Agilent

Agilent InfinityLab LC Series

1260 Infinity III Bio-inert LC System

System Manual

Notices

Document Information

The information in this document also applies to 1260 Infinity II and 1290 Infinity II modules.

Document No: SD-29000129 Rev. C
Edition: 10/2024

Copyright

Agilent Technologies
Hewlett-Packard-Strasse 8
76337 Waldbronn, Germany

Warranty

The material contained in this document is provided "as is," and is subject to being changed, without notice, in future editions. Further, to the maximum extent permitted by applicable law, Agilent disclaims all warranties, either express or implied, with regard to this manual and any information contained herein, including but not limited to the implied warranties of merchantability and fitness for a particular purpose. Agilent shall not be liable for errors or for incidental or consequential damages in connection with the furnishing, use, or performance of this document or of any information contained herein. Should Agilent and the user have a separate written agreement with warranty terms covering the material in this document that conflict with these terms, the warranty terms in the separate agreement shall control.

Technology Licenses

The hardware and/or software described in this document are furnished under a license and may be used or copied only in accordance with the terms of such license.

Restricted Rights Legend

U.S. Government Restricted Rights. Software and technical data rights granted to the federal government include only those rights customarily provided to end user customers. Agilent provides this customary commercial license in Software and technical data pursuant to FAR 12.211 (Technical Data) and 12.212 (Computer Software) and, for the Department of Defense, DFARS 252.227-7015 (Technical Data - Commercial Items) and DFARS 227.7202-3 (Rights in Commercial Computer Software or Computer Software Documentation).

Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Contents

In This Book

This manual covers the Agilent 1260 Infinity III Bio-inert LC System.

1 Introduction

This chapter gives an introduction to the Agilent 1260 Infinity III Bio-inert LC System, the underlying concepts and features.

Product Description of the 1260 Infinity III Bio-inert LC System

The Agilent 1260 Infinity III Bio-inert LC System is a dedicated solution for large bio-molecule analysis. The design of new metal-free components in the sample flow-path and the absence of iron and steel in solvent delivery ensures the integrity of bio-molecules, minimizes unwanted surface interactions and increases column life-time. This is ideal when working under harsh solvent or extreme pH conditions. The power range expands from lowest pressure for traditional bio-purification columns up to high pressure STM analytical bio-columns.

Together with the Agilent AdvanceBio column portfolio you can advance your biopharmaceutical discovery, development and QA/QC utilizing conventional and ultra-performance LC systems for analysis of intact and fragmented monoclonal antibodies. Enable advanced SEC of large biomolecules with the new Agilent 1260 Infinity Multi-Detector Bio-SEC Solution.

Designed for use with InfinityLab Assist, Level Sensing and Sample ID Reader.

Features of the 1260 Infinity III Bio-inert LC System

- *Reliable analysis of biological samples* – the metal-free sample flow path at 600 bar means that none of your precious sample touches metal surfaces and minimizes unwanted surface interactions while increasing column lifetime.
- *Instrument variety* – inert flow-cells for UV and fluorescence detection and inert solvent and column selection valves for multi-method/multi-attribute analysis.
- *Increased flexibility* – with high salt tolerance (2 M) and wide pH range (1–13, short term 14).
- *Increased adjustability* – flow rates up to 10 mL/min enable power ranges from lowest pressure for traditional biopurification columns up to high pressure STM analytical bio-columns.
- Active seal wash and quaternary solvent blending included.
- *Ultralow carryover* – the 1260 Infinity III Bio-inert Multisampler is designed for low carryover using multiwash capability, to reduce carryover to less than 9 ppm.
- *Bio-inert capillaries and connections* – novel bio-inert capillary and connection design and InfinityLab QuickConnect/QuickTurn Fittings offering.
- *Faster pH scouting and easy buffer/solvent preparation* – for ion exchange chromatography with Agilent Buffer Advisor software.
- *Upgrade to Multi-Detector Bio-SEC Solution* – for reproducible advanced analysis of accurate molecular weights and size information of protein-based pharmaceuticals.
- *AdvanceBio column portfolio* – large portfolio of Bio-HPLC columns for SEC, IEX, reversed phase and peptide mapping.
- *Equipped with InfinityLab Assist* - adds an Intuitive User Interface, Automated Workflows, Predictive Maintenance and Assisted Troubleshooting

System Components

The Agilent 1260 Infinity III Bio-inert LC System consists of the following components:

- Bio-inert Pump (G5654A)
- Bio-inert Multisampler (G5668A)
- Multicolumn Thermostat (G7116A) with Bio-inert Heat Exchanger
- Diode Array Detector (G7115A), Multiple Wavelength Detector (G7165A), or Fluorescence Detector (G7121A) with respective Bio-inert flow cell
- Bio-inert Manual Injector (G1328B)
- Solvent Cabinet

The Agilent 1260 Infinity III Bio-inert LC System is described in more detail in the following sections. All modules are stackable, see Optimizing the Stack Configuration on page 18.

For specifications, please refer to the individual module user documentation.

Product Description of the 1260 Infinity III Bio-Inert Pump (G5654A)

The 1260 Infinity III Bio-Inert Pump is the pump of choice for all your biological and extreme pH applications. The titanium-based pump offers highest corrosion resistance against high salt concentration (2 M) and offers a handling of a wide pH range (1 – 13 , short term 14). It features a pressure range of up to 600 bar and a flow rate up to 10 mL/min (at 200 bar). Which enables the use of almost any column: conventional, sub-2 µm particle, or superficially porous columns.

Together with the Agilent Bio-HPLC column portfolio for SEC and IEX, the highest resolution per time is achieved for protein and NBE characterization. The Agilent Buffer Advisor software allows fast pH scouting and easy buffer/solvent preparation in ion exchange chromatography.

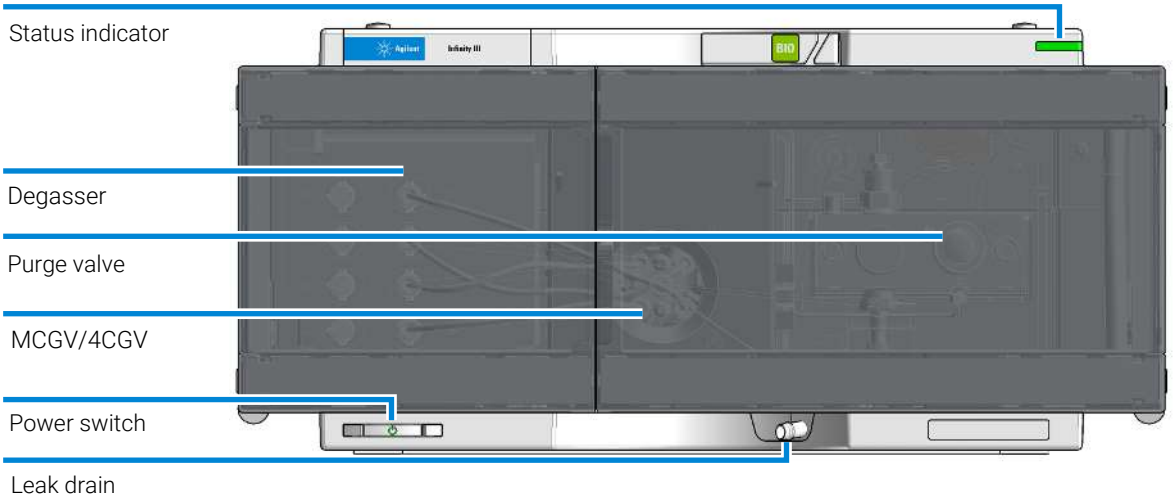


Figure 1: Overview of the Bio-inert Pump

Product Description of the 1260 Infinity III Bio-Inert Multisampler (G5668A)

The Agilent 1260 Infinity III Bio-Inert Multisampler features a 100 % metal-free sample flow path and is therefore the ideal injector for all biorelated applications, including analysis of mAbs, proteins in general and oligonucleotides. The ceramic needle, PEEK needle seat, and stainless steel-clad PEEK capillaries ensure highest injection accuracy and precision and are rated for a maximum system pressure of 600 bar allowing the use of highest performance columns. With multiwash capability, you can reduce carryover to less than 9 ppm. This design offers highest flexibility by handling both vials and microtiter plates and can house up to 6144 samples. For temperature-sensitive samples, simply add Agilent's highly efficient compressor-based thermostatting system. It allows you to maintain perfect temperature control on all vials and plates inserted.

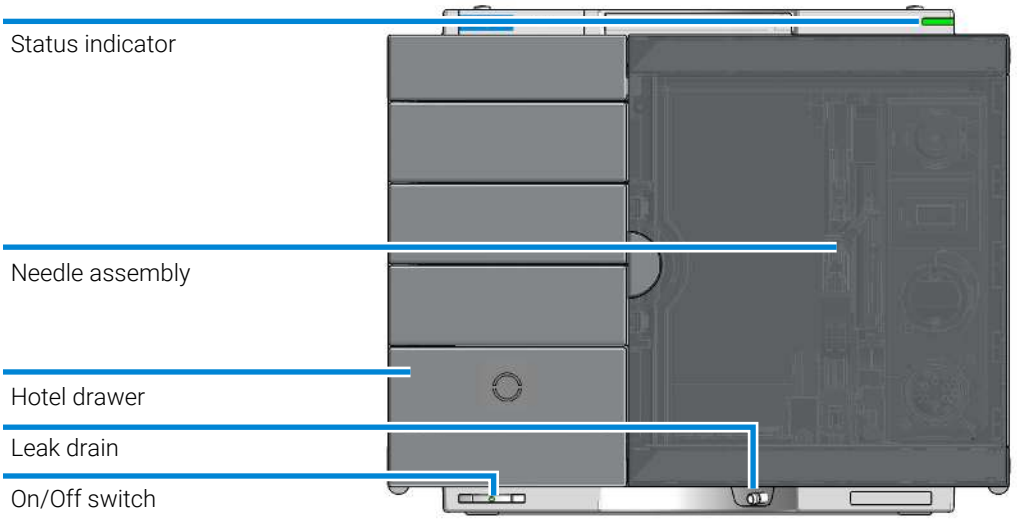


Figure 2: Overview of the Bio-inert Multisampler

Product Description of the 1260 Infinity III Multicolumn Thermostat (G7116A)

The Agilent 1260 Infinity III Multicolumn Thermostat (MCT) facilitates precise column thermostatting over a broad temperature range with cooling down to 10 °C below ambient temperature and heating up to 85 °C.

This capability provides robust and reliable separations for maximum application flexibility. Exchangeable high-pressure valves enable a wide range of applications such as column selection of up to four columns, sample preparation for analyte enrichment or matrix removal, or alternating column regeneration.

The MCT matches perfectly with all InfinityLab LC Series systems and can also be combined with 1290 Infinity III Series modules as well as with previous 1260 and 1290 Series modules.

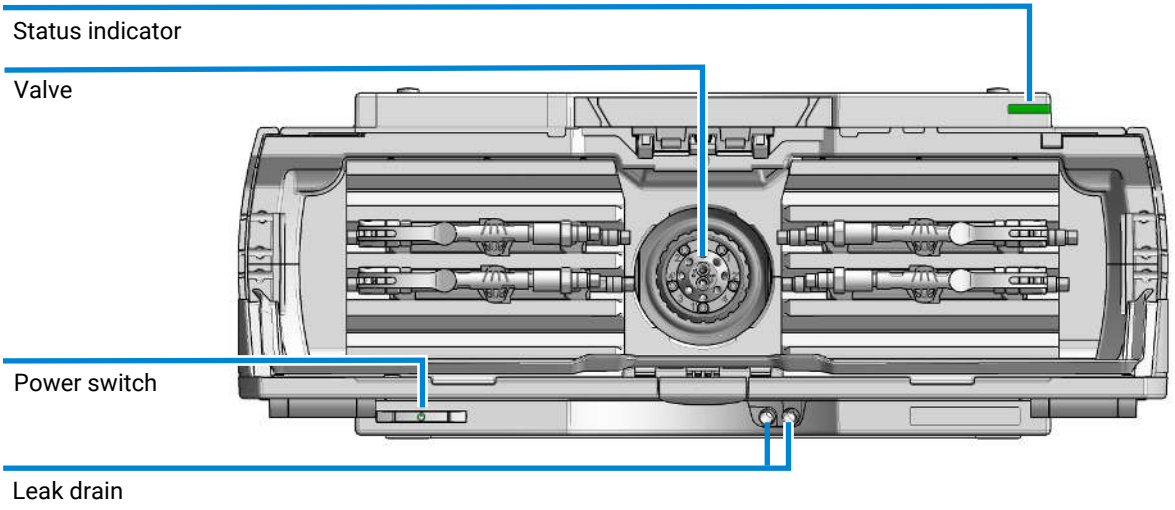


Figure 3: Overview of the Multicolumn Thermostat

Product Description of the 1260 Infinity III Diode Array Detector WR (G7115A)

The Agilent 1260 Infinity III DAD WR Detector is designed for highest optical performance, GLP compliance and easy maintenance. With its 120 Hz data acquisition rate the detector is perfectly suited for fast LC applications. The long –life deuterium lamps allow highest intensity and lowest detection limits over a wavelength range of 190 – 950 nm. The use of RFID tags for all flow cells and UV-lamps provides traceable information about these assemblies.

The built-in holmium oxide filter features the fast wavelength accuracy verification, while the built-in temperature controls improves the baseline stability. Additional diagnostic signals for temperature and lamp voltage monitoring are available.

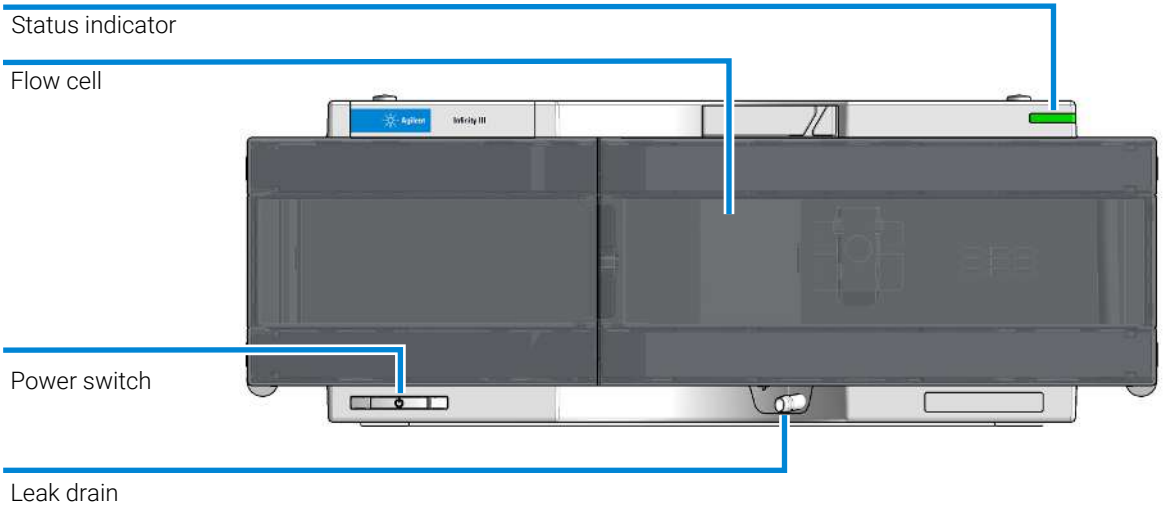


Figure 4: Overview of the G7115A Detector

Product Description of the 1260 Infinity III Fluorescence Detector (G7121A)

The proven optical and electronic design of the Agilent 1260 Infinity III Fluorescence Detector provides highest sensitivity for the analysis of trace-level components. Time-programmable excitation and emission wavelength switching allows you to optimize the detection sensitivity and selectivity for your specific applications. High-speed detection with up to 74 Hz data rates keeping you pace with the analysis speed of fast LC.

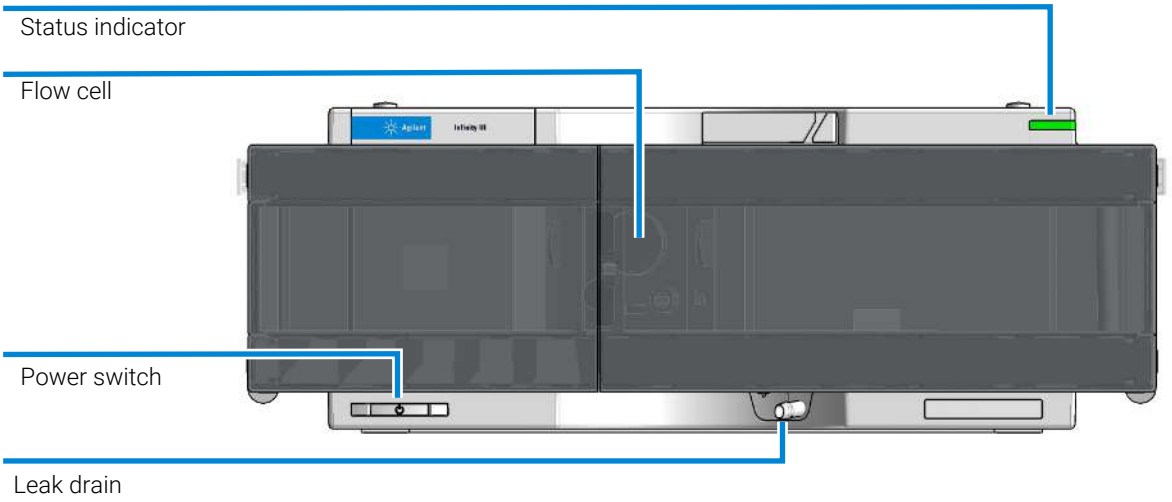


Figure 5: Overview of the G7121A Detector

Bio-inert Manual Injector (G1328B)

The Agilent 1260 Infinity III Bio-inert Manual Injector can be used for manual operation or use of large injection volumes. It offers a standard injection volume of 20 µL (optional 5 µL to 5 mL) and ensures highest injection accuracy.

The Manual Injector uses a 5067-4158 (Bio-inert 6-port sample injection valve) . Sample is loaded into the external 20 µL sample loop through the injection port at the front of the valve. The valve has a PEEK™ injection seal. A make-before-break passage in the stator ensures that the flow is not interrupted when the valve is switched between the INJECT and LOAD positions, and back again.

The Agilent 1260 Infinity III Manual Injector is based on the Manual Injector (G1328C). For further information refer to the *Agilent InfinityLab LC Series Manual Injector User Manual (G1328CUser.pdf)* (G1328B, G5628A).

1260 Infinity Multi-Detector Bio-SEC Solution

The Agilent 1260 Infinity Multi-Detector Bio-SEC System is a dedicated solution for reproducible advanced analysis of protein-based pharmaceuticals. Size-exclusion chromatography (SEC) is the standard method to determine and quantitate monomers, dimers, aggregates, and potential degradants and is a common requirement for regulatory approval. Advanced light scattering detectors enable biochemists to determine accurate molecular weights and size in solution, while providing more sensitive aggregation detection for analysis of large bio-molecules. Superior reproducibility is derived from Agilent's robust instrumentation and size-exclusion column technology.

