# Getting the Most From Your Agilent HPLC Pump

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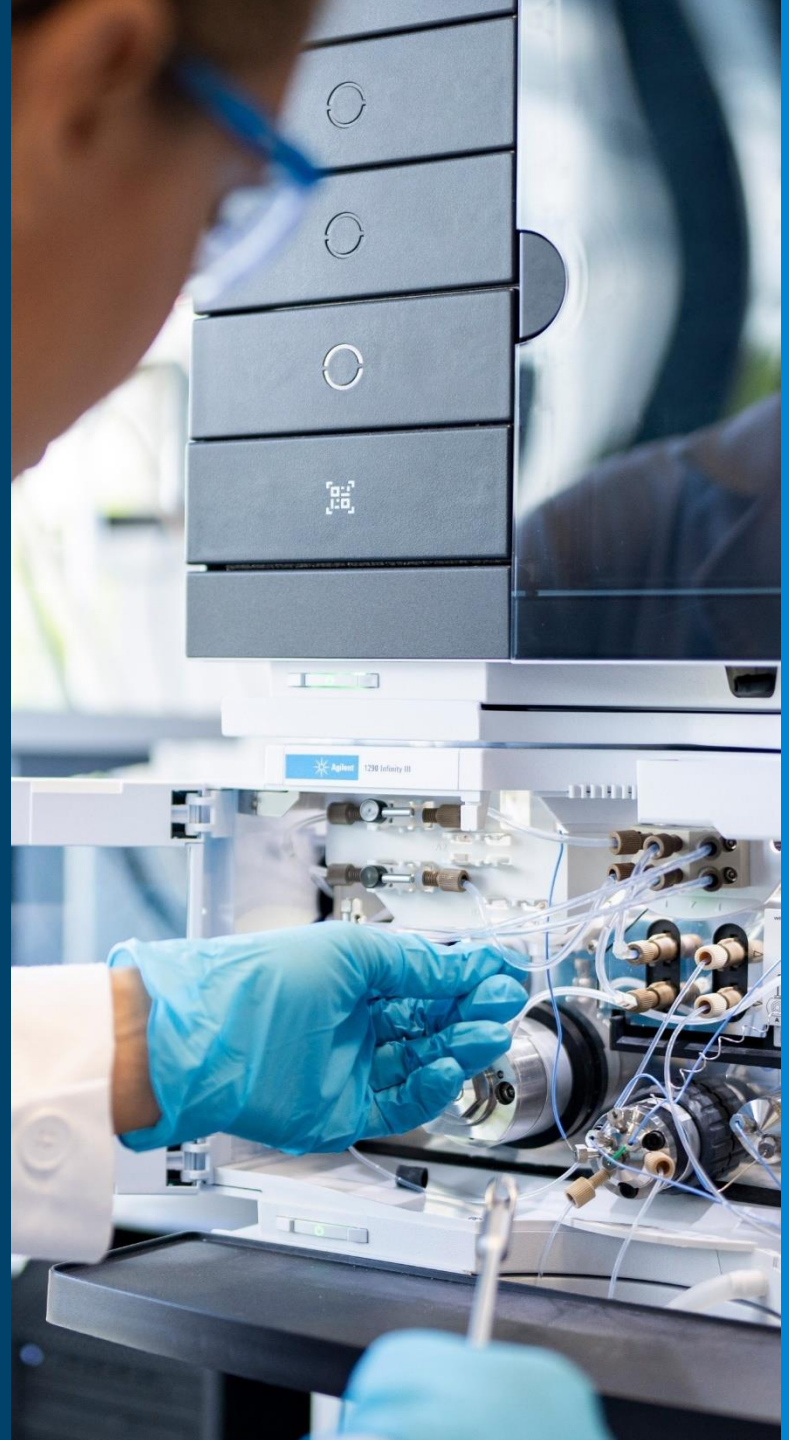
Columns and Supplies Technical Support
January 14, 2025



