Agilent InfinityLab LC Series

1260 Infinity II Bio-inert LC System

Manual and Quick Guide

Agilent Technologies

Notices

Manual Part Number

G5654-90300 Rev. B

Edition

05/2016

Printed in Germany

Agilent Technologies
Hewlett-Packard-Strasse 8
76337 Waldbronn

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In This Book

This book describes the Agilent 1260 Infinity II Bio-inert LC System.

1 Introduction

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

2 Install the Modules

This chapter provides information on how to install the modules and the capillaries.

3 Configuration Settings

This chapter describes how to configure the system.

4 Quick Start Guide

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

5 Typical Bio-inert LC Applications

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Contents

1 Introduction

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

Product Description

The Agilent 1260 Infinity II 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.

Features of the Agilent 1260 Infinity II Bio-inert LC

- *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 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.

System Components

The Infinity II Bio-inert LC System consists of the following components:

- Bio-inert Pump
- Bio-inert Multisampler (MLS)
- Multicolumn Thermostat (MCT) with Bio-inert Heat Exchanger
- Diode Array Detector (DAD), Multiple Wavelength Detector (MWD), or Fluorescence Detector (FLD) with respective Bio-inert flow cell
- Bio-inert Manual Injector
- Solvent Cabinet

The Agilent 1260 Infinity II Bio-inert LC is described in more detail in the following sections. All modules are stackable, see "Optimize the Stack Configuration" on page 24.

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

Bio-inert Pump

The 1260 Infinity II 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

Bio-inert Multisampler

Agilent 1260 Infinity II 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. 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 cooling system. It allows you to maintain perfect temperature control on all vials and plates inserted.

Figure 2 Overview of the Bio-inert Multisampler

Multicolumn Thermostat with Bio-inert Heat exchangers

The Agilent 1260 Infinity II Multicolumn Thermostat (MCT) allows precise column thermostatting over a broad temperature range: from cooling down to 10 degrees below ambient temperature up to 85 °C, providing high flexibility for optimized speed and selectivity of LC separation.

Ultrahigh pressure valves enable a wide range of applications such as column selection from 4 columns in a single MCT, sample preparation for analyte enrichment or matrix removal, alternating column regeneration, etc.

For bio-inert applications bio-inert heat exchangers and a selection of bio-inert valves are offered.

The MCT fits with all 1260 Infinity II modules and can also be combined with modules of the Agilent 1260 and 1290 Infinity II Series.

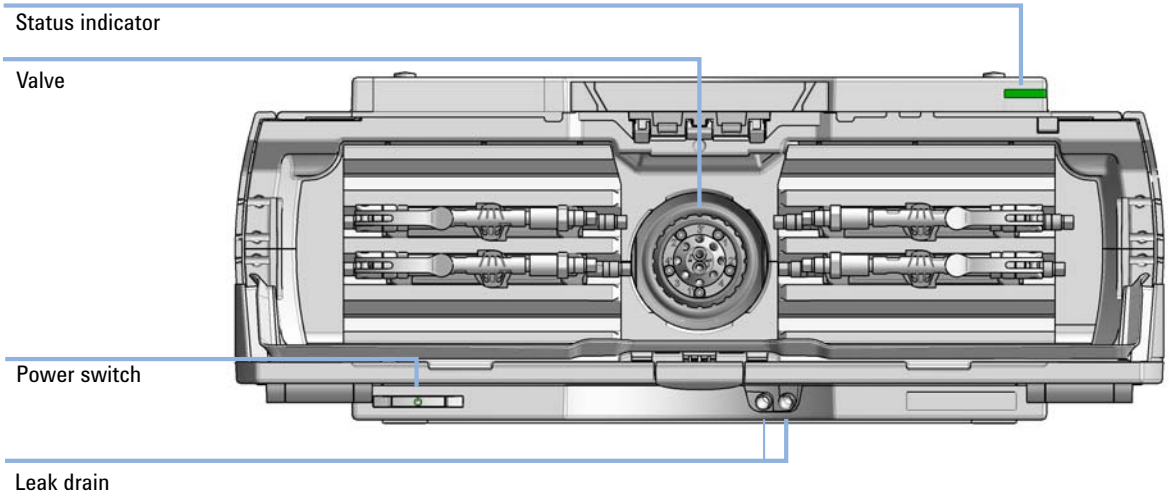


Figure 3 Overview of the Multicolumn Thermostat

Diode Array Detector (DAD) WR with Bio-inert Flow Cell

The 1260 Infinity II 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.

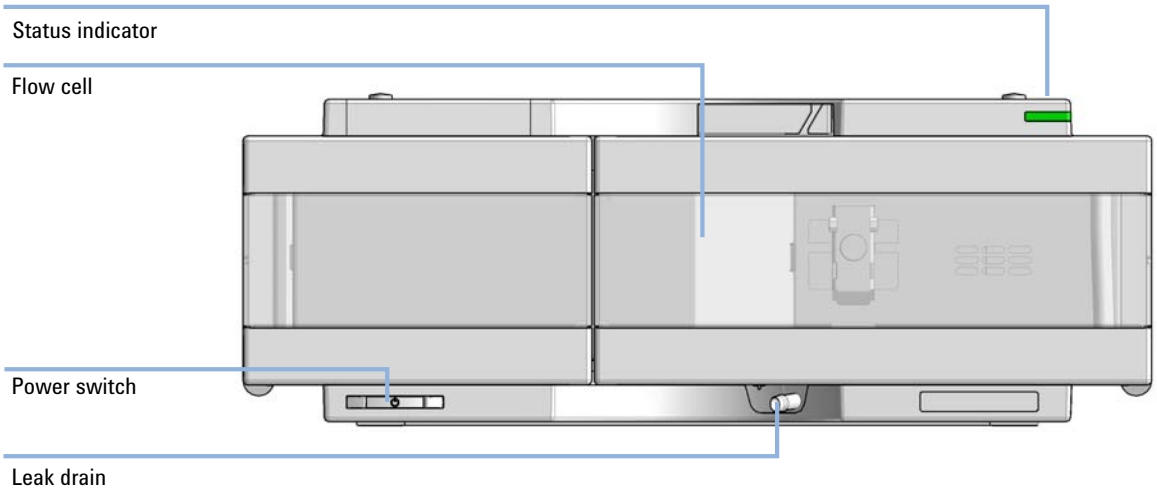


Figure 4 Overview of the detector

Fluorescence Detector (FLD) with Bio-inert flow cell

The proven optical and electronic design of the Agilent 1260 Infinity II 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.

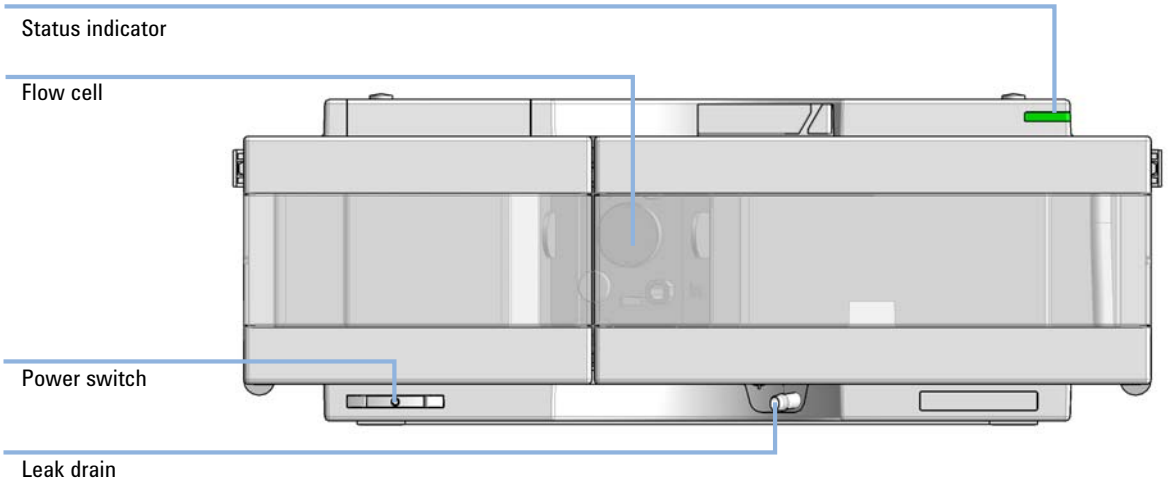


Figure 5 Overview of the detector

Bio-inert Manual Injector

The Agilent 1260 Infinity II 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 Bio-inert 6-port sample injection valve (5067-4158). 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 II Manual Injector is based on the Manual Injector (G1328C). For further information refer to the *Agilent InfinityLab LC Series Manual Injector User Manual (G1328B, G5628A)*.

Bio-inert Materials

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 1 Used Bio-inert materials

Module	Materials
Agilent 1260 Infinity II Bio-inert Pump (G5654A)	Titanium, gold, platinum-iridium, ceramic, ruby, PTFE, PEEK
Agilent 1260 Infinity II Bio-inert Multisampler (G5668A)	Upstream of sample introduction: • Titanium, gold, PTFE, PEEK, ceramic Downstream of sample introduction: • PEEK, ceramic
Agilent 1260 Infinity II Bio-inert Manual Injector (G5628A)	PEEK, ceramic
Bio-inert Flow Cells:	
Standard flow cell bio-inert, 10 mm, 13 µL, 120 bar (12 MPa) for MWD/DAD, includes Capillary Kit Flow Cells BIO (p/n G5615-68755) (G5615-60022) (*for Agilent 1260 Infinity II Diode Array Detectors DAD G7115A*)	PEEK, ceramic, sapphire, PTFE
Bio-inert flow cell, 8 µL, 20 bar (pH 1–12) includes Capillary Kit Flow Cells BIO (p/n G5615-68755) (G5615-60005) (*for Agilent 1260 Infinity Fluorescence Detector FLD G7121A/B*)	PEEK, fused silica, PTFE
Bio-inert Heat Exchangers, Valves and Capillaries:	
Quick-Connect Heat Exchanger Bio-inert (G7116-60041) (*for Agilent 1260 Infinity II 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 to more than 600 bar.

NOTE To ensure optimum bio-compatibility of your Agilent 1260 Infinity II 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 "Material Information" on page 17.

Solvent Information

Observe the following recommendations on the use of solvents.

- Follow recommendations for avoiding the growth of algae, see pump manuals.
- 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 like flow cells, valve materials etc. and recommendations in subsequent sections.

Material Information

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 were collected from external resources and are 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 can also not 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 non-conductive 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.

