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.

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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.

The instrument may only be operated by appropriately qualified and trained personnel.

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 or InfinityLab Level Sensing (G7175A)

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 20.

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.

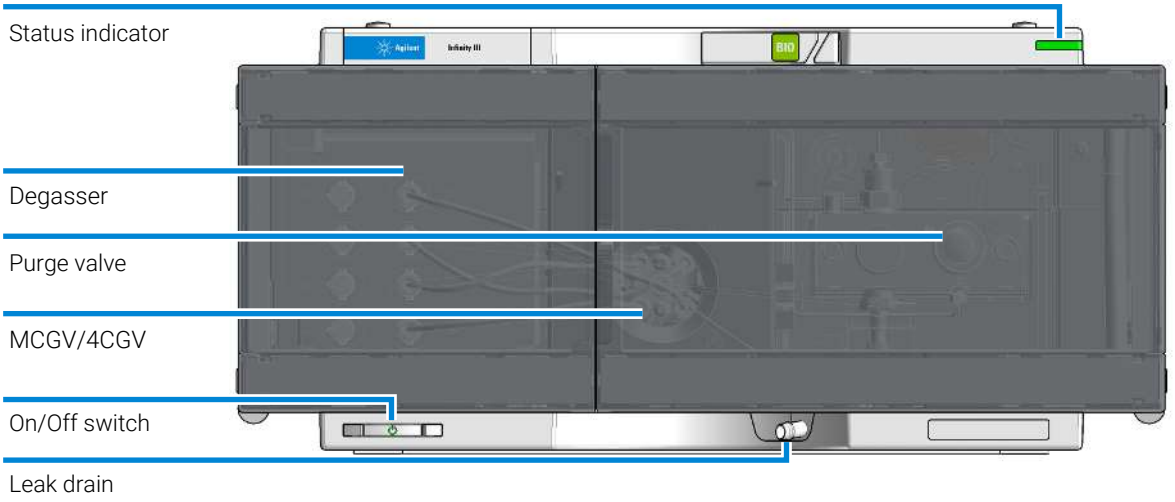


Figure 1: Overview of the Bio-inert Pump

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.

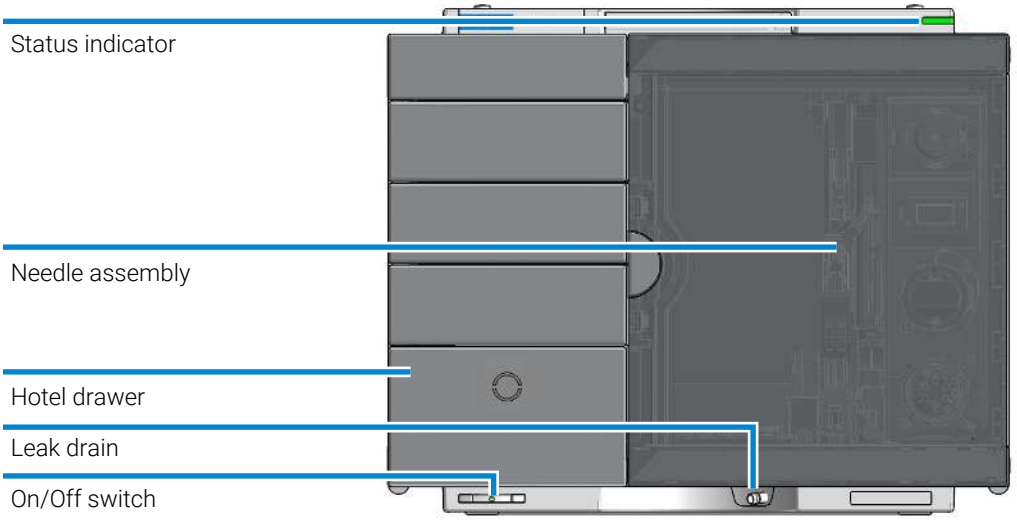


Figure 2: Overview of the Bio-inert Multisampler

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.

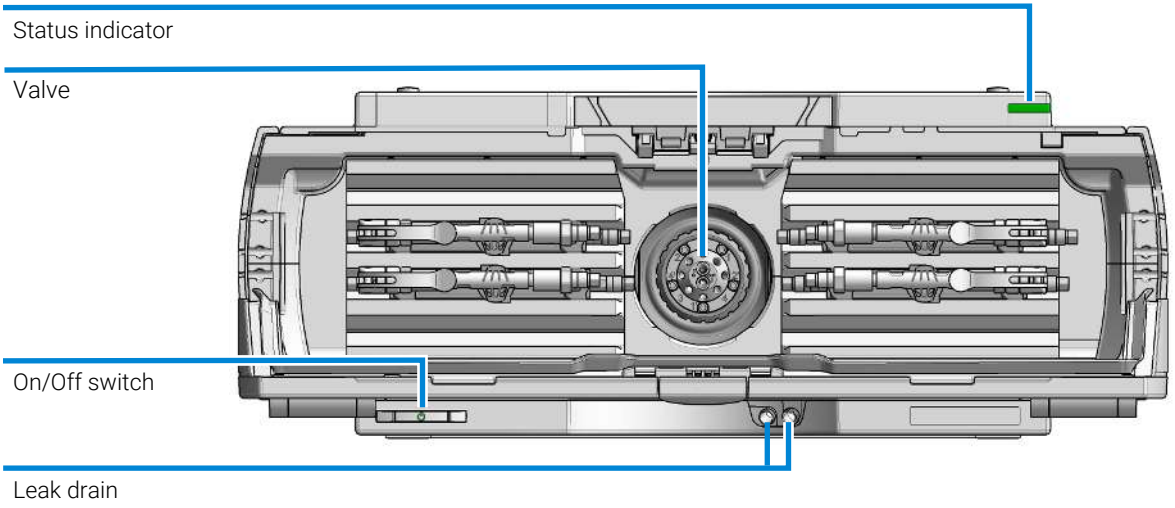


Figure 3: Overview of the Multicolumn Thermostat

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.

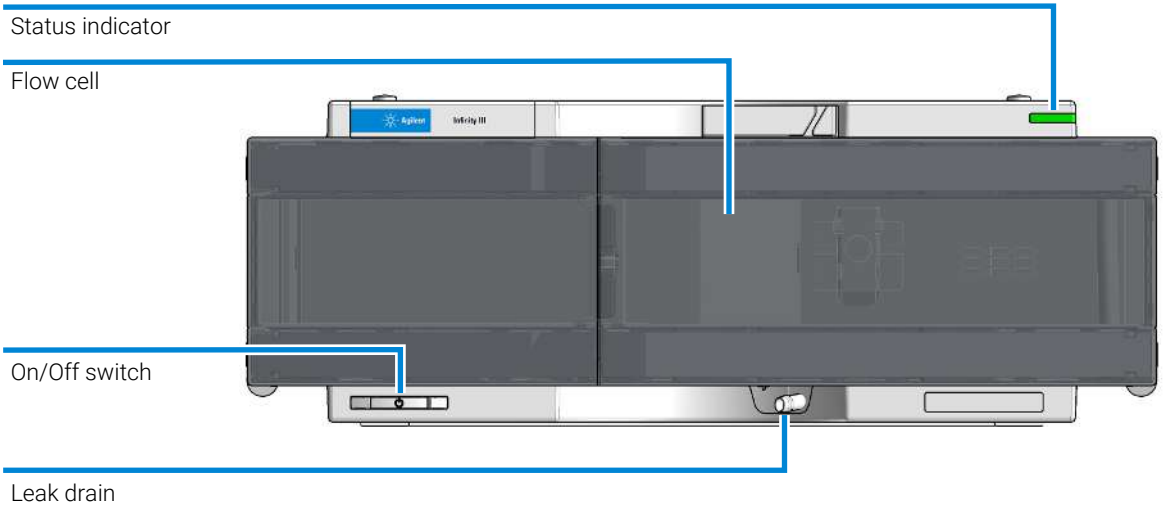


Figure 4: Overview of the G7115A Detector

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.

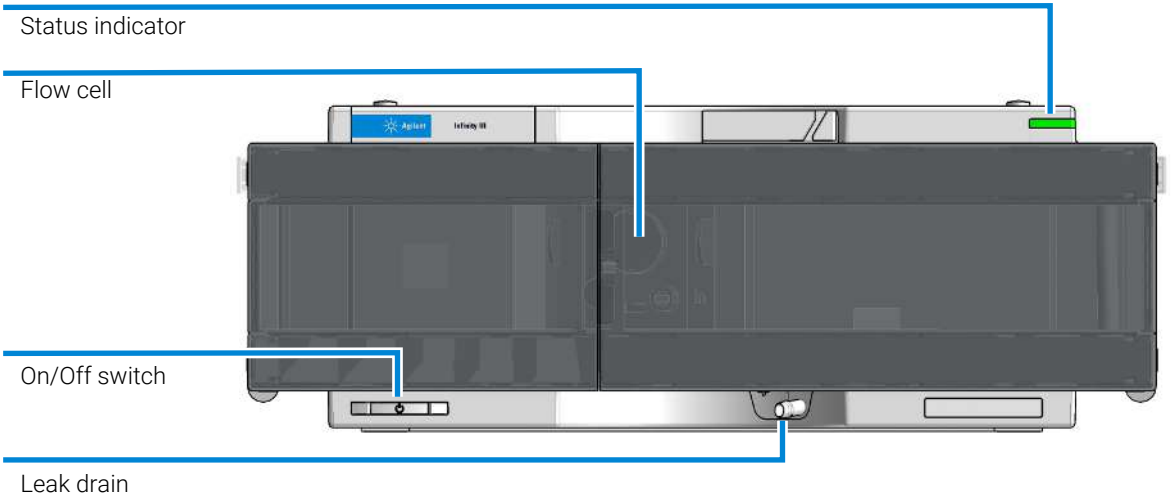


Figure 5: Overview of the G7121A Detector

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).

Product Description of the InfinityLab Level Sensing (G7175A)

The Agilent InfinityLab Level Sensing prevents HPLC instrument or column damage, and time-consuming re-analysis because the mobile phase has run dry. This easy, straightforward system, when used together with OpenLab CDS, also offers a solvent prediction. This feature predicts your HPLC solvent consumption for a complete sequence and warns you before you start so that the injections complete successfully.

The InfinityLab Level Sensing module simplifies the process of LC system preparation and control during analysis. Accurate monitoring of solvent levels ensures optimal productivity.