- Reproducible and accurate molecular weights and size information
- Sensitive detection of aggregates with market-leading low dead volume light scattering detection
- Accuracy for size and molecular weight due to advanced detection
- Excellent repeatability and transferability minimizes effort on data review and potential rework
- High system uptime due to robustness of a fully tested single vendor solution
- Metal-free sample flow path for lowest surface activity and high salt tolerance and sub-ambient thermostatting
- Easy to use software simplifies workflow with routine and expert mode
- High efficiency Bio-SEC columns provide maximum resolution

2 Installation

This chapter provides information on unpacking, checking on completeness, stack considerations and installation of the module.

Installing the System Modules

For details of installation procedures for the modules, refer to the individual module manuals. These manuals also contain information on specifications, maintenance and parts.

Optimizing the Stack Configuration

You can ensure optimum performance by installing the system in one of the following configurations. These configurations optimize the system flow path, ensuring minimum delay volume.

The following configurations are possible:

- InfinityLab Flex Bench
- Single Stack (maximal 4 modules, in a bench rack or directly on the bench)
- InfinityLab Benchtop, providing more flexibility
- Two Stacks (in a bench rack or directly on the bench)

The table below summarizes the advantages of the different prescribed configurations.

Table 1: Overview on pros and cons of different stack configurations

Modules in a stack	InfinityLab Flex Bench Configuration	Single Stack Configuration	Two Stacks Configuration
fewer than 5	Pros • no bench required • mobile • optimal access to the modules, solvent bottles, pumps, columns, and accessories • integrated waste concept	Pros • minimal bench space required Cons • high stack	Pros • lower stacks • flexible combinations Cons • maximum bench space required
5 and more	+ possible	- not possible	+ possible

Agilent InfinityLab Flex Bench

Agilent recommends using the InfinityLab Flex Bench for all Agilent LC systems.

Main features:

- Increases flexibility in the lab
- Safe moving of LC
- Easy stack customization
- Included waste management

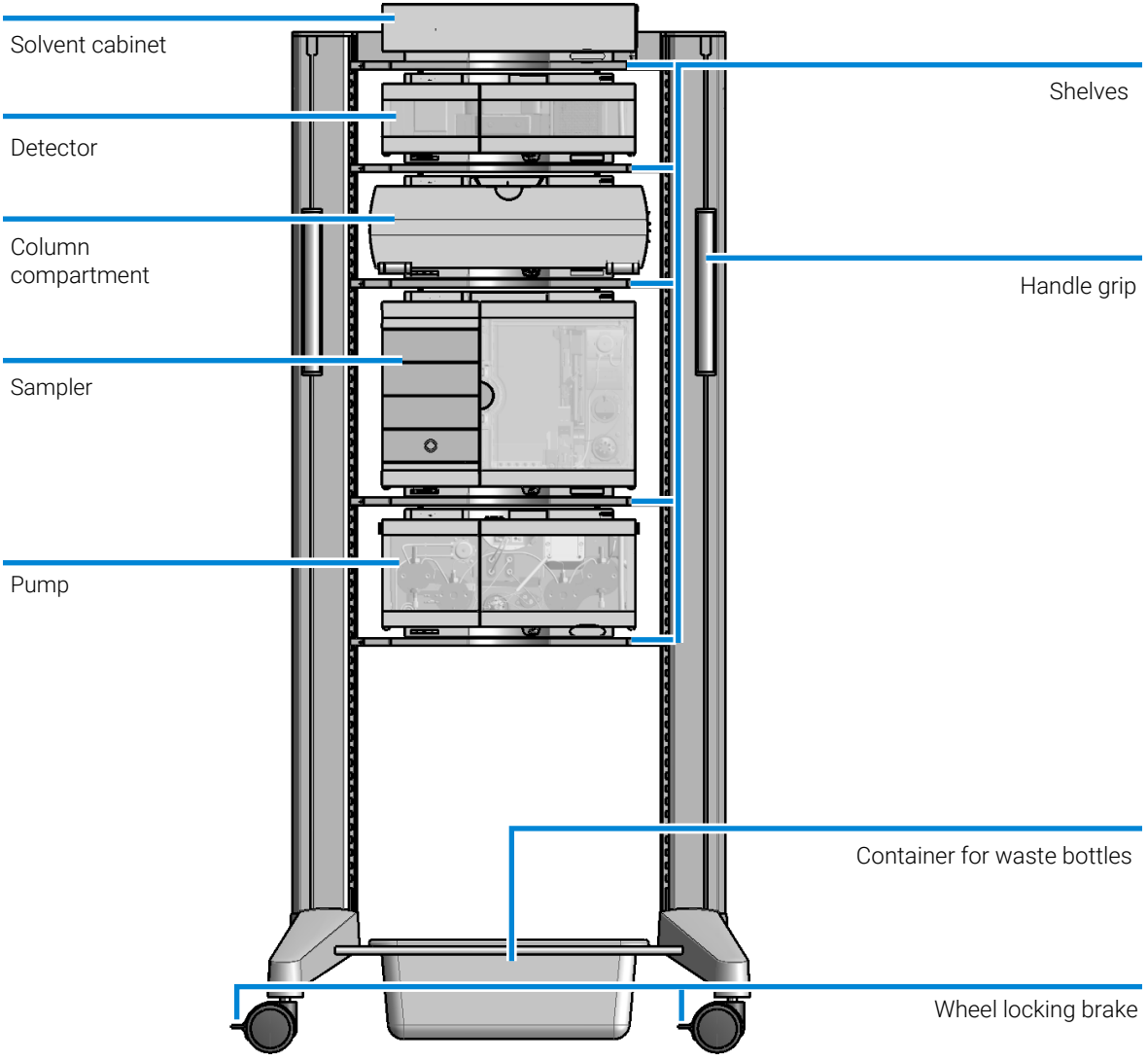


Figure 6: Agilent InfinityLab Flex Bench

One Stack Configuration

Ensure optimum performance by stacking the modules as shown exemplarily in Figure 7 on page 21. This configuration optimizes the flow path for minimum delay volume and minimizes the bench space required.

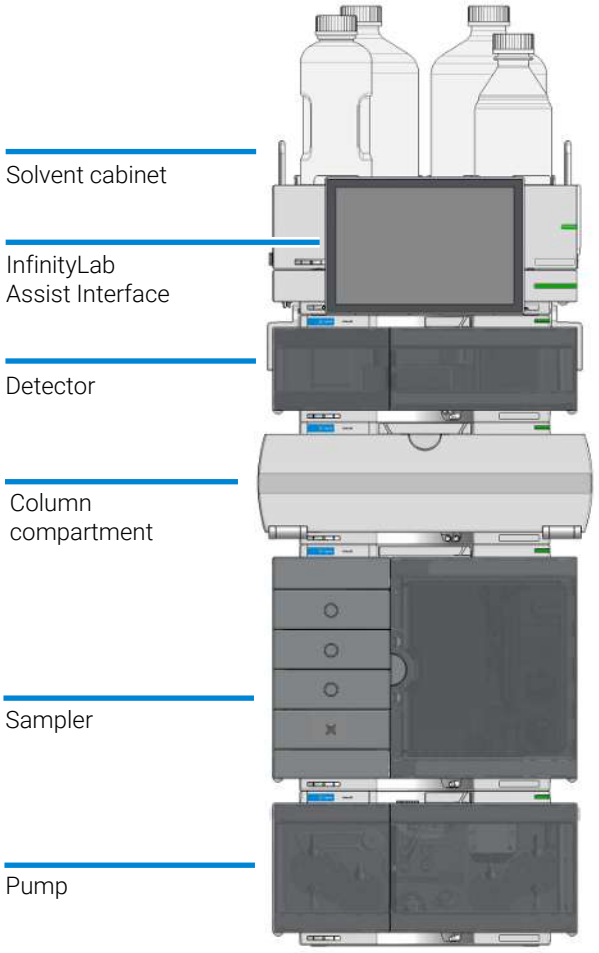


Figure 7: Single stack configuration (bench installation, example shows a Multisampler)

Two Stack Configuration

To avoid excessive height of the stack (for example when using the system in combination with an additional detector), it is recommended to form two stacks.

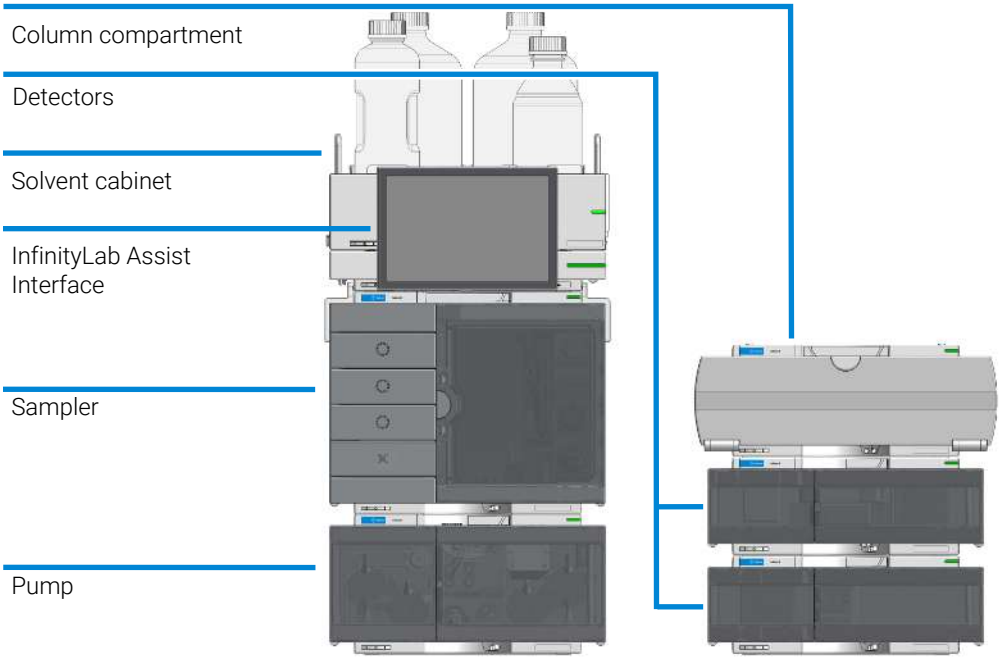


Figure 8: Two stack configuration (bench installation, example shows a Multisampler)

Integration Into the Network

For network integration of your system refer to user manuals of your modules (chapter *LAN Configuration*).

Capillary and Tubing Connections in Flow Path

Figure 9 on page 24 shows capillary and tubing connections in the flow path. For details and necessary parts, refer to the individual module manuals.

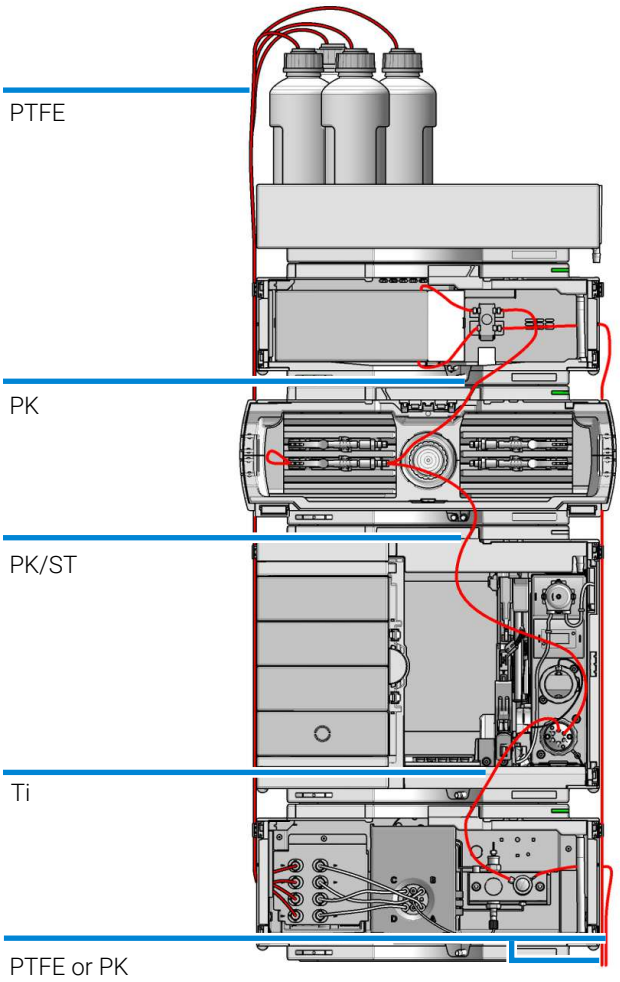


Figure 9: Capillary and tubing connections in flow path

NOTE

Normal PEEK capillaries are very sensitive to high pressures. For the bio-inert LC system, Agilent uses stainless-steel cladded PEEK capillaries (PK/ST), which keep the flow path free of metal as required for bio-inert applications and ensure pressure stability to at least 600 bar. These capillaries are equipped with UHP-FF fittings, see Install UHP-FF Fittings on page 26 for handling instructions.

Depending on the system configuration, one may need capillaries of different lengths. To achieve optimal LC-results, the following different bio-inert capillaries are available:

Bio-inert capillaries

p/n	Description
5500-1264	Capillary Ti 0.17 mm x 500 mm, SL/SLV Pump to multisampler
G5667-81005	Capillary PK/ST 0.17 mm x 500 mm, RLO/RLO (Bio-inert) Multisampler to MCT
G5615-68755	Capillary Kit Flow Cells BIO includes Capillary PK 0.18 mm x 1.5 m and PEEK Fittings 10/PK (p/n 5063-6591) MCT to DAD
G5664-68712	Analytical tubing kit 0.25 mm i.d. PTFE-ESD DAD to fraction collector

For other connections, following stainless-steel cladded PEEK capillaries are available for bio-inert applications:

PK/ST capillaries

p/n	Description
G5667-60502	Capillary, PK/ST, 0.17 mm x 100 mm, male to male, pre-swaged
G5667-60503	Capillary, PK/ST, 0.17 mm x 150 mm, male to male, pre-swaged
G5667-60504	Capillary, PK/ST, 0.17 mm x 200 mm, male to male, pre-swaged
G5667-60505	PEEK/SST cap 0.17 mm ID, 300 mm long (bio-inert)
G5667-60500	PEEK/SST capillary (bio-inert) 0.17 mm ID, 400 mm long
G5667-60501	PEEK/SST cap 0.17 mm ID, 500 mm long

See module manuals for module-internal capillary and tubing connections.

Installing Capillaries

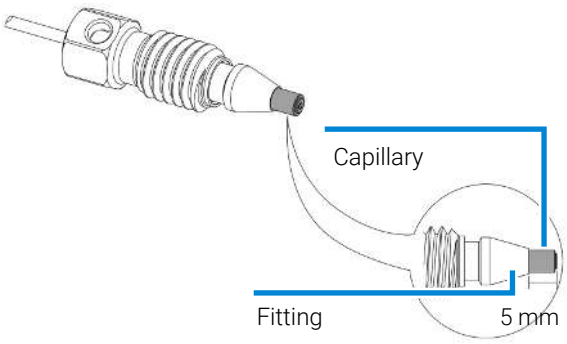
Install UHP-FF Fittings

Tools required

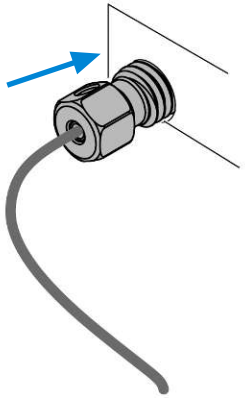
Qty.	p/n	Description
1	5043-0915	Fitting mounting tool

For details on necessary capillaries and fittings, see the part section of the manual.

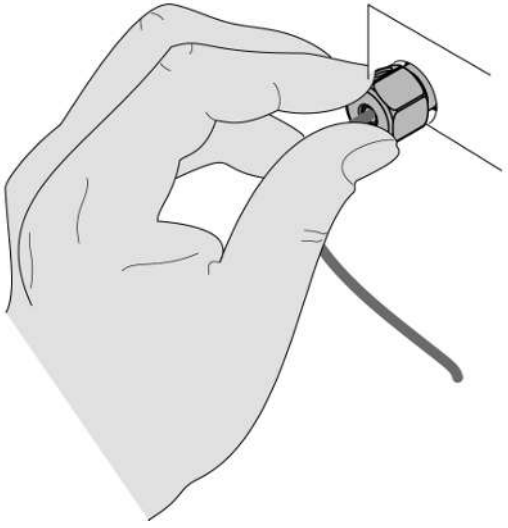
1 Slide the fitting on the capillary. Let the capillary jut out 5 mm.



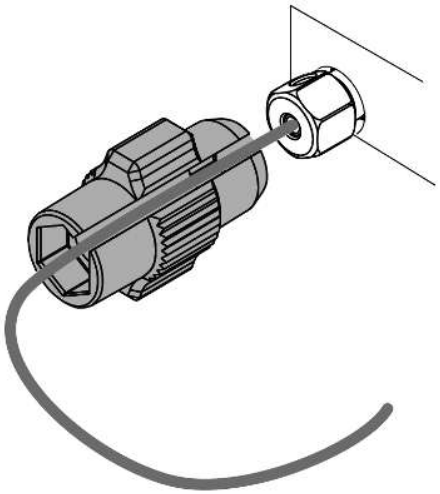
2 Insert the fitting to the receiving port and push the capillary to the bottom of the port.



3 Finger tighten the nut into the port until snug.



4 Use 5043-0915 (Fitting mounting tool) or a 5 mm hex wrench for fixing the fitting (maximum torque 0.8 Nm).



CAUTION

Potential damage of capillaries

— Do not remove fittings from used capillaries.

5 When using UHP-FF fittings with bio-inert capillaries, do not try to remove fittings from these capillaries. Bio-inert capillaries are using a PEEK front end, which may expand under pressure especially when being in contact with some organic solvents. If a fitting is moved across an expanded PEEK end,

there is a risk of damaging the capillary by ripping off its end. Before re-installing such capillaries, push the ferrule towards the rear site for a small distance.

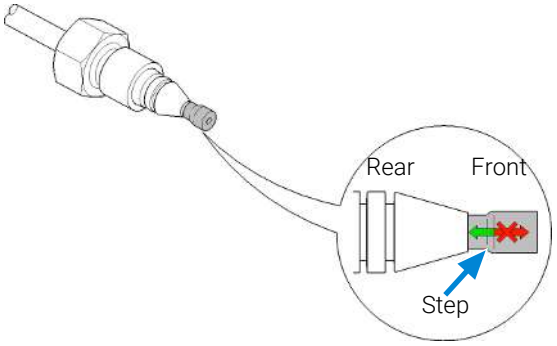


Figure 10: Capillary fitting

Install the Bio-Inert Zero Dead Volume (ZDV) Union

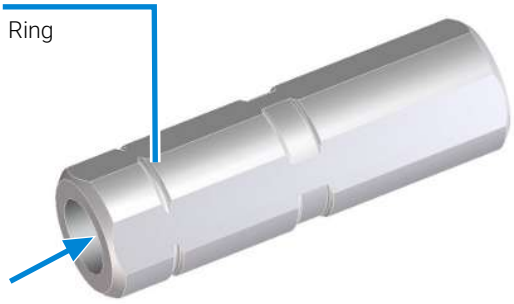
The 5067-4741 (ZDV union (Bio-inert)) has two different connectors where capillaries need to be installed in the correct sequence. Otherwise, an inset of the union may be damaged and the connection may not be tight.