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 is still a number of known incompatibilities with chemicals such as chloroform, methylene chloride, THF, DMSO, strong acids (nitric acid > 10 %, sulphuric acid > 10 %, sulfonic acids, trichloroacetic acid), halogenes or aequous halogene solutions, phenol and derivatives (cresols, salicylic acid etc.).

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 very sensitive to high pressure. Therefore Agilent uses stainless-steel cladded PEEK capillaries in bio-inert systems. The use of stainless steel cladded PEEK capillaries keeps the flow path free of steel and ensures pressure stability to at least 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 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 to 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 (ST)

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 so on) 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, di-isopropylether). Such ethers should be filtered through dry aluminium 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, ethylene diamine tetra-acetic acid).
- Mixtures of carbon tetrachloride with 2-propanol 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 1290 Infinity Flow Cells and capillaries. 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)

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 1322A, 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.

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:

- Standard flow cell bio-inert, 10 mm, 13 µL, 120 bar (12 MPa) for MWD/DAD, includes Capillary Kit Flow Cells BIO (p/n G5615-68755) (G5615-60022) (PEEK, ceramic, sapphire, PTFE) for 1260 Infinity II Diode Array Detectors (G7115A):
  - The recommended pH range of the cell is 1 – 13 (short term 14)
- Bio-inert flow cell, 8 µL, 20 bar (pH 1–12) includes Capillary Kit Flow Cells BIO (p/n G5615-68755) (G5615-60005), (PEEK, fused silica, PTFE) for 1260 Infinity II 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).

Solutions

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

Optimize the Stack Configuration

To ensure safe operation and optimum performance of an Agilent InfinityLab LC System, Agilent Technologies prescribe stack configurations. The following configurations are possible:

- A-Line Flex Bench
- Single Stack (maximal 4 modules, in a bench rack or directly on the bench)
- Two Stacks (in a bench rack or directly on the bench)

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

Table 2 Overview on pros and cons of different stack configurations

modules in a stack	Rack 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 A-Line LC Flex Bench

Agilent recommends to use the A-Line LC Flex Bench for all Agilent-LC-Systems.

Main features:

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

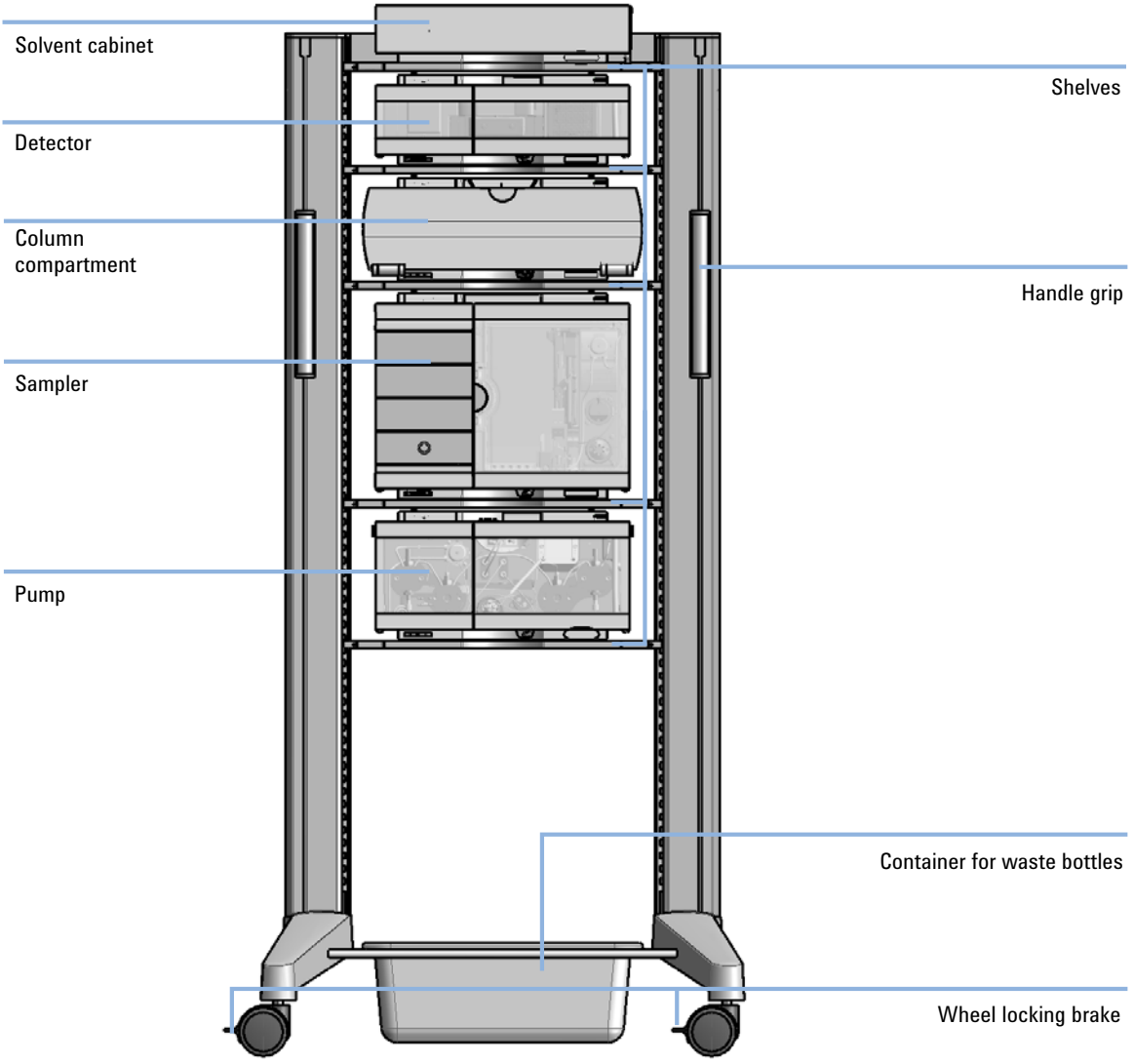


Figure 6 Agilent A-Line LC Flex Bench

One Stack Configuration

Ensure optimum performance by stacking the modules as shown exemplarily in Figure 7 on page 26. This configuration optimizes the flow path for minimum delay volume and minimizes the bench space required.

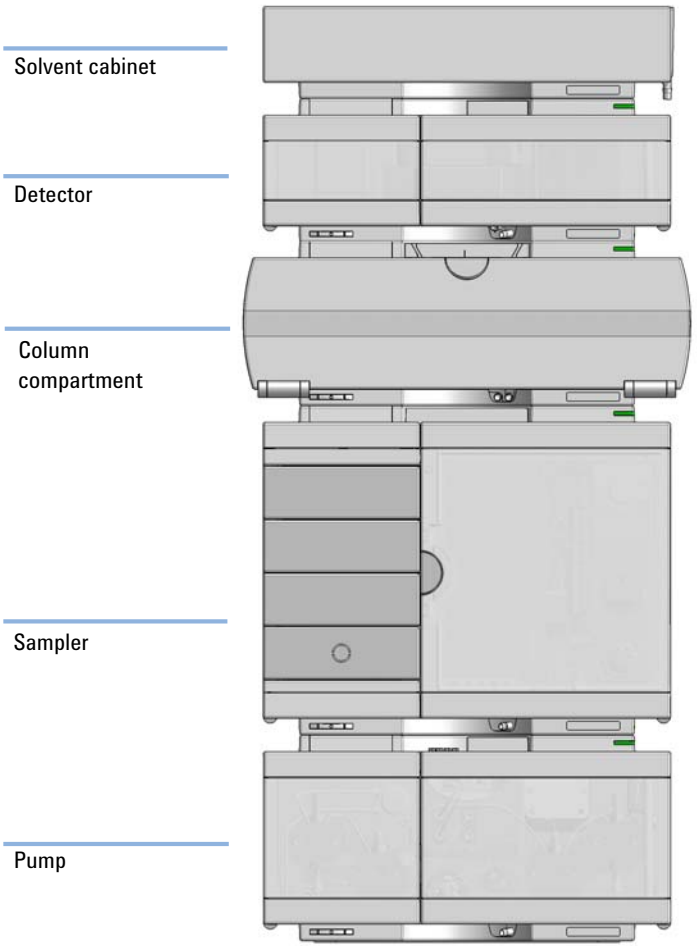


Figure 7 Single stack configuration (bench installation, example shows a multisampler)

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.

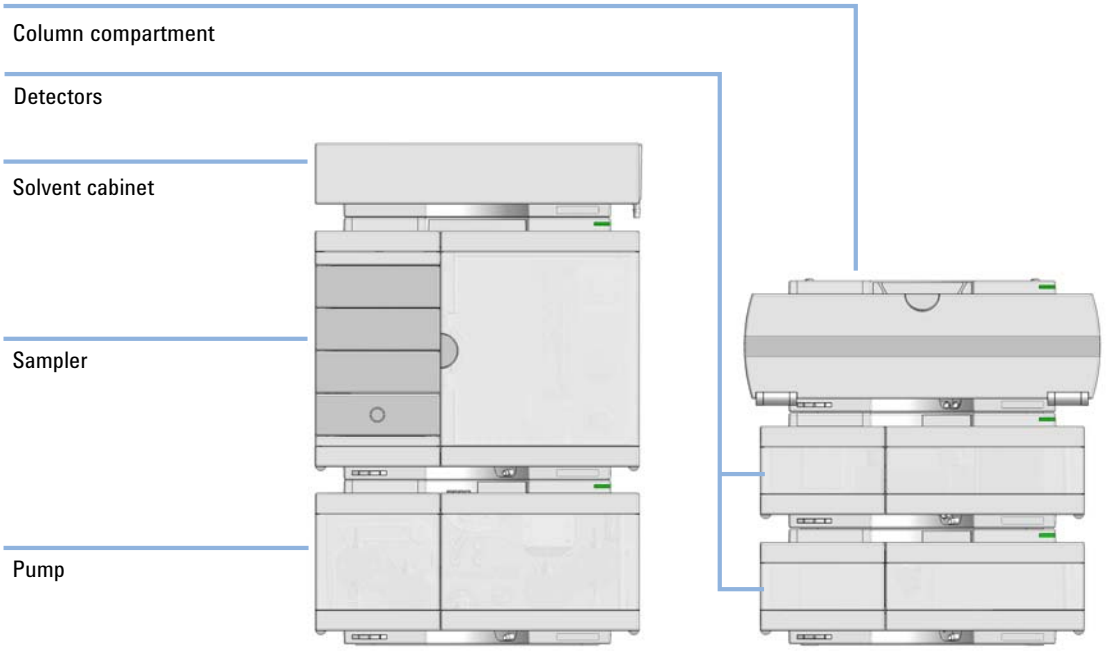


Figure 8 Two stack configuration (bench installation, example shows a multisampler)