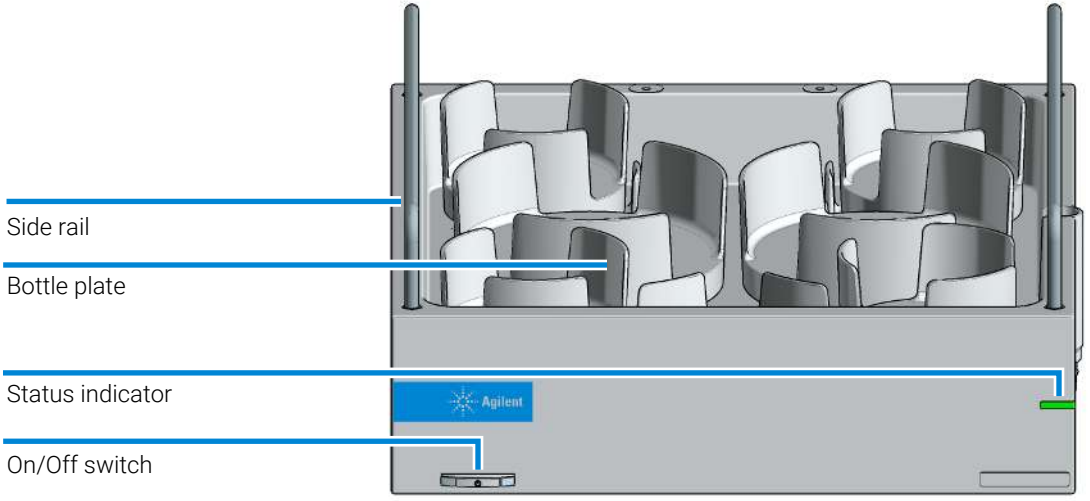


Figure 6: Overview of the Level Sensing module

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

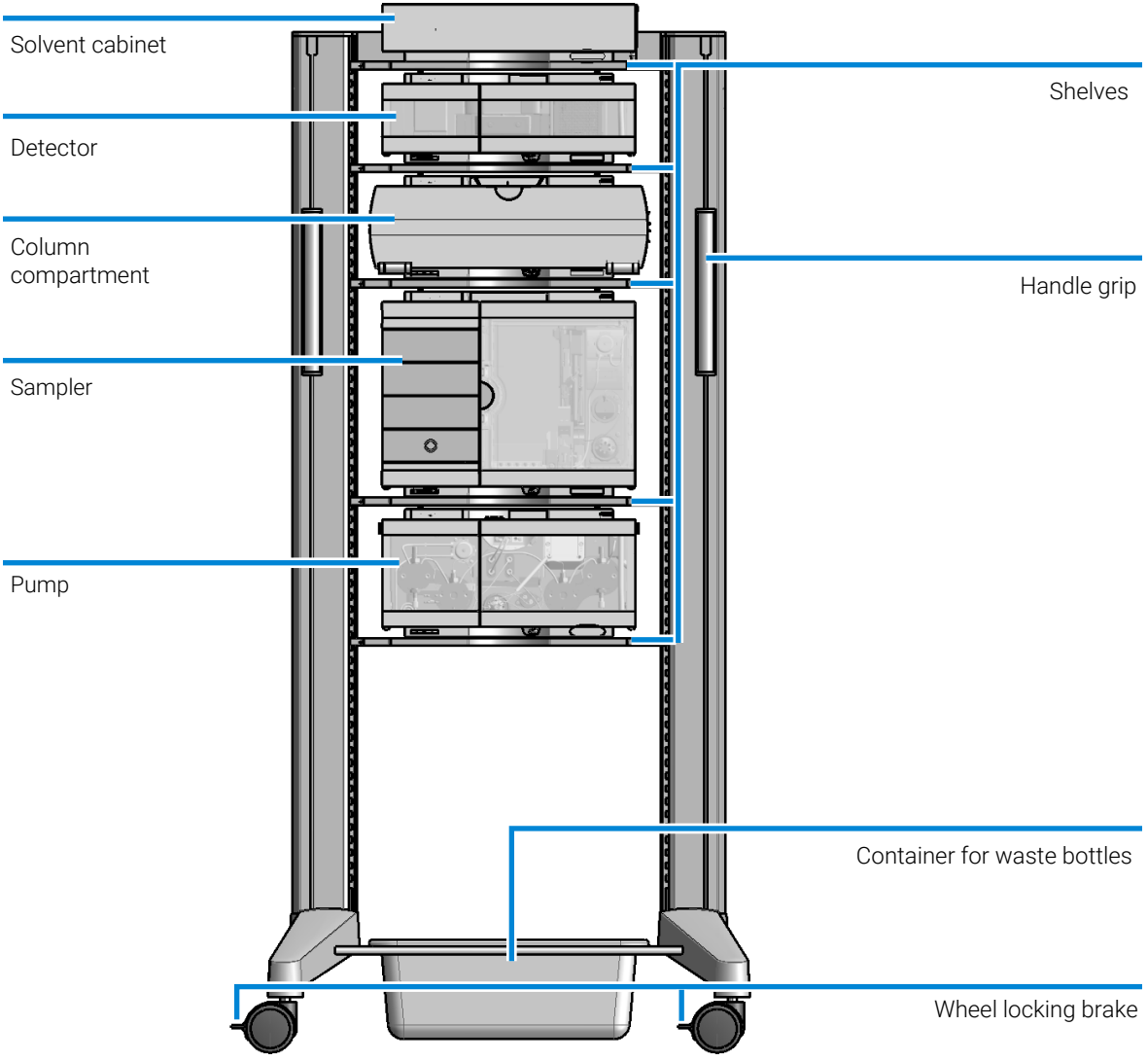


Figure 7: Agilent InfinityLab Flex Bench

One Stack Configuration

Ensure optimum performance by stacking the modules as shown exemplarily in Figure 8 on page 23. This configuration optimizes the flow path for minimum delay volume and minimizes the bench space required.

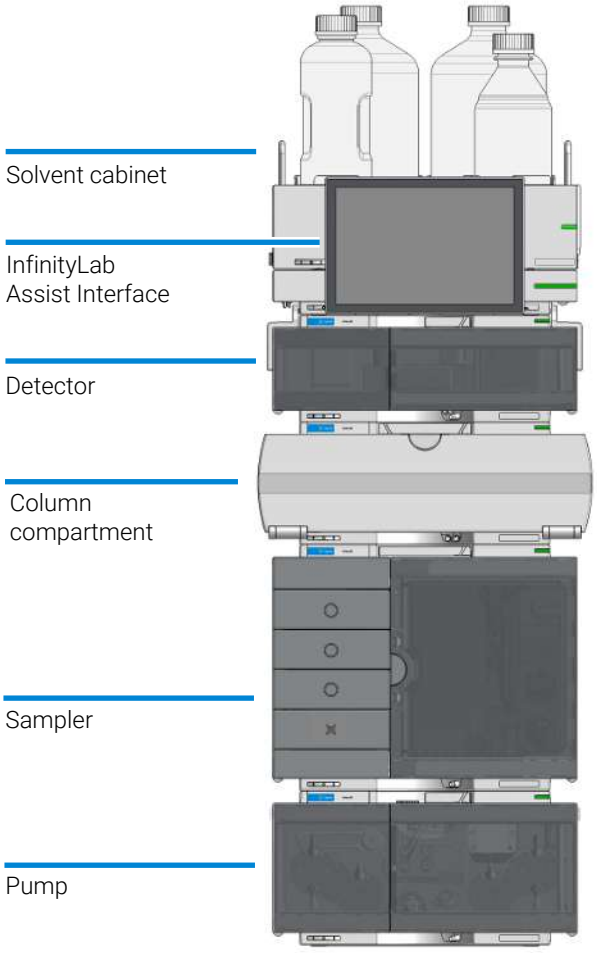


Figure 8: Single stack configuration (bench installation, example shows a Multisampler)

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.

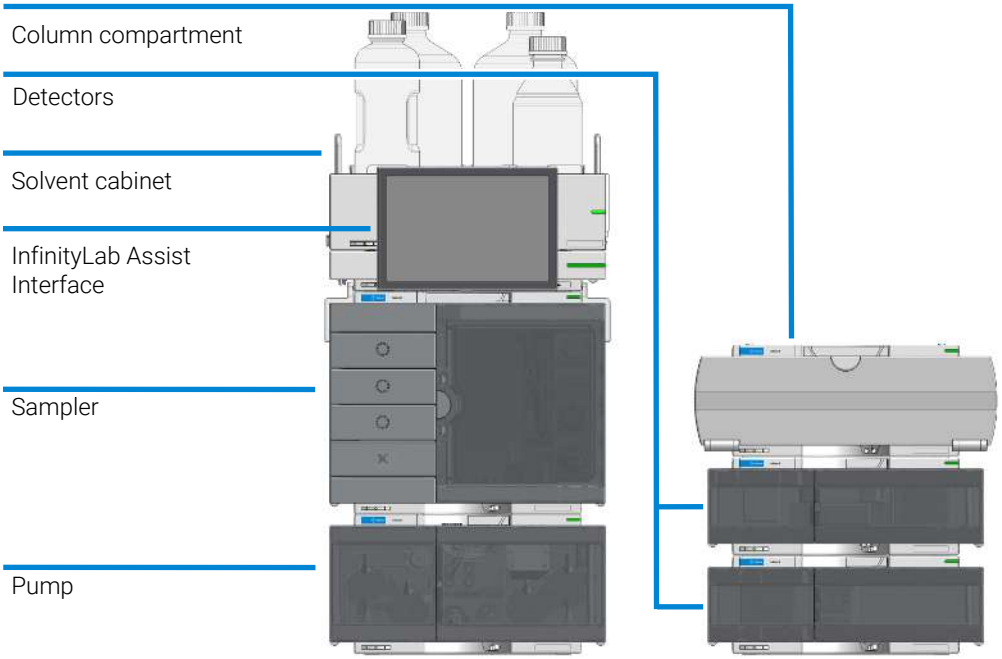


Figure 9: Two stack configuration (bench installation, example shows a Multisampler)

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 10 on page 26 shows capillary and tubing connections in the flow path. For details and necessary parts, refer to the individual module manuals.

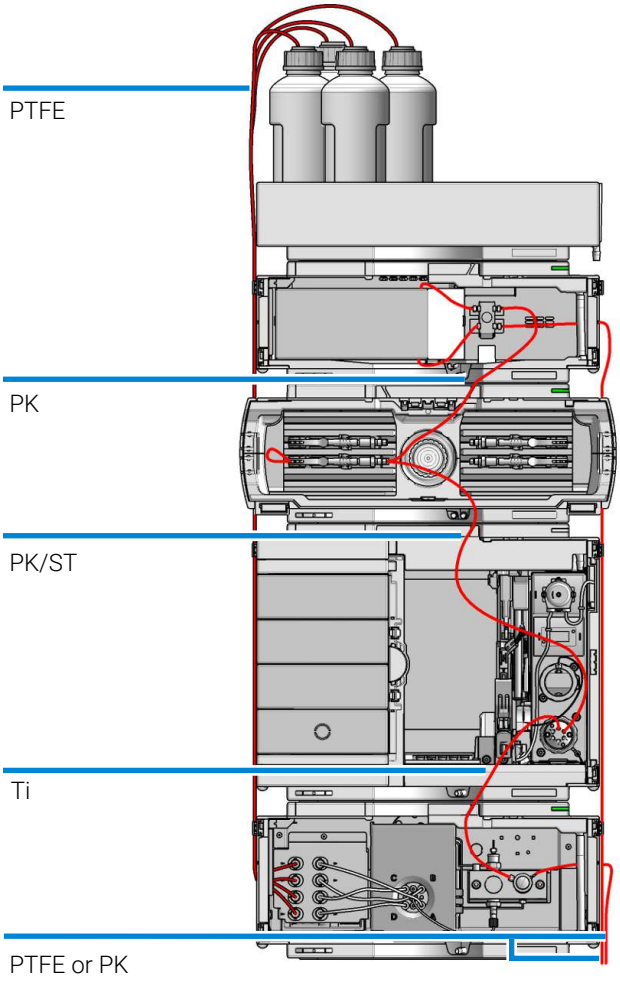


Figure 10: Capillary and tubing connections in flow path

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 28 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

This procedure describes the installation of bio-inert capillaries with UHP-FF fitting.