CAUTION

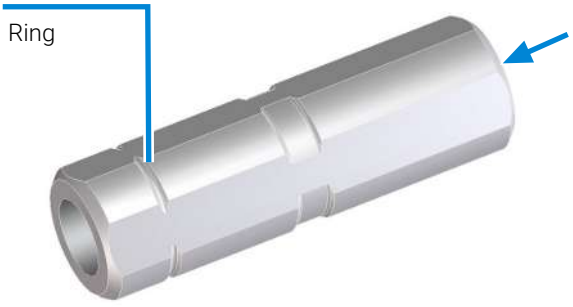
Potential leak or damage of the Bio-inert ZDV Union.

— To avoid leaks or a damage to the Bio-inert ZDV union, follow the procedure below in the prescribed sequence.

1 Install the capillary at the end marked with a ring/indentation.



2 Install the second capillary at the other end.



Handling Leak and Waste

The Agilent InfinityLab LC Series has been designed for safe leak and waste handling. It is important that all security concepts are understood and instructions are carefully followed.

The solvent cabinet is designed to store a maximum volume of 8 L solvent. The maximum volume for an individual bottle stored in the solvent cabinet should not exceed 2 L. For details, see the usage guideline for the Agilent Infinity III Solvent Cabinets (a printed copy of the guideline has been shipped with the solvent cabinet, electronic copies are available on the Internet).

All leak plane outlets are situated in a consistent position so that all Infinity and Infinity II/III modules can be stacked on top of each other. Waste tubes are guided through a channel on the right hand side of the instrument, keeping the front access clear from tubes.

The leak plane provides leak management by catching all internal liquid leaks, guiding them to the leak sensor for leak detection, and passing them on to the next module below, if the leak sensor fails. The leak sensor in the leak plane stops the running system as soon as the leak detection level is reached.

Solvent and condensate is guided through the waste channel into the waste container:

- from the detector's flow cell outlet
- from the Multisampler needle wash port
- from the Sample Thermostat (condensate)
- from the pump's Seal Wash Sensor (if applicable)
- from the pump's Purge Valve or Multipurpose Valve

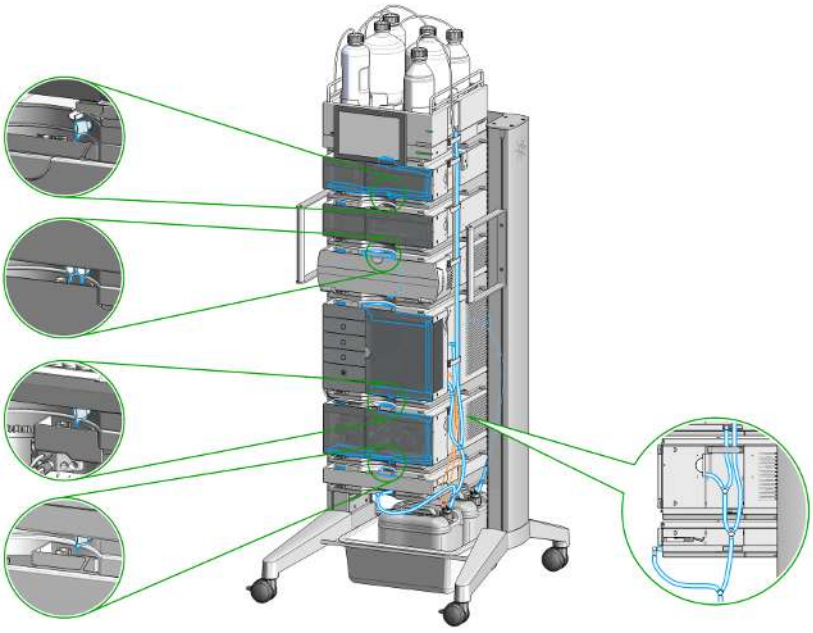


Figure 11: Infinity III Leak Waste Concept (Flex Bench installation)

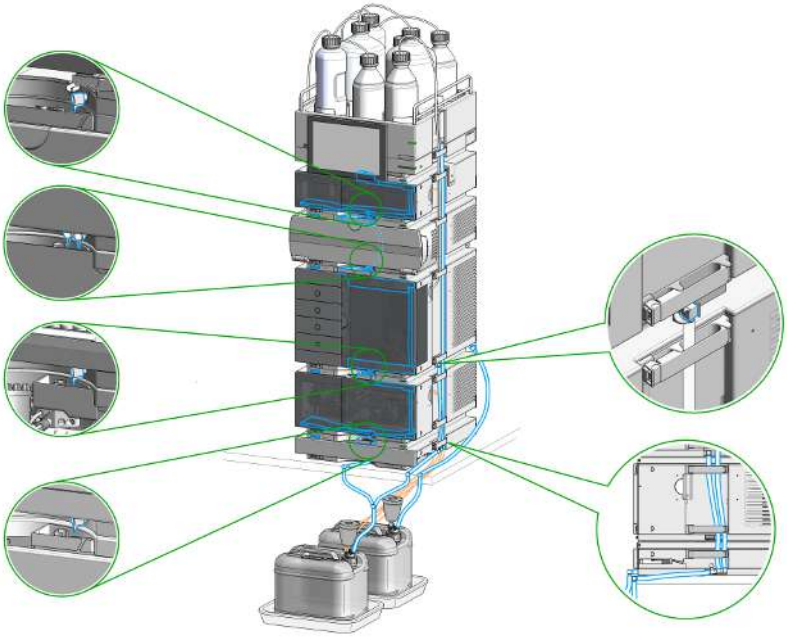


Figure 12: Infinity III Single Stack Leak Waste Concept (bench installation)

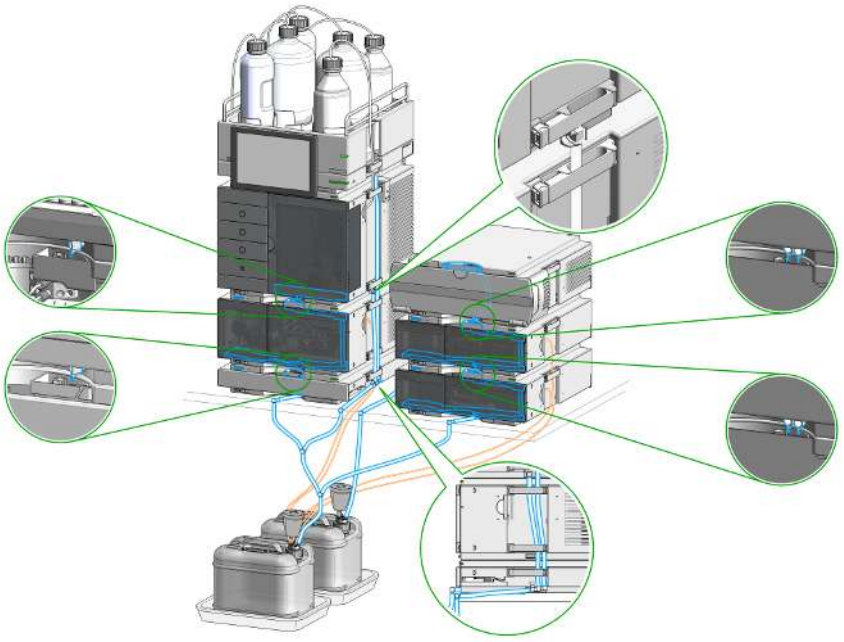


Figure 13: Infinity III Two Stack Leak Waste Concept (bench installation)

The waste tube connected to the leak plane outlet on each of the bottom instruments guides the solvent to a suitable waste container.

Drain Connectors Installation

Drain Connectors have been developed to improve leak drainage for low flow leaks of high viscosity solvents (for example, isopropanol) in Agilent InfinityLab LC Series Systems. Install these parts to modules where they are missing (usually preinstalled).

- Make sure that dripping adapters are correctly installed on each module in the LC stack, excluding lowest module.
- Remove the dripping adapter if it is appeared to be installed on the lowest module in the LC stack and connect waste tube instead.
- Consider 5004-0000 (Drain Connectors Kit) if drain adaptor is missing on some module(s).

For illustration, see Handling Leak and Waste on page 31.

Parts required

Qty.	p/n	Description
	5004-0000	Drain Connectors Kit

Content of Drain Connectors Kit (p/n 5004-0000)

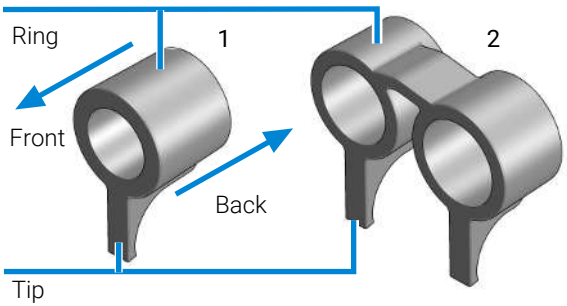


Figure 14: Overview of Drain Connectors: Single (left) and Double (right)

Qty.	p/n	Description
Parts can be ordered only as a complete kit.		
3	5043-1834	Single Drain Connector ID3.0-Long
1	5043-1836	Double Drain Connector-Long

Table 2: Compatibility of drain connectors and modules

Drain Connector Type	Compatible Module	Compatible Module Type
Double	G7116A/B	Column Compartment
Single	G7114A/B	Detector
	G7115A	
	G7117A/B/C	
	G7121A/B	
	G7162A/B	
	G7165A	
	G7129A/B/C	Sampler
	G7167A/B/C	
	G5668A	
	G7137A	
	G7157A	
	G4767A	
	G7122A	Degasser
	G7104A/C	Pump
	G7110B	
	G7111A/B	
	G7112B	
	G7120A	
	G7131A/C	
	G7132A	
	G5654A	
	G4782A	

Preparations

- Leak drains of LC modules are clean and free of salt or solvent residuals.

NOTE

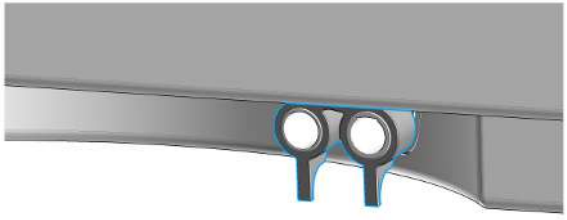
Do not install drain connectors on the bottom modules of the stack. Drain outlet of the bottom module has to be connected via waste tubing to a suitable waste container (see Leak and Waste Handling in the manual for a respective module).

NOTE In case of incorrect installation, drain connectors cannot fully perform the intended function.

NOTE It is not required to power off the HPLC stack to install Single and Double Drain Connectors. The installation of the connectors does not affect the analysis performed during the installation.

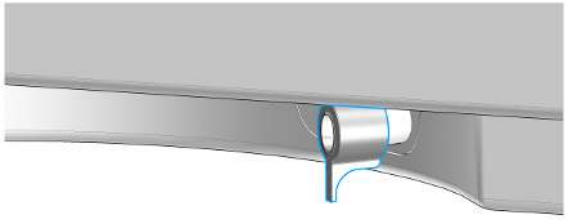
Install the Double Drain Connector on the leak drain of the 1260 Infinity III Multicolumn Thermostat (G7116A)/ 1290 Infinity III Multicolumn Thermostat (G7116B)

1 Align the rings with the leak drain outlets of the module, press slightly with the fingers, and slide the connector along the leak drain outlets until it is aligned with the front of the leak drain.



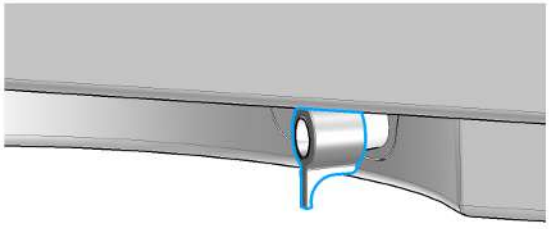
Install Single Drain Connectors on other modules in the LC stack

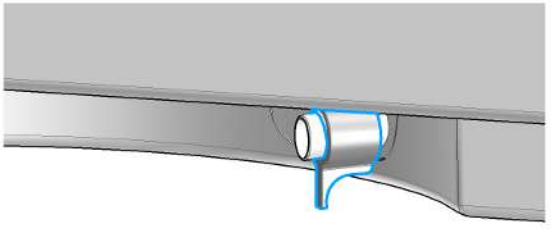
1 Align the ring with the leak drain outlet of the module, press slightly with the fingers, and slide the connector along the leak drain outlet until it is aligned with the front of the leak drain.



Make sure that the following requirements are covered:

- The tip of the drain connector points straight down.
- The leak drain outlets and the drain connectors are aligned properly.

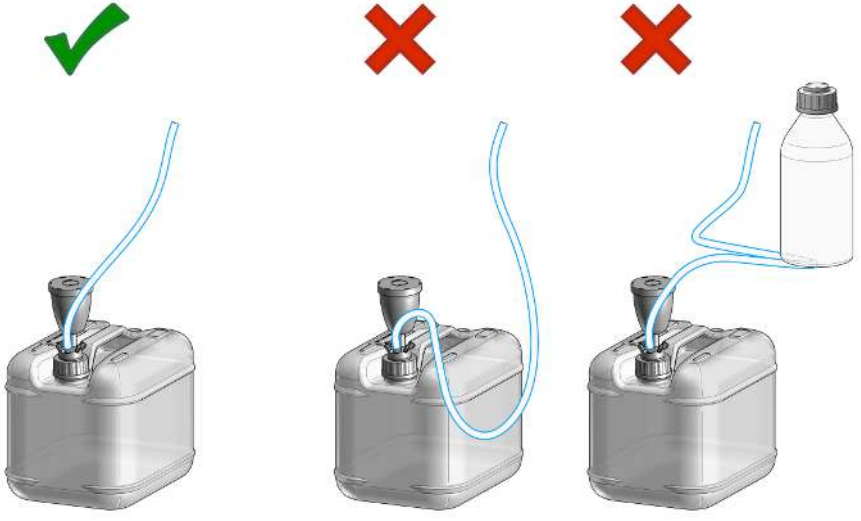




Waste Concept

Agilent recommends using the 5043-1221 (6 L waste can with 1 Stay Safe cap GL45 with 4 ports) for optimal and safe waste disposal. If you decide to use your own waste solution, make sure that the tubes don't immerse in the liquid.

Waste Guidance



NOTE The waste drainage must go straight into the waste containers. The waste flow must not be restricted at bends or joints.

Leak Sensor

CAUTION Solvent incompatibility

The solvent DMF (dimethylformamide) leads to corrosion of the leak sensor. The material of the leak sensor, PVDF (polyvinylidene fluoride), is incompatible with DMF.

- Do not use DMF as mobile phase.
- Check the leak sensor regularly for corrosion.

Handling Leak and Waste in a Mixed Configuration

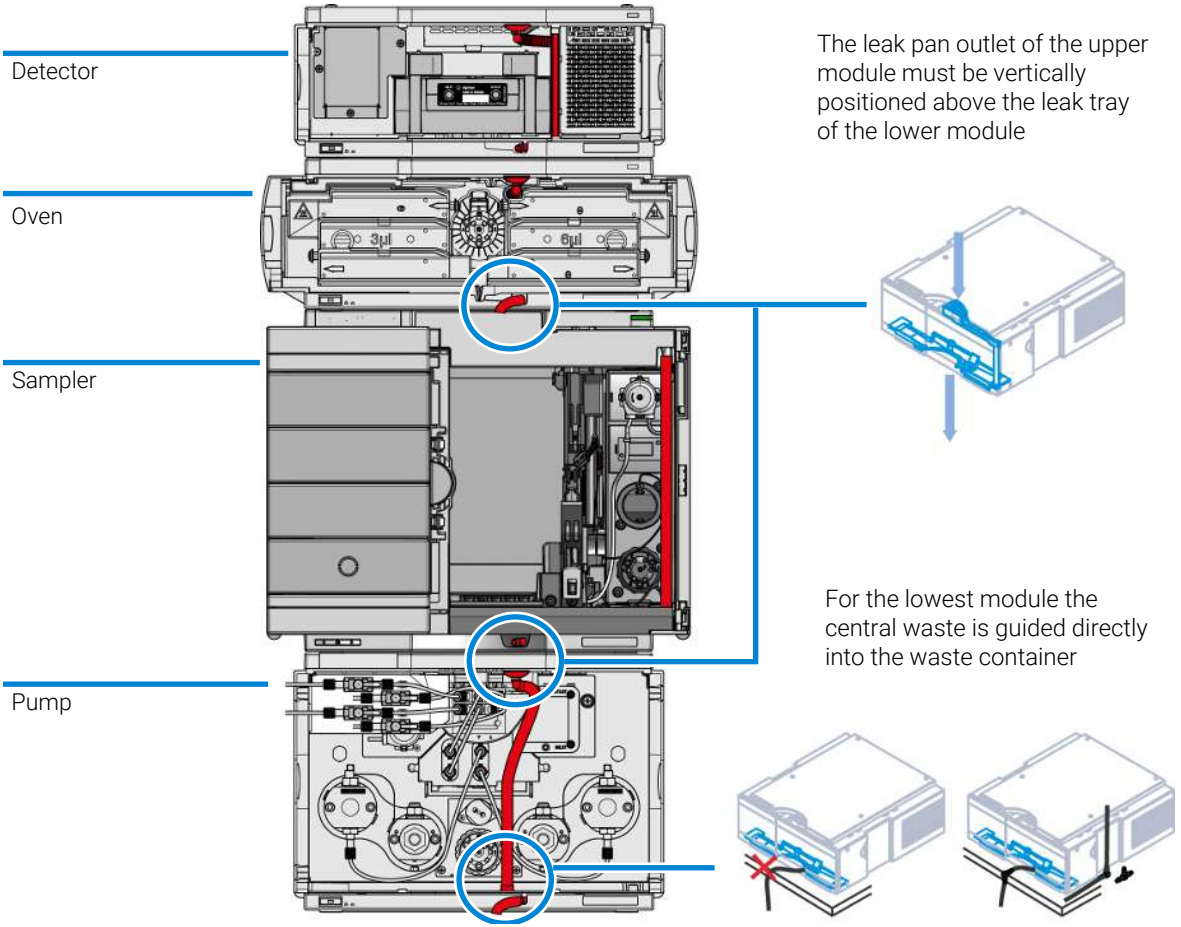


Figure 15: Leak and waste handling with multisampler in a mixed configuration as an example

NOTE Flush solvent from the washport of the multisampler is guided out to the right of the instrument.