Leak and Waste Handling

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 1200 Infinity Series 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 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 Cooler (condensate)
- from the Seal Wash Sensor
- from the pump's Purge Valve or Multipurpose Valve

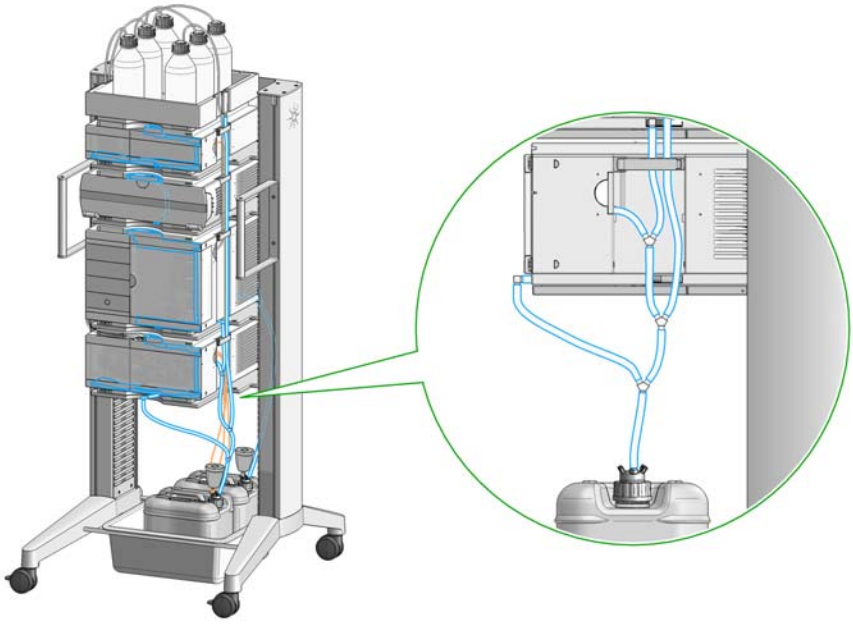


Figure 9 Infinity II Leak Waste Concept (flexible rack installation)

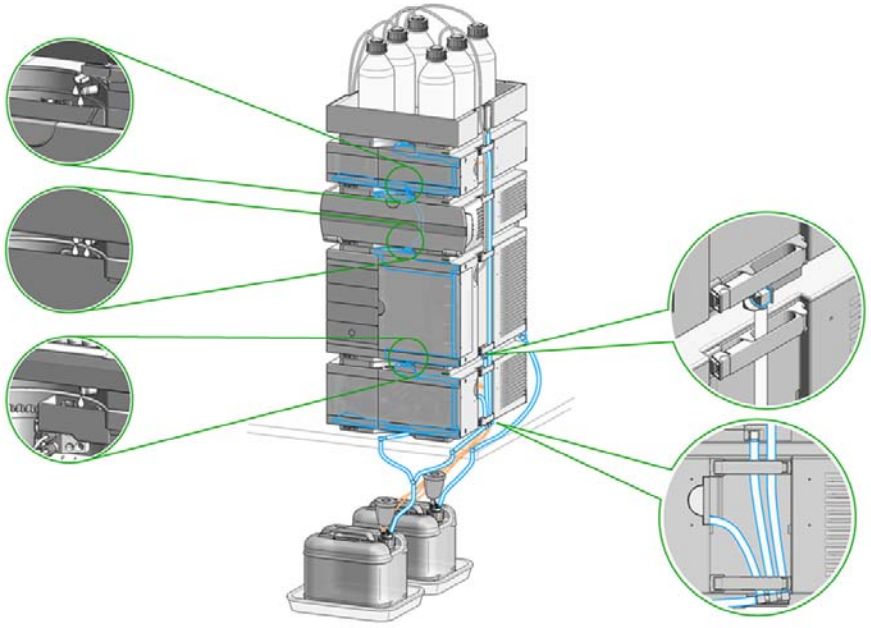


Figure 10 Infinity II Single Stack Leak Waste Concept (bench installation)

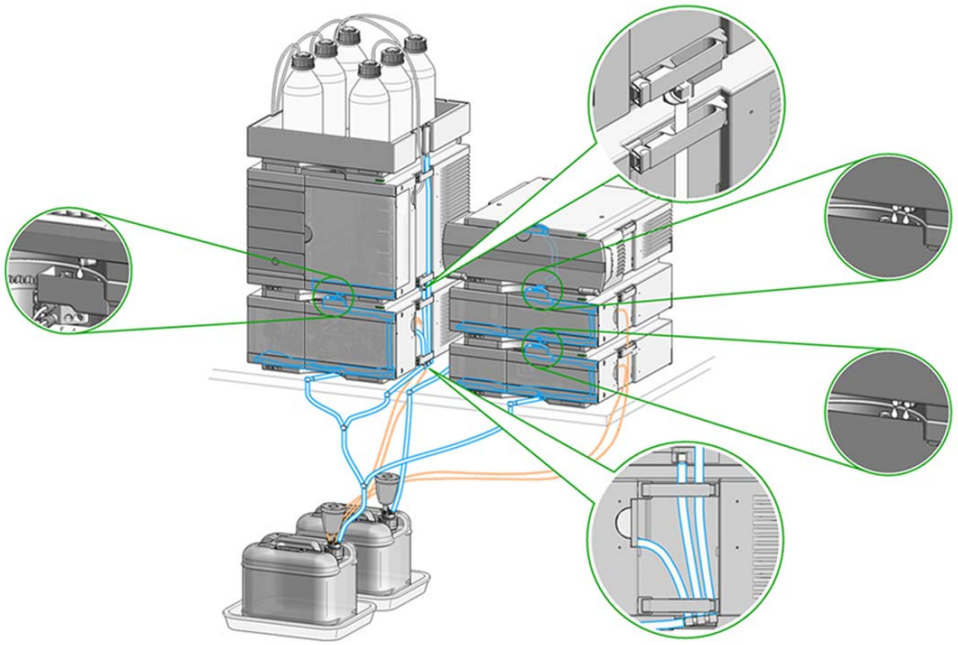
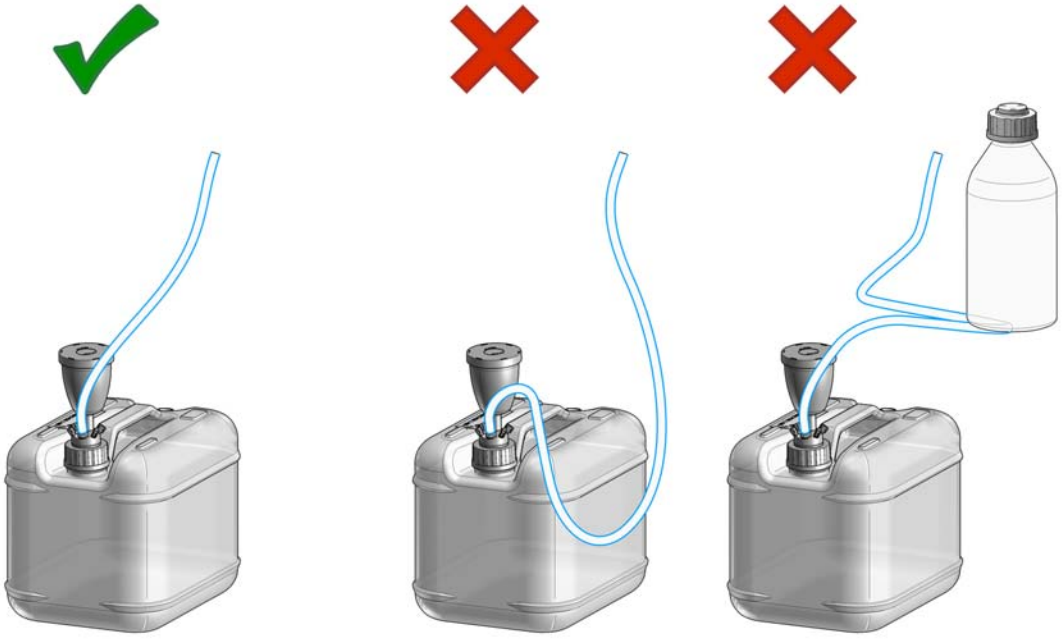


Figure 11 Infinity II Two Stack Leak Waste Concept (bench installation)

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

Waste Guidance



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

Waste Concept

1 Agilent recommends using the 6 L waste can with 1 Stay Safe cap GL45 with 4 ports (5043-1221) 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.

2
Install the Modules

This chapter provides information on how to install the modules and the capillaries.

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.

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 12 on page 38 shows capillary and tubing connections in the flow path. For details and necessary parts, refer to the individual module manuals.

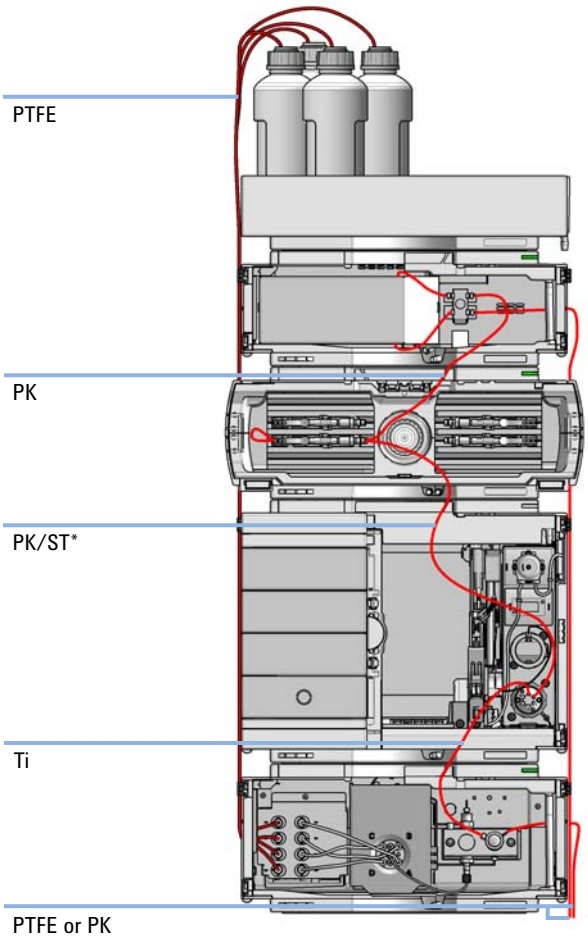


Figure 12 Capillary and tubing connections in flow path

1* 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 "Installing UHP-FF Fittings" on page 40 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	Capillary, PK/ST, 0.17 mm x 300 mm, male to male, pre-swaged
G5667-60500	Capillary, PK/ST, 0.17 mm x 400 mm, male to male, pre-swaged
G5667-60501	Capillary, PK/ST, 0.17 mm x 500 mm, male to male, pre-swaged