Agilent InfinityLab

Agilent

# Overview

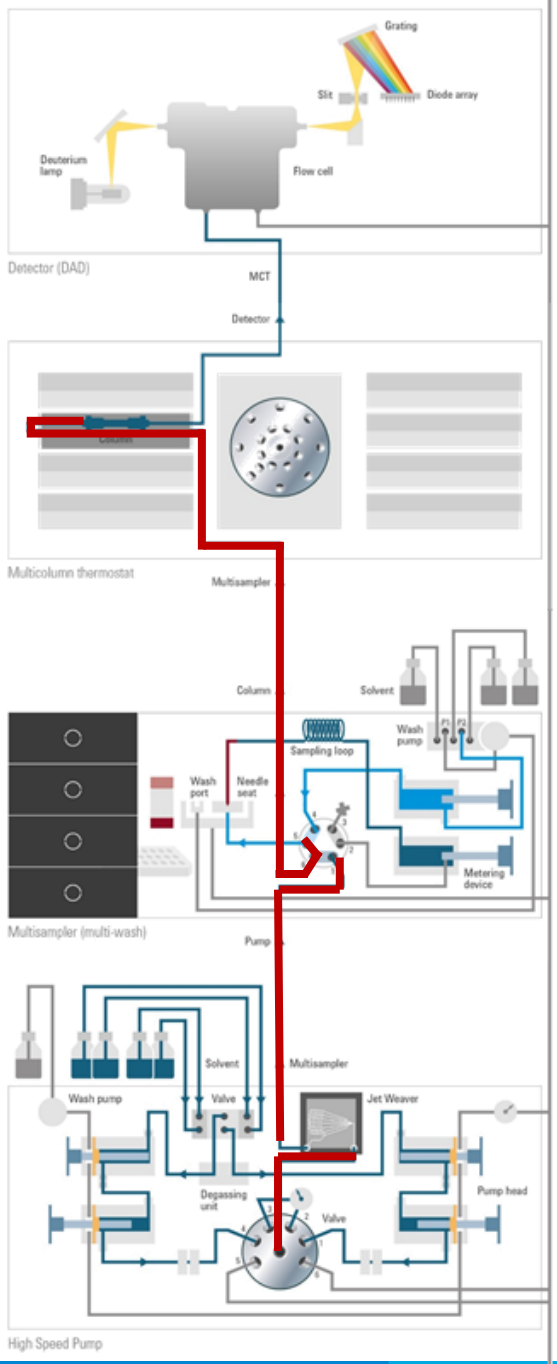
- Pump overview
  - High pressure vs. low pressure mixing
  - Delay or dwell volume
- Selecting a column for your pump
- Mobile phase
  - Housekeeping
  - Daily/weekly tasks
  - Seal wash
  - Flushing for buffers, acetonitrile, warm water
  - Normal phase and GPC
- Purging vs conditioning vs priming
- Pump shutdown
- Pump benchmarking
  - Restriction capillary
  - InfinityLab Performance Standard
- Points of maintenance
- How-to videos on maintenance
  - YouTube
  - Agilent Community
- Lab Advisor
- Infinity III Assist Hub

# System Considerations

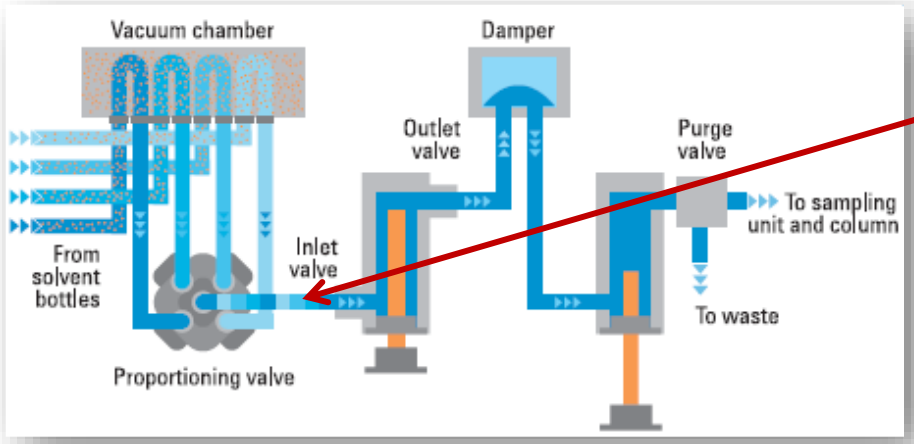
**Dwell or Delay Volume:** System volume from the formation of the gradient to the top of the column

Can affect:

- Retention time
- Resolution
- Peak width