Multisampler

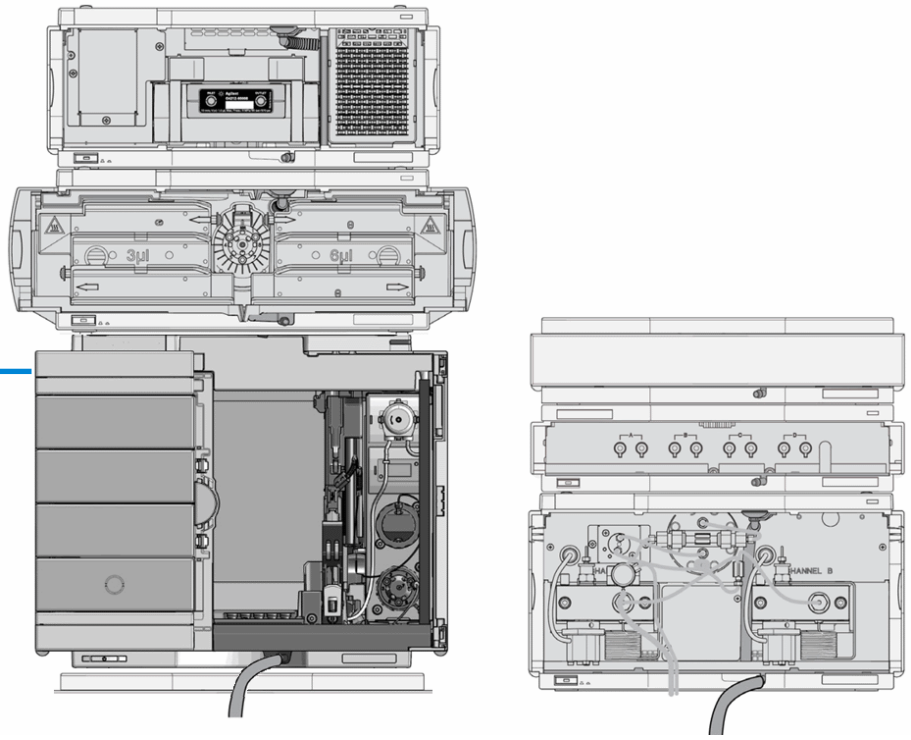


Figure 16: Leak and waste handling with multisampler in a mixed configuration as an example (two stack configuration)

NOTE Do not place the multisampler directly on the bench if a sample cooler or sample thermostat is installed.

3 Configuration Settings

This chapter describes how to configure the system.

General Information on LAN Configuration

LAN configuration is executed from the module with direct LAN connection to the controller software. This must be the module (usually the detector) with the highest data rate.

Instrument Configuration

Example shows an instrument configuration with a Diode Array Detector.

1 Set the switches of the Configuration switch at the rear of the module:

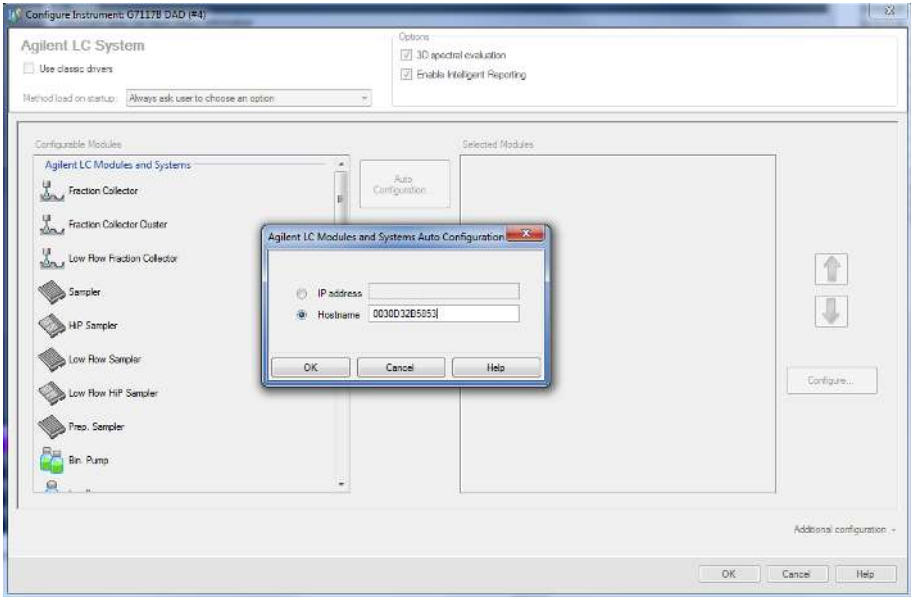
   a All switches DOWN: module uses the default IP address 192.168.254.11.

   b Switch 4 UP and others DOWN: module uses DHCP.

   c Switch 5 UP and others DOWN: modules uses STORED address.

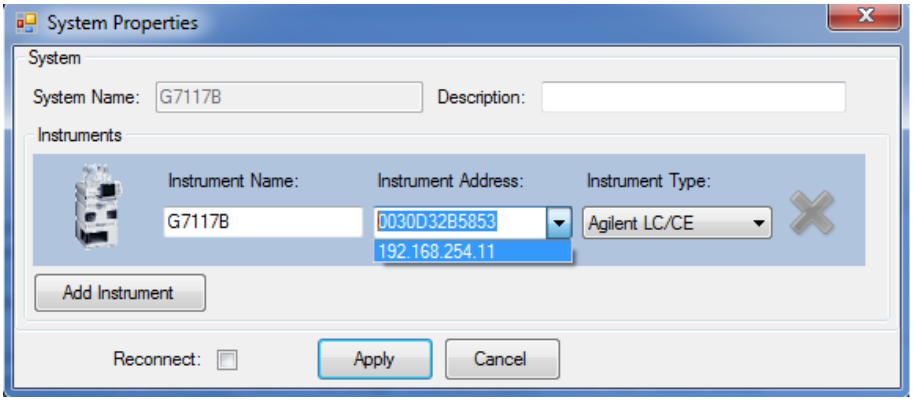
2 Enter the setup information (MAC [1] / IP address and/or Instrument Name).

   a Agilent OpenLab ChemStation (Configure Instrument):



1 MAC address can only be used in DHCP DIP-switch configuration.

b Lab Advisor (Instrument Overview - Add Instrument):



4 Quick Start Guide

This chapter provides information on running an Agilent 1260 Infinity III Bio-inert LC System.

Best Practices

For best practices, refer to the *Agilent Information Center* on Agilent InfinityLab LC Series User Documentation (G4800-64600), Best Practices for Using an Agilent LC System (01200-90090), or the 1290 Infinity II Bio and 1260 Infinity II Prime Bio LC Quick Reference Sheet (G7132-90110).

Prepare a Run

This procedure examplarily shows how to prepare a run. Parameters as shown in the screenshots may vary, depending on the system installed.

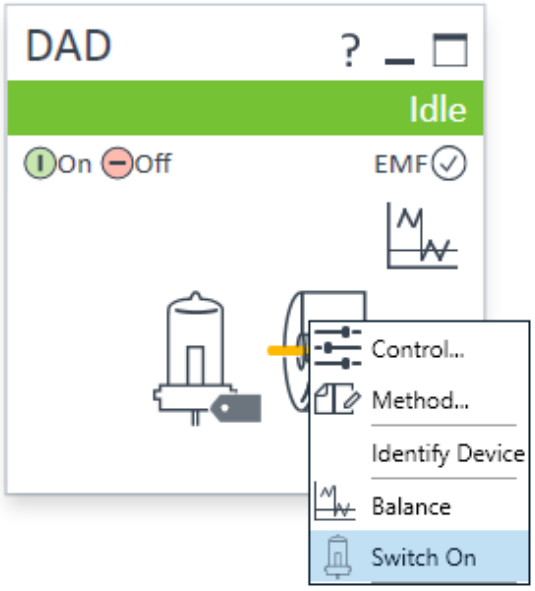
WARNING

Toxic, flammable and hazardous solvents, samples and reagents

The handling of solvents, samples and reagents can hold health and safety risks.

- **When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.**
- **Do not use solvents with an auto-ignition temperature below 200 °C (392 °F). Do not use solvents with a boiling point below 56 °C (133 °F).**
- **Avoid high vapor concentrations. Keep the solvent temperature at least 40 °C (72 °F) below the boiling point of the solvent used. This includes the solvent temperature in the sample compartment. For the solvents methanol and ethanol keep the solvent temperature at least 25 °C (45 °F) below the boiling point.**
- **Do not operate the instrument in an explosive atmosphere.**
- **Do not use solvents of ignition Class IIC according IEC 60079-20-1 (for example, carbon disulfide).**
- **Reduce the volume of substances to the minimum required for the analysis.**
- **Never exceed the maximum permissible volume of solvents (8 L) in the solvent cabinet. Do not use bottles that exceed the maximum permissible volume as specified in the usage guideline for solvent cabinet.**
- **Ground the waste container.**
- **Regularly check the filling level of the waste container. The residual free volume in the waste container must be large enough to collect the waste liquid.**
- **To achieve maximal safety, regularly check the tubing for correct installation.**

1 Switch on the detector.

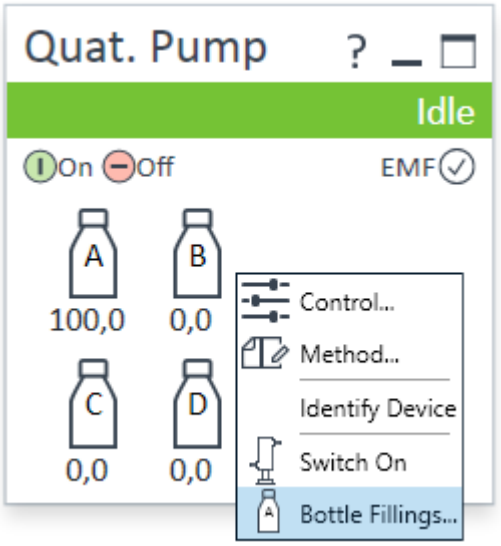


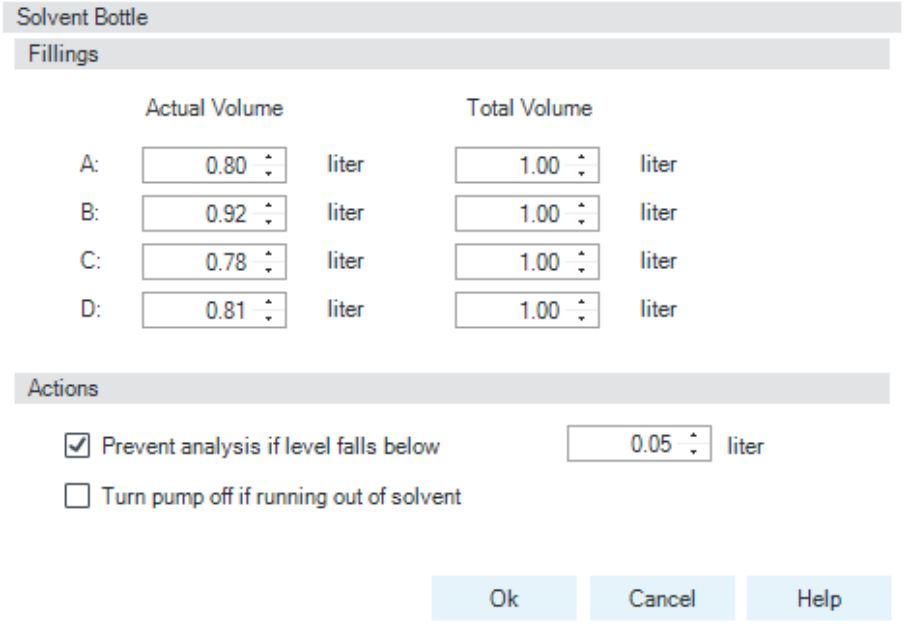
2 Fill the solvent bottles with adequate solvents for your application.

3 Place solvent tubings with bottle head assemblies into the solvent bottles.

4 Place solvent bottles into the solvent cabinet.

5 Solvent bottle filling dialog (in the software).





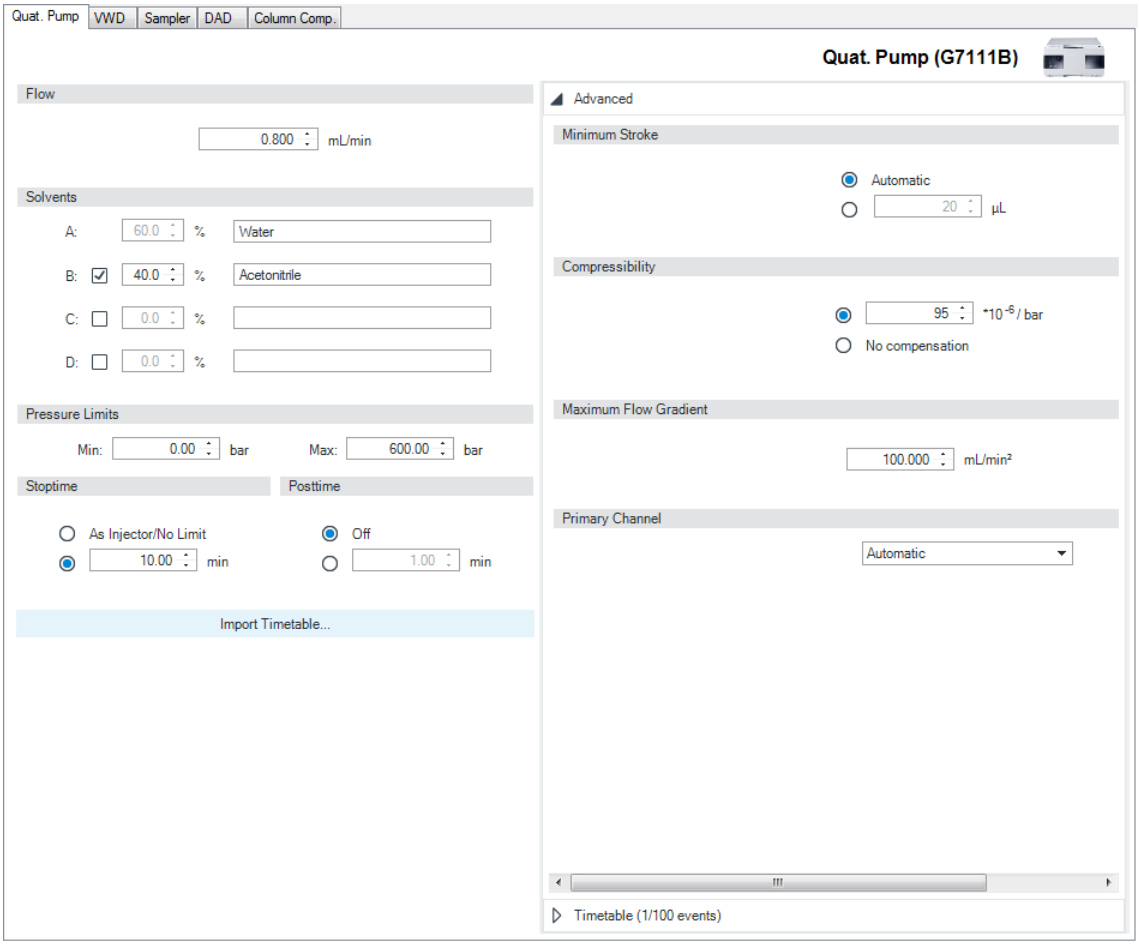
6 Purge the pump (in normal usage scenario).

OR: Prime the pump (after installation of the system).

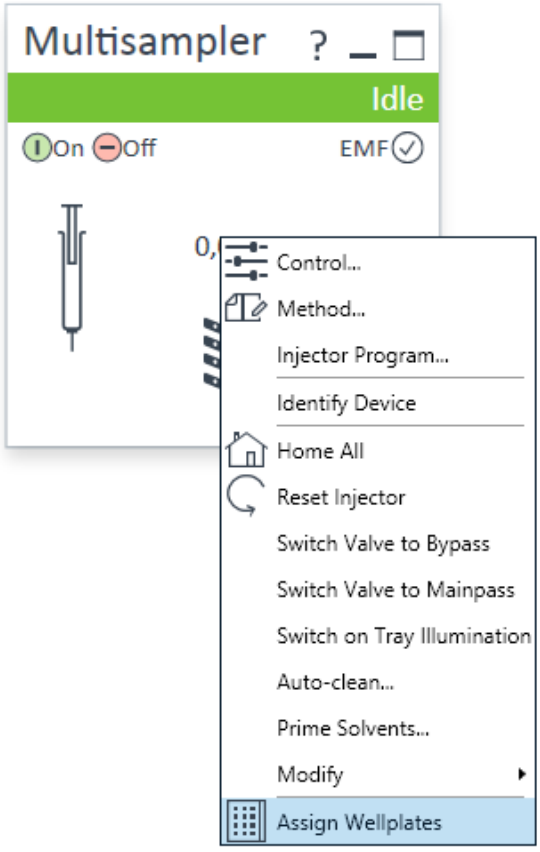
NOTE

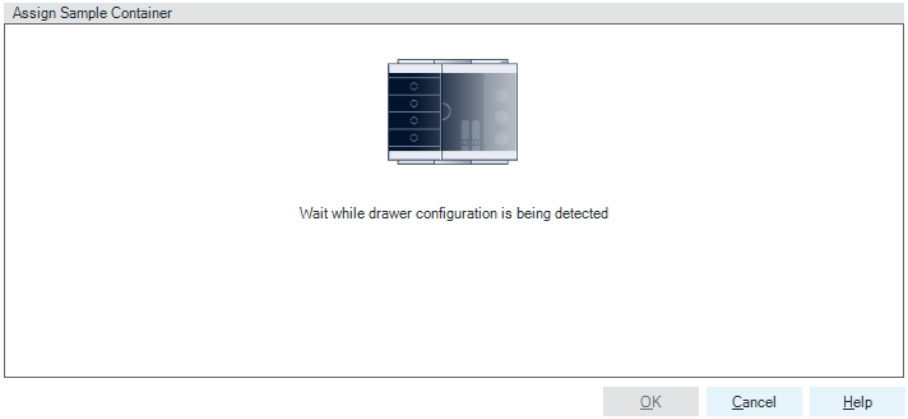
For details on priming and purging, refer to the technical note *Best Practices for Using an Agilent LC System Technical Note (InfinityLab-BestPractice-en-SD-29000194.pdf, SD-29000194)*.