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

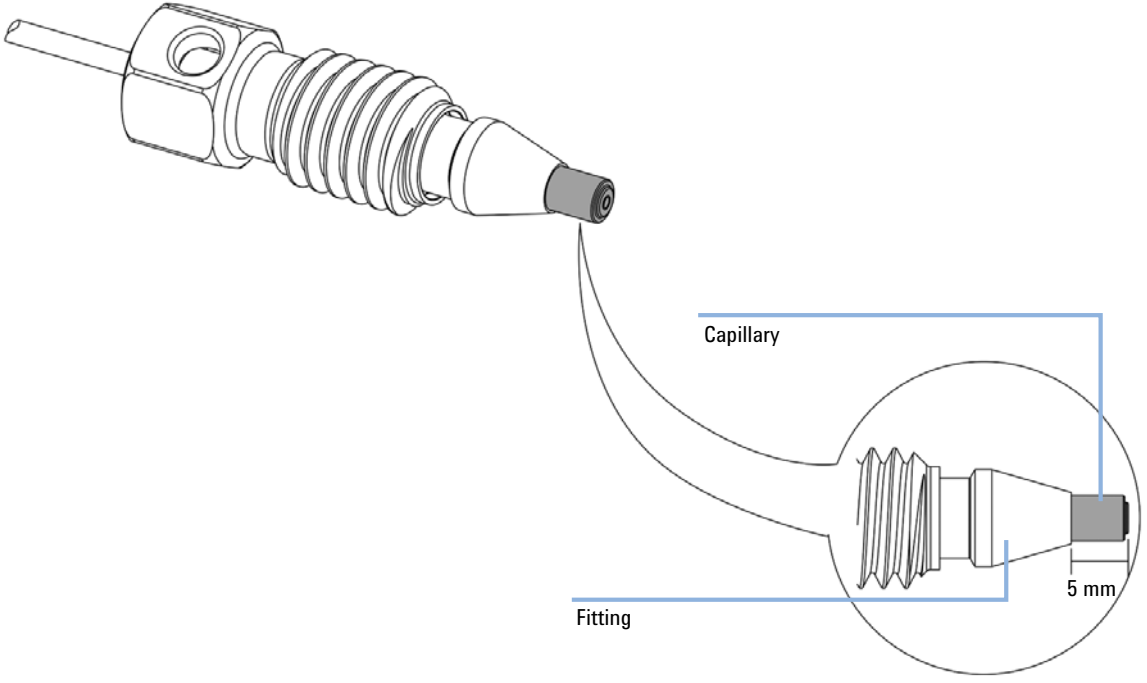
Installing Capillaries

Installing UHP-FF Fittings

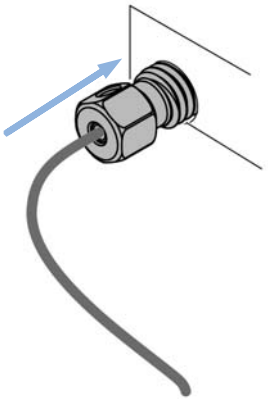
Tools required	p/n	Description
	5043-0915	Fitting mounting tool for bio-inert capillaries

Parts required	p/n	Description
	Capillaries and Fittings	For details refer to the part section of the manual.

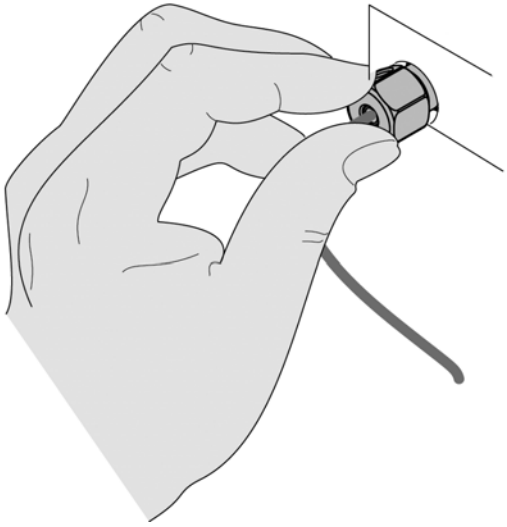
1 Slide the fitting on the capillary. Let the capillary jut out 5 mm.



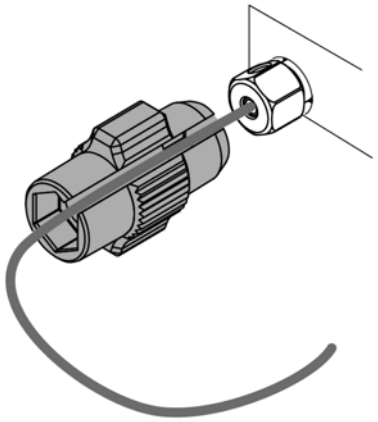
2 Insert the fitting to the receiving port and push the capillary to the bottom of the port.



3 Finger tighten the nut into the port until snug.



4 Use Fitting mounting tool (5043-0915) 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 bioinert 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.

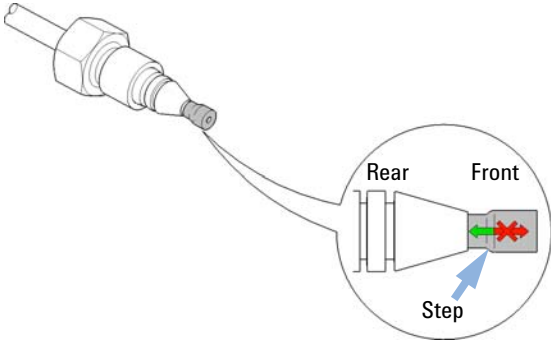


Figure 13 Capillary fitting

Installation of the Bio-inert Zero Dead Volume (ZDV) Union

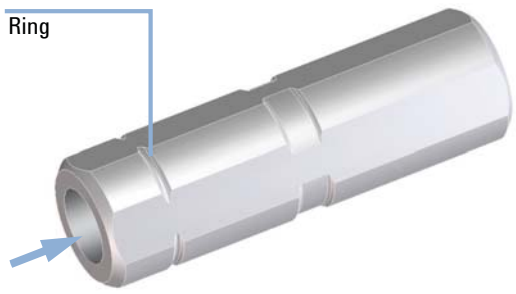
The Bio-inert ZDV (p/n 5067-4741) union has two different connectors where capillaries need to be installed in the correct sequence. Otherwise, an inset of the union may be damaged and the connection may not be tight.

CAUTION

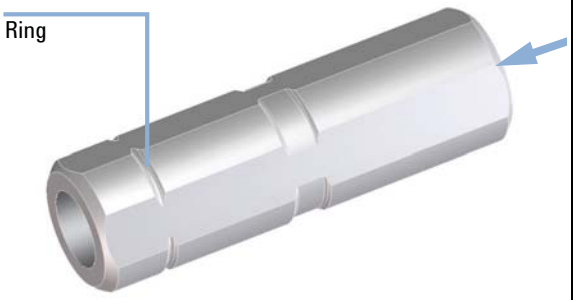
Potential leak or damage of the Bio-inert ZDV Union.

➔ 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.



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.

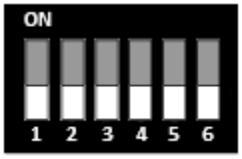
Instrument Configuration

Example shows an instrument configuration with a Diode Array Detector.

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

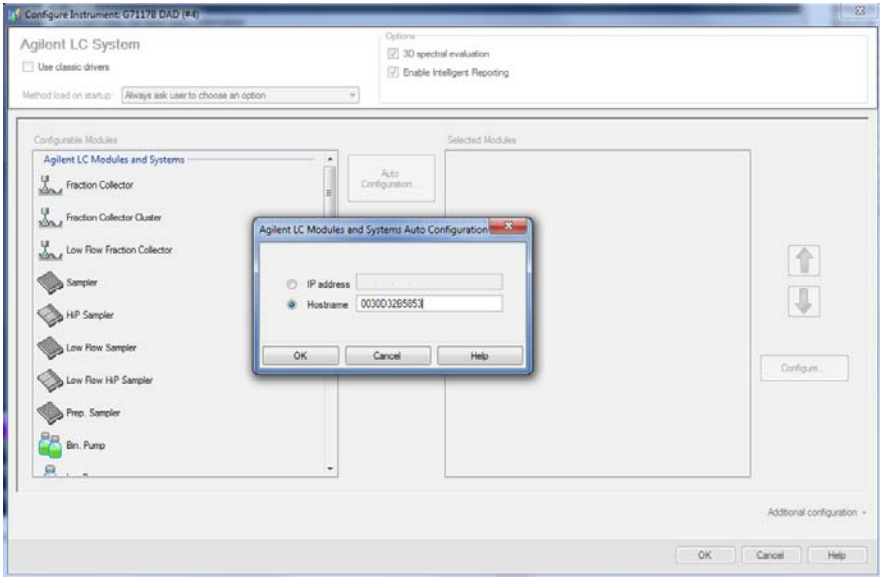
   a All switches DOWN: module uses the default IP address 192.168.254.11.

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

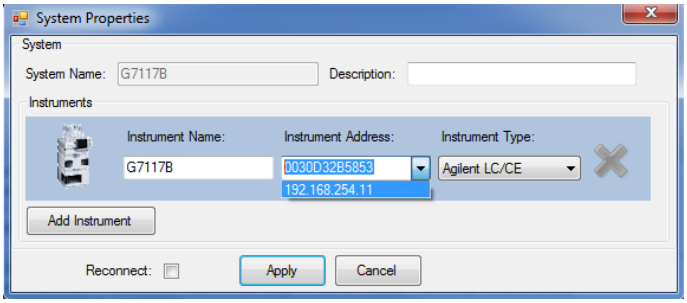


2 Enter the setup information (MAC / IP address and/or Instrument Name).

   a Agilent OpenLab ChemStation (Configure Instrument):



b Lab Advisor (Instrument Overview - Add Instrument):



4
Quick Start Guide

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

Best Practices

NOTE For best practices, refer to the *Agilent Information Center* on Agilent InfinityLab LC Series User Documentation (G4800-64600) or the 1290 Infinity Pump Quick Reference Sheet (01200-90091).

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.

