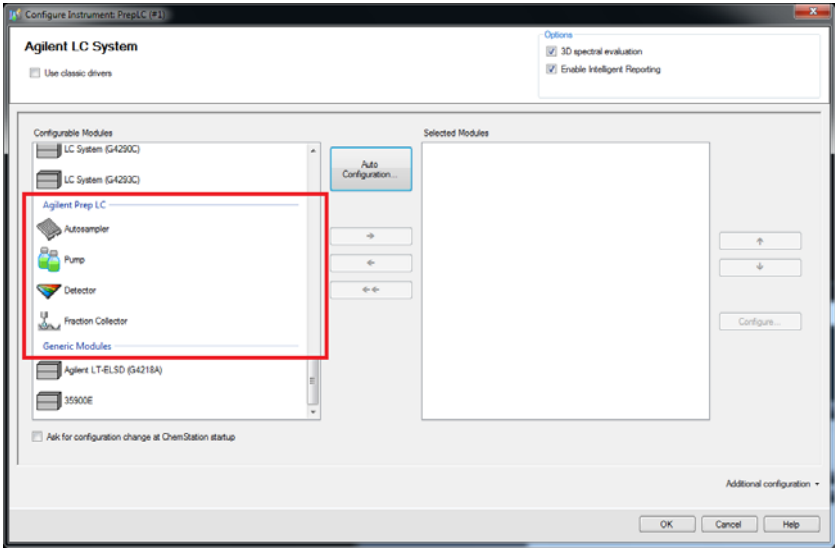
4 Select **Configure Instrument**.



5 Select **No** to avoid the auto-configuration (It doesn't work with this version).



6 The left panel of the **Configurable Modules** contains the **Agilent Prep LC**.



7 Select the module you have available in your system.

8 Click → to add the modules to the **Selected Modules**.

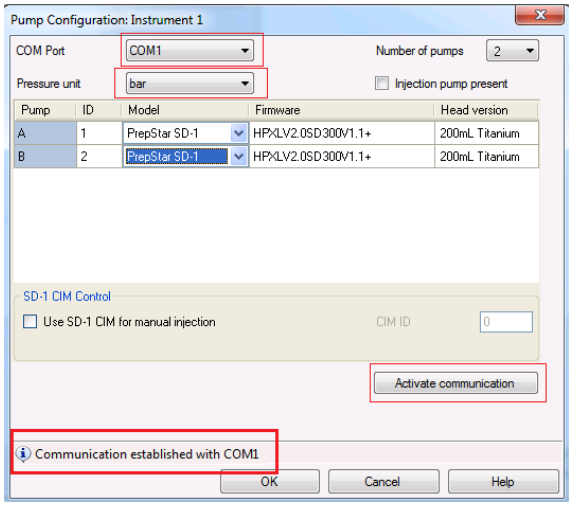
The configuration dialog for the selected module opens.

Pump Configuration

1 Doubleclick in **Configurable Modules** on Pump.

Pump Configuration dialog opens.

2 Select the COM Port as COM 1 and the desired Pressure unit bar (available kPa, PSI, bar, MPa).



3 Verify the connection by clicking on **Activate communication**.

Information **Communication established with COM1** is visible on the screen.

4 Click **OK**.

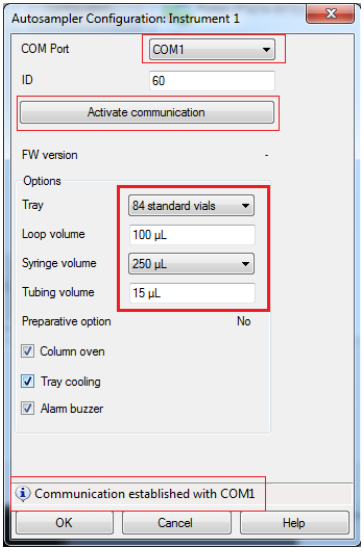
Configuration dialog closes.

Autosampler configuration

1 Doubleclick in **Configurable Modules** on Autosampler.

Autosampler Configuration dialog opens.

2 Select the **COM Port** as **COM 1**.



3 Select the **Tray84 standard vials** as an example.

4 Double check your actual configured Tray.

5 Check the other variables present in the **Autosampler Configuration** screen.

6 Verify the connection by clicking on **Activate communication**.

Information **Communication established with COM1** is visible on the screen.

7 Click **OK**.

Configuration dialog closes.

Detector Configuration

1 Doubleclick in **Configurable Modules** on Detector.

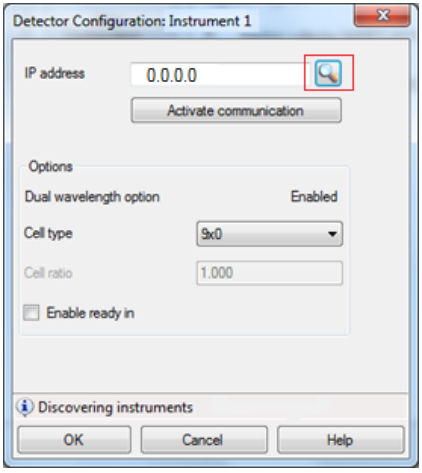
Detector Configuration dialog opens.

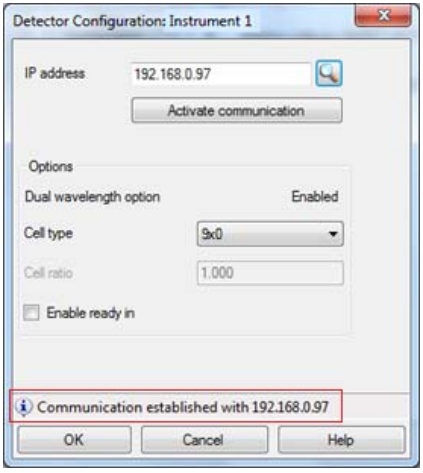
2 Select the Browse function  to start discovery your Detector

The IP address will be populated by the searching results.

Example:

IP address 192.168.0.97





3 Verify the connection by clicking on **Activate communication**.

Information **Communication established with COM1** is visible on the screen.

4 Click **OK**.

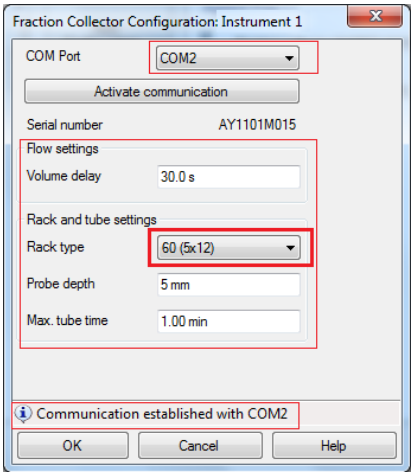
Configuration dialog closes.

Fraction Collector Configuration

1 Doubleclick in **Configurable Modules** on Fraction Detector.

Fraction Collector Configuration dialog opens.