7 Change solvent (if necessary).

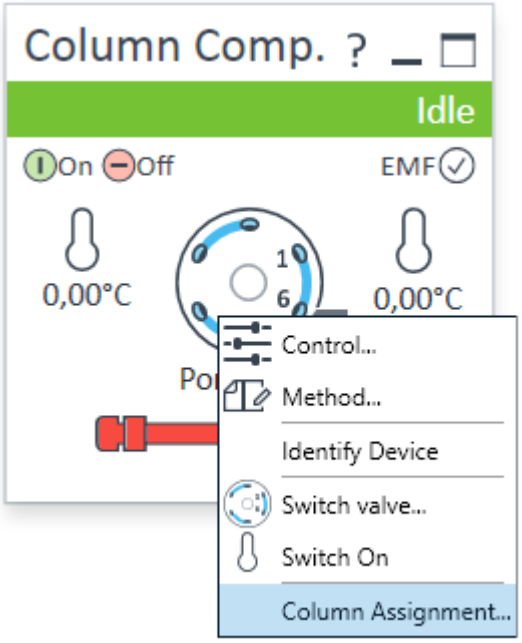


8 Choose the tray format of the sampler.





9 Add a new column.



10 Enter the column information.

Plumbing

Valve Position	Location
1	Left 1
2	Left 2

Visualization

Valve Type: 2-pos/6-port valve 600 bar (5067-4137)

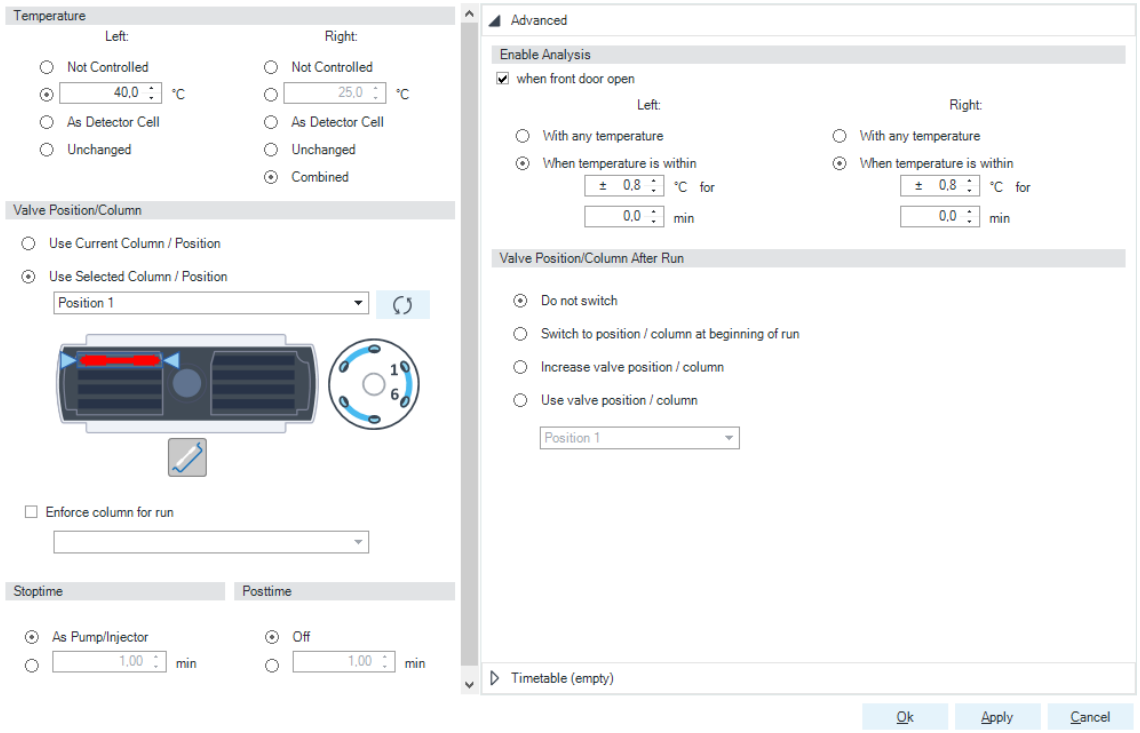
Column Tag Information

>>

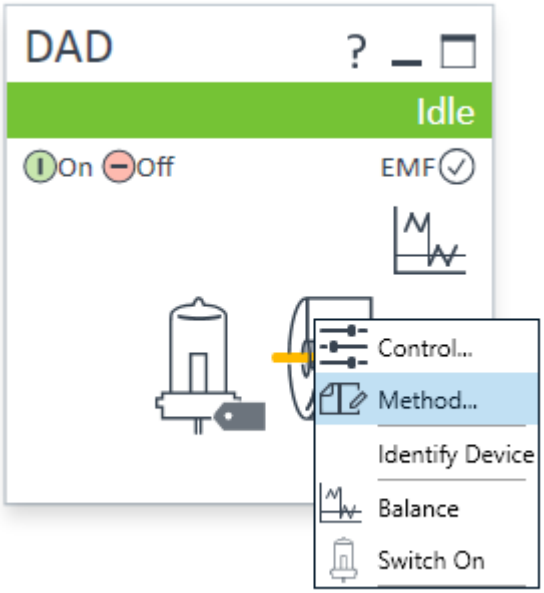
Location	Color Code	Description	Length [mm]	Diameter [mm]	Particle Size [µm]	Max. Pressure [bar]	Injections
Left 1	Red		0	0,0	0,0	0	0
Left 2	Blue		0	0,0	0,0	0	0
Left 3	None		0	0,0	0,0	0	0
Left 4	None		0	0,0	0,0	0	0
Right 1	None		0	0,0	0,0	0	0
Right 2	None		0	0,0	0,0	0	0
Right 3	None		0	0,0	0,0	0	0
Right 4	None		0	0,0	0,0	0	0

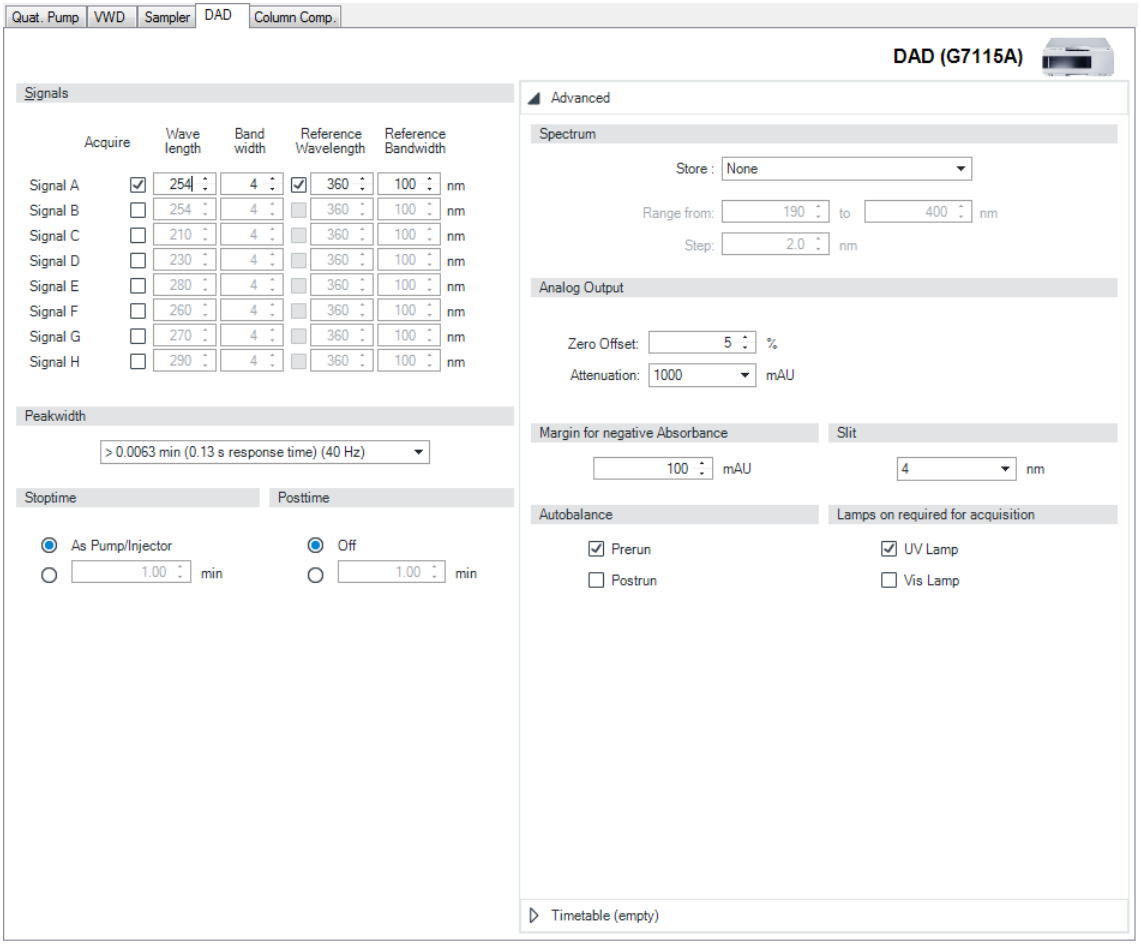
Ok/Write Tag Cancel Help

11 Select the column position.



12 Set the detector according to the needs of your method.





NOTE For details on running a method, see Setup the Checkout Method (Bio-inert) as an example.

Installation Checkout (Customer presence recommended)

For instruments where the checkout columns mentioned in Overview of column options for different pumps are included, the checkout is run after the complete installation of the module stack to affirm the functionality of all modules.

The checkout confirms that each module performs and is connected correctly. The chromatography should show a single peak for FLD, RID or ELSD or four or nine separated peaks, respectively, but is not a substitute for system suitability tests or qualifications.

The checkout for UV and Fluorescent Light Detectors should be run with one of the checkout columns supplied with the pump or with an equivalent column to ensure separation of the compounds.

The checkout for RIDs and ELSDs is done with a 5022-2159 (Restriction capillary, SST 0.12 mm ID, 2 m long) (use 5005-0046 (Capillary MP35N 0.12 mm x 2 m) for 1290 Infinity II Bio LC System).

When Analytical Fraction Collector is installed in the LC System, acetonitrile and water mobile phases with 0.1 % formic acid (as needed in the FC checkout procedure) can be used for the system checkout procedure without any further method modifications.

NOTE If a system is equipped with multiple detectors, only run one checkout run and always use the column based procedure for system checkout.

Checkout Columns

The information in this table applies to Infinity II and Infinity III modules.

Table 3: Overview of column options for different pumps

Pump	Option 1	Option 2	Option 3
1260 Isocratic, Quaternary and Binary Pump (G7110B/ G7111B/G7112B)	695975-902T, InfinityLab Poroshell 120 EC-C18, 4.6 x 100 mm, 2.7 µm 600 bar	693975-302T, InfinityLab Poroshell 120 EC-C18, 3.0 x 150 mm, 2.7 µm, 600 bar	699975-302T, InfinityLab Poroshell 120 EC-C18, 3.0 x 50 mm, 2.7 µm, 600 bar
1260 Quaternary Pump VL (G7111A)	695970-902T, InfinityLab Poroshell 120 EC-C18, 4.6 x 100 mm, 4 µm, 600 bar	699975-902T, InfinityLab Poroshell 120 EC-C18, 4.6 x 50 mm, 2.7 µm, 600 bar	693970-902T, InfinityLab Poroshell 120 EC-C18, 4.6 x 150 mm, 4 µm, 600 bar
1260 Flexible Pump (G7104C)	693575-302, InfinityLab Poroshell 120 EC-C18, 3.0 x 150 mm, 2.7 µm, 1000 bar	695575-302, InfinityLab Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm, 1000 bar	699675-902, InfinityLab Poroshell 120 EC-C18, 2.1 x 50 mm, 1.9 µm, 1300 bar
1290 Pumps (G7120A and G7104A)	699675-902, InfinityLab Poroshell 120 EC-C18, 2.1 x 50 mm, 1.9 µm, 1300 bar		
1260 Bio-inert Pump (G5654A)	653750-902, AdvanceBio Peptide Mapping 120Å, 2.1 x 150 mm, 2.7 µm, 600 bar		
1260 and 1290 Bio Flexible Pump and 1290 Bio High-Speed Pump (G7131A/C and G7132A)	691975-302T, InfinityLab Poroshell 120 EC-C18, 3.0 x 30 mm, 2.7 µm, 600 bar		

Checkout Samples

Checkout Sample for UV Detection at 600 bar or higher

The 5188-6529 (RRLC checkout sample) serves as standard for systems with 600 bar or higher and contains 100 ng/µL each of nine components dissolved in water / acetonitrile (65/35). The nine components are:

- Acetanilide
- Acetophenone
- Propiophenone
- Butyrophenone
- Benzophenone
- Valerophenone
- Hexanophenone
- Heptanophenone
- Octanophenone

Checkout Sample for UV Detection at Maximum 400 bar and FL Detection

The 01080-68704 (Isocratic standard) serves as standard for isocratic systems, systems with a FLD as only detector, and for systems with 400 bar pumps. It contains each of four components dissolved in methanol.

- Dimethylphthalate
- Diethylphthalate
- Biphenyl
- o-Terphenyl

Checkout Sample for ELSD

The 5190-0488 (Caffeine standards kit) serves as standard for several instrument related procedures. The 200 µg/mL standard is used for checkout of ELSD systems. The standard kit contains calibrated amounts of caffeine in water with concentrations of:

- 0.5 µg/mL
- 1 µg/mL
- 2 µg/mL
- 5 µg/mL
- 25 µg/mL
- 50 µg/mL
- 100 µg/mL
- 200 µg/mL

Checkout Sample for RID

The 5064-8220 (OQ/PV test sample) serves as standard for several instrument related procedures. The 15 mg/mL sample is used for checkout of RID only systems. The standard kit contains calibrated amounts of glycerol in water with concentrations of:

- 5 mg/mL
- 10 mg/mL
- 15 mg/mL
- 25 mg/mL
- 50 mg/mL

Checkout Sample for Fraction Collection

The 5190-8223 (Delay and checkout calibrant) is used for delay calibration and for the checkout of the Fraction Collectors. It contains three dyes dissolved in DMSO, which can be separated chromatographically, resulting in three differently colored fractions. To successfully separate the dyes, acidification of the mobile phase is necessary. If not present at the customer, G2453-85060 (Formic acid) can be used to prepare the mobile phase for checkout.

Checkout Method for Isocratic, 400 bar and FLD only systems

This checkout method covers configurations with isocratic or 400 bar pumps or systems with FLD as only detectors. For column options and checkout samples, see Table 3 on page 60, and Checkout Sample for UV Detection at Maximum 400 bar and FL Detection on page 61.

1 Section NOT Applicable

2 Install the checkout column.

3 Setup the system.

a Set parameters for the pump.

OR

Table 4: Checkout method parameter settings G7111A/B, G7112B, G7120A, G7132A, G7104A/C, G7131A/C, or G5654A

Parameter	Value
Flow	1 mL/min (0.8 mL/min for G5654A)
Solvent A	Water
Solvent B	ACN
Compressibility	Use solvent types (Use 95 for G7111A/B) for Compressibility
Composition	35 % A (Water)
Composition	65 % B (ACN)
Stoptime	10 min
Pressure Limit	400 bar
Minimum Stroke	Automatic

b Set parameters for the injector.

Table 5: Checkout method parameter settings G7129A/B, G7167A/B, G7137A, or G5668A

Parameter	Value
Injection	1 µL
Stoptime	as pump
Draw speed	100 µL/min

OR

c Set parameters for the multicolumn thermostat.

Table 6: Checkout method parameter settings G7116A/B, or G7130A

Parameter	Value
Temperature (left)	40 °C
Temperature (right)	combined
Stoptime	as pump

d Set parameters for the detector.

Table 7: Checkout method parameter settings G7115A, G7165A, or G7117A/B/C

Parameter	Value
Signal A	254 /4 nm
Ref A	360 /100 nm
Peakwidth	40 Hz
Stoptime	as pump
Spectrum	None
Autobalance	Prerun

OR

OR

Table 8: Checkout method parameter settings G7121A/B

Parameter	Value
Excitation Wavelength	246 nm
Emission Wavelength	317 nm
PMT gain	Starting at a value of 9, decrease/increase the PMT to bring the highest peak on the FLD to a value between 1 and 100 LU
Response time	4 s

4 Start the system.

5 Equilibrate the system until the pressure signal and the detector baseline are stable.

6 Run the checkout sample and check the chromatogram for obvious abnormalities.

Checkout Method for 1260 and 1290 Systems with UV Detectors

This checkout method covers configurations with gradient pumps with pressures of 600 bar and above with UV detectors. For column options and checkout samples, see Table 3 on page 60 and Checkout Sample for UV Detection at 600 bar or higher on page 61.

1 Section NOT Applicable

2 Install the checkout column.

3 Setup the system.

a Set parameters for the pump.

Table 9: Checkout method parameter settings G7111A

Parameter	Value
Flow	1 mL/min
Solvent A	Water
Solvent B	ACN
Compressibility	75
Composition	35 % A (Water)
Composition	65 % B (ACN)
Stoptime	10 min
Pressure Limit	400 bar
Minimum Stroke	Automatic

OR

Table 10: Checkout method parameter settings G7111B, G7112B, G7120A, G7132A, G7104A/C, G7131A/C, or G5654A

Parameter	Value
Flow	0.8 mL/min (0.6 mL/min for G5654A)
Solvent A	Water
Solvent B	ACN
Compressibility	Use solvent types (use 95 for G7111B)

Parameter	Value
Composition	60 % A (Water)
Composition	40 % B (ACN)
Stoptime	10 min
Minimum Stroke	Automatic
Timetable	2.5 min, 80 %B

b Set parameters for the injector.

Table 11: Checkout method parameter settings G7129A/B, G7167A/B, G7137A, or G5668A

Parameter	Value
Injection	1 µL
Stoptime	as pump
Draw speed	100 µL/min

OR

Table 12: Checkout method parameter settings G7167C

Parameter	Value
Injection	1 µL
Stoptime	as pump
Draw speed	100 µL/min
Feed Injection Mode	**mandatory**
Feed Speed	Adaptive: 80 % of the pump flow
Flush-out	automatic
Flow-through Injection Mode	**optional** [2]
Flush-out Factor	3.5
Delay Volume Reduction	Enabled

2 Flow-through Injection is optional. It can be done as an extra to the Feed Injection by customer request.

c Set parameters for the multicolumn thermostat.

Table 13: Checkout method parameter settings G7116A/B, or G7130A

Parameter	Value
Temperature (left)	40 °C
Temperature (right)	combined
Stoptime	as pump

d Set parameters for the multicolumn thermostat.

Table 14: Checkout method parameter settings G7116A/B, or G7130A

Parameter	Value
Temperature (left)	40 °C
Temperature (right)	combined
Stoptime	as pump

e Set parameters for the detector.

Table 15: Checkout method parameter settings G7115A, G7165A, or G7117A/B/C

Parameter	Value
Signal A	254 /4 nm
Ref A	360 /100 nm
Peakwidth	40 Hz
Stoptime	as pump
Spectrum	None
Autobalance	Prerun

OR

Table 16: Checkout method parameter settings G7114A/B

Parameter	Value
Wavelength	254 nm
Peakwidth	40 Hz
Stoptime	as pump
Autobalance	Prerun

4 Start the system.

5 Equilibrate the system until the pressure signal and the detector baseline are stable.

6 Run the checkout sample and check the chromatogram for obvious abnormalities.

Checkout Method for 1260 and 1290 Systems with ELSD or RID

This checkout method covers configurations with RI or ELS detectors as only detectors. For column options and checkout samples (keep in mind the samples are different for ELSD and RID), see Table 3 on page 60, Checkout Sample for ELSD on page 62, and Checkout Sample for RID on page 62.

1 Section NOT Applicable

2 Install the restriction capillary in the MCT.

3 Setup the system.

a Set parameters for the pump.

Table 17: Checkout method parameter settings G7110B, G7111A/B, G7112B, G7120A, G7132A, G7104A/C, G7131A/C, or G5654A with ELSD or RID

Parameter	Value
Flow	1 mL/min
Solvents	Water
Compressibility	46
Stoptime	5 min
Pressure Limit	400 bar
Minimum Stroke	Automatic

b Set parameters for the injector.