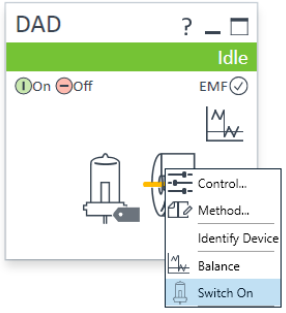
WARNING

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. Always keep the temperature in the sample compartment at least 25 K below the boiling point of the solvent used.
- ➔ Do not operate the instrument in an explosive atmosphere.
- ➔ 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.

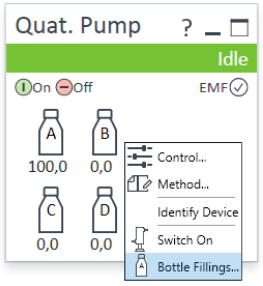


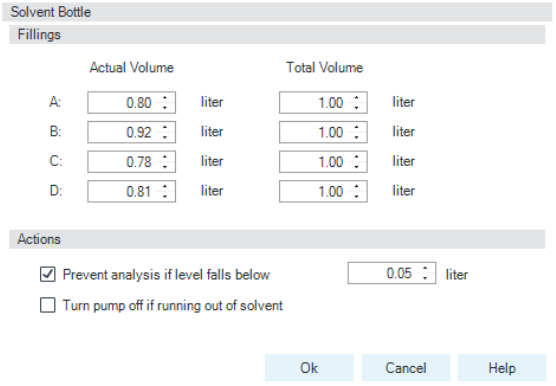
2 Fill the solvent bottles with adequate solvents for your application.

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).





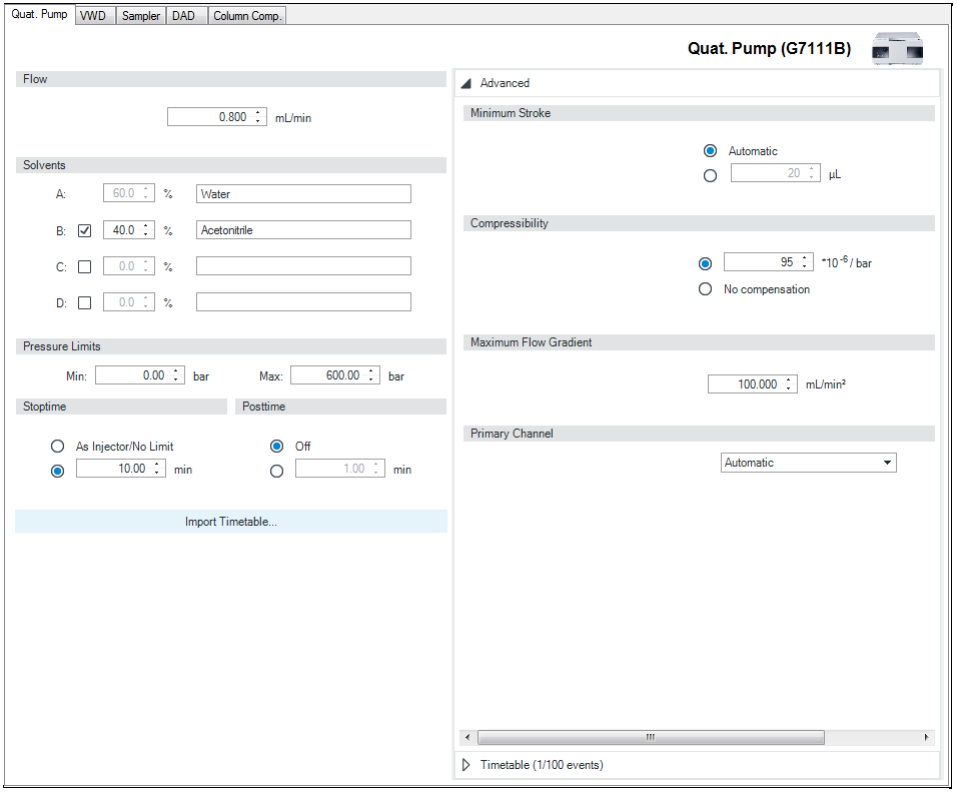
6 Purge the pump (in normal usage scenario).

OR

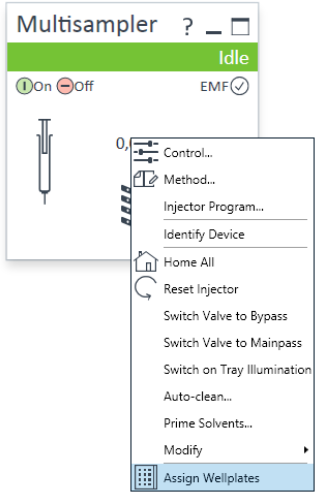
Prime the pump (after installation of the system).

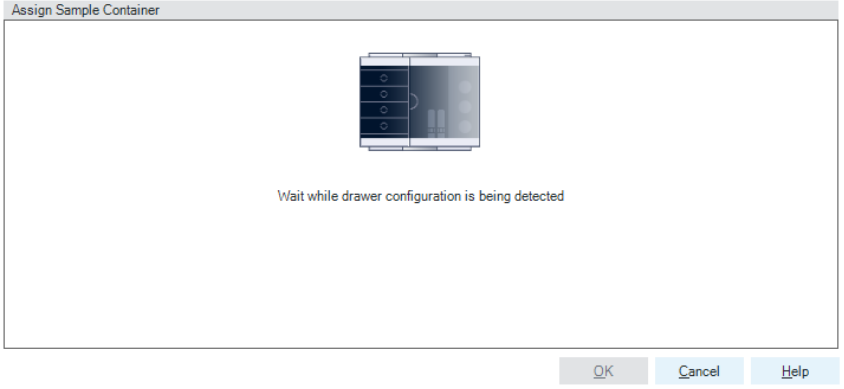
NOTE For details on priming and purging, refer to the technical note *Best Practices for Using an Agilent LC System* .

7 Change solvent (if necessary).

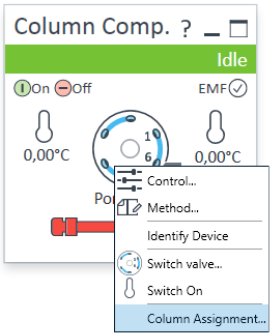


8 Choose the tray format of the sampler.

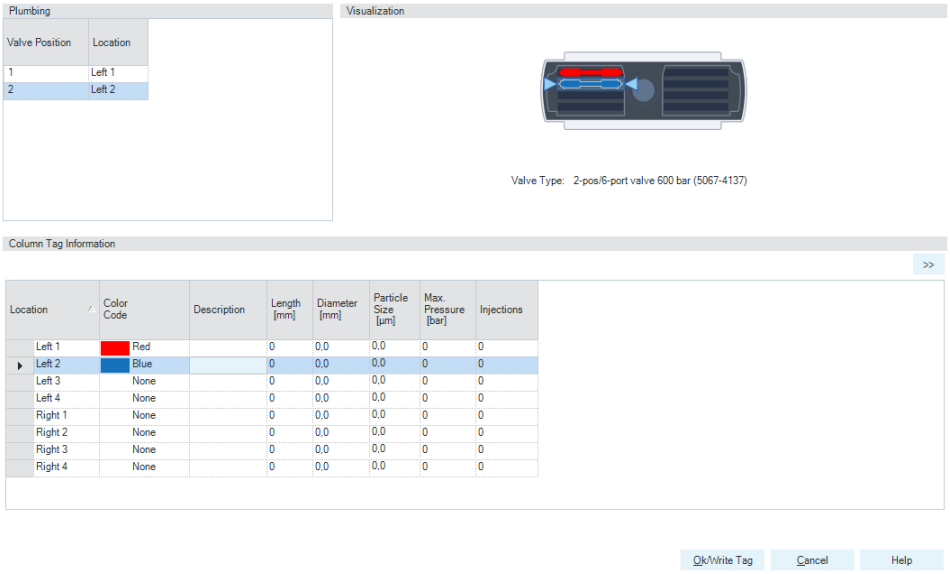




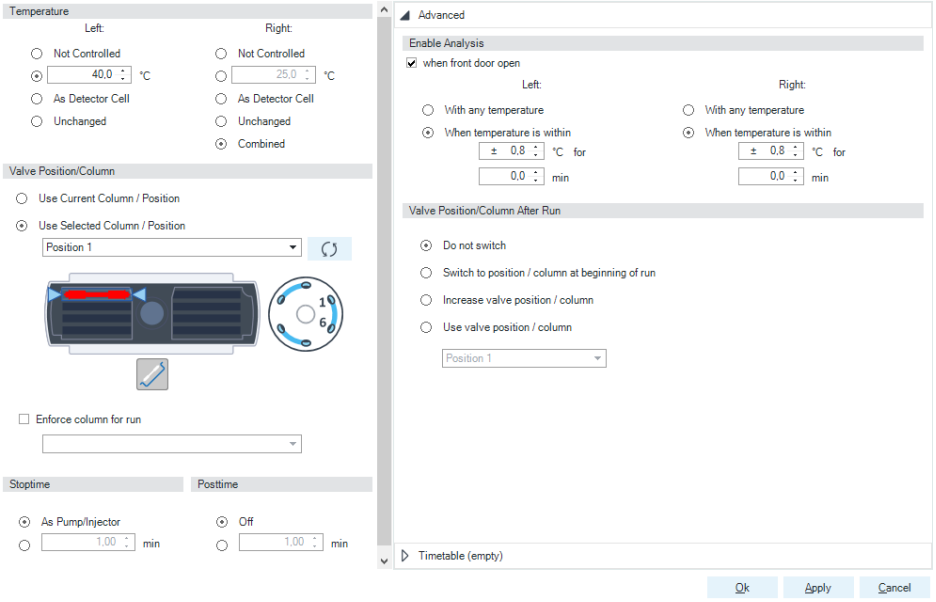
9 Add a new column.



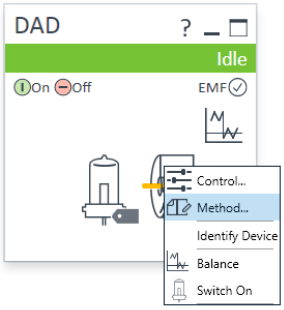
10 Enter the column information.