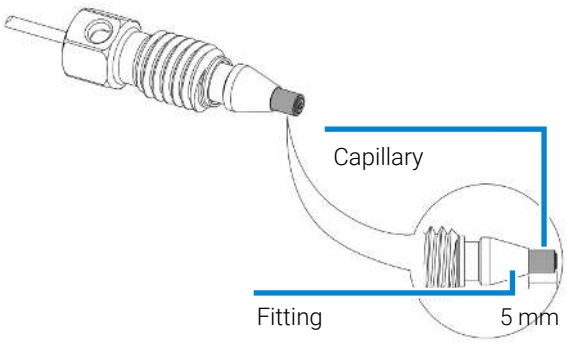
Tools required

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

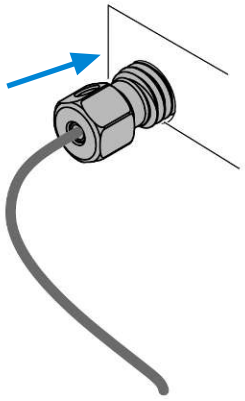
For details on necessary capillaries and fittings, see the part section of the manual.

Install UHP-FF Fittings

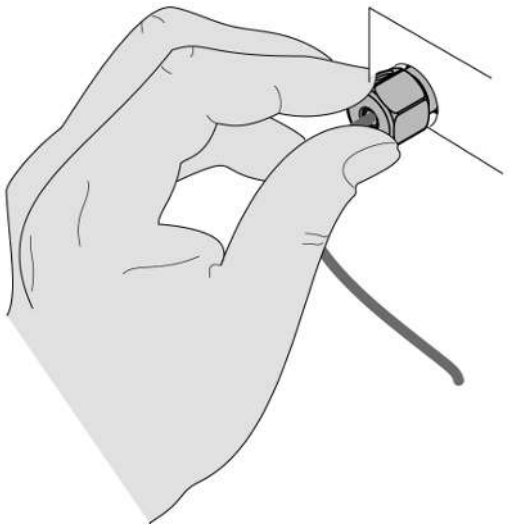
1 Slide the fitting on the capillary. Let the capillary jut out 5 mm.



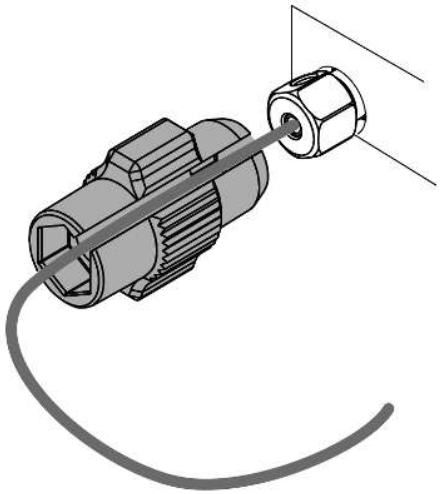
2 Insert the fitting to the receiving port and push the capillary to the bottom of the port.



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.

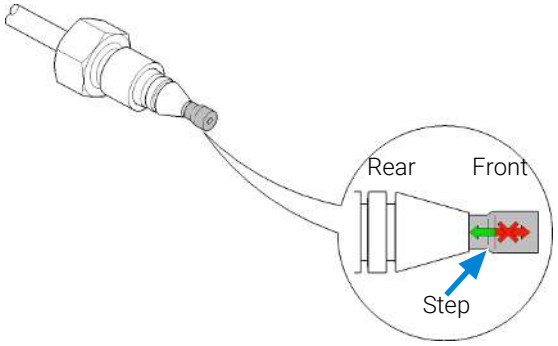


Figure 11: Capillary fitting

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

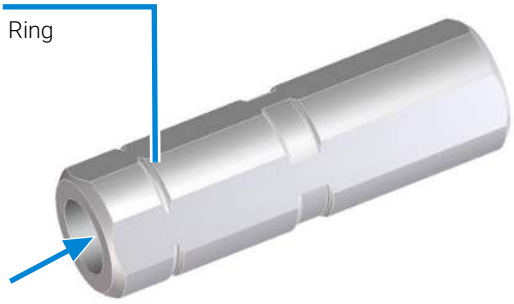
The 5067-4741 (ZDV union (Bio-inert)) has two different connectors where capillaries need to be installed in the correct sequence. Otherwise, an inset of the union may be damaged and the connection may not be tight.

CAUTION

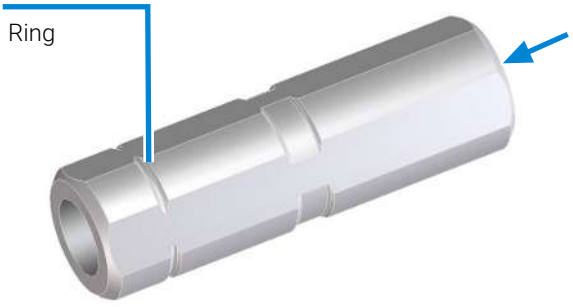
Potential leak or damage of the Bio-inert ZDV Union.

— 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

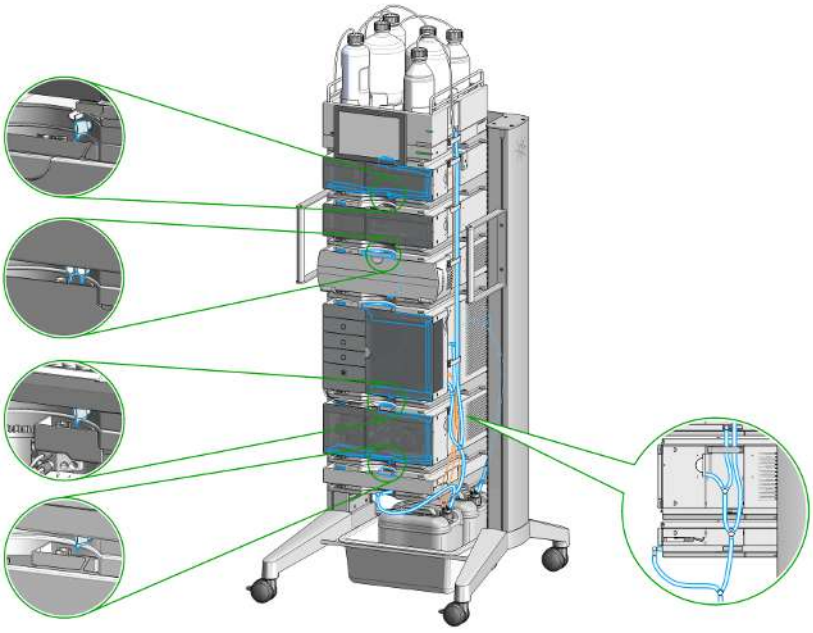


Figure 12: Infinity III Leak Waste Concept (Flex Bench installation)

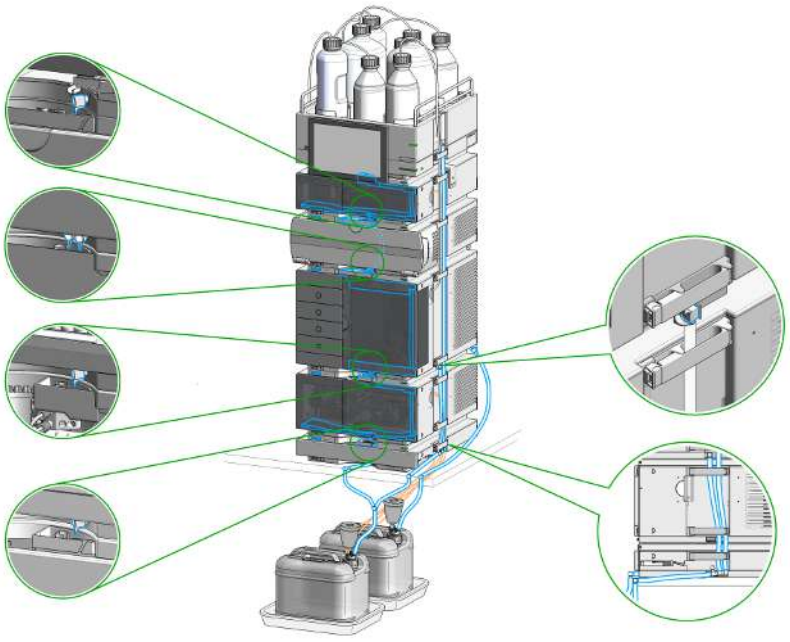


Figure 13: Infinity III Single Stack Leak Waste Concept (bench installation)

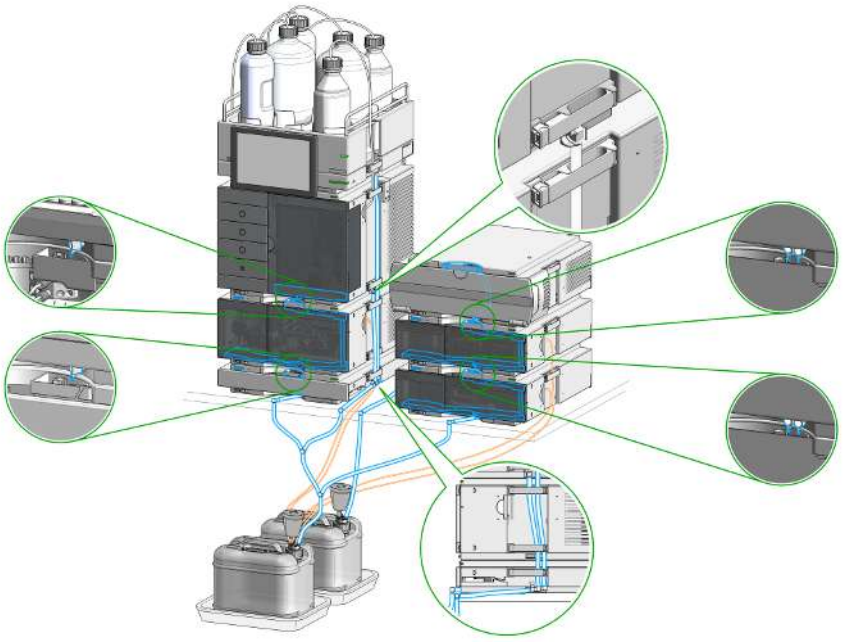


Figure 14: Infinity III Two Stack Leak Waste Concept (bench installation)

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 33.