Table 18: Checkout method parameter settings G7129A/B, G7167A/B, G7137A, or G5668A with ELSD or RID

Parameter	Value
Injection	20 µL
Stoptime	as pump
Draw speed	100 µL/min

OR

Table 19: Checkout method parameter settings G7167C

Parameter	Value
Injection	20 µL
Stoptime	as pump
Draw speed	100 µL/min
Feed Injection Mode	**mandatory**
Feed Speed	Adaptive: 80 % of the pump flow
Flush-out	automatic
Flow-through Injection Mode	**optional** [3]
Flush-out Factor	3.5
Delay Volume Reduction	Enabled

c Set parameters for the multicolumn thermostat.

Table 20: Checkout method parameter settings G7116A/B with ELSD, RID

Parameter	Value
Temperature (left)	35 °C
Temperature (right)	combined
Stoptime	as pump

d Set parameters for the detector.

Table 21: Checkout method parameter settings G7102A, G426XA/B

Parameter	Value
Nebulizer	Temperature: 70 °C
Evaporator	Temperature: 70 °C
Gas Flow	1.60 SLM
Data Rate	10 Hz
Smoothing	1 s
PMT Gain	1 (No PMT Gain setting for G7102A)
LED Intensity	100 % (No Laser setting for the Agilent G7102A)

OR

3 Flow-through Injection is optional. It can be done as an extra to the Feed Injection by customer request.

Table 22: Checkout method parameter settings G7162A/B

Parameter	Value
Optical Unit Temperate	35 °C
Signal	Acquire
Response time	4 s
Signal Polarity	positive
Automatic Zero	on
Automatic Recycling	off

4 Start the system.

5 Equilibrate the system until the pressure signal and the detector baseline are stable.

6 Run the checkout sample and check the chromatogram for obvious abnormalities.

5 Typical Bio-inert LC Applications

This chapter gives an overview on typical Bio-inert LC application examples in protein characterization.

Characterization of Bio-Therapeutics

Physico-chemical characterization and confirmation plays a crucial role in the New Biological Entity (NBE) and biotherapeutics workflow to ensure drug safety and efficacy. Agilent offers a broad spectrum of tools for all assays in order to fulfill the regulatory requirements. For the LC-based tests the Agilent 1260 Infinity III Bio-inert LC System offers a high degree of flexibility to address the needs of a quality control environment as well as flexibility for method development in SEC, ion-exchange, peptide mapping, confirmation and glycan analysis. Therapeutic monoclonal antibodies (mABs, Figure 17 on page 74) are characterized by a variety of assays to ensure drug safety and efficacy. The Bio-inert LC System is a flexible tool addressing the major requirements.

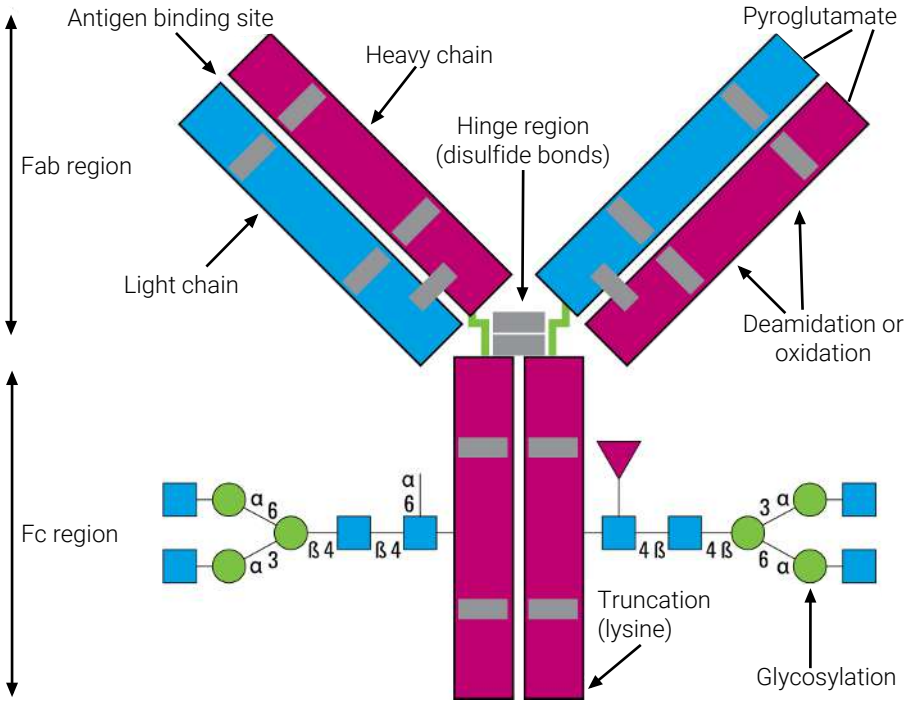


Figure 17: General structure of therapeutic antibodies

Application Examples

Ion Chromatography of Charge Variants of Monoclonal Antibodies

With the Agilent 1260 Infinity III Bio-inert LC System and the polymeric BioMab WCX columns, Agilent offers a unique single-vendor solution for highest performance requirements. The combination of column technology from 10 µm particles to 1.7 µm particles and biocompatibility results in increased robustness, superior resolution and prolonged column lifetime.

In ion-exchange applications, the LC system is often operated at high salt concentrations or in extreme pH ranges. Agilent recommends that solutions be replaced after usage to prevent crystallization of salts or to avoid additional stress on system components.

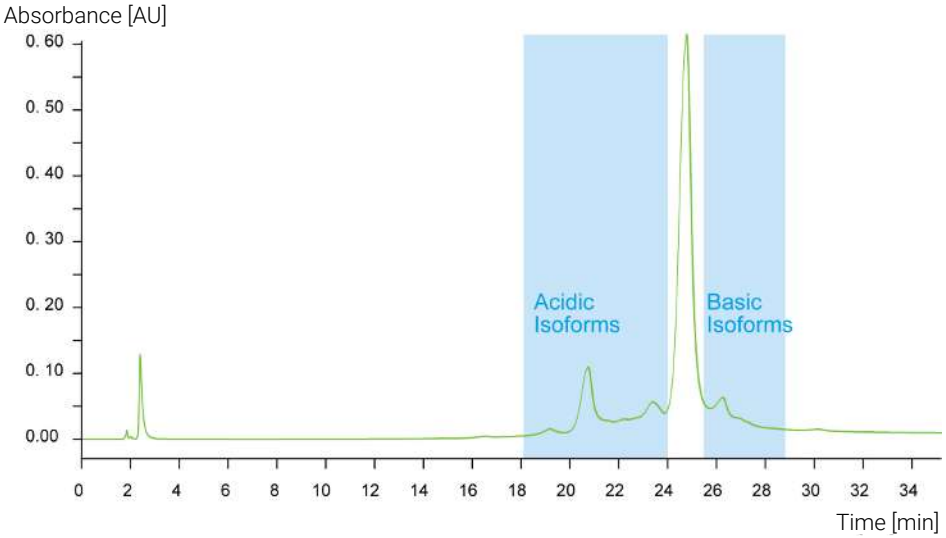


Figure 18: High resolution separation of acidic and basic charge variants using the Agilent Bio MabNP10 column

Size Exclusion Chromatography for Purity and Aggregation Analysis

With the Agilent 1260 Infinity III Bio-inert LC System and Bio SEC 5 and Bio SEC 3 columns, robust performance and high reproducibility are obtained under different buffer conditions with or without detergents. In combination with a variety of detectors such as UV and fluorescence, impurities can be easily resolved and detected with superior sensitivity.

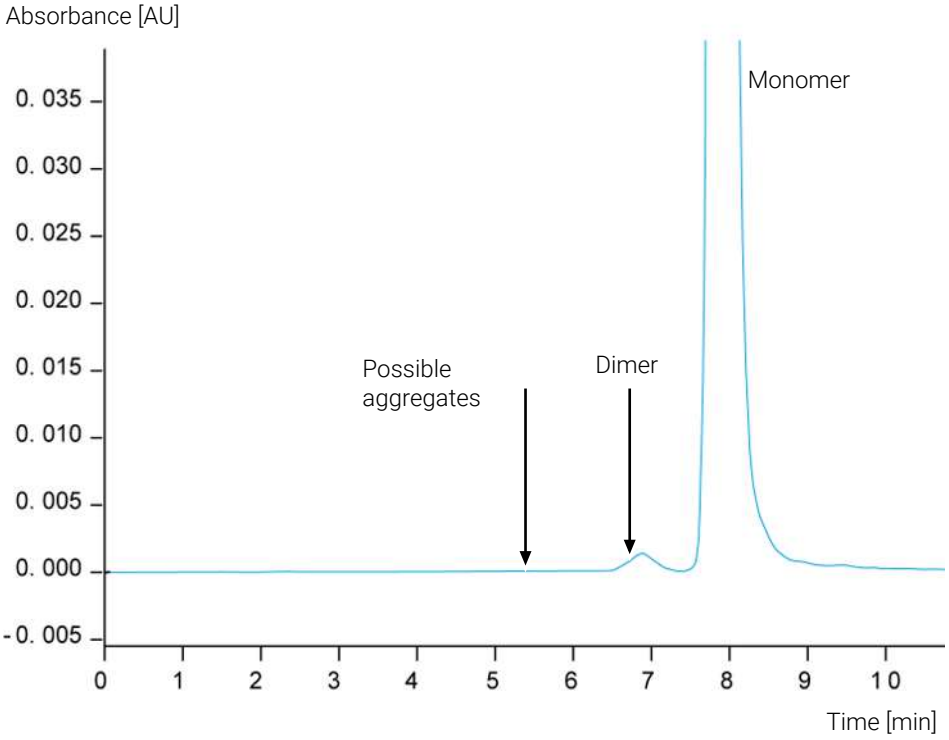


Figure 19: Aggregation analysis of a monoclonal antibody

Peptide Mapping (UHPLC at 600 bar)

The Agilent 1260 Infinity III Bio-inert LC System offers UHPLC capability combined with low surface activity, especially for critical samples. In combination with the Agilent Eclipse Plus 1.8 µm particle columns or with the Poroshell 120 stationary phases, high resolution and superior peak capacities are achieved in order to confirm the identity of the analyzed NBE drug in a QA/QC environment.

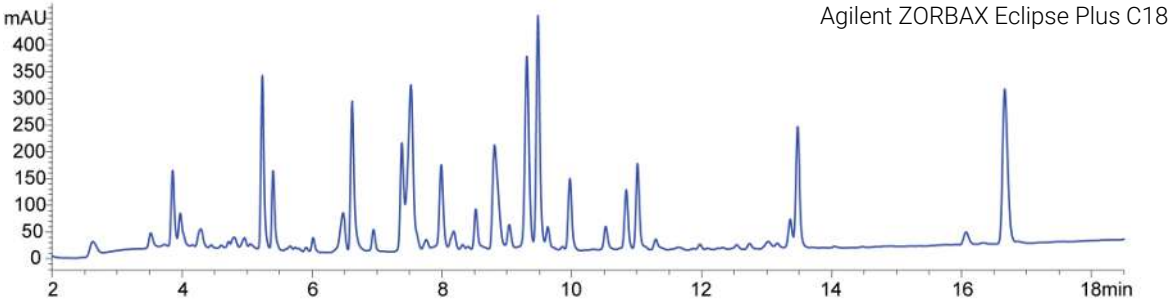
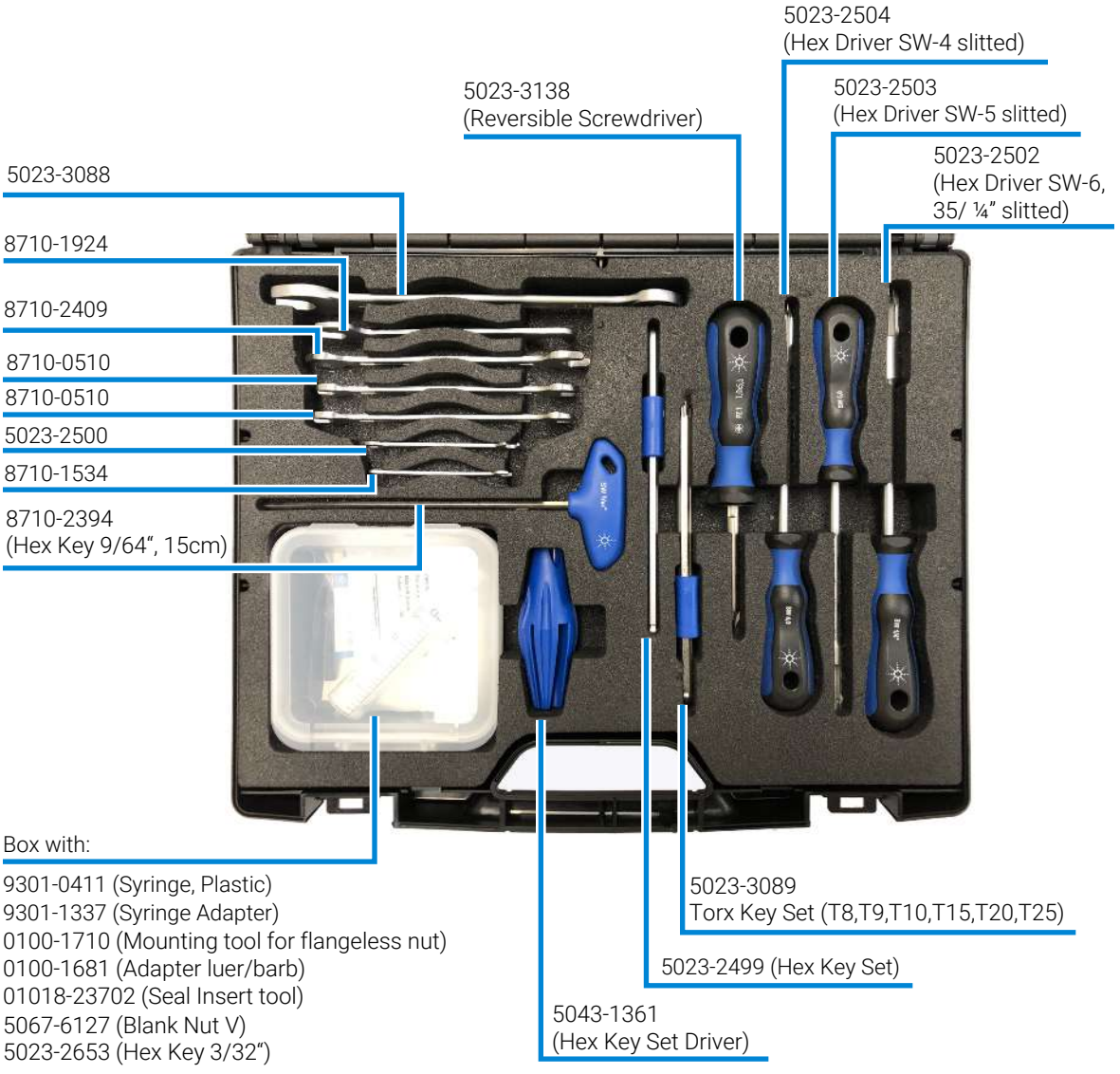


Figure 20: Peptide map with Agilent Zorbax Eclipse Plus C18 Column

6 Parts and Consumables

This chapter provides information on additional parts and consumables.

HPLC System Tool Kit



Additional Heater Devices

For biocompatible modules use bio / biocompatible parts only!

Do not mix with bio-inert parts.

For bio-inert modules use bio-inert parts only!

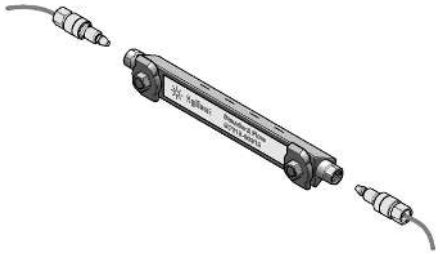
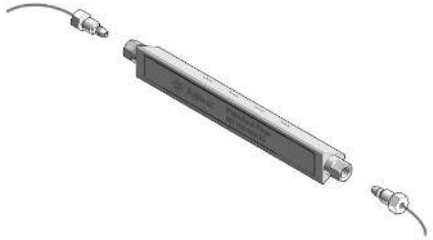
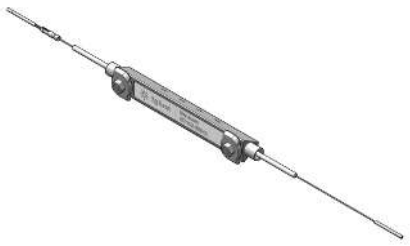
Do not mix with bio / biocompatible parts.

Table 23: Heat Exchanger overview

Flow rate	0.075 mm i.d. capillary	0.12 mm i.d. capillary	0.17 mm i.d. capillary
< 2 mL/min	*Ultra-low Dispersion* G7116-60021 (Internal volume: 1.0 µL)	*Standard Flow* G7116-60015 (Internal volume: 1.6 µL)	*Large ID* G7116-60051 (Internal volume: 3.0 µL)
> 2 mL/min		*High Flow* G7116-60031 (Internal volume: 3.0 µL)	*Large ID High Flow* G7116-60061 (Internal volume: 6.0 µL)
BIO Bio, all flow rates	*Bio Ultra-low Dispersion* G7116-60091 (Internal volume: 1.0 µL)	*Bio Standard Flow* G7116-60071 (Internal volume: 1.6 µL) *Bio High Flow* G7116-60081 (Internal volume: 3.0 µL)	
BIO INERT Bio-inert, all flow rates			*Bio-inert* G7116-60041 (Internal volume: 9.0 µL)

Quick Connect Heat Exchangers with 0.12 mm and 0.075 mm i.d. capillary are suitable for G7116B. Quick Connect Heat Exchangers with 0.17 mm i.d. are suitable for G7116A.

Table 24: InfinityLab Quick Connect Heat Exchangers

Item	Description
	Old design Quick Connect Heat Exchanger: twist lock clips of the Quick Connect Heat Exchanger are used to secure the Quick Connect Heat Exchanger in the heater block of the MCT.
	New design Quick Connect Heat Exchanger: column holders (lamella type G7116-68003 or clamp type G7116-68004) are used to secure the Quick Connect Heat Exchanger in the heater block of the MCT. G7116-68003 lamella type column holders are delivered as a part of the Quick Connect Heat Exchanger and do not need to be ordered separately.
	Design has been changed for all Quick Connect Heat Exchangers except G7116-60041, Quick Connect Heat Exchanger Bio-inert.

1260 Infinity II Max Uptime Kit Bio

G5654-68707 (1260 Infinity II & III Max Uptime Kit Bio-inert) :

Qty.	p/n	Description
1	0890-1763	Capillary PEEK 0.18 mm x 1.5 m
3	5067-4741	ZDV union (Bio-inert)
1	5062-2418	1/16in Fittings and Ferrules, 10/Pk
1	5065-4426	Colored finger-tight PEEK fittings, 10/PK
1	5971-5210	Assortment Box
1	8710-1930	Plastic and PEEK tubing cutter
1	8710-2391	Rheotool socket wrench ¼ inch
2	5041-2168	Glass filter, solvent inlet, 20 µm
1	01018-22707	PTFE Frit (5/Pk)
1	5043-0915	Fitting mounting tool
1	G5611-60502	Capillary Ti 0.17 mm x 900 mm, L (Bio-inert) Connection from Pump to Sampler
1	5500-1264	Capillary Ti 0.17 mm x 500 mm, SL/SLV Connection from Pump to Sampler
1	G5667-81005	Capillary PK/ST 0.17 mm x 500 mm, RLO/RLO (Bio-inert) Connection from Sampler to Bio Heat Exchanger
1	G5654-90130	Technical Note 1260 Infinity II & III Max Uptime Kit Bio-inert

Valve Kits

For bio-inert modules use bio-inert parts only!