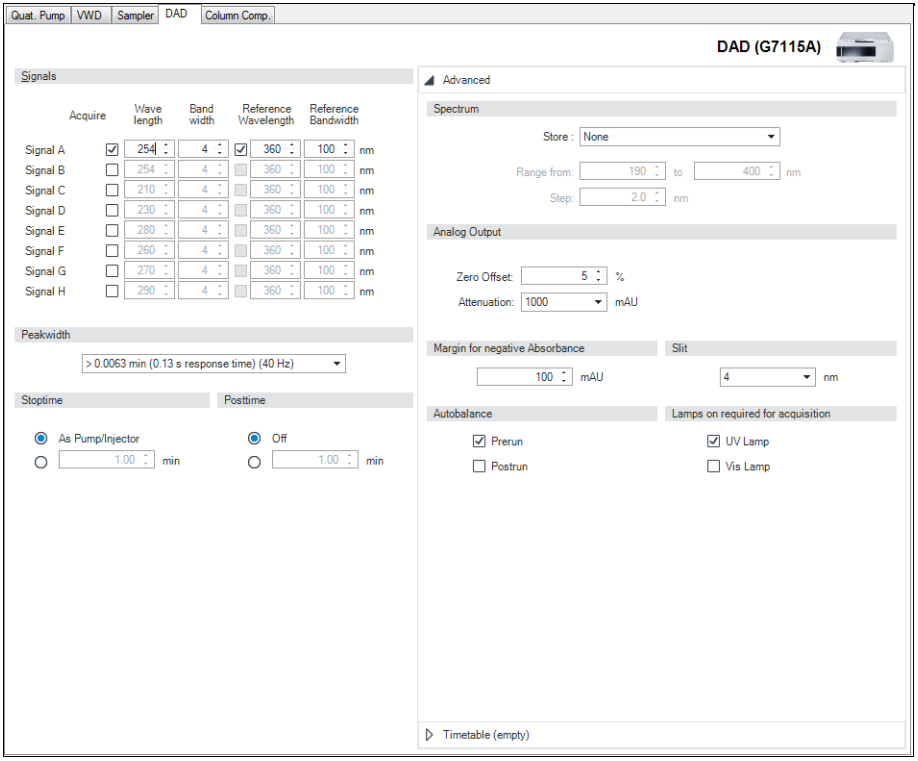


11 Select the column position.



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





NOTE For details on running a method, see "Setup the Checkout Method" on page 61 as an example.

Check Out the System

Checkout Method

Configuration:

- Pump G5654A
- Multisampler G5668A
- Multicolumn Thermostat G7116A
- Diode Array Detector WR G7115A

The RRLC checkout sample (5188-6529) serves as standard for systems with 600 bar 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

NOTE

Find the correct settings for the individual modules here:

- Checkout method parameter settings Bio-Inert Pump (G5654A)(Table 3 on page 59)
- Checkout method parameter settings Bio-Inert Multisampler (G5668A)(Table 4 on page 59)
- Checkout method parameter settings Multicolumn Thermostat (G7116A)(Table 5 on page 59)
- Checkout method parameter settings Diode Array Detector HS (G7117C)(Table 6 on page 60)

Table 3 Checkout method parameter settings Bio-Inert Pump (G5654A)

Parameter	Value
Flow	0.8 mL/min
Solvent A	Water
Solvent B	ACN
Compressibility	95
Composition	40 %B (ACN)
Composition	60 %A (Water)
Stoptime	10 min
Pressure Limit	600 bar
Minimum Stroke	Automatic
Timetable	2.5 min80 %B

Table 4 Checkout method parameter settings Bio-Inert Multisampler (G5668A)

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

Table 5 Checkout method parameter settings Multicolumn Thermostat (G7116A)

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

Table 6 Checkout method parameter settings Diode Array Detectors (G7115A)

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

Setup the Checkout Method

NOTE The setup of the checkout method in this procedure is an example. For the individual module parameters, see "Checkout Method" on page 58.

1 Turn on the lamp.

2 Load the the default method DEF_LC.M

3 Change the method and timetable settings for the 1260 Infinity II Bio-inert Pump (G5654A).

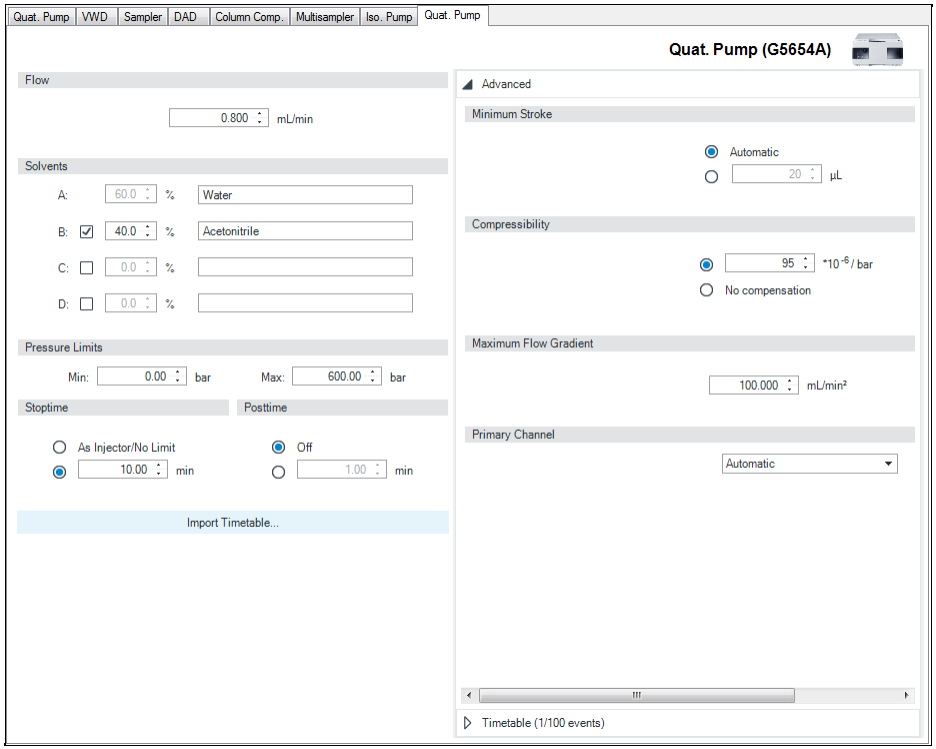


Figure 14 Method settings 1260 Infinity II Bio-inert Pump (G5654A)

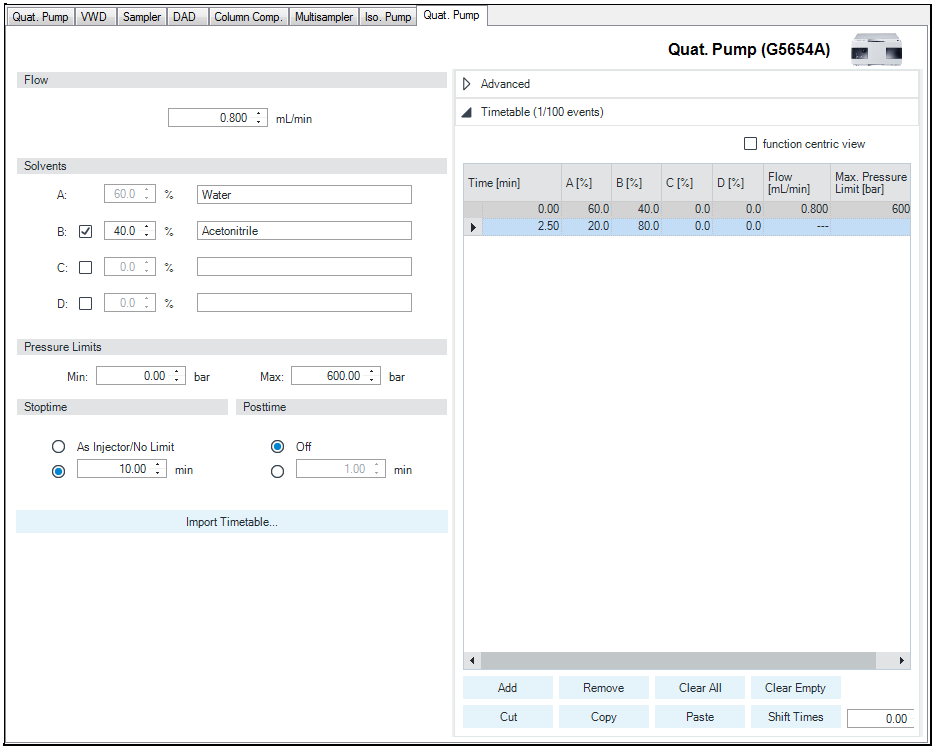


Figure 15 Timetable settings 1260 Infinity II Bio-inert Pump (G5654A)

4 Change the method settings for the 1260 Infinity II Bio-inert Multisampler (G5668A).

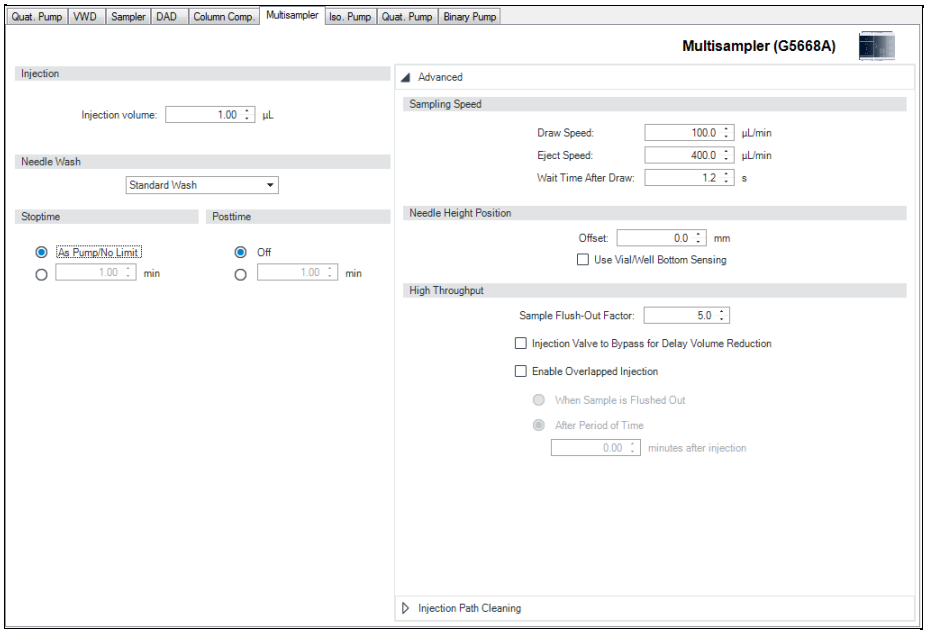


Figure 16 Method setting 1260 Infinity II Bio-inert Multisampler (G5668A)