Parts required

Qty.	p/n	Description
1	5004-0000	Drain Connectors Kit

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

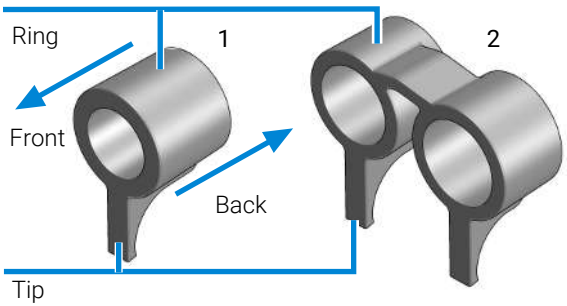


Figure 15: Overview of Drain Connectors: Single (left) and Double (right)

#	Qty.	p/n	Description
Parts can be ordered only as a complete kit.			
1	3	5043-1834	Single Drain Connector ID3.0-Long
2	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	
	G7123B	
	G7162A/B	
	G7165A	
	G7129A/B/C	Sampler
	G7167A/B/C	
	G5668A	
	G7137A/B	
	G7157A	
	G4767A	
	G7122A	Degasser
	G7104A/C	Pump
	G7110B	
	G7111A/B	
	G7112B	
	G7120A	
	G7131A/C	
	G7132A	
	G5654A	
	G4782A	

Prerequisites

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

NOTE

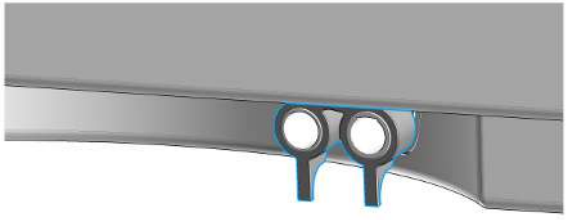
Do not install drain connectors on the bottom modules of the stack. Drain outlet of the bottom module has to be connected via waste tubing to a suitable waste container (see Leak and Waste Handling in the manual for a respective module).

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.

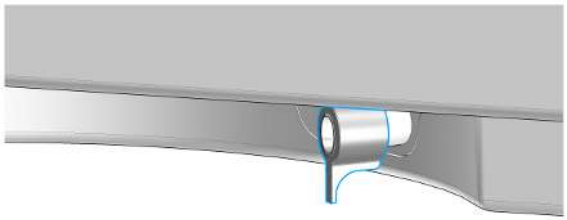
Install the Double Drain Connector on the leak drain of the 1260 Infinity III Multicolumn Thermostat (G7116A)/ 1290 Infinity III Multicolumn Thermostat (G7116B)

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.



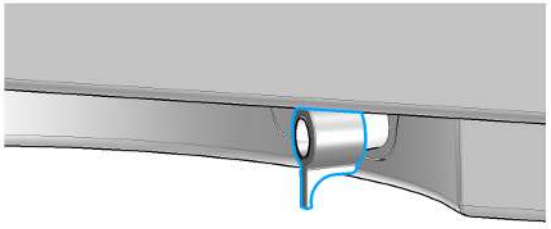
Install Single Drain Connectors on other modules in the LC stack

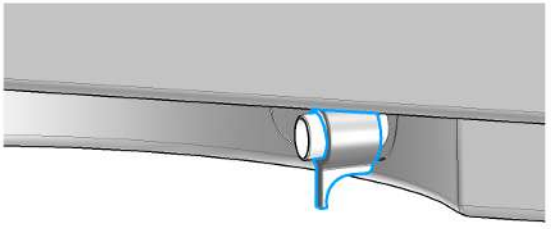
1 Align the ring with the leak drain outlet of the module, press slightly with the fingers, and slide the connector along the leak drain outlet until it is aligned with the front of the leak drain.



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.

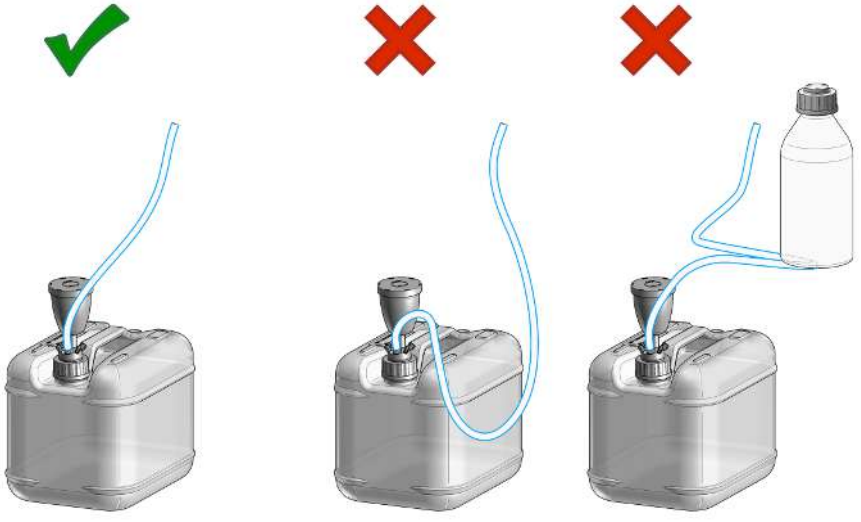




Waste Concept

Agilent recommends using 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.
- Do not place metal parts on the leak panel or the leak sensor.

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

NOTE

If the system in use supports the InfinityLab Assist, follow the instructions provided.

Else use LabAdvisor, or OpenLab CDS.

Example shows an instrument configuration with a Diode Array Detector in LabAdvisor.

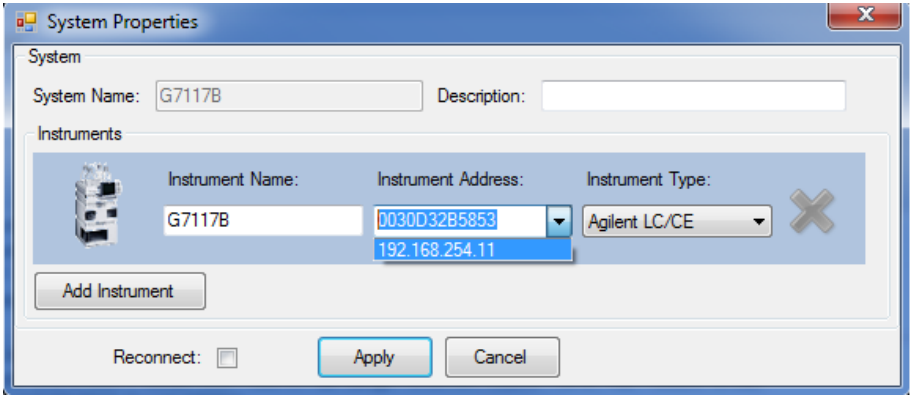
1 Set the switches of the Configuration switch at the rear of the module:

   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).

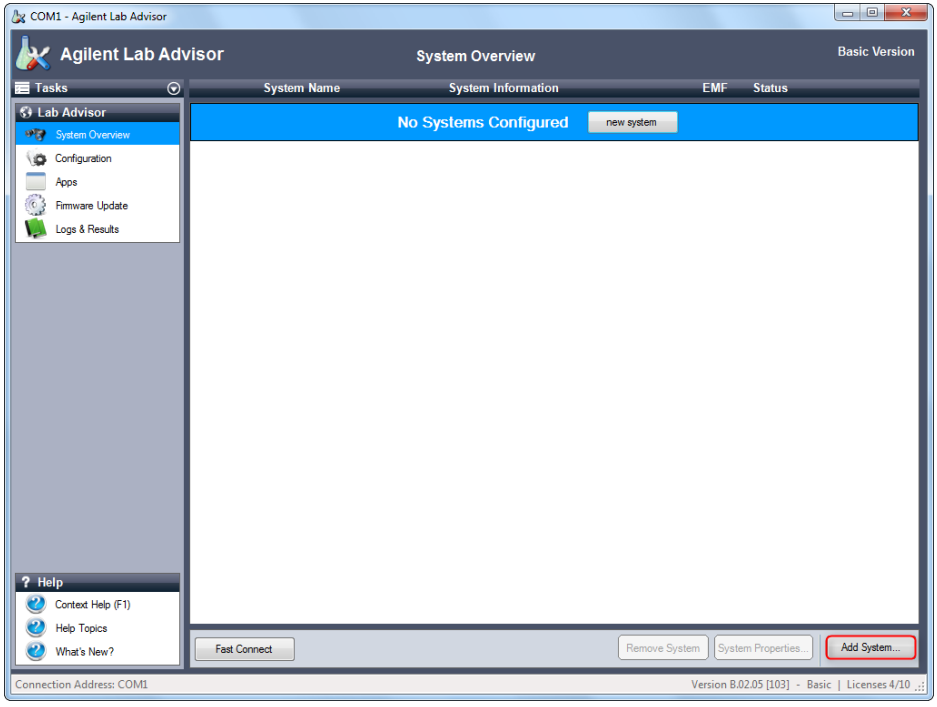


1 MAC address can only be used in DHCP DIP-switch configuration.

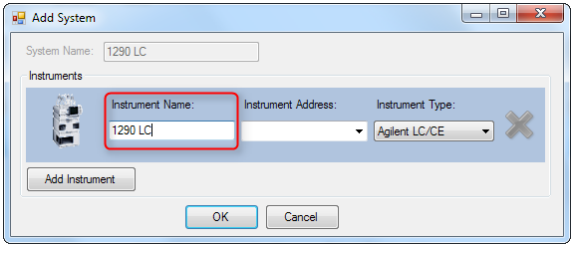
Lab Advisor

Adding a New System

1 In the Action Panel of the **System Overview**, click **Add System**.



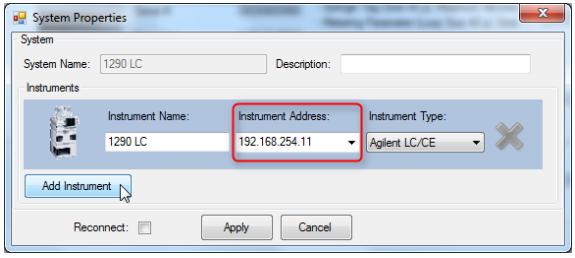
The **Add System** dialog box is displayed.



2 Enter a name in the **Instrument Name** field.

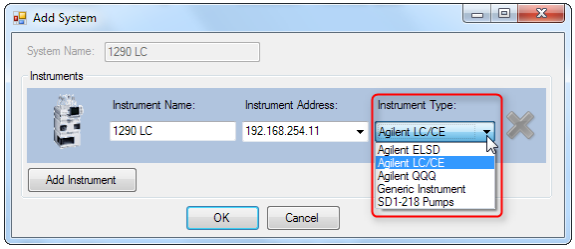
NOTE If your system comprises just one instrument, the **Instrument Name** is copied to the **System Name** field.