Do not mix with bio / biocompatible parts.

G5631A Bio-inert 2pos/6port Valve Head kit

Table 25: G5631A Bio-inert 2pos/6port Valve Head kit

Description	Amount	Unit	Comment
5067-4148 (2-position/6-port Bio-inert valve, 600 bar)	1	ea	
5067-4767 (Bio-inert Capillary kit for 2 pos/6 port valve)	1	ea	

G5632 Bio-inert 2pos/10port Valve Head kit

Table 26: G5632A Bio-inert 2pos/10port Valve Head kit

Description	Amount	Unit	Comment
5067-4132 (2-position/10-port Bio-inert valve, 600 bar)	1	ea	
5067-5419 (Capillary kit for 2 position/10 port bio-inert valve)	1	ea	Optional

G5639A Bio-inert 4 Column Selector Valve Kit

Table 27: G5639A Bio-inert 4 Column Selector Valve Kit

Description	Amount	Unit	Comment
5067-4134 (4-position/10-port Bio-inert valve, 600 bar)	1	ea	
5067-4769 (Bio-inert Capillary kit for 4 Column Selector)	1	ea	

G4235A Solvent Selection Valve Head, Bio-inert - Stand Alone Valve

Table 28: G4235A Solvent Selection Valve Head, Bio-inert - Stand Alone Valve

Description	Amount	Unit	Comment
5067-4159 (12-position/13-port selector valve head, 210 bar, bio-inert)	1	ea	
5067-4601 (Solvent selection tubing kit, 4 solvents)	up to 4	ea	optional

7 Appendix

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

7 Appendix

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.**

Safety Standards

This is a Safety Class I instrument (provided with terminal for protective earthing) and has been manufactured and tested according to international safety standards.

General

Do not use this product in any manner not specified by the manufacturer. The protective features of this product may be impaired if it is used in a manner not specified in the operation instructions.

Before Applying Power

WARNING Wrong voltage range, frequency or cabling

Personal injury or damage to the instrument

- Verify that the voltage range and frequency of your power distribution matches to the power specification of the individual instrument.
- Never use cables other than the ones supplied by Agilent Technologies to ensure proper functionality and compliance with safety or EMC regulations.
- Make all connections to the unit before applying power.

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.

NOTE Note the instrument's external markings described under Safety Symbols on page 96.

Ground the Instrument

WARNING Missing electrical ground

Electrical shock

- If your product is provided with a grounding type power plug, the instrument chassis and cover must be connected to an electrical ground to minimize shock hazard.
- The ground pin must be firmly connected to an electrical ground (safety ground) terminal at the power outlet. Any interruption of the protective (grounding) conductor or disconnection of the protective earth terminal will cause a potential shock hazard that could result in personal injury.

Do Not Operate in an Explosive Atmosphere

WARNING

Presence of flammable gases or fumes

Explosion hazard

- **Do not operate the instrument in the presence of flammable gases or fumes.**

Do Not Remove the Instrument Cover

WARNING

Instrument covers removed

Electrical shock

- **Do Not Remove the Instrument Cover**
- **Only Agilent authorized personnel are allowed to remove instrument covers. Always disconnect the power cables and any external circuits before removing the instrument cover.**

Do Not Modify the Instrument

Do not install substitute parts or perform any unauthorized modification to the product. Return the product to an Agilent Sales and Service Office for service and repair to ensure that safety features are maintained.

In Case of Damage

WARNING

Damage to the module

Personal injury (for example electrical shock, intoxication)

- **Instruments that appear damaged or defective should be made inoperative and secured against unintended operation until they can be repaired by qualified service personnel.**

Solvent Information

WARNING **Toxic, flammable and hazardous solvents, samples and reagents**

The handling of solvents, samples and reagents can hold health and safety risks.

- **When working with these substances observe appropriate safety procedures (for example by wearing goggles, safety gloves and protective clothing) as described in the material handling and safety data sheet supplied by the vendor, and follow good laboratory practice.**
- **Do not use solvents with an auto-ignition temperature below 200 °C (392 °F). Do not use solvents with a boiling point below 56 °C (133 °F).**
- **Avoid high vapor concentrations. Keep the solvent temperature at least 40 °C (72 °F) below the boiling point of the solvent used. This includes the solvent temperature in the sample compartment. For the solvents methanol and ethanol keep the solvent temperature at least 25 °C (45 °F) below the boiling point.**
- **Do not operate the instrument in an explosive atmosphere.**
- **Do not use solvents of ignition Class IIC according IEC 60079-20-1 (for example, carbon disulfide).**
- **Reduce the volume of substances to the minimum required for the analysis.**
- **Never exceed the maximum permissible volume of solvents (8 L) in the solvent cabinet. Do not use bottles that exceed the maximum permissible volume as specified in the usage guideline for solvent cabinet.**
- **Ground the waste container.**
- **Regularly check the filling level of the waste container. The residual free volume in the waste container must be large enough to collect the waste liquid.**
- **To achieve maximal safety, regularly check the tubing for correct installation.**

NOTE For details, see the usage guideline for the solvent cabinet. A printed copy of the guideline has been shipped with the solvent cabinet, electronic copies are available in the Agilent Information Center or via the Internet.

Recommendations on the Use of Solvents

Observe the following recommendations on the use of solvents.

- Brown glass ware can avoid growth of algae.
- Follow the recommendations for avoiding the growth of algae, see the pump manuals.
- Follow the recommendations for avoiding the growth of algae, see Algae Growth in HPLC Systems on page 93
- Small particles can permanently block capillaries and valves. Therefore, always filter solvents through 0.22 µm filters.
- Avoid or minimize the use of solvents that may corrode parts in the flow path. Consider specifications for the pH range given for different materials such as flow cells, valve materials etc. and recommendations in subsequent sections.
- Avoid the use of the following steel-corrosive solvents:
  - solutions of alkali halides and their respective acids (for example, lithium iodide, potassium chloride, and so on),
  - high concentrations of inorganic acids like sulfuric acid and nitric acid, especially at higher temperatures (if your chromatography method allows, replace by phosphoric acid or phosphate buffer which are less corrosive against stainless steel),
  - halogenated solvents or mixtures which form radicals and/or acids, for example:

    $2CHCl_3 + O_2 \rightarrow 2COCl_2 + 2HCl$

    This reaction, in which stainless steel probably acts as a catalyst, occurs quickly with dried chloroform if the drying process removes the stabilizing alcohol,
  - chromatographic grade ethers, which can contain peroxides (for example, THF, dioxane, diisopropyl ether) should be filtered through dry aluminium oxide which adsorbs the peroxides,
  - solvents containing strong complexing agents (e.g. EDTA),
  - mixtures of carbon tetrachloride with 2-propanol or THF.
- Avoid the use of dimethyl formamide (DMF). Polyvinylidene fluoride (PVDF), which is used in leak sensors, is not resistant to DMF.

Recommended Wash Solvents

- water

- ethanol
- methanol
- water/acid (especially for basic compounds)
- water/base (especially for acidic compounds)
- water/acetonitrile

NOTE For different wash solvents as mentioned above, verify that the wash solvent is suitable for the silicone wash tubing.

Solvent Compatibility of Tubings for Peristaltic Pumps

The table shows the chemical resistance properties of Silicone and PharMed tubing to different needle wash solvents:

Table 29: Solvent Compatibility of Silicone and PharMed Tubing

	Silicone	PharMed
Acids		
• weak	• good	• very good
• medium	• unsatisfactory	• good
• strong	• not recommended	• not recommended
Alkaline solution		
• weak	• good	• very good
• medium	• unsatisfactory	• very good
• strong	• not recommended	• good
Hydrocarbons		
• aliphatic	• not recommended	• not recommended
• aromatizised	• not recommended	• not recommended
• halogenated	• not recommended	• not recommended

Flow cell

To protect optimal functionality of your flow-cell:

- Avoid the use of alkaline solutions (pH > 9.5) which can attack quartz and thus impair the optical properties of the flow cell.

Algae Growth in HPLC Systems

The presence of algae in HPLC systems can cause many problems that may be incorrectly diagnosed as instrument or application problems. Algae grow in aqueous media, preferably in a pH range from 4 to 8. Their growth is accelerated by buffers, for example phosphate or acetate. Since algae grow through photosynthesis, light will also stimulate their growth. Even in distilled water small-sized algae grow after some time.

Instrumental Problems Associated With Algae

Algae deposit and grow everywhere within the HPLC system, causing the following problems:

- Blocked solvent filters, or deposits on inlet or outlet valves, resulting in unstable flow, composition or gradient problems, or a complete failure of the pump.
- Plugging of small-pore, high-pressure solvent filters, usually placed before the injector, resulting in high system pressure.
- Blockage of PTFE frits, leading to increased system pressure.
- Plugging of column filters, giving high system pressure.
- Dirty flow cell windows of detectors, resulting in higher noise levels (since the detector is the last module in the flow path, this problem is less common).

How to Prevent and/or Reduce the Algae Problem

- Always use freshly prepared solvents, especially use demineralized water, which was filtered through 0.2 µm filters.
- Never leave mobile phase in the instrument for several days without flow.
- Always discard old mobile phase.
- Use the amber solvent bottle (9301-6526 (Solvent bottle, amber, 1000 mL)) supplied with the instrument for your aqueous mobile phase.
- If possible add a few mg/L sodium azide or a few percent organic solvent to the aqueous mobile phase.

Refrigerant

Table 30: Physical properties of refrigerant R600a (isobutane)

Molecular weight	58.12
Critical temperature	134.98 °C
Critical pressure	36.6 bar
Boiling point	-11.7 °C

The refrigerant HFC-134a is used only in the Agilent Infinity II Sample Cooler.

Table 31: Physical properties of refrigerant HFC-134a

Molecular weight	102
Critical temperature	101.1 °C
Critical pressure	40.6 bar
Boiling point	-26.5 °C

WARNING **Refrigerant**

Refrigerant HFC-134a is known as a safe refrigerant, however accidents can occur if it is handled incorrectly. For this reason, the following instructions must be observed:

- **Avoid contact with liquid refrigerant HFC-134a. At atmospheric pressure HFC-134a evaporates at approximately -26 °C and causes frost bite.**
- **After skin contact, rinse the affected area with water.**
- **After eye contact, rinse the eye(s) with plenty of water for at least 15 minutes and consult a doctor.**
- **HFC-134a must not be allowed to escape in enclosed areas. Although HFC-134a is not toxic, there is a danger of suffocation as gaseous refrigerant is heavier than air.**
- **Please observe the following first aid instructions. After inhalation, move the affected person to fresh air, keep him warm and allow him to rest. If necessary, he should be supplied with oxygen. If he has stopped breathing**

or is breathing erratically, he should be given artificial respiration. In the case of cardiac arrest, carry out heart massage. Send for a doctor immediately.

- **Moreover, it must be noted that HFC-134a must always be extracted from the system and collected. It must never be discharged into the atmosphere on environmental grounds (greenhouse effect).**

CAUTION

General hazards and improper disposal

Improper disposal of the media and components used pollutes the environment.

- The disposal or scrapping of the Sample Cooler/Sample Thermostat must be carried out by a qualified disposal company.
- The disposal or scrapping of the Sample Thermostat must be carried out by a qualified disposal company.
- All media must be disposed of in accordance with national and local regulations.
- Please contact your local Agilent Service Center in regard to safe environmental disposal of the appliance or check www.agilent.com for more info.

CAUTION

Risk of fire or explosion

- Dispose of properly in accordance with federal or local regulations. Flammable Refrigerant Used.
- Do not dispose of in domestic household waste.
- To return unwanted products, contact your local Agilent office, or see http://www.agilent.com for more information.

Magnets

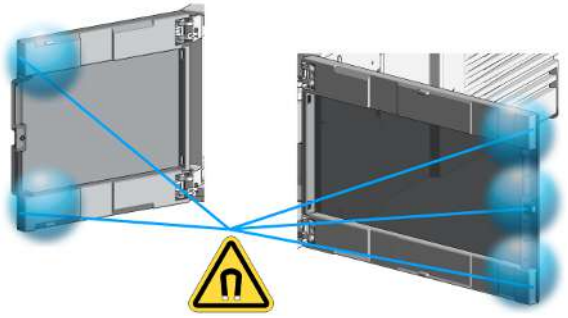


Figure 21: Magnets in doors of pumps, autosamplers, detectors, and fraction collectors

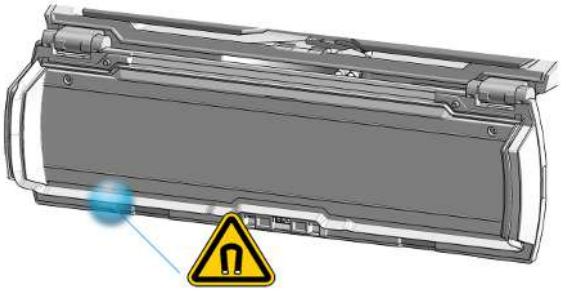
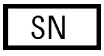


Figure 22: Magnet in the front door of the Multicolumn Thermostat

Safety Symbols

Table 32: Symbols

	The apparatus is marked with this symbol when the user shall refer to the instruction manual in order to protect risk of harm to the operator and to protect the apparatus against damage.
	Indicates dangerous voltages.

	Indicates a protected ground terminal.
	The apparatus is marked with this symbol when hot surfaces are available and the user should not touch it when heated up.
	Indicates flammable material used. Consult the Agilent Information Center / User Manual before attempting to install or service this equipment. Follow all safety precautions.
	Confirms that a manufactured product complies with all applicable European Community directives. The European Declaration of Conformity is available at: http://regulations.corporate.agilent.com/DoC/search.htm
	Manufacturing date.
	Product Number
	Serial Number
	Power symbol indicates On/Off. The apparatus is not completely disconnected from the mains supply when the on/off switch is in the Off position
	Pacemaker Magnets could affect the functioning of pacemakers and implanted heart defibrillators. A pacemaker could switch into test mode and cause illness. A heart defibrillator may stop working. If you wear these devices keep at least 55 mm distance to magnets. Warn others who wear these devices from getting too close to magnets.
	Magnetic field Magnets produce a far-reaching, strong magnetic field. They could damage TVs and laptops, computer hard drives, credit and ATM cards, data storage media, mechanical watches, hearing aids and speakers. Keep magnets at least 25 mm away from devices and objects that could be damaged by strong magnetic fields.
	Indicates a pinching or crushing hazard

Indicates a piercing or cutting hazard.

Sample Cooler unit is designed as vapor-compression refrigeration system. Contains fluorinated greenhouse gas (refrigerant) according to the Kyoto protocol.
For specifications of refrigerant, charge capacity, carbon dioxide equivalent (CDE), and global warming potential (GWP) see instrument label.

WARNING

A WARNING

alerts you to situations that could cause physical injury or death.

- **Do not proceed beyond a warning until you have fully understood and met the indicated conditions.**

CAUTION

A CAUTION

alerts you to situations that could cause loss of data, or damage of equipment.

- Do not proceed beyond a caution until you have fully understood and met the indicated conditions.

Material Information

This section provides detailed information about materials used in the HPLC system and general information about solvent/material compatibility.

Materials Used in the Bio-inert LC System

For the Bio-inert LC system, Agilent Technologies uses highest-quality materials in the flow path (also referred to as wetted parts), which are widely accepted by life science scientists, as they are known for optimum inertness to biological samples and ensure best compatibility with common samples and solvents over a wide pH range. Explicitly, the complete flow path is free of stainless steel and free of other alloys containing metals such as iron, nickel, cobalt, chromium, molybdenum, or copper, which can interfere with biological samples. The flow downstream of the sample introduction contains no metals whatsoever.

Table 33: Used bio-inert materials

Module	Materials
Agilent 1260 Infinity III Bio-inert Pump (G5654A)	Titanium, gold, platinum-iridium, ceramic, ruby, PTFE, PEEK
Agilent 1260 Infinity III Bio-inert Multisampler (G5668A)	Upstream of sample introduction: • Titanium, gold, PTFE, PEEK, ceramic Downstream of sample introduction: • PEEK, ceramic
Agilent 1260 Infinity III Bio-inert Manual Injector (G5628A)	PEEK, ceramic
Agilent 1260 Infinity III Bio-inert Analytical Fraction Collector (G5664B)	PEEK, ceramic, PTFE
Bio-inert Flow Cells:	
G5615-60022 (Standard flow cell bio-inert, 10 mm, 13 µL, 120 bar (12 MPa) for MWD/DAD, includes 0890-1763 – 0.18 x 1500 mm PEEK capillary and 5063-6591 – PEEK fittings) (*for Agilent 1260 Infinity III DAD G7115A, and MWD G7165A*)	PEEK, ceramic, sapphire, PTFE
G5615-60005 (Bio-inert flow cell, 8 µL, 20 bar) (*for Agilent 1260 Infinity III FLD G7121A/B*)	PEEK, fused silica, PTFE
Bio-inert Heat Exchangers, Valves and Capillaries:	
G7116-60041 (Quick Connect Heat Exchanger Bio-inert) (*for Agilent 1260 Infinity III Multicolumn Thermostat G7116A*)	PEEK (steel-cladded)
Bio-inert Valve heads (G4235A, G5631A, G5632A, G5639A)	PEEK, ceramic (Al_2O_3 based)
Bio-inert Connection capillaries	Upstream of sample introduction: • Titanium Downstream of sample introduction: • Agilent uses stainless-steel-cladded PEEK capillaries, which keep the flow path free of steel and provide pressure stability up to 600 bar.

NOTE To ensure optimum biocompatibility of your Bio-inert LC system, do not include non-inert standard modules or parts to the flow path. Do not use any parts that are not labeled as Agilent "Bio-inert". For solvent compatibility of these materials, see General Information About Solvent/Material Compatibility on page 101.

Materials in Flow Path

Following materials are used in the flow path of this module:

Table 34: Materials in flow path (G5654A)

Part	Materials
Degasser chamber	TFE/PDD Copolymer, PFA (internal tubings), PEEK (inlets), FEP (tubings), ETFE (fittings)
MCGV	Platinum-iridium, titanium, PTFE, PEEK, FEP, PFA, ceramic, sapphire, ruby
4CGV	PEEK, FFKM
Active inlet valve	Platinum-iridium, titanium, gold, sapphire, ruby, ceramic, PTFE
Outlet valve	Titanium, gold, ruby, ZrO_2-based ceramic, tantalum
Adapter	Titanium, gold
Pump head (body)	Titanium
Pistons	Sapphire
Piston seals/wash seals	PTFE, gold
Pressure sensor	Titanium
Purge valve	Titanium, gold, PTFE, ceramic
Damping unit	Titanium, gold
Capillaries/fittings	Titanium
Tubings	PTFE

General Information About Solvent/Material Compatibility

Materials in the flow path are carefully selected based on Agilent's experiences in developing highest-quality instruments for HPLC analysis over several decades. These materials exhibit excellent robustness under typical HPLC conditions. For any special condition, please consult the material information section or contact Agilent.