2 Select the **COM Port** as **COM 2**



3 Fill in the **Flow settings** and the **Rack and tube settings**.

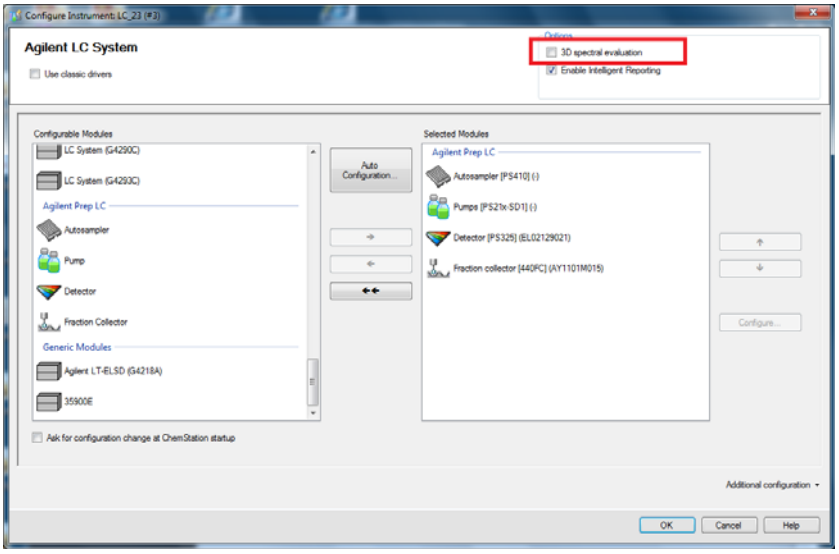
4 Verify the connection by clicking on **Activate communication**.

5 Verify the connection by clicking on **Activate communication**.

Information **Communication established with COM2** is visible on the screen.

Terminating configuration

1 Before closing the **Configure Instrument** windows deselect the **3D spectral evaluation** check box.

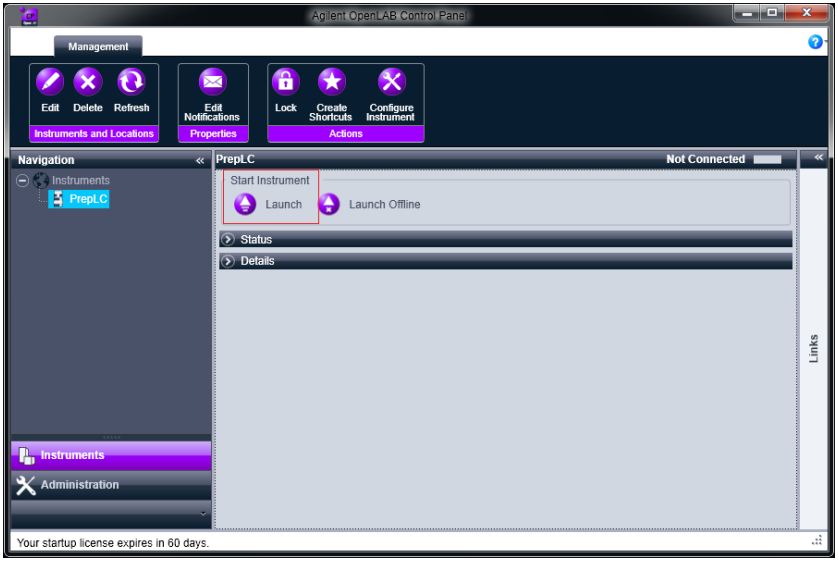


2 Click **OK**.

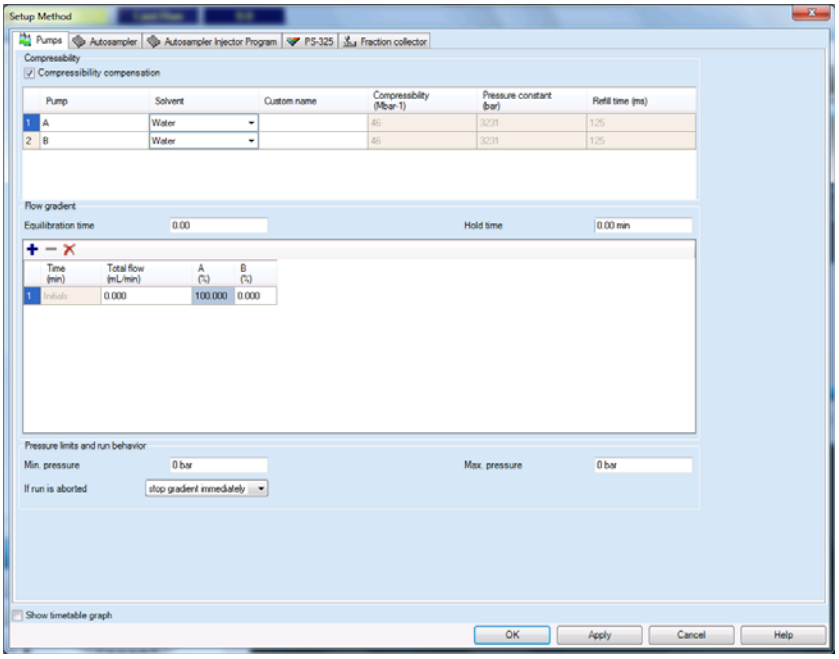
You're back in Agilent OpenLAB Control Panel.

Setup Method

1 Select **Launch** to start the OpenLab CDS Chemstation Edition.

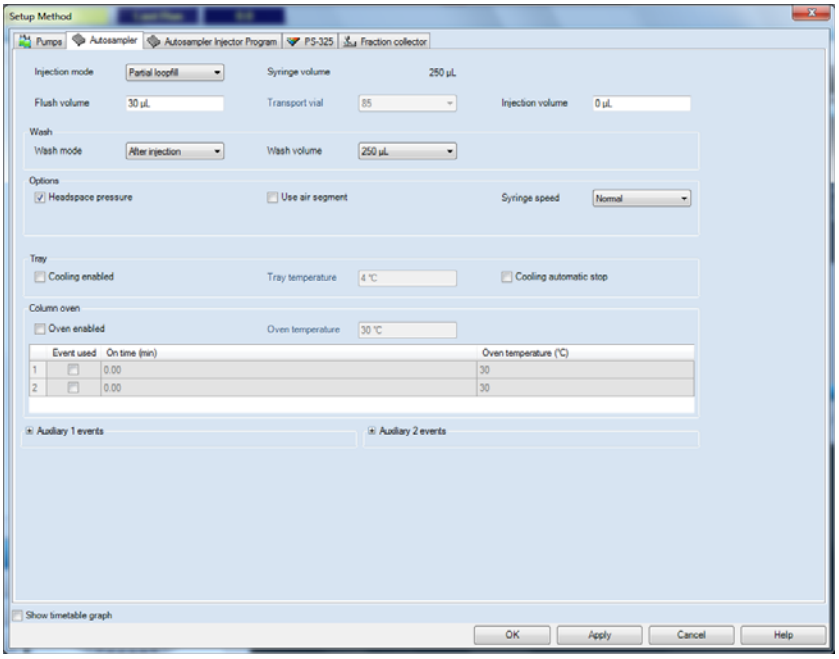


2 Set **Pumps** parameters.



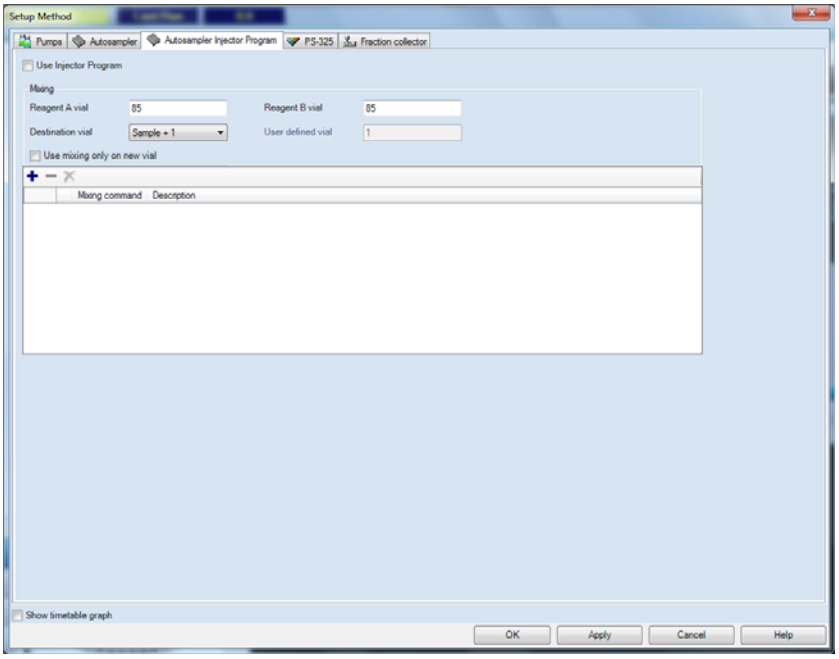
3 Click **OK** to confirm method.