3 Enter the connection details in the **Instrument Address** field.



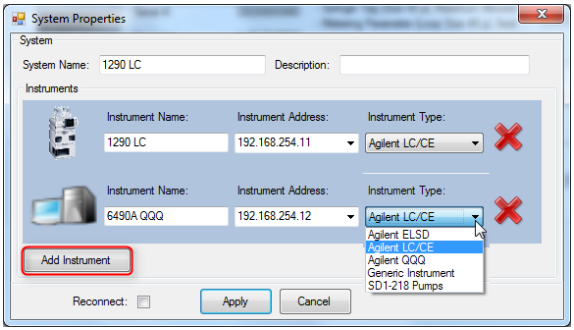
NOTE The **Instrument Address** can be an IP address, the host name or, if you are connecting using a serial cable, the COM port.

4 Click the **Instrument Type** down-arrow and select the type of instrument you are adding from the list. The default setting is **Agilent LC/CE**. Additional instrument types become available when the respective add-ons are installed.



NOTE By default, the **Instrument Type** drop-down list contains only the entry **Agilent LC/CE**. Addition instrument types can be added by installing the respective add-ons (see Installing Add-ons on page 49).

5 If your system comprises more than one instrument, click **Add Instrument** and complete the details as above.

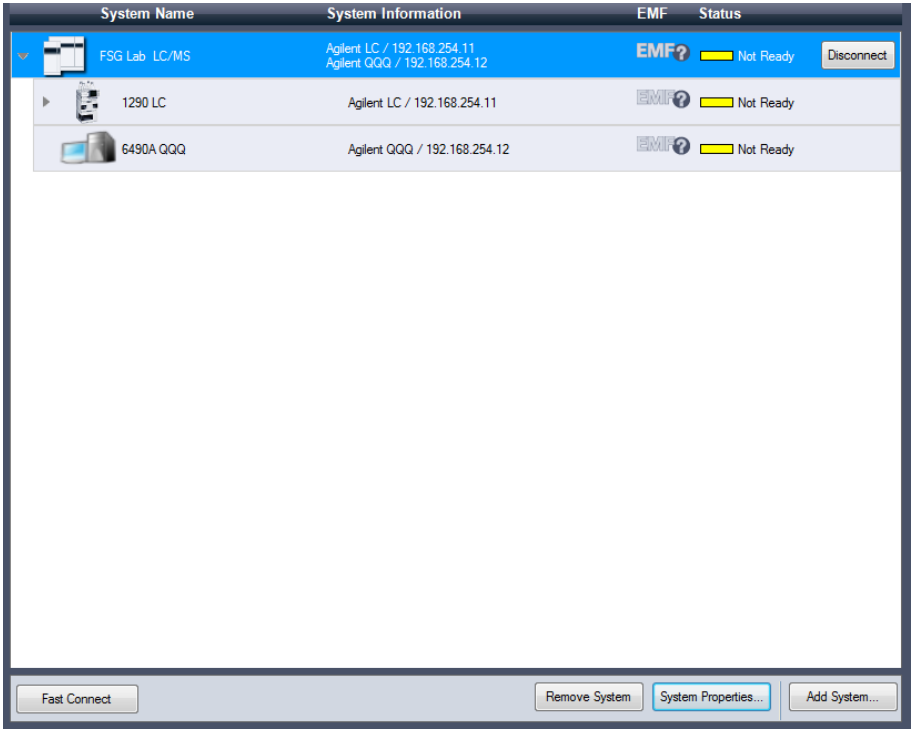


NOTE

As soon as you add a second instrument, the **System Name** field is activated to allow you to edit the system name.

6 Click **OK** to finish adding the system and close the **Add System** dialog box.

The system becomes visible in the **System Overview**, and Lab Advisor tries to connect to it.



Installing Add-ons

Add-ons are installed from the **Configuration** screen, using a Lab Advisor Extension file with the with the extension .LAX.

NOTE You need Administrator rights in order to install Add-ons.

1 In the Global Tasks section of the Navigation Panel, click **Configuration**.

The **Configuration** screen is displayed.

2 Click **Add-ons** to navigate to the **Configuration - Add-ons** screen.

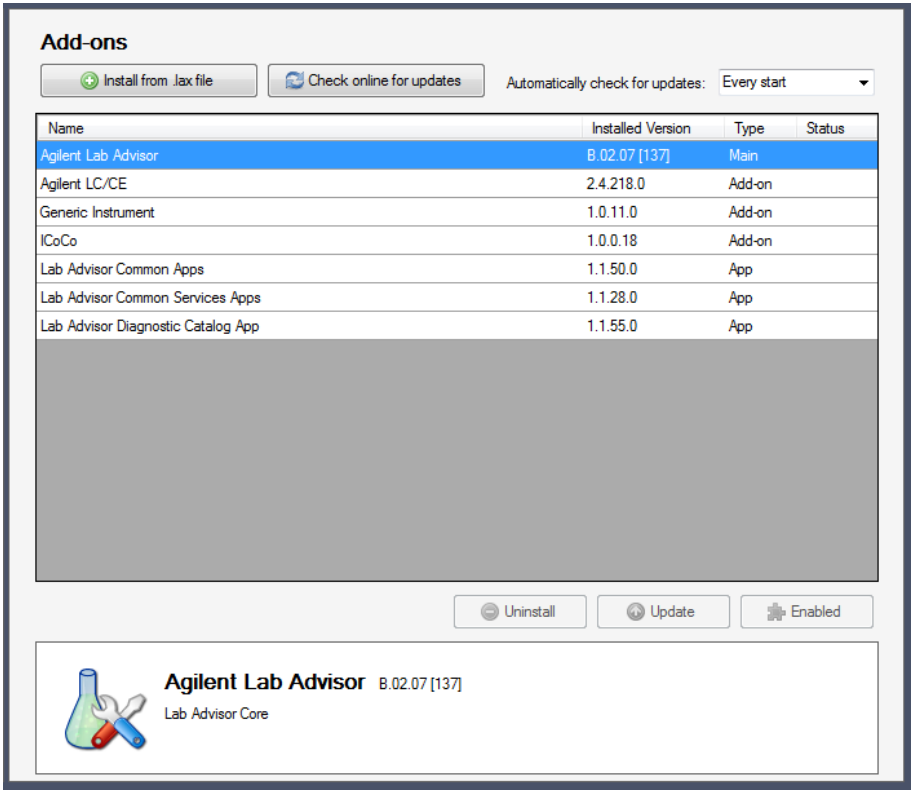


Figure 16: Add-ons in Configuration

The **Configuration - Add-ons** screen contains a table listing all the Add-ons that are already installed.

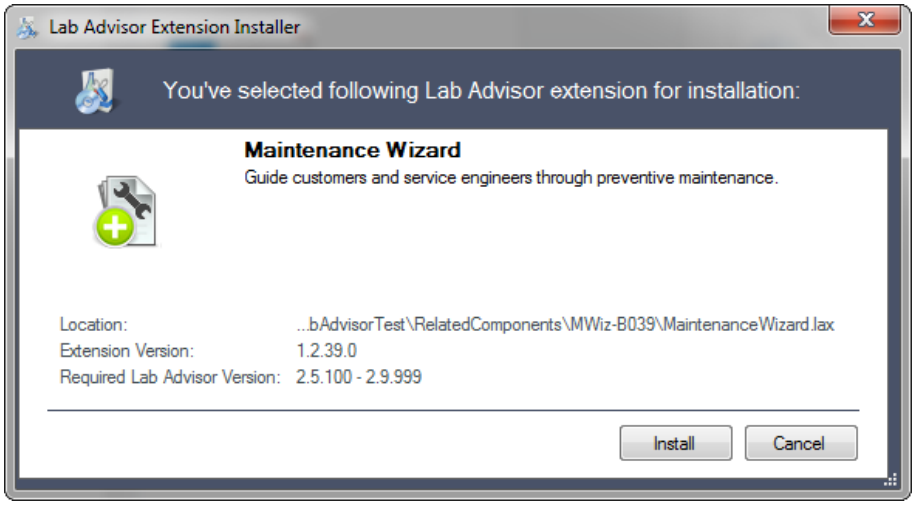
3 Click **Install from .lax file**.

A file selection dialog box is displayed to allow you to select the App or Add-on to install.

4 Navigate to the folder containing the Add-on files, select the .lax file and click **Open** to install the Add-on.

5 Click **Yes** when the request to shut down Lab Advisor appears.

Lab Advisor shuts down and the Add-on installation is started.



When the installation is finished, the newly installed Add-on is included in the table in the **Configuration - Add-ons** screen.

4 Quick Start Guide

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

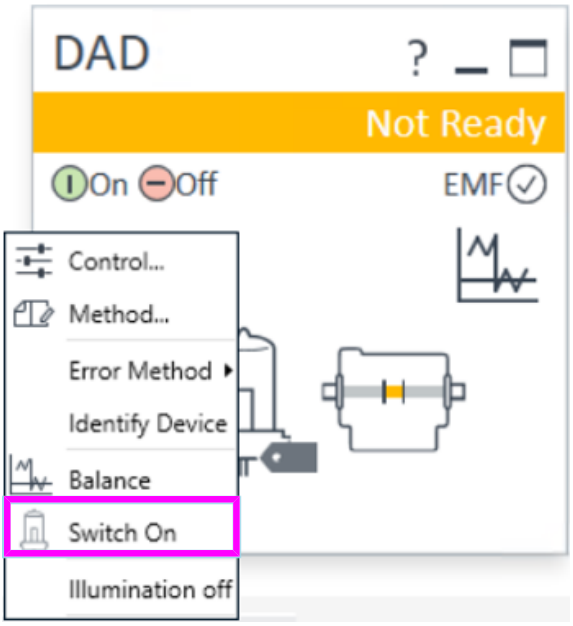
Best Practices

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.