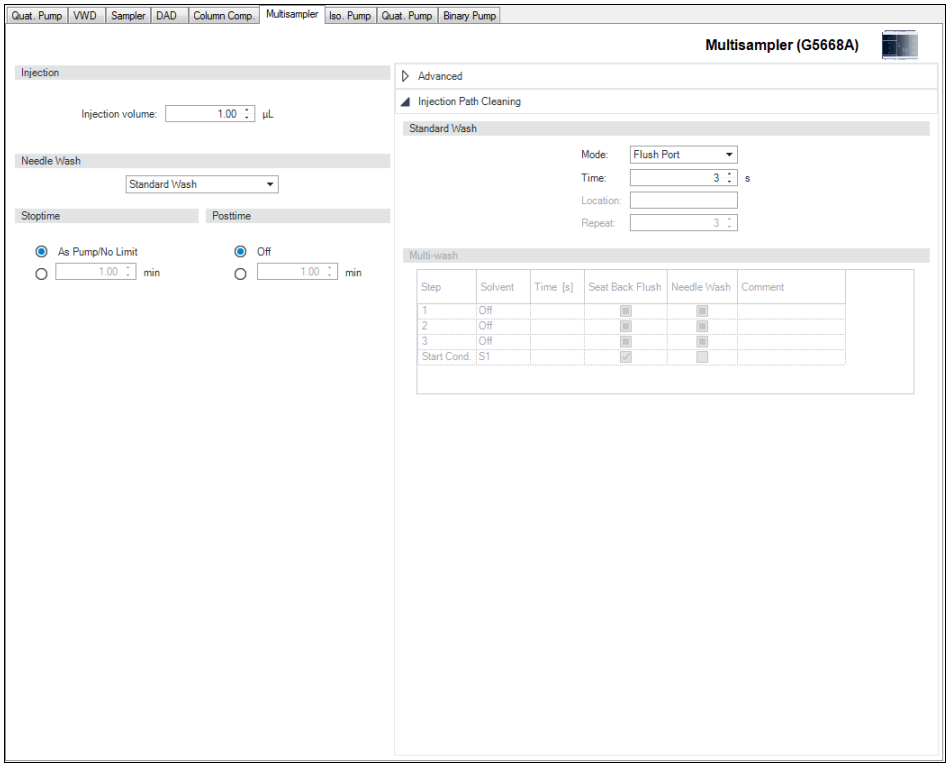


Figure 17 Method setting 1260 Infinity II Bio-inert Multisampler (G5668A) - Injection Path Cleaning

5 Change the method settings for the 1260 Infinity II Multicolumn Thermostat (G7116A)

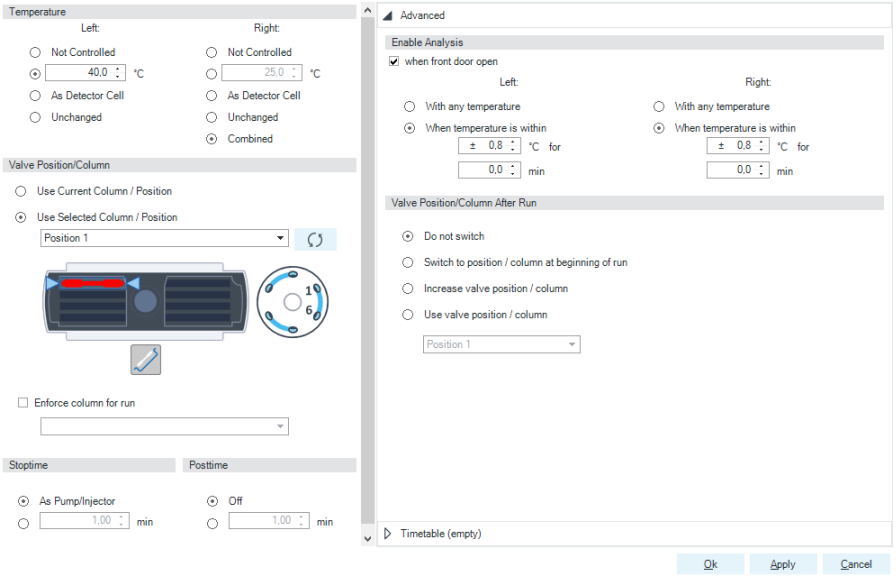


Figure 18 Method setting 1260 Infinity II Multicolumn Thermostat (G7116A)

6 Change the method settings for the 1260 Infinity II Diode Array Detector WR (G7115A)

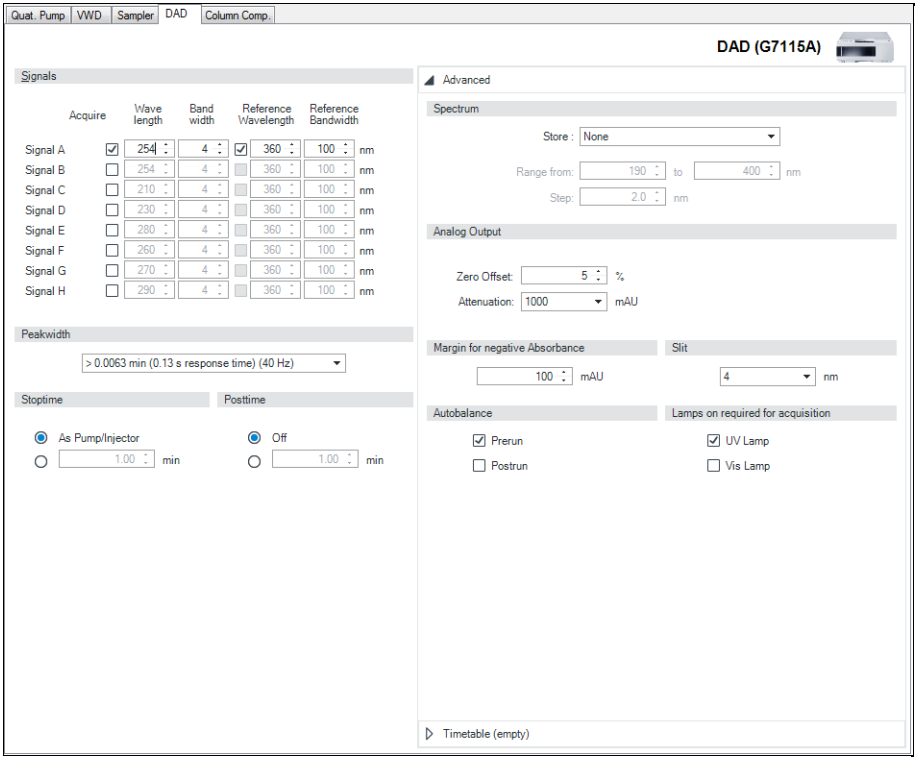


Figure 19 Method setting 1260 Infinity II Diode Array Detector WR (G7115A)

7 Save the method as GRAD-1.M

8 Equilibrate the system for 10 min under checkout conditions

9 Run and evaluate the checkout method

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 II 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 20 on page 68) are characterized by a variety of assays to ensure drug safety and efficacy. The Agilent 1260 Infinity II Bio-inert LC System is a flexible tool addressing the major requirements.

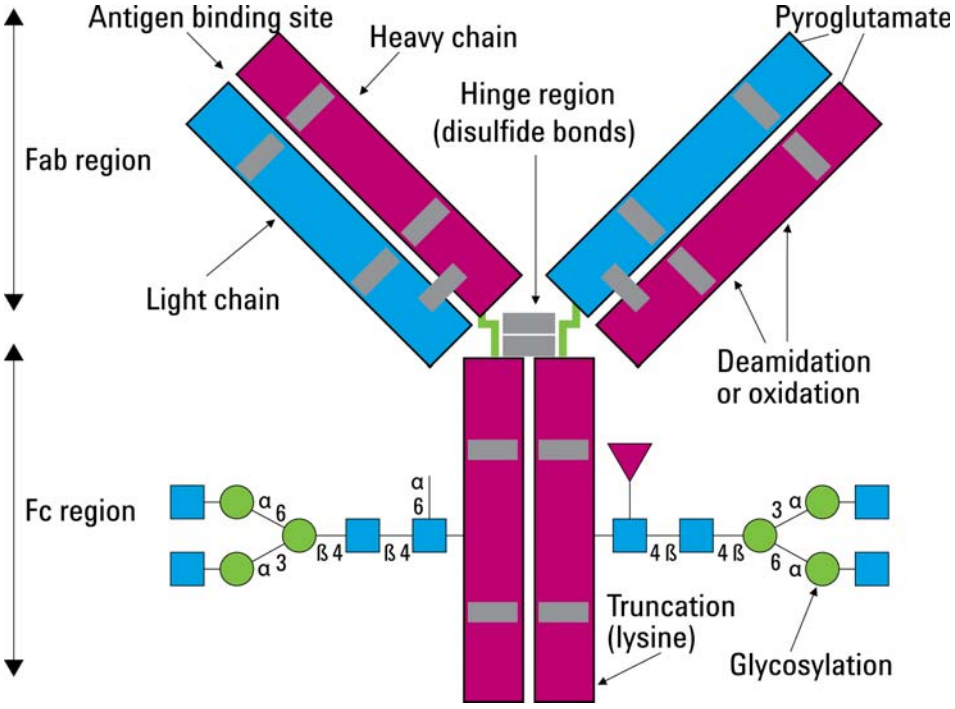


Figure 20 General structure of Therapeutic Antibodies

Application Examples

Ion chromatography of charge variants of monoclonal antibodies

With the Agilent 1260 Infinity II 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.

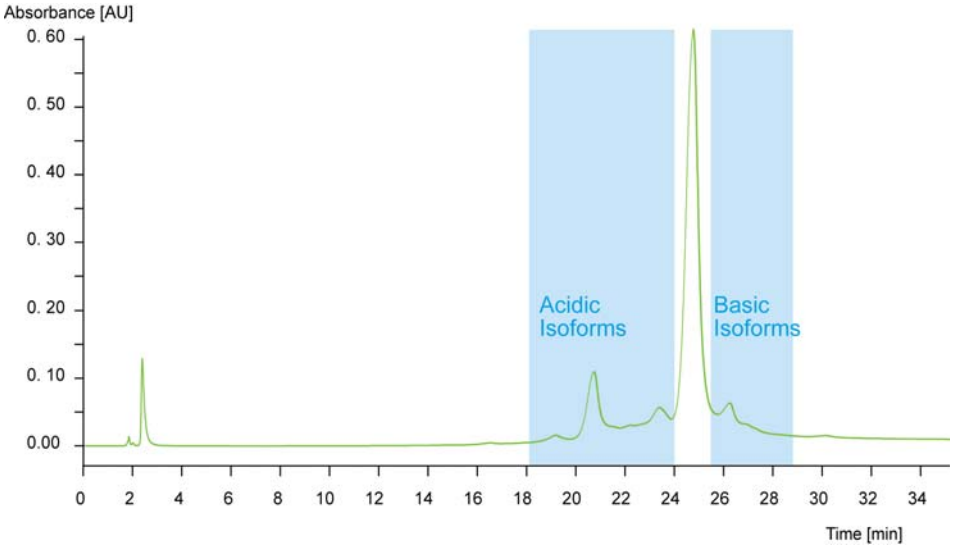


Figure 21 High resolution separation of acidic and basic charge variants using the Agilent Bio MabNP10 column.