4 Set **Autosampler** parameters.



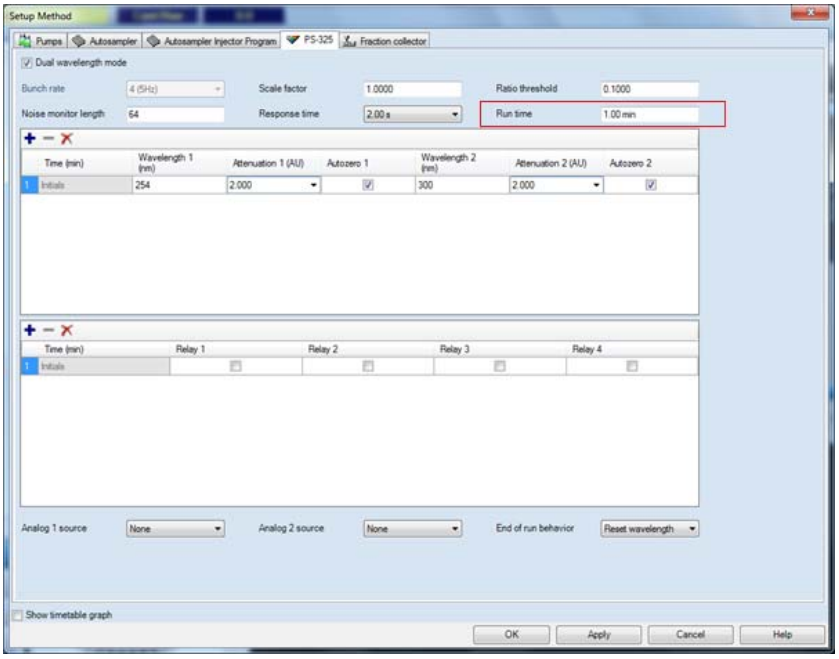
5 Click **OK** to confirm method.

6 Set **Autosampler Injector Program**.



7 Click **OK** to confirm method.

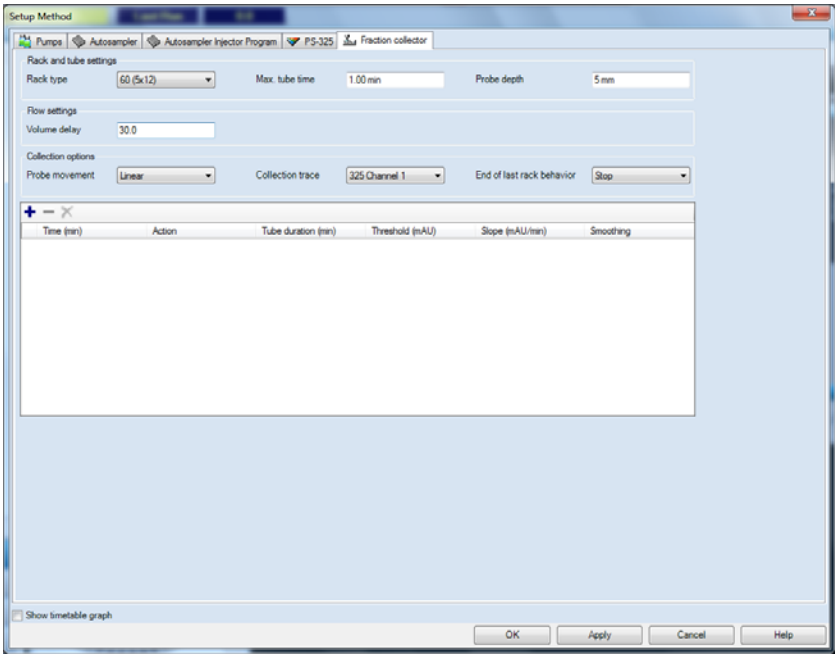
8 Set **PS-325** parameters.



NOTE The **Run time** must be defined to the detector parameters.

9 Click **OK** to confirm method.

10 Set **Fraction collector**.



11 Click **OK** to confirm method.

Setup is completed.

Setup the CIM in the OpenLAB CDS ChemStation PrepLC Drivers

The Control Interface Module (CIM) in the OpenLAB CDS ChemStation PrepLC Drivers can be used for acquiring signals from extern detectors. If 218/SD-1 and 1260 modules are connected via remote cable, error messages will be properly relayed. With the remote cable it is also possible to run 218/SD-1 preparative HPLC system with any Agilent 1200 Infinity Series detector using the CIM as trigger source for fractionation. For this analog input interface one or two 218/SD-1 pump modules can be mixed.

The CIM offers different features:

- from the ability to configure the module,
- to establish communication with the module and re-establish in case of communication loss,
- to edit the CIM detector method,
- to perform a run, and acquire data,
- to view the CIM status in the RC.NET Status Dashboard,
- to activate the CIM relays manually,
- to monitor the CIM signal to fractionate, and
- to detect the 440 Fraction Collector peaks on the CIM signal.