1 Switch on the detector.

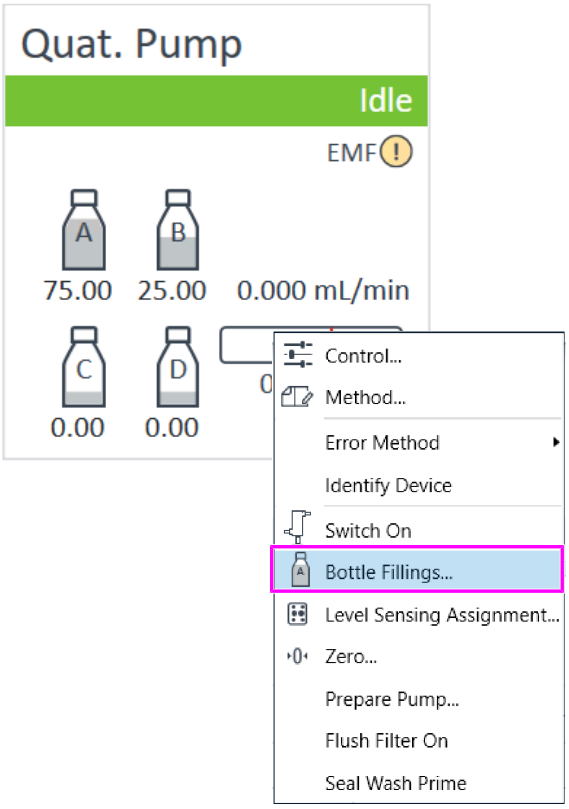


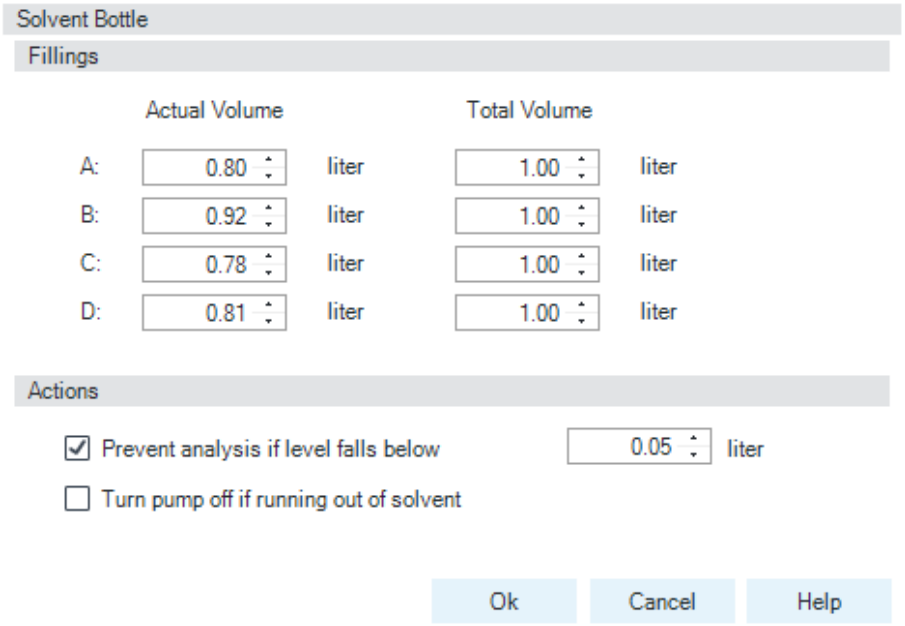
2 Fill the solvent bottles with adequate solvents for your application.

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).



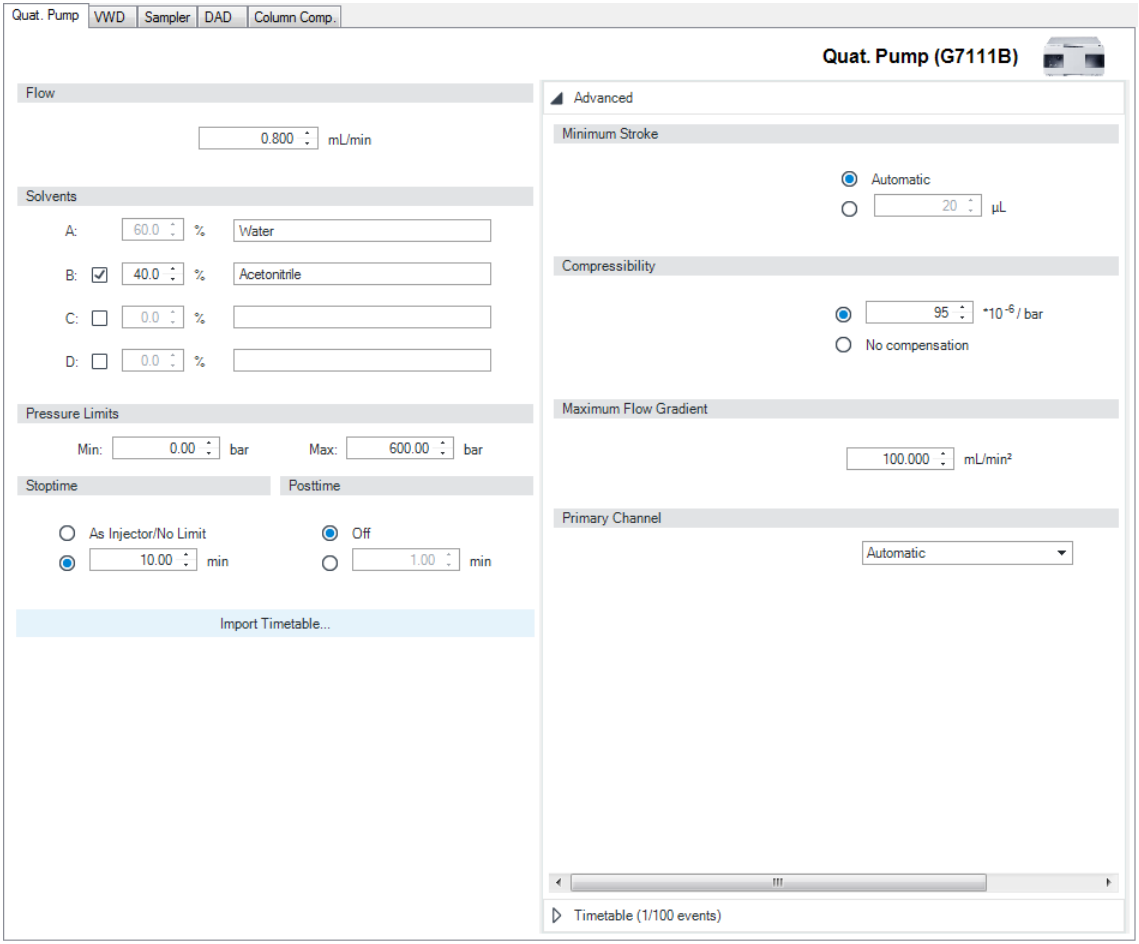


6 Purge the pump (in normal usage scenario).

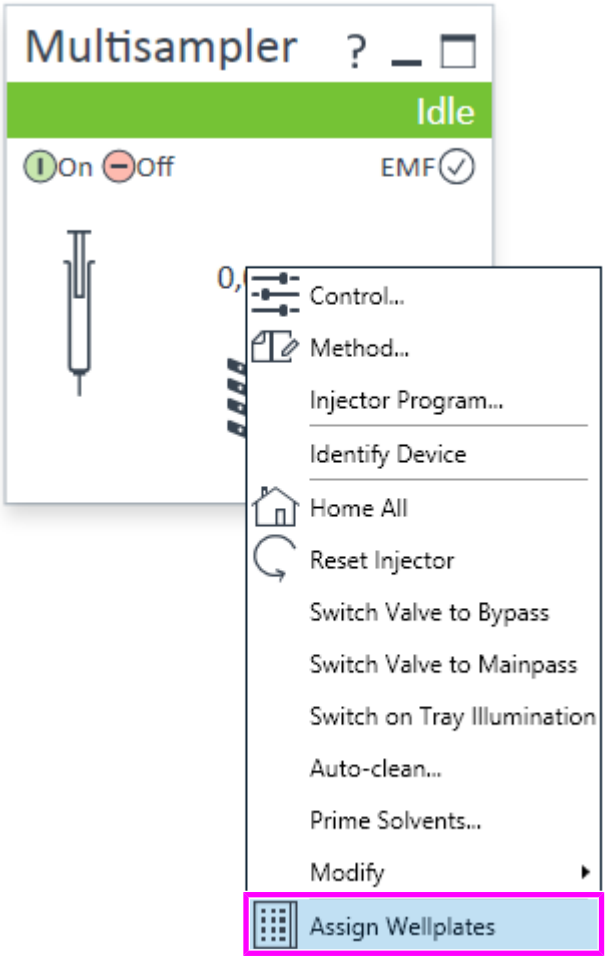
OR: Prime the pump (after installation of the system).

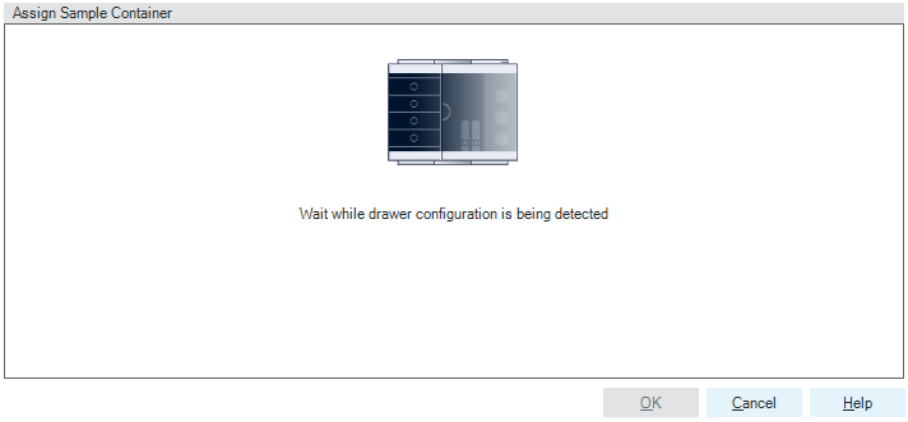
NOTE For details on priming and purging, refer to the technical note *Best Practices for Using an Agilent LC System Technical Note (InfinityLab-BestPractice-en-SD-29000194.pdf, SD-29000194)*.

7 Change solvent (if necessary).

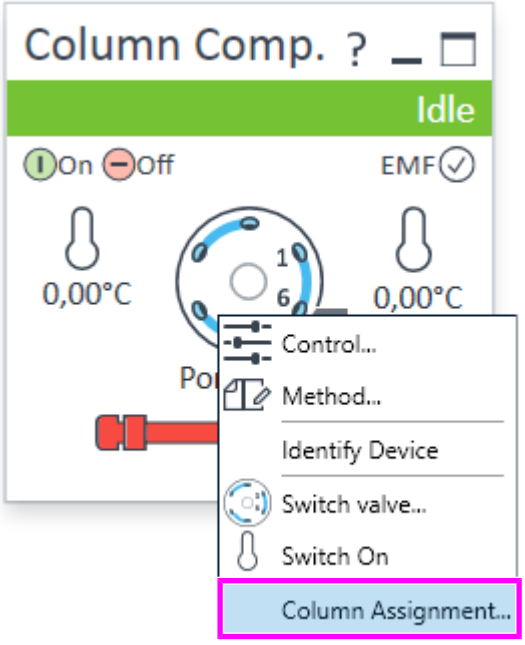


8 Choose the tray format of the sampler.





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)