Size exclusion chromatography for purity and aggregation analysis

With the Agilent 1260 Infinity II 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.

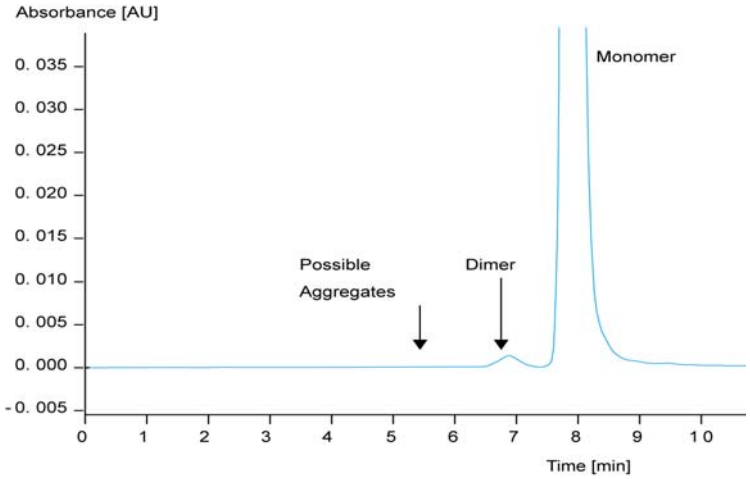


Figure 22 Aggregation analysis of a monoclonal antibody

Peptide Mapping (UHPLC at 600 bar)

The Agilent 1260 Infinity II 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.

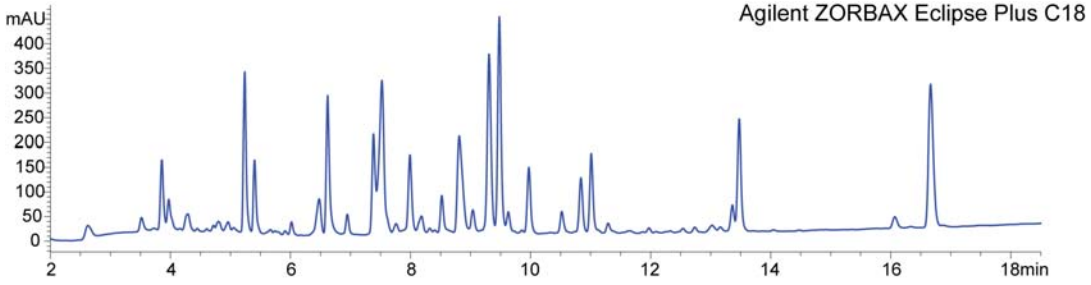


Figure 23 Peptide map with Agilent Zorbax Eclipse Plus C18 Column

6
Parts and Consumables

This chapter provides information on additional parts and consumables.

Tool Kit

The HPLC System Tool Kit contains all tools to operate and maintain a 1260 Infinity II LC system.

The High quality tools are packed in an appealing tool case.

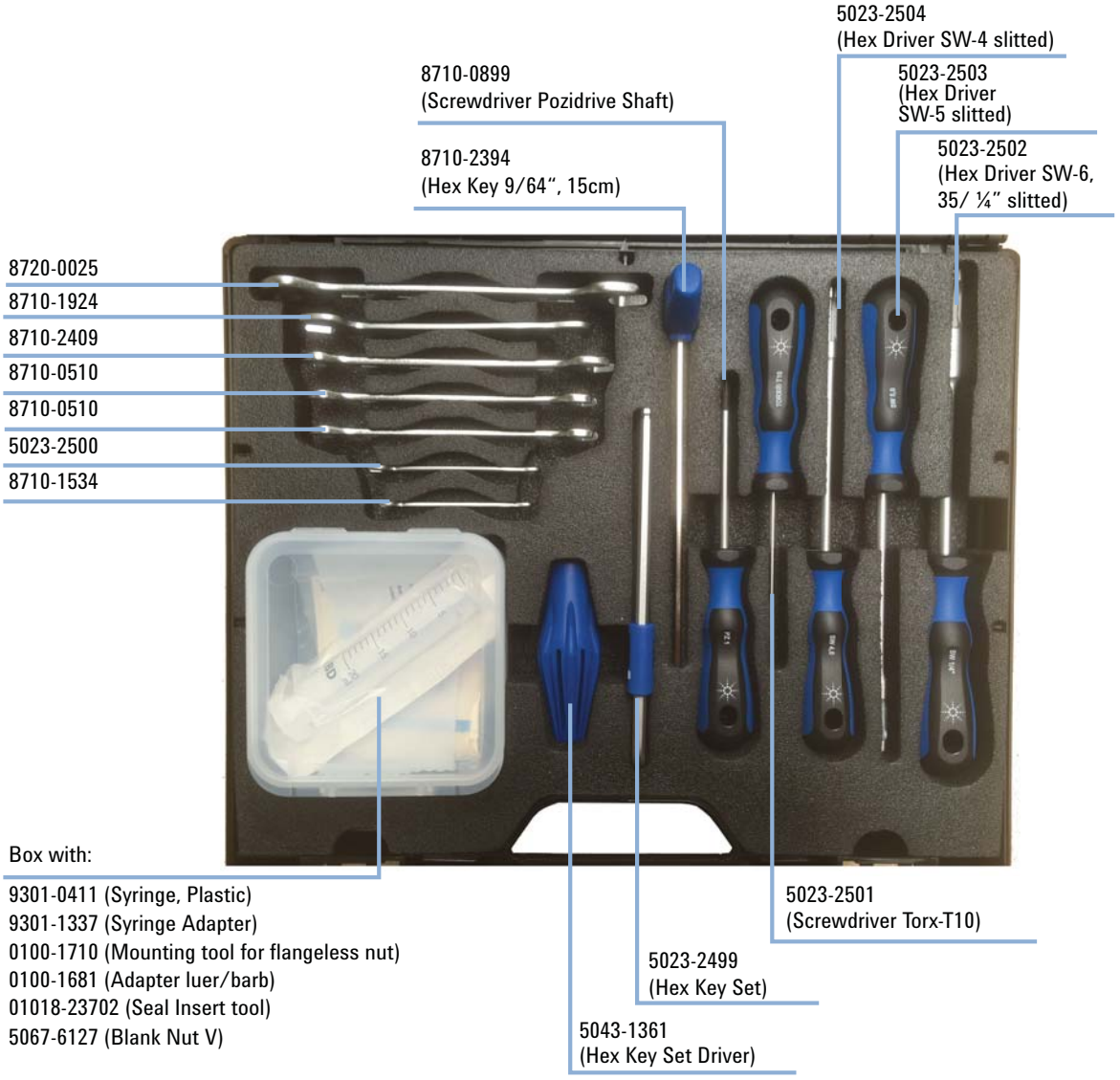


Figure 24 Tool kit

Heat Exchanger for MCT

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

p/n	Description
G7116-60041	Quick-Connect Heat Exchanger Bio-inert

1260 Infinity II Max Uptime Kit Bio

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

Item	#	p/n	Description
1	1	0890-1763	PEEK tubing 1/16" i.d. 0.18 mm, 1500 mm lg
2	3	5067-4741	ZDV union (Bio-inert)
3	1	5062-2418	1/16" fittings and ferrules 10/pk
4	1	5065-4426	Colored finger-tight PEEK fittings, 10/PK
5	1	5971-5210	Assortment Box
6	1	8710-1930	Plastic tubing cutter
7	1	8710-2391	Rheotool socket wrench ¼ inch
8	2	5041-2168	Solvent inlet filter, 20 µm pore size
9	1	01018-22707	PTFE frits (pack of 5)
10	1	5043-0915	Fitting mounting tool for bio-inert capillaries
11	1	G5611-60502	Capillary 900 x 0.17 mm, titanium (Bio-inert) Connection from Pump to Sampler
12	1	5500-1264	Capillary Ti 0.17 mm x 500 mm SL/SLV Connection from Pump to Sampler
13	1	G5667-81005	Capillary PK/ST 0.17 mm x 500 mm RLO/RLO (bio-inert) Connection from Sampler to Bio Heat Exchanger
14	1	G5654-90130	Technical Note 1260 Infinity II Max Uptime Kit Bio

Valve Kits

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

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

Table 7 G5631A Bio-inert 2pos/6port Valve Head kit

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

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

Table 8 G5632 Bio-inert 2pos/10port Valve Head kit

Description	Amount	Unit	Comment
2 pos/10 port valve bio-inert, 600 bar (5067-4132)	1	ea	
2-10 POD Bio Capillary Kit (5067-5419)	1	ea	

G5639A Bio-inert 4 Column Selector Valve Kit

Table 9 G5639A Bio-inert 4 Column Selector Valve Kit

Description	Amount	Unit	Comment
Bio-inert valve head 4 column selector (600 bar) (5067-4134)	1	ea	
Bio-inert Capillary kit for 4 Column Selector (5067-4769)	1	ea	

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

Table 10 G4235A Solvent Selection Valve Head, Bio-inert - Stand Alone Valve

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

7
Appendix

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

Safety

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.

NOTE

Note the instrument's external markings described under "Symbols" on page 82.

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.

Solvents

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. Always keep the temperature in the sample compartment at least 25 K below the boiling point of the solvent used.
- ➔ Do not operate the instrument in an explosive atmosphere.
- ➔ 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.

Symbols

Table 11 Symbols

	The apparatus is marked with this symbol when the user should 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.
	Cooling 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.
	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.
	Power symbol indicates On/Off. The apparatus is not completely disconnected from the mains supply when the power 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.

Table 11 Symbols

	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.

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.

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In This Book

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

The manual describes the following:

- introduction,
- specifications,
- install the modules,
- configuration settings,
- quick start guide,
- typical Bio-inert LC applications.
- parts and consumables.

Printed in Germany
05/2016

G5654-90300
Rev. B

Agilent Technologies