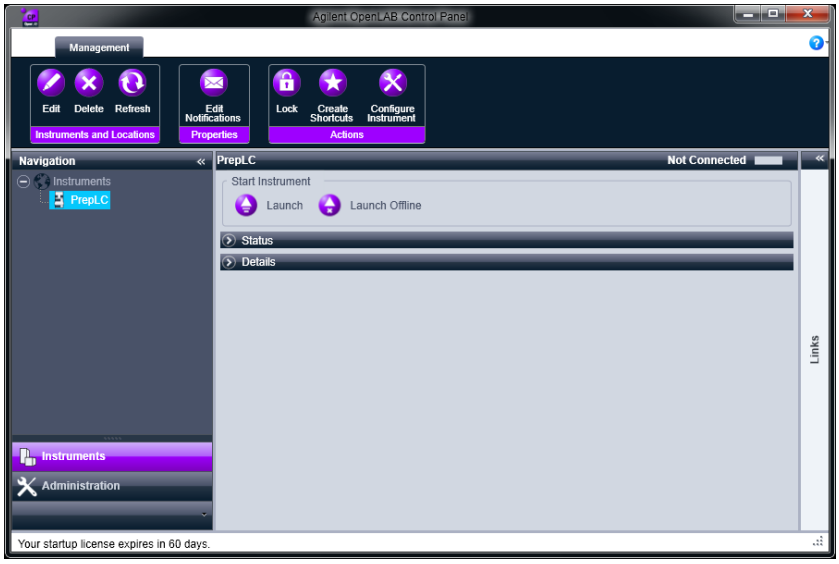
Parts required

p/n	Description
5062-3378	Remote cable

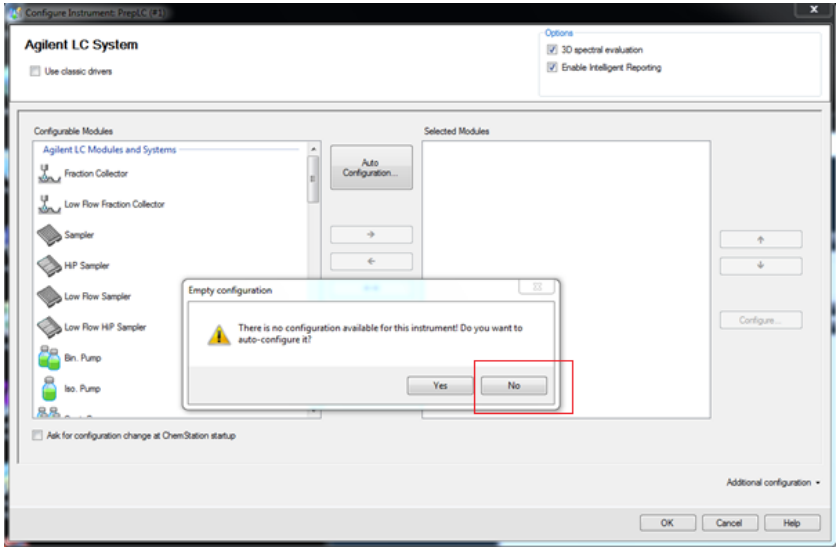
Preparations

- OpenLab CDS Chemstation Control Panel is configured as described in "Setup the CIM in the OpenLAB CDS ChemStation PrepLC Drivers" on page 116

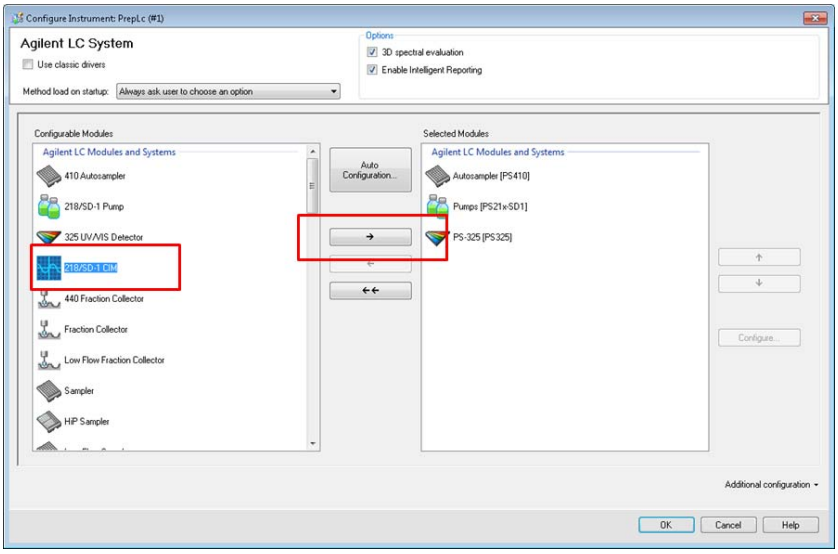
1 Select **Configure Instrument**.



2 Select **No** to avoid the auto-configuration (It doesn't work with this version).



3 Select the **218/SD-1 CIM** on the left panel of the **Configurable Modules**.



4 Click → to add the modules to the **Selected Modules**.

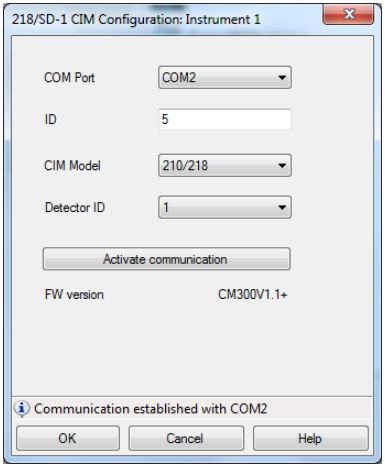
The configuration dialog for the selected module opens.

5 Doubleclick in **Configurable Modules** on **218/SD-1 CIM**.

218/SD-1 CIM Configuration dialog opens.

6 Select the **COM Port**, the **CIM Model**, **Detector ID** and enter the **ID** value.

7 Verify the connection by clicking on **Activate communication**.



Information **Communication established with COMn** is visible on the screen.

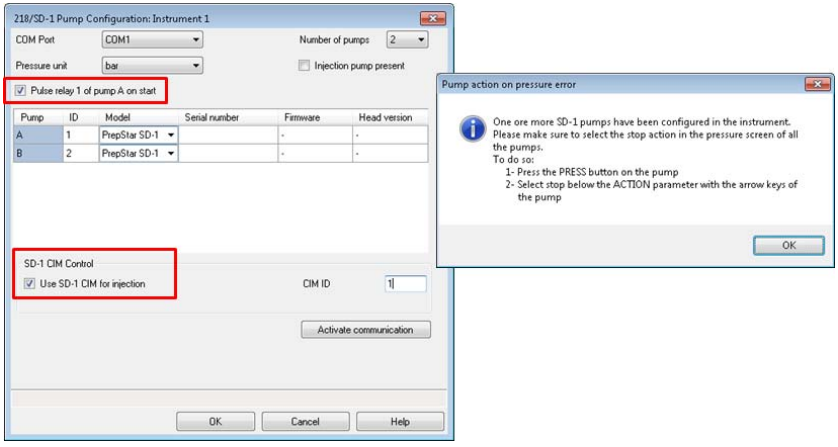
8 Click **OK**.

Configuration dialog closes.

9 Doubleclick in **Configurable Modules** on **218/SD-1 Pump**.

218/SD-1 Pump Configuration dialog opens.

10 Select the check box **Pulse relay 1 of pump A on start**.



The CIM is able to send a start or stop pulse.

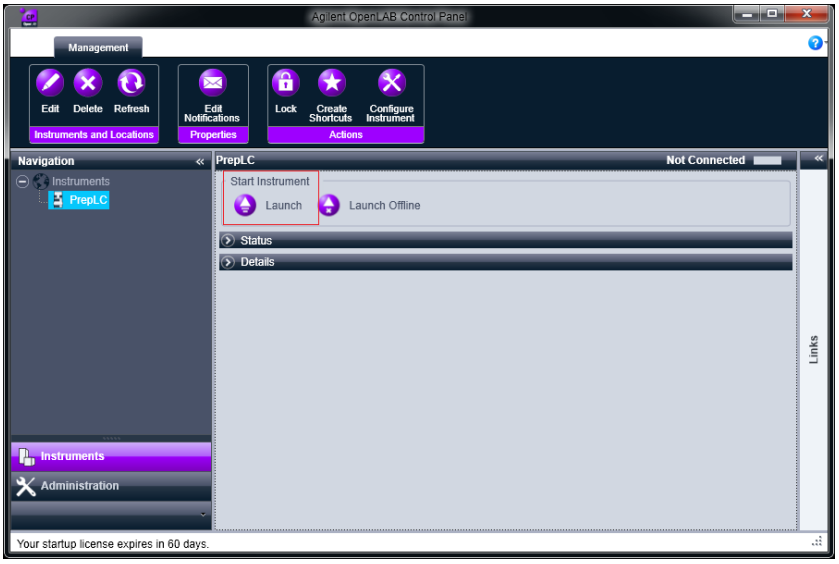
11 Click **OK**.

Configuration dialog closes.