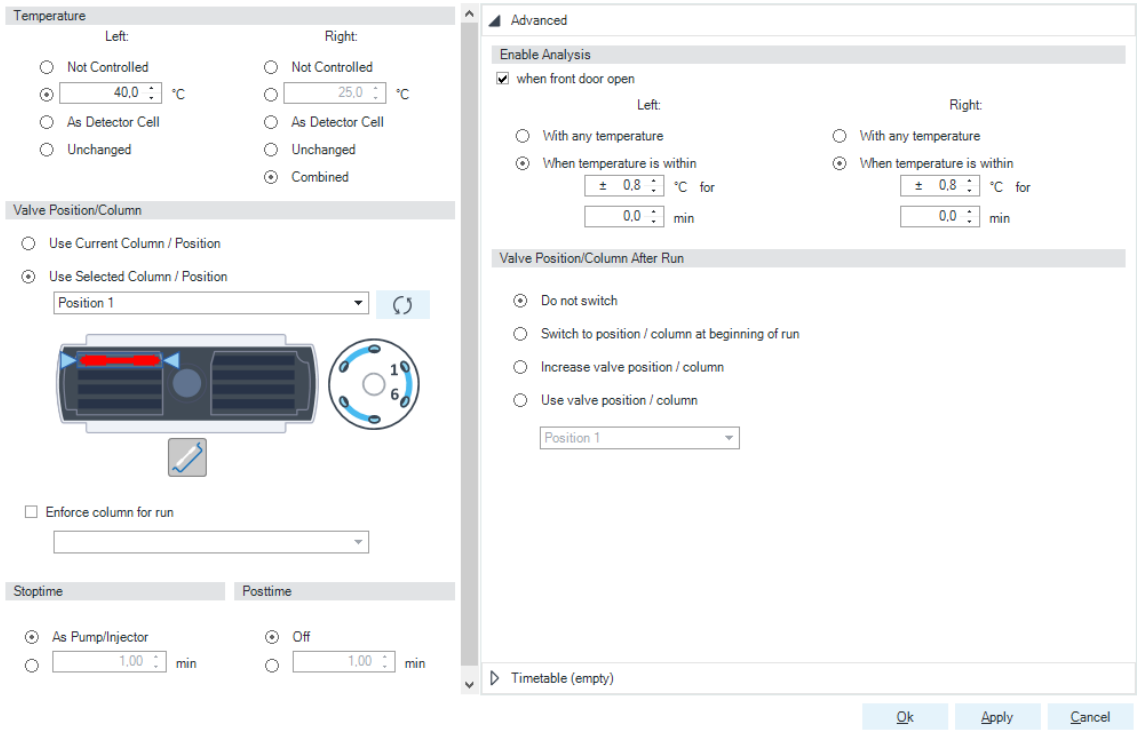
Column Tag Information

>>

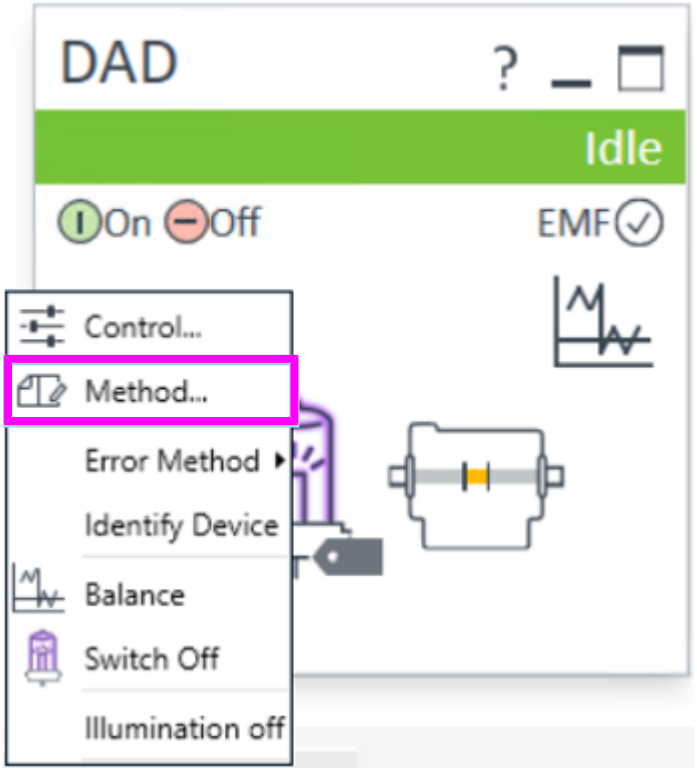
Location	Color Code	Description	Length [mm]	Diameter [mm]	Particle Size [µm]	Max. Pressure [bar]	Injections
Left 1	Red		0	0,0	0,0	0	0
Left 2	Blue		0	0,0	0,0	0	0
Left 3	None		0	0,0	0,0	0	0
Left 4	None		0	0,0	0,0	0	0
Right 1	None		0	0,0	0,0	0	0
Right 2	None		0	0,0	0,0	0	0
Right 3	None		0	0,0	0,0	0	0
Right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

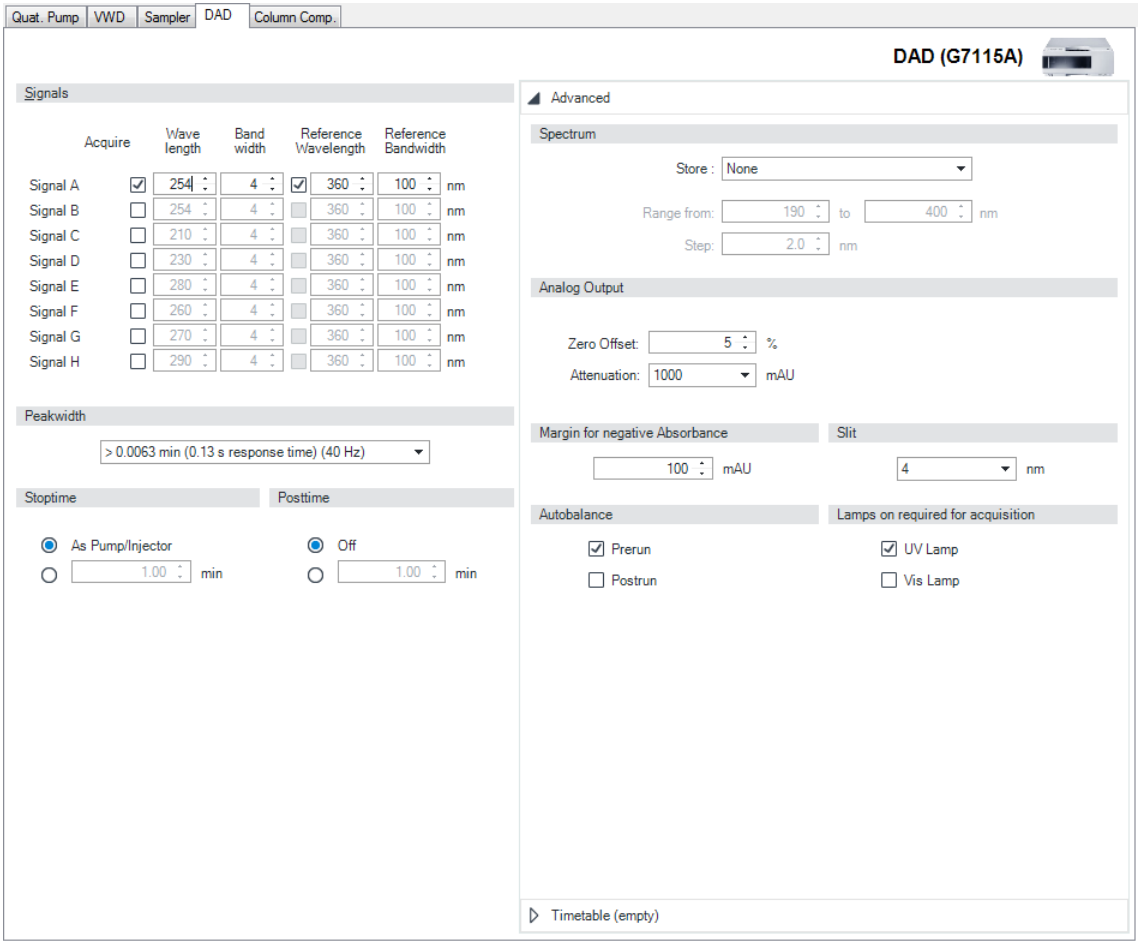
Ok/Write Tag Cancel Help

11 Select the column position.



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




Quat. Pump
VWD
Sampler
DAD
Column Comp.
DAD (G7115A)
Signals
Acquire
Wave length
Band width
Reference Wavelength
Reference Bandwidth
Signal A 254 4 360 100 nm
Signal B 254 4 360 100 nm
Signal C 210 4 360 100 nm
Signal D 230 4 360 100 nm
Signal E 280 4 360 100 nm
Signal F 260 4 360 100 nm
Signal G 270 4 360 100 nm
Signal H 290 4 360 100 nm
Peakwidth
> 0.0063 min (0.13 s response time) (40 Hz)
Stoptime
As Pump/Injector
1.00 min
Posttime
Off
1.00 min
Advanced
Spectrum
Store : None
Range from: 190 to 400 nm
Step: 2.0 nm
Analog Output
Zero Offset: 5 %
Attenuation: 1000 mAU
Margin for negative Absorbance
100 mAU
Slit
4 nm
Autobalance
Prerun
Postrun
Lamps on required for acquisition
UV Lamp
Vis Lamp
Timetable (empty)

InfinityLab LC Performance Check

The InfinityLab LC Performance Check is run after the complete installation of the module stack to affirm the functionality of all modules and the system. The InfinityLab LC Performance Check confirms that each module performs and is connected correctly.

For more information, refer to Agilent CrossLab Start Up Installation, or Introduction.

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 65) 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.

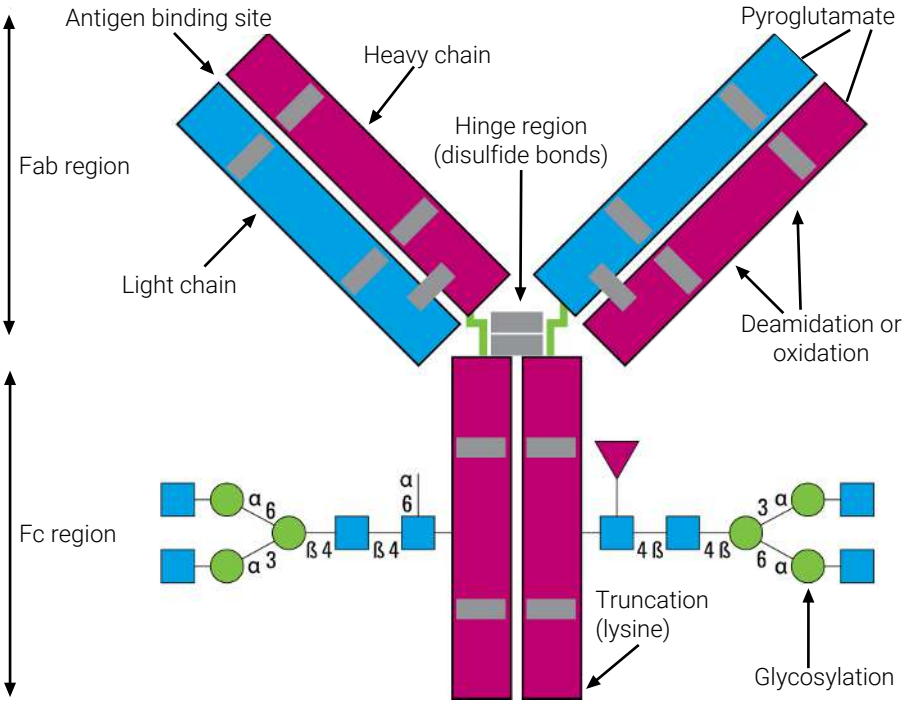


Figure 17: General structure of therapeutic antibodies

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.