Disclaimer

Subsequent data was collected from external resources and is meant as a reference. Agilent cannot guarantee the correctness and completeness of such information. Data is based on compatibility libraries, which are not specific for estimating the long-term life time under specific but highly variable conditions of UHPLC systems, solvents, solvent mixtures, and samples. Information also cannot be generalized due to catalytic effects of impurities like metal ions, complexing agents, oxygen etc. Apart from pure chemical corrosion, other effects like electro corrosion, electrostatic charging (especially for nonconductive organic solvents), swelling of polymer parts etc. need to be considered. Most data available refers to room temperature (typically 20 – 25 °C, 68 – 77 °F). If corrosion is possible, it usually accelerates at higher temperatures. If in doubt, please consult technical literature on chemical compatibility of materials.

MP35N

MP35N is a nonmagnetic, nickel-cobalt-chromium-molybdenum alloy demonstrating excellent corrosion resistance (for example, against nitric and sulfuric acids, sodium hydroxide, and seawater) over a wide range of concentrations and temperatures. In addition, this alloy shows exceptional resistance to high-temperature oxidation. Due to excellent chemical resistance and toughness, the alloy is used in diverse applications: dental products, medical devices, nonmagnetic electrical components, chemical and food processing equipment, marine equipment. Treatment of MP35N alloy samples with 10 % NaCl in HCl (pH 2.0) does not reveal any detectable corrosion. MP35N also demonstrates excellent corrosion resistance in a humid environment. Although the influence of a broad variety of solvents and conditions has been tested, users should keep in mind that multiple factors can affect corrosion rates, such as temperature, concentration, pH, impurities, stress, surface finish, and dissimilar metal contacts.

Polyphenylene Sulfide (PPS)

Polyphenylene sulfide has outstanding stability even at elevated temperatures. It is resistant to dilute solutions of most inorganic acids, but it can be attacked by some organic compounds and oxidizing reagents. Nonoxidizing inorganic acids, such as sulfuric acid and phosphoric acid, have little effect on polyphenylene sulfide, but at high concentrations and temperatures, they can still cause material damage. Nonoxidizing organic chemicals generally have little effect on polyphenylene sulfide stability, but amines, aromatic compounds, and halogenated compounds may cause some swelling and softening over extended

periods of time at elevated temperatures. Strong oxidizing acids, such as nitric acid (> 0.1 %), hydrogen halides (> 0.1 %), peroxy acids (> 1 %), or chlorosulfuric acid degrade polyphenylene sulfide. It is not recommended to use polyphenylene sulfide with oxidizing material, such as sodium hypochlorite and hydrogen peroxide. However, under mild environmental conditions, at low concentrations and for short exposure times, polyphenylene sulfide can withstand these chemicals, for example, as ingredients of common disinfectant solutions.

PEEK

PEEK (Polyether-Ether Ketones) combines excellent properties regarding biocompatibility, chemical resistance, mechanical and thermal stability. PEEK is therefore the material of choice for UHPLC and biochemical instrumentation.

It is stable in the specified pH range (for the Bio-Inert LC system: pH 1 – 13 , see bio-inert module manuals for details), and inert to many common solvents.

There are still some known incompatibilities with chemicals such as chloroform, methylene chloride, THF, DMSO, strong acids (nitric acid > 10 %, sulfuric acid > 10 %, sulfonic acids, trichloroacetic acid), halogens or aqueous halogen solutions, phenol and derivatives (cresols, salicylic acid, and so on).

When used above room temperature, PEEK is sensitive to bases and various organic solvents, which can cause it to swell. Under such conditions, normal PEEK capillaries are sensitive to high pressure. Therefore, Agilent uses stainless steel clad PEEK capillaries in bio-inert systems. The use of stainless steel clad PEEK capillaries keeps the flow path free of steel and ensures pressure stability up to 600 bar. If in doubt, consult the available literature about the chemical compatibility of PEEK.

Polyimide

Agilent uses semi-crystalline polyimide for rotor seals in valves and needle seats in autosamplers. One supplier of polyimide is DuPont, which brands polyimide as Vespel, which is also used by Agilent.

Polyimide is stable in a pH range between 1 and 10 and in most organic solvents. It is incompatible with concentrated mineral acids (e.g. sulphuric acid), glacial acetic acid, DMSO and THF. It is also degraded by nucleophilic substances like ammonia (e.g. ammonium salts in basic conditions) or acetates.

Polyethylene (PE)

Agilent uses UHMW (ultra-high molecular weight)-PE/PTFE blends for yellow piston and wash seals, which are used in 1290 Infinity pumps, 1290 Infinity II/III pumps, the G7104C and for normal phase applications in 1260 Infinity pumps.

Polyethylene has a good stability for most common inorganic solvents including acids and bases in a pH range of 1 to 12.5 . It is compatible with many organic solvents used in chromatographic systems like methanol, acetonitrile and isopropanol. It has limited stability with aliphatic, aromatic and halogenated hydrocarbons, THF, phenol and derivatives, concentrated acids and bases. For normal phase applications, the maximum pressure should be limited to 200 bar.

Tantalum (Ta)

Tantalum is inert to most common HPLC solvents and almost all acids except fluoric acid and acids with free sulfur trioxide. It can be corroded by strong bases (e.g. hydroxide solutions > 10 %, diethylamine). It is not recommended for the use with fluoric acid and fluorides.

Stainless Steel (SST)

Stainless steel is inert against many common solvents. It is stable in the presence of acids and bases in a pH range of 1 to 12.5 . It can be corroded by acids below pH 2.3 . It can also corrode in following solvents:

- Solutions of alkali halides, their respective acids (for example, lithium iodide, potassium chloride) and aqueous solutions of halogens.
- High concentrations of inorganic acids like nitric acid, sulfuric acid, and organic solvents especially at higher temperatures (replace, if your chromatography method allows, by phosphoric acid or phosphate buffer, which are less corrosive against stainless steel).
- Halogenated solvents or mixtures, which form radicals and/or acids, for example:

  $2\ CHCl_3 + O_2 \rightarrow 2\ COCl_2 + 2\ HCl$

  This reaction, in which stainless steel probably acts as a catalyst, occurs quickly with dried chloroform if the drying process removes the stabilizing alcohol.
- Chromatographic grade ethers, which can contain peroxides (for example, THF, dioxane, diisopropyl ether). Such ethers should be filtered through dry aluminum oxide, which adsorbs the peroxides.

- Solutions of organic acids (acetic acid, formic acid, and so on) in organic solvents. For example, a 1 % solution of acetic acid in methanol will attack steel.
- Solutions containing strong complexing agents (for example, EDTA, ethylenediaminetetraacetic acid).
- Mixtures of carbon tetrachloride with isopropanol or THF.

Titanium (Ti)

Titanium is highly resistant to oxidizing acids (for example, nitric, perchloric and hypochlorous acid) over a wide range of concentrations and temperatures. This is due to a thin oxide layer on the surface, which is stabilized by oxidizing compounds. Non-oxidizing acids (for example, hydrochloric, sulfuric and phosphoric acid) can cause slight corrosion, which increases with acid concentration and temperature. For example, the corrosion rate with 3 % HCl (about pH 0.1) at room temperature is about 13 µm/year. At room temperature, titanium is resistant to concentrations of about 5 % sulfuric acid (about pH 0.3). Addition of nitric acid to hydrochloric or sulfuric acids significantly reduces corrosion rates. Titanium is sensitive to acidic metal chlorides like $FeCl_3$ or $CuCl_2$. Titanium is subject to corrosion in anhydrous methanol, which can be avoided by adding a small amount of water (about 3 %). Slight corrosion is possible with ammonia > 10 %.

Diamond-Like Carbon (DLC)

Diamond-Like Carbon is inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

Fused Silica and Quartz (SiO_2)

Fused silica is used in Max Light Cartridges. Quartz is used for classical flow cell windows. It is inert against all common solvents and acids except hydrofluoric acid and acidic solvents containing fluorides. It is corroded by strong bases and should not be used above pH 12 at room temperature. The corrosion of flow cell windows can negatively affect measurement results. For a pH greater than 12, the use of flow cells with sapphire windows is recommended.

Gold

Gold is inert to all common HPLC solvents, acids, and bases within the specified pH range. It can be corroded by complexing cyanides and concentrated acids like aqua regia.

Zirconium Oxide (ZrO_2)

Zirconium Oxide is inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

Platinum/Iridium

Platinum/Iridium is inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

Fluorinated Polymers (PTFE, PFA, FEP, FFKM, PVDF)

Fluorinated polymers like PTFE (polytetrafluorethylene), PFA (perfluoroalkoxy), and FEP (fluorinated ethylene propylene) are inert to almost all common acids, bases, and solvents. FFKM is perfluorinated rubber, which is also resistant to most chemicals. As an elastomer, it may swell in some organic solvents like halogenated hydrocarbons.

TFE/PDD copolymer tubings, which are used in all Agilent degassers except G1322A/G7122A, are not compatible with fluorinated solvents like Freon, Fluorinert, or Vertrel. They have limited life time in the presence of hexafluoroisopropanol (HFIP). To ensure the longest possible life with HFIP, it is best to dedicate a particular chamber to this solvent, not to switch solvents, and not to let dry out the chamber. For optimizing the life of the pressure sensor, do not leave HFIP in the chamber when the unit is off.

The tubing of the leak sensor is made of PVDF (polyvinylidene fluoride), which is incompatible with the solvent DMF (dimethylformamide).

Sapphire, Ruby, and Al_2O_3-Based Ceramics

Sapphire, ruby, and ceramics based on aluminum oxide Al_2O_3 are inert to almost all common acids, bases, and solvents. There are no documented incompatibilities for HPLC applications.

Flow Cell

To protect optimal functionality of your flow cell:

- G5615-60022 (Standard flow cell bio-inert, 10 mm, 13 µL, 120 bar (12 MPa) for MWD/DAD, includes 0890-1763 – 0.18 x 1500 mm PEEK capillary and 5063-6591 – PEEK fittings) (PEEK, ceramic, sapphire, PTFE) for 1260 Infinity III Diode Array Detectors (G7115A):

  The recommended pH range of the cell is 1 – 13 (short term 14)

- G5615-60005 (Bio-inert flow cell, 8 µL, 20 bar) , (PEEK, fused silica, PTFE) for 1260 Infinity III Fluorescence Detector (G7121A/B)

  The recommended pH range of the cell is 1 – 12 (solvent dependent).

- If the flow cell is transported while temperatures are below 5 °C, it must be ensured that the cell is filled with alcohol to avoid damage by freezing water.
- Aqueous solvents in the flow cell can build up algae. Therefore, do not leave aqueous solvents sitting in the flow cell. Add a small percentage of organic solvents (for example, about 5 % of acetonitrile or methanol).

At-a-Glance Details About Agilent Capillaries

The following section provides useful information about Agilent capillaries and its characteristics.

Syntax for capillary description

Type - Material - Capillary dimensions - Fitting Left/Fitting right

Table 35: Example for a capillary description

Code provided with the part	Meaing of the code
Color code: 	Material of the product is MP35N, the inner diameter is 0.20 or 0.25 mm
Capillary	The part is a connection capillary
MP35N	Material of the part is MP35N
0.25 x 80 mm	The part has an inner diameter of 0.25 mm and a length of 80 mm
SI/SI	Left fitting: Swagelok + 1.6 mm Port id, Intermediate Right fitting: Swagelok + 1.6 mm Port id, Intermediate

To get an overview of the code in use, see

- Color: Table 36 on page 109
- Type: Table 37 on page 109
- Material: Table 38 on page 110
- Dimension: Table 39 on page 110
- Fittings: Table 40 on page 111

Color Coding Guide

Table 36: Color-coding key for Agilent capillary tubing

Internal diameter in mm		Color code
0.015		Orange
0.025		Yellow
0.05		Beige
0.075		Black
0.075	MP35N	Black with orange stripe
0.1		Purple
0.12		Red
0.12	MP35N	Red with orange stripe
0.17		Green
0.17	MP35N	Green with orange stripe
0.20 /0.25		Blue
0.20 /0.25	MP35N	Blue with orange stripe
0.3		Grey
0.50		Bone White

NOTE As you move to smaller-volume, high efficiency columns, you'll want to use narrow id tubing, as opposed to the wider id tubing used for conventional HPLC instruments.

Abbreviation Guide for Type

Table 37: Type (gives some indication on the primary function, like a loop or a connection capillary)

Key	Description
Capillary	Connection capillaries
Loop	Loop capillaries
Seat	Autosampler needle seats

Key	Description
Tube	Tubing
Heat exchanger	Heat exchanger

Abbreviation Guide for Material

Table 38: Material (indicates which raw material is used for the capillary)

Key	Description
ST	Stainless steel
Ti	Titanium
PK	PEEK
FS/PK	PEEK-coated fused silica [4]
PK/ST	Stainless steel-coated PEEK [5]
PFFE	PTFE
FS	Fused silica
MP35N	Nickel-cobalt-chromium-molybdenium alloy

Abbreviation Guide for Capillary Dimensions

Table 39: Capillary dimensions (indicates inner diameter (id), length, and volume of the capillary)

Description
id (mm) x Length (mm)
Volume (µL)

4 Fused silica in contact with solvent

5 Stainless steel-coated PEEK

Abbreviation Guide for Fitting Left/Fitting Right

Table 40: Fitting left/fitting right (indicates which fitting is used on both ends of the capillary)

Key	Description
W	Swagelok + 0.8 mm Port id
S	Swagelok + 1.6 mm Port id
M	Metric M4 + 0.8 mm Port id
E	Metric M3 + 1.6 mm Port id
U	Swagelok union
L	Long
X	Extra long
H	Long head
G	Small head SW 4
N	Small head SW 5
F	Finger-tight
V	1200 bar
B	Bio
P	PEEK
I	Intermediate

Waste Electrical and Electronic Equipment (WEEE) Directive

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

NOTE

Do not dispose of in domestic household waste
To return unwanted products, contact your local Agilent office, or see https://www.agilent.com for more information.

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.

RFID Statement

Brasil

Este equipamento não tem direito à proteção contra interferência prejudicial e não pode causar interferência em sistemas devidamente autorizados. Para mais informações, consulte o site da Anatel: https://www.gov.br/anatel/pt-br.

Este produto não é apropriado para uso em ambientes domésticos, pois poderá causar interferências eletromagnéticas que obrigam o usuário a tomar medidas necessárias para minimizar estas interferências.

Canada

Statement according to RSS GEN Issue 5:

This device contains licence-exempt transmitter(s)/receiver(s) that comply with Innovation, Science and Economic Development Canada's licence-exempt RSS(s). Operation is subject to the following two conditions:

1. This device may not cause interference
2. This device must accept any interference, including interference that may cause undesired operation of the device.

Cet appareil contient des émetteurs / récepteurs exemptés de licence conformes aux RSS (RSS) d'Innovation, Sciences et Développement économique Canada. Le fonctionnement est soumis aux deux conditions suivantes:

1. Cet appareil ne doit pas causer d'interférences
2. Cet appareil doit accepter toutes les interférences, y compris celles susceptibles de provoquer un fonctionnement indésirable de l'appareil.

Mexico

La operación de este equipo está sujeta a las siguientes dos condiciones:

1. es posible que este equipo o dispositivo no cause interferencia perjudicial y
2. este equipo o dispositivo debe aceptar cualquier interferencia, incluyendo la que pueda causar su operación no deseada.

Thailand

เครื่องโทรคมนาคมและอุปกรณ์นี้มีความสอดคล้องตามมาตรฐานหรือข้อกำหนดทางเทคนิคของ กสทช.

This telecommuinication equipment conforms to NTC/NBTC technical requirement.

USA

1. User Information according to FCC 15.21:Changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.
2. Part 15 Statement according to FCC 15.19:

   This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions:

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

CAUTION Do not change or modify the equipment.

Changes or modifications not expressly approved by Agilent could void your authority to operate the equipment.

NOTE This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules.

These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Table 41: Operating frequencies and maximum power levels

Technology	Operating Frequencies/ Bands	Maximum Transmit Power Level
RFID	125 kHz	26.8 dBm

Sound Emission

Sound Pressure

Sound pressure Lp < 70 db(A) according to DIN EN ISO 7779

Schalldruckpegel

Schalldruckpegel Lp < 70 db(A) nach DIN EN ISO 7779

UV-Radiation

NOTE

This information is only valid for UV-lamps without cover (e.g. 2140-0590 and 2140-0813).

Emissions of ultraviolet radiation (200-315 nm) from this product is limited such that radiant exposure incident upon the unprotected skin or eye of operator or service personnel is limited to the following TLVs (Threshold Limit Values) according to the American Conference of Governmental Industrial Hygienists:

Table 42: UV-Radiation Limits

Exposure/day	Effective Irradiance
8 hours	0.1 µW/cm2
10 minutes	5.0 µW/cm2

Typically the radiation values are much smaller than these limits:

Table 43: UV-Radiation Typical Values

Position	Effective Irradiance
Lamp installed, 50 cm distance	Average 0.016 µW/cm2
Lamp installed, 50 cm distance	Maximum 0.14 µW/cm2

Declaration of Conformity for HOX2 Filter

Declaration of Conformity

We herewith inform you that the

Holmium Oxide Glass Filter

used in Agilents absorbance detectors listed in the table below meets the requirements of National Institute of Standards and Technology (NIST) to be applied as certified wavelength standard.

According to the publication of NIST in J. Res. Natl. Inst. Stand. Technol. 112, 303-306 (2007) the holmium oxide glass filters are inherently stable with respect to the wavelength scale and need no recertification. The expanded uncertainty of the certified wavelength values is 0.2 nm.

Agilent Technologies guarantees, as required by NIST, that the material of the filters is holmium oxide glass representing the inherently existent holmium oxide absorption bands.

Test wavelengths:

Where "x" can be any alphanumeric character

Product Number	**Series**	**Measured Wavelength ***	**Wavelength Accuracy**	**Optical Bandwidth**
G1315x, G1365x	1100, 1200, 1260	361.0 nm 418.9 nm 453.7 nm 536.7 nm	+/- 1 nm	2 nm
G7115x, G7165x	1260			
G1600x, G7100x	CE			
G1314x	1100, 1200, 1260, 1290	360.8nm 418.5nm 536.4nm	+/- 1 nm	6 nm
G7114x	1260, 1290			
G4286x,..., 94x	1120, 1220			

*) The variation in Measured Wavelength depends on the different Optical Bandwidth.

28-Oct-2014

(Date)

Thomas Jöris
(R&D Manager)

Stephan Bayerbach
(Quality Manager)

P/N 89550-90501

Revision: G
Effective by: 28-Oct-2014

Agilent Technologies on Internet

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

https://www.agilent.com

In This Book

This manual contains technical reference information about the Agilent 1260 Infinity III Bio-inert LC System.

The manual describes the following:

- introduction,
- install the modules,
- configuration settings,
- quick start guide,
- typical bio-inert LC applications,
- parts and consumables,
- safety and related information.

www.agilent.com

Edition: 10/2024

Document No: SD-29000129 Rev. C

Agilent