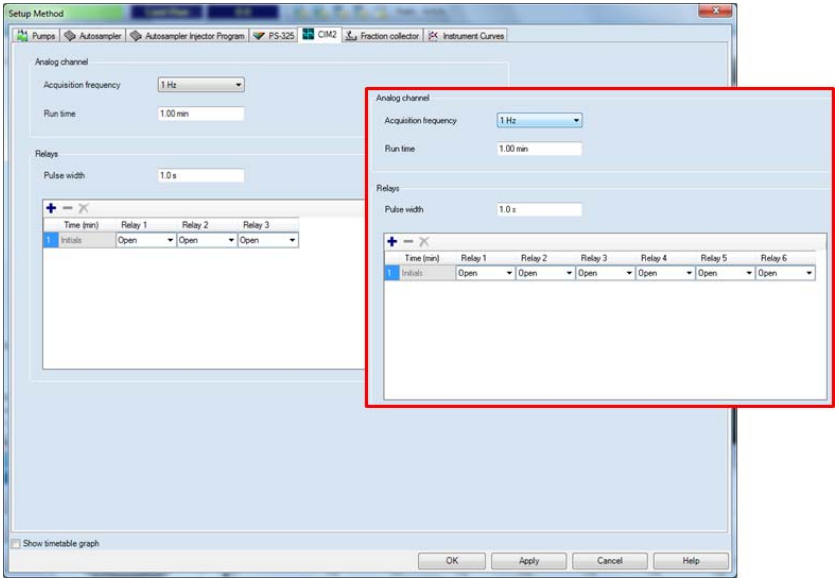
12 Click **OK**.

You're back in Agilent OpenLAB Control Panel.

1 Select **Launch** to start the OpenLab CDS Chemstation Edition.



2 Set **CIM** parameters.



3 Click **OK** to confirm method.

4
Using, Troubleshooting, Maintenance and Parts

This chapter provides information on how to access further details on the system components.

Using, Troubleshooting, Maintenance and Parts

For details on using, troubleshooting, maintenance and necessary parts for the individual modules, please refer to the according manuals:

- Agilent SD-1 Isocratic Solvent Delivery Module - User Manual (G9302-90001)
- Agilent 325 UV/VIS Dual Wavelength Detector - User Manual (G9309-90000)
- Agilent 410 Autosampler - User Manual (G9331-90000)
- Agilent 440 Fraction Collector - User Manual (G9340-90000)
- Agilent 218 Solvent Delivery Module - User Manual (G9300-90001)

5
Cables

This chapter provides information on cables used with the instrument.

Cable Overview

Necessary cables

p/n	Description	
	392612901	Ethernet cable (for use in a *network*)
OR	5023-0203	Ethernet cable (cross-over, for *standalone* use)
	392607969	Inject marker cable
	392607975	Next injection cable
	393546291	Serial communication ribbon
	393597601	Converter RS-232 to RS-422
	7910046300	Serial cable

Optional cables

p/n	Description
110743800	Relay interface cable (for relay interface board, one relay contact per cable)
110744200	Analog signal cable

Cable Connections

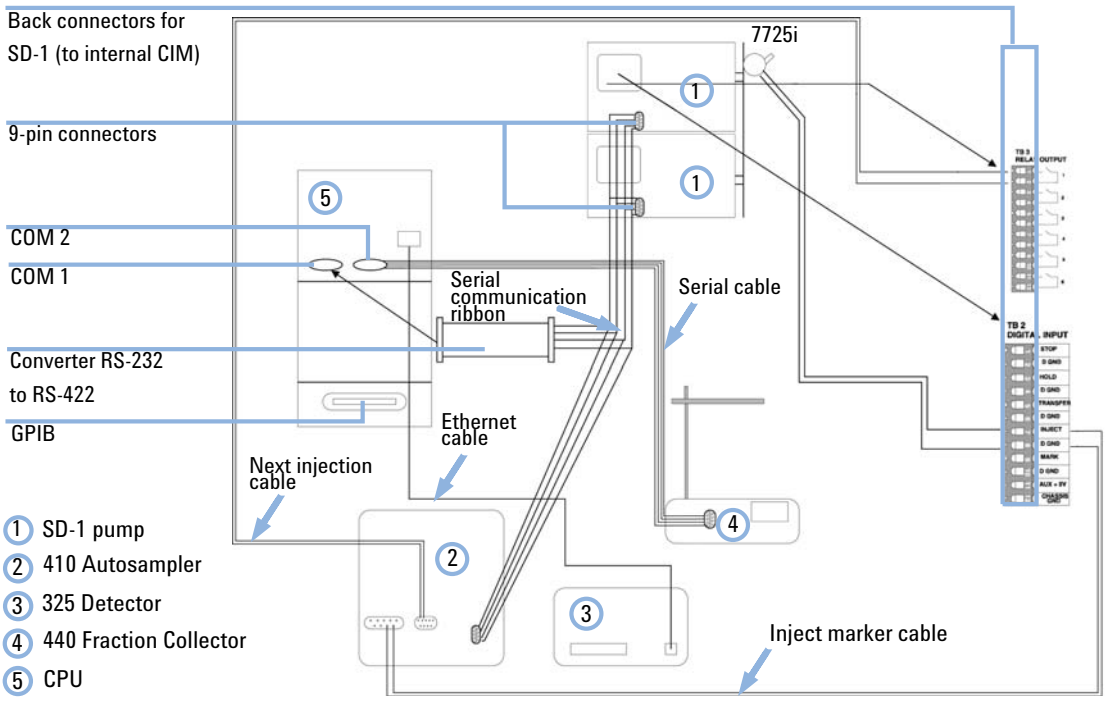


Figure 23 Cable connections for workstation control of Agilent SD-1 Solvent Delivery Modules, Agilent 325 Detector, Agilent 410 Autosampler and Agilent 440 Fraction Collector

Analog Output

For analog output signals, install the optional Analog signal cable (110744200) into the J14 receptacle. Pin designations are shown below.

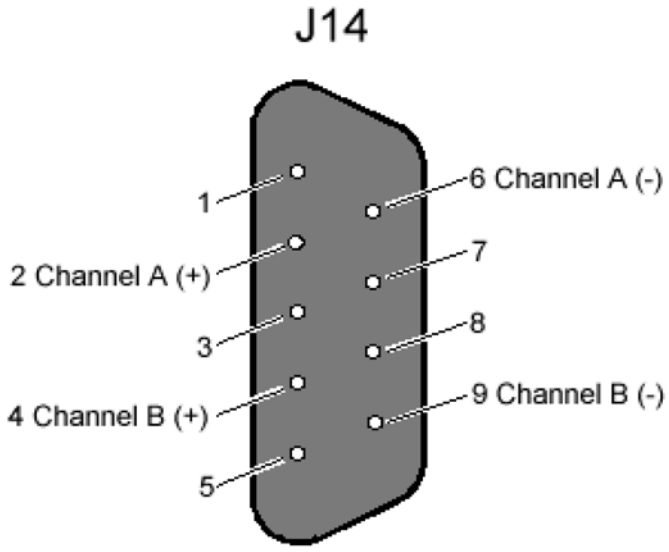


Figure 24 Pin designation for J14

The open ends of the analog output cable have labels with the signal names (Channel A +, Channel A - and Channel B +, Channel B -).

Relay Output

For time programming external events, a contact closure Relay output is available. To configure the Relay output, install the optional Assy PWB relay interface 325 (210187590) into the J4 receptacle. Pin designations are shown below.

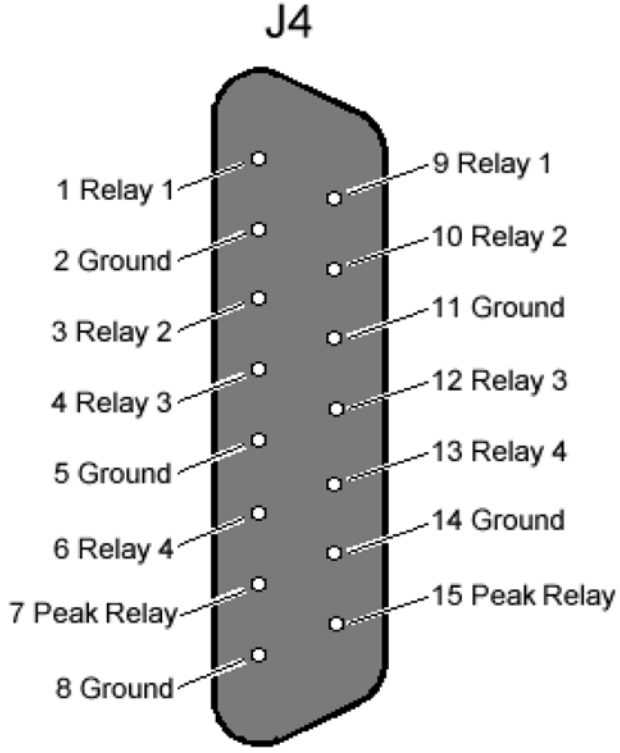


Figure 25 Pin designation for J4

There are four general purpose output relays and one dedicated Peak relay. Each output uses a DIP relay that is capable of handling 500 mA of contact current. At reset or power up, the output relay contacts are set to the default parameters (open). After loading a method they will be set as defined in the method's **time=0 parameters**.

The Peak relay is software programmable for duration, delay and active sense. At power up, the relay contact will be set to the inactive state (as defined by the value of the **active sense** parameter stored in the detector). Upon being triggered, relay activation will occur for the time interval equal to the **Peak Sense duration** parameter as stored in the method.

The Peak relay can be activated from any of the following sources (only one source can be active at any one time):

- Time Slice event – Once time slice has been turned on, it will provide a periodic activation of the Peak Sense relay at an interval defined in **Time Slice period** within the method. Time Slice can be turned on and off by time.
- Pulse event – A single timed programmed activation of the Peak Sense relay as defined in the method.
- Peak sense has been turned on.

To connect open-ended wires to the relay signals available at J4, use the optional Assy PWB relay interface 325 (210187590) (see Figure 26 on page 128). Simply plug this PWB into the rear panel connector.

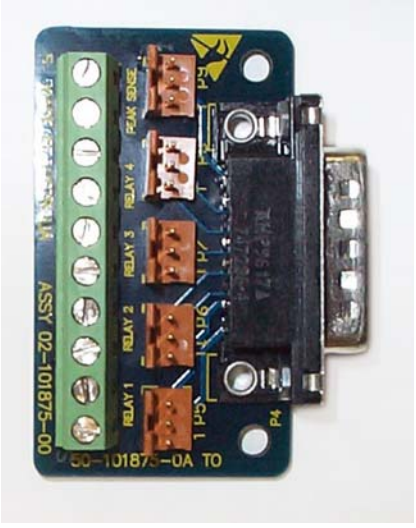


Figure 26 Relay output board

The Relay interface cable (for relay interface board, one relay contact per cable) (110743800) is used to attach to the 3 pin connectors at the relay output board.

With the 3 pin connectors, the contact closure is between pins 1 and 2 of the plugs. Pin 3 is connected to ground. The relay interface cable has three open-ended wires. The relay contact is connected between the clear and the black wire. The green wire is connected to ground.

Desktop PC Communications

Communication between the detector and a desktop PC occurs by an Ethernet connection. Communication by an Ethernet connection is required to control the detector remotely by OpenLAB. When the Workstation provides HPLC system control, the synchronization cables from P9 and J10 are not used.

To create an Ethernet connection, insert an RJ45 cable included in the ship kit into the J1 receptacle and into the PC. The Ethernet cable that comes with the detector is a cross-over cable, which is appropriate for connecting the detector directly to a PC. Connecting the detector to a network or a hub will usually require a patch cable. A Ethernet cable (for use in a network) (392612901) can be purchased from Agilent or either locally.

Most PCs come pre-configured with an Ethernet connection, which is usually built into the motherboard, or with an Ethernet network card installed. However, if you have a PC that has no network interface, you will need to install and configure a Network Interface Card (PCI bus). The PC must have a spare PCI slot for the installation of this device. You are also responsible for setting up and maintaining any LAN configuration where a detector may be used. All network issues are to be dealt with by the user.

Synchronization Signals

The synchronization signals at P9 and J10 are used to synchronize the operation of a group of instruments that are not interfaced to OpenLAB. The synchronization signals come in four pairs and define how the detector will operate in a HPLC system. These signals are important for controlling timing and synchronization of the detector with the other devices in the system. Synchronization signals are closely tied to the detector states and transitions. P9 and J10 pin designations are shown in Figure 27 on page 131.

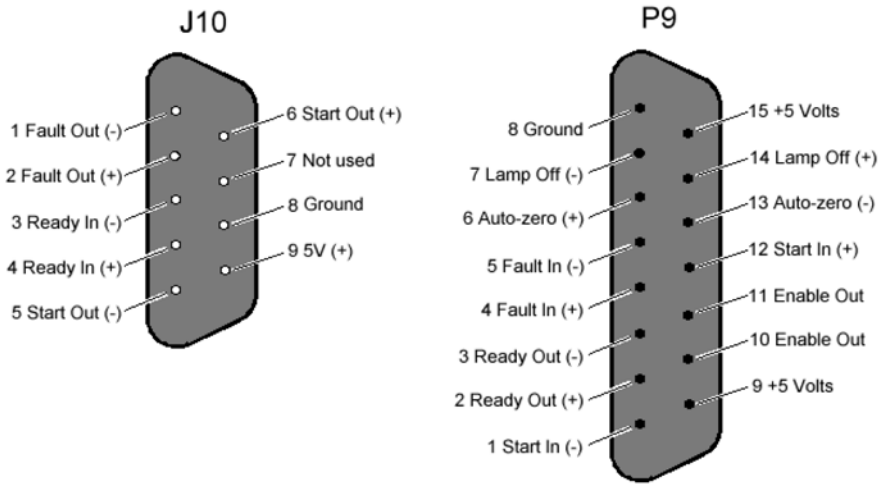


Figure 27 Pin designations for J10 and P9

P9 provides connections when the Agilent 325 UV/VIS Dual Wavelength Detector acts as a “slave” and receives control from another module. J10 provides connections when the detector acts as a “master” and sends control to another device.

An input is activated or said to be present when its two signal wires are connected together. This can be done with a relay contact closure. If the inputs are driven from another instrument with optical isolators or other polarized devices, then attention must be paid to the polarity of the signal wire

connections. The positive (+) output signal must be connected to the positive (+) input signal and the negative (-) output to the negative (-) input.

The color coding and physical design of the cable connectors ensure that correct signals and polarity are matched.

The outputs are optical isolators and simulate a relay contact closure when they are activated (see Figure 28 on page 132). The minimum requirement for an input signal to be detected is 200 ms.

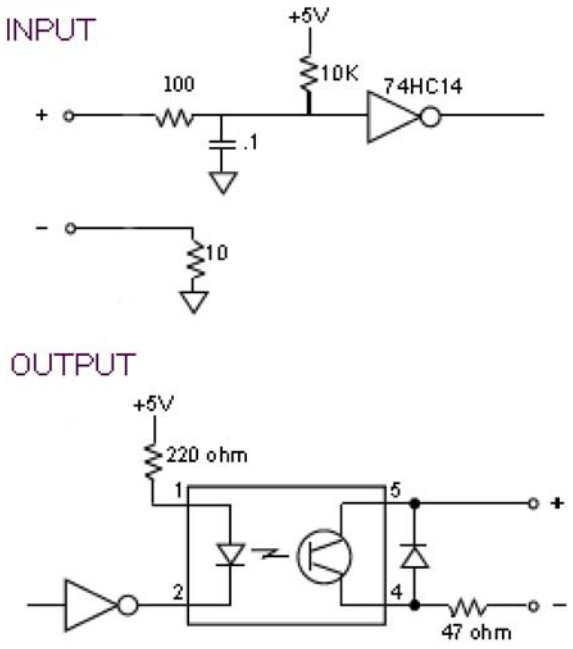


Figure 28 Input/output schematics

Table 7 Signal Description J10 and P9

Signal	Description
Enable Out	A non-polarized constantly active output (a short). This output can be used to activate Enable In on the next instrument.
Ready In	When Enable Ready In is set (software switch), this polarized input signal must be present before the Agilent 325 Detector can go to the Ready state. Specifically, when the Agilent 325 Detector is in the NOT Ready Lamp On state, on receiving a Ready In signal, a monitor period will occur after which the Agilent 325 Detector goes to the Ready state. It must stay active until the Agilent 325 Detector starts. Ready In will be ignored in all other states.
Ready Out	This polarized output signal indicates that the Agilent 325 Detector is in the Ready state and is ready to start a time program.
Start In	This polarized edge triggered input signal will start the active method if the Agilent 325 Detector is in the Ready state.
Start Out	This polarized output signal will be activated for 600 ms when the Agilent 325 Detector starts a time program.
Fault In	This polarized edge triggered input signal informs the Agilent 325 Detector that a fault condition exists in another instrument in the system. The Agilent 325 Detector halts the time program and sends a Fault Out signal. The lamp can be programmed to either remain on or turn off upon receiving a fault signal.
Fault Out	This polarized output signal will activate for 600 ms when either of the following conditions occurs: • The Agilent 325 Detector discovers an internal fault condition that warrants aborting the run. • The Agilent 325 Detector receives a Fault In signal and it has no internal fault condition itself.
Auto-zero	This edge-triggered contact closure causes an auto-zero adjustment.
Lamp off	This edge-triggered contact closure switches the lamp off. It is possible to turn the lamp back on manually if the contact is still closed.

An optional Assy PWB sync. interface 325 (210186590) is available to interface between the Agilent 325 UV/VIS Dual Wavelength Detector synchronization signals and other devices. This board is inserted into the P9 and J10 connectors and connects to a terminal strip on the adapter board. This terminal strip accepts bare wire leads from cables connecting other devices. These cables may originate from the other device, or a dedicated cable can be used, if available for the particular application.

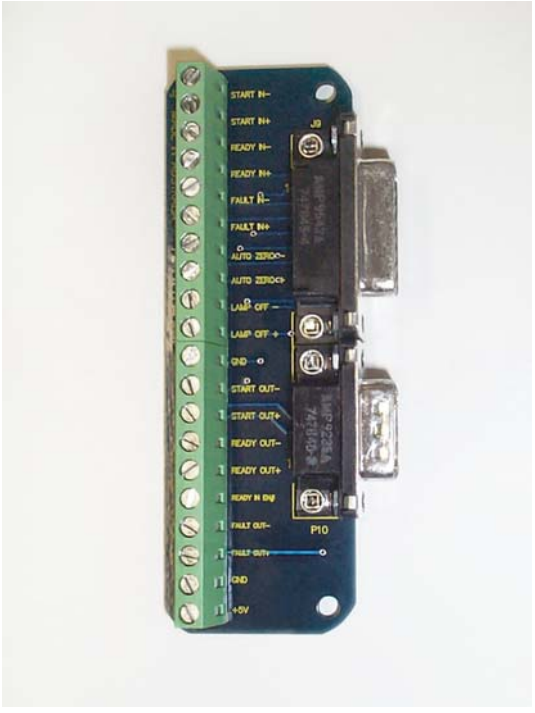


Figure 29 The I/O adapter board

6
Appendix

This chapter provides addition information on safety, legal and web.

General Safety Information

General Safety Information

The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. Agilent Technologies assumes no liability for the customer's failure to comply with these requirements.

WARNING

Ensure the proper usage of the equipment.

The protection provided by the equipment may be impaired.

➔ The operator of this instrument is advised to use the equipment in a manner as specified in this manual.

Information Symbols

The following is a list of symbols that appear with warnings in this manual or on the liquid chromatograph. The hazard they describe is also shown.

A triangular symbol indicates a warning. The meanings of the symbols that may appear alongside warnings in the documentation or on the instrument itself are as follows:

Table 8 Warning symbols

 Broken glass	 Chemical hazard	 Electrical shock
 Explosion hazard	 Eye hazard	 Fire hazard
 Heavy weight (danger to feet)	 Heavy weight (danger to hands)	 Hot surface
 Moving parts	 Respiratory hazard	 Attention[1]

[1] The symbol may be used on warning labels attached to the instrument. When you see this symbol, refer to the relevant operation or service manual for the correct procedure referred to by that warning label.

Table 9 Information symbols

Symbol	Description
I	Mains power on
o	Mains power off
	Fuse
	Single phase alternating current
	Direct current
	Confirms that the product complies with the requirements of all applicable European Community directives

Solvent Hazards

WARNING

Explosion, fire, asphyxiation

This instrument is not explosion-proof.

Certain solvents may cause weakening and leaks of tubings or fitthings with possible bursting.

Even small leaks in solvent supply systems can be dangerous.

- ➔ Only use solvents compatible with the HPLC system tubings and fittings.
- ➔ Employ static measuring and static discharge devices to safeguard against the buildup of static electricity.
- ➔ In unattended operation, do not use organic solvents having an ignition point below 70 °C.
- ➔ Do not bring a heat or flame source near the instrument.
- ➔ The area in which solvents are stored and the area surrounding the instrument must be adequately ventilated to prevent accumulations of gas.
- ➔ Always check the condition of the instrument (leakage of solvent or waste solution, leakage of solvent inside the instrument). If an abnormality is found, stop operation immediately.
- ➔ When using flammable chemicals, be careful about possible ignition due to static electricity. To prevent the build-up of static electricity, use a conductive container for waste.
- ➔ Use only approved regulator and hose connectors (refer to the supplier's instructions).
- ➔ Keep solvents cool and properly labeled. Ensure that you have the correct solvent before connecting it to the instrument.

WARNING

Inflammation or injury due to toxic, corrosive or stimulative solvent

- ➔ Do not contact toxic, corrosive or stimulative solvent.
- ➔ For details of the properties of each solvent and how to handle it, refer to the relevant Material Safety Data Sheets (MSDS).
- ➔ Be sure to handle each solvent properly.
- ➔ Wear proper personal protective clothes (e.g., safety goggles) so that a solvent will not come into direct contact with the skin.
- ➔ Ventilate the laboratory room adequately to prevent accidental inhalation of harmful solvent vapor.

WARNING

Cuts

- ➔ When working with glass or quartz parts take care to prevent breakage.

Other Precautions

Airflow to the cooling fans of the liquid chromatograph must be unobstructed. Do not block the ventilation grills on the liquid chromatograph and accessories.

Consult the manuals supplied with your PC, monitor and for their specific ventilation requirements.

High Pressure Hazards

WARNING

High velocity stream of volatile and/or toxic liquids.

If a line ruptures, a relief device opens, or a valve opens accidentally under pressure, potentially hazardous high liquid pressures can be generated by the pump.

- ➔ Wear personal protective equipment when you inject samples or perform routine maintenance.
- ➔ Never open a solvent line or valve under pressure. Stop the pump first and let the pressure drop to zero.
- ➔ Always keep the doors and covers closed during operation.
- ➔ Read and adhere to all Notes, Cautions, and Warnings in the manual.

Ultraviolet Radiation

WARNING

Irritation to the skin, eyes and upper respiratory system

- ➔ Ensure that protective lamp covers of variable and fixed wavelength detectors are in place during operation.
- ➔ Do not look directly into detector fluid cells or at the UV light source. When inspecting the light source or fluid cell, always use protective eye covering such as borosilicate glass or polystyrene.
- ➔ Ventilate the area surrounding the detector such that the concentration of ozone does not exceed the maximum permissible level. All venting must be to outside air, never within the building.

Ozon generation

Ozone can be generated by radiation from the source lamps. The maximum permissible exposure level is 0.1 ppm (0.2 mg/m^3).

Solvent Miscibility

Solvents should mix with each other in all proportions. This is important during elution and during solvent changeover. Refer to Figure 30 on page 142 for miscibility of some common HPLC solvents.

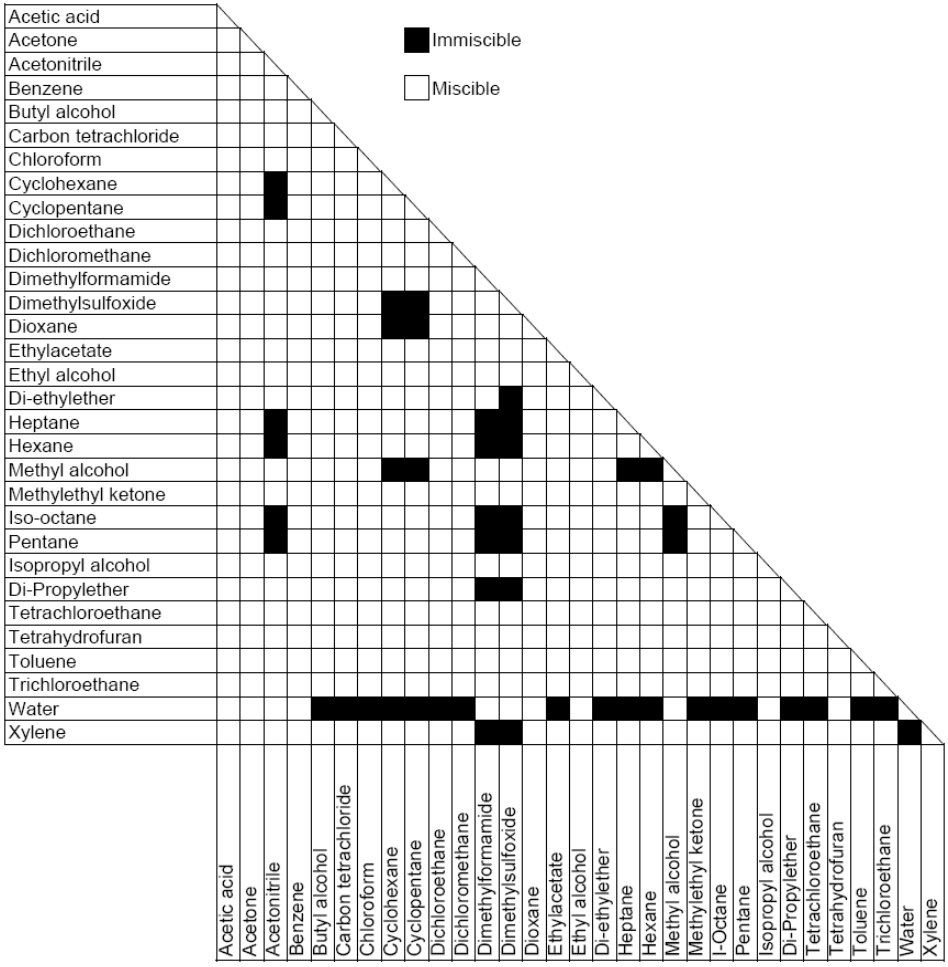


Figure 30 Solvent miscibility of some common solvents

The Waste Electrical and Electronic Equipment Directive

Abstract

The Waste Electrical and Electronic Equipment (WEEE) Directive (2002/96/EC), adopted by EU Commission on 13 February 2003, is introducing producer responsibility on all electric and electronic appliances starting with 13 August 2005.

NOTE

This product complies with the WEEE Directive (2002/96/EC) marking requirements. The affixed label indicates that you must not discard this electrical/electronic product in domestic household waste.

Product Category:

With reference to the equipment types in the WEEE Directive Annex I, this product is classed as a Monitoring and Control Instrumentation product.

NOTE

Do not dispose off in domestic household waste

To return unwanted products, contact your local Agilent office, or see www.agilent.com for more information.

Batteries Information

WARNING

Lithium batteries may not be disposed-off into the domestic waste. Transportation of discharged Lithium batteries through carriers regulated by IATA/ICAO, ADR, RID, IMDG is not allowed.

Danger of explosion if battery is incorrectly replaced.

➔ Discharged Lithium batteries shall be disposed off locally according to national waste disposal regulations for batteries.

➔ Replace only with the same or equivalent type recommended by the equipment manufacturer.

WARNING

Lithiumbatteri - Eksplosionsfare ved fejlagtig håndtering.

Udskiftning må kun ske med batteri af samme fabrikat og type.

➔ Lever det brugte batteri tilbage til leverandøren.

WARNING

Lithiumbatteri - Eksplosionsfare.

Ved udskiftning benyttes kun batteri som anbefalt av apparatfabrikanten.

➔ Brukt batteri returneres appararleverandoren.

NOTE

Bij dit apparaat zijn batterijen geleverd. Wanneer deze leeg zijn, moet u ze niet weggooien maar inleveren als KCA.

Radio Interference

Cables supplied by Agilent Technologies are screened to provide optimized protection against radio interference. All cables are in compliance with safety or EMC regulations.

Test and Measurement

If test and measurement equipment is operated with unscreened cables, or used for measurements on open set-ups, the user has to assure that under operating conditions the radio interference limits are still met within the premises.

Electromagnetic Compatibility

EN55011/CISPR11

Group 1 ISM equipment: group 1 contains all ISM equipment in which there is intentionally generated and/or used conductively coupled radio- frequency energy which is necessary for the internal functioning of the equipment itself.

Class A equipment is equipment suitable for use in all establishments other than domestic and those directly connected to a low voltage power supply network which supplies buildings used for domestic purposes.

This device complies with the requirements of CISPR11, Group 1, Class A as radiation professional equipment. Therefore, there may be potential difficulties in ensuring electromagnetic compatibility in other environments, due to conducted as well as radiated disturbances.

Operation is subject to the following two conditions:

- This device may not cause harmful interference.
- This device must accept any interference received, including interference that may cause undesired operation.

If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try one or more of the following measures:

- Relocate the radio or antenna.
- Move the device away from the radio or television.
- Plug the device into a different electrical outlet, so that the device and the radio or television are on separate electrical circuits.
- Make sure that all peripheral devices are also certified.
- Make sure that appropriate cables are used to connect the device to peripheral equipment.
- Consult your equipment dealer, Agilent Technologies, or an experienced technician for assistance.
- Changes or modifications not expressly approved by Agilent Technologies could void the user's authority to operate the equipment.

ICES/NMB-001

This ISM device complies with Canadian ICES- 001.

Cet appareil ISM est conforme à la norme NMB-001 du Canada.

Agilent Technologies on Internet

For the latest information on products and services visit our worldwide web site on the Internet at:

http://www.agilent.com

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www.agilent.com

In This Book

This manual provides setup information on following modules:

- Agilent SD-1 Solvent Delivery Module (G9302A/G9303A)
- Agilent 325 UV/VIS Dual WL Detector (G9309A)
- Agilent 410 Autosampler (G9331A/G9332A)
- Agilent 218 Solvent Delivery Module (G9300A/G9301A)
- Agilent 440 Fraction Collector (G9340A)

The manual describes the following:

- Optimizing stack configuration
- Setup hardware
- Setup software
- Setup methods

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Agilent Technologies