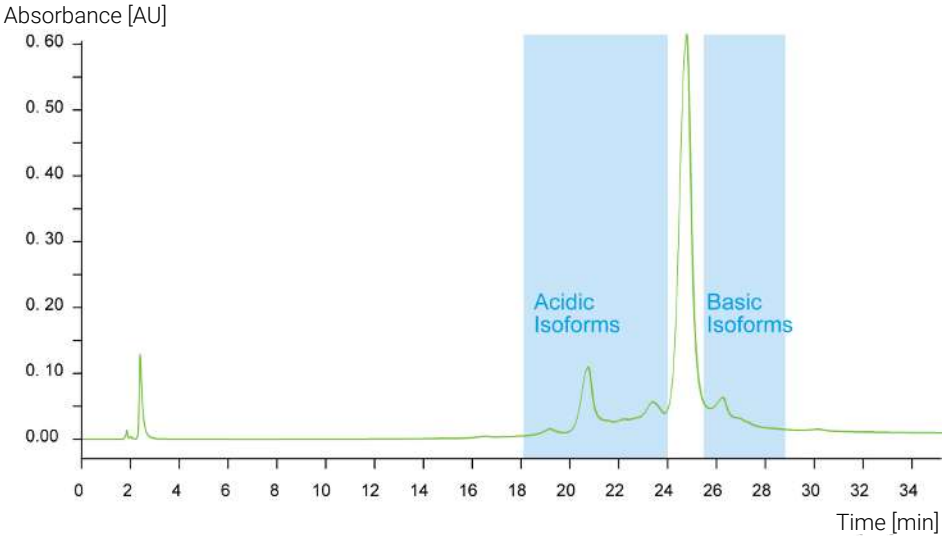


Figure 18: High resolution separation of acidic and basic charge variants using the Agilent Bio MabNP10 column

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.

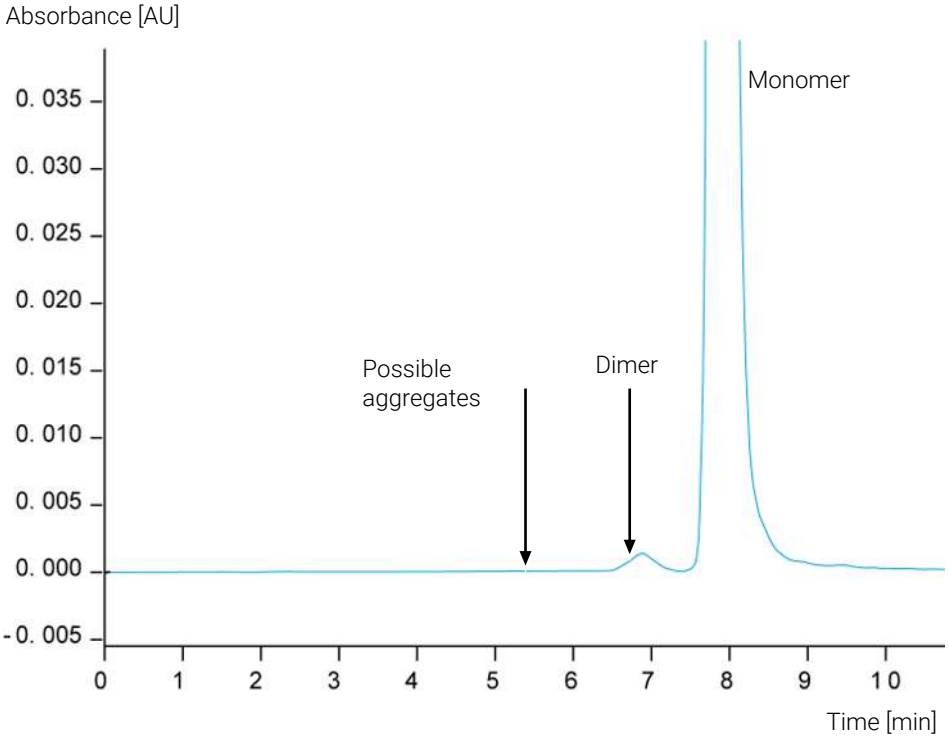


Figure 19: Aggregation analysis of a monoclonal antibody

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.

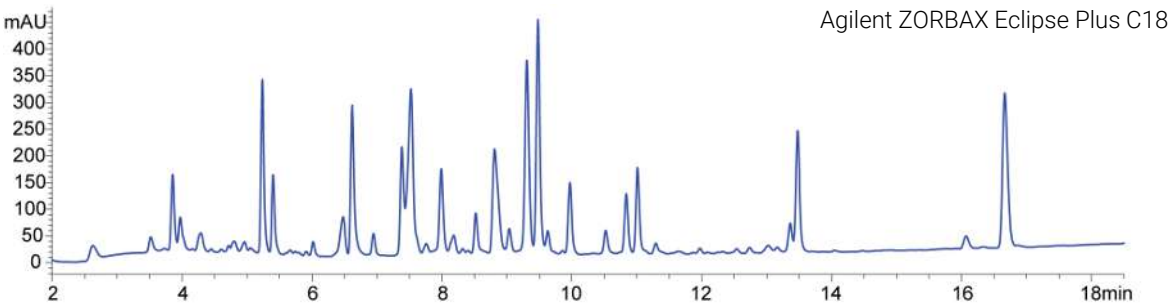
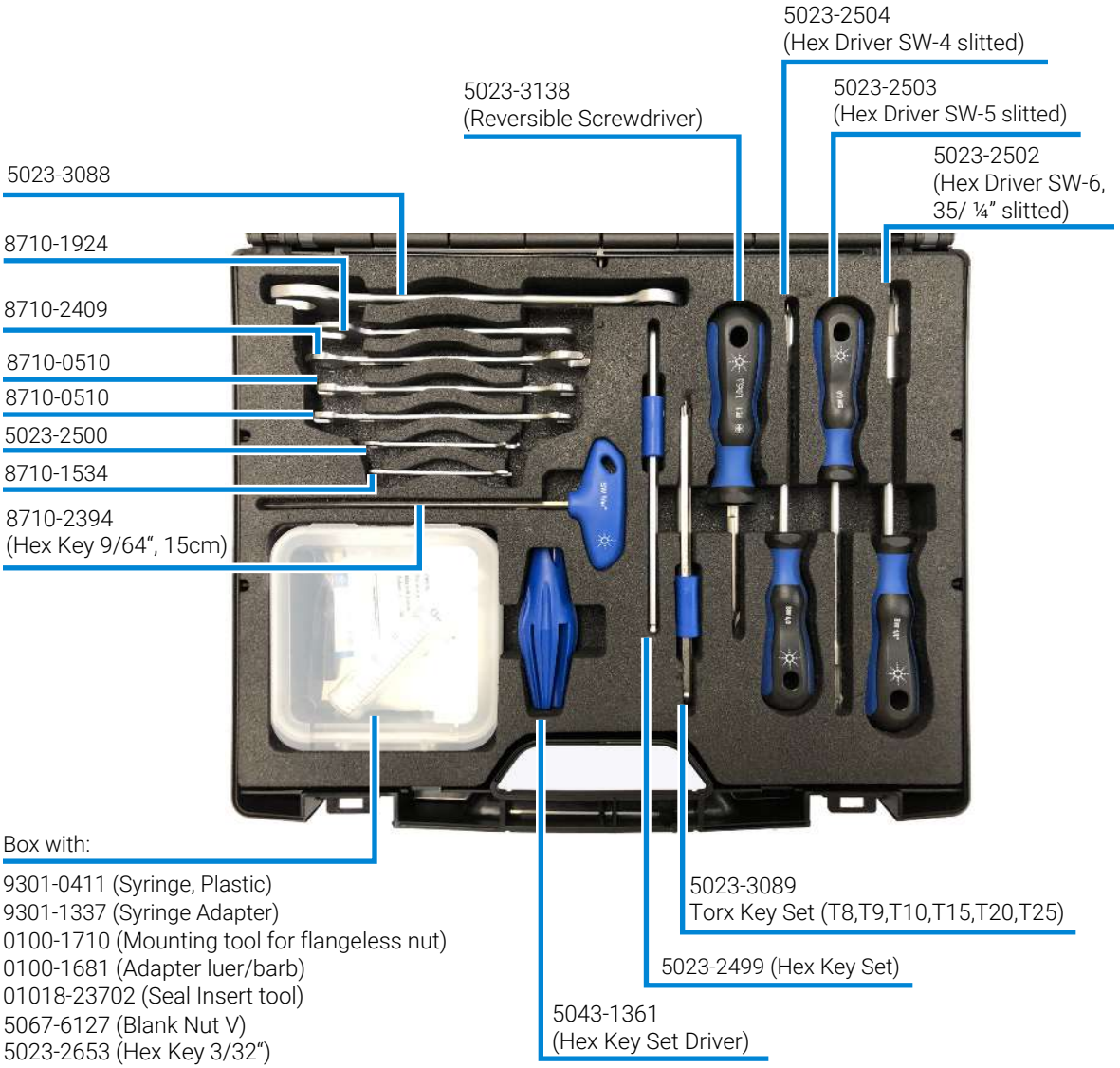


Figure 20: Peptide map with Agilent Zorbax Eclipse Plus C18 Column

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!

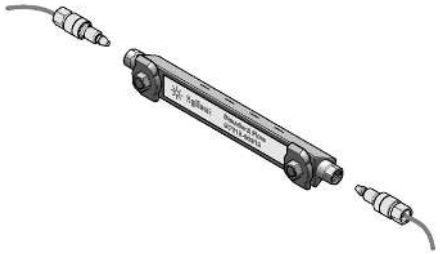
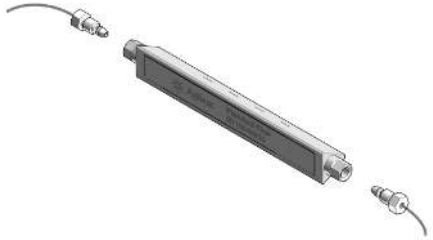
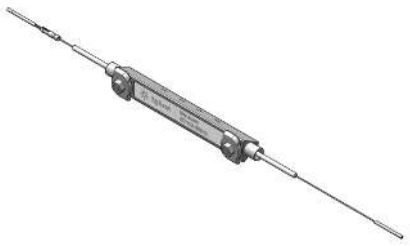
Do not mix with bio / biocompatible parts.

Table 3: 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 4: 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	Solvent inlet filter (glass), 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 5: 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 6: 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 7: 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 8: 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

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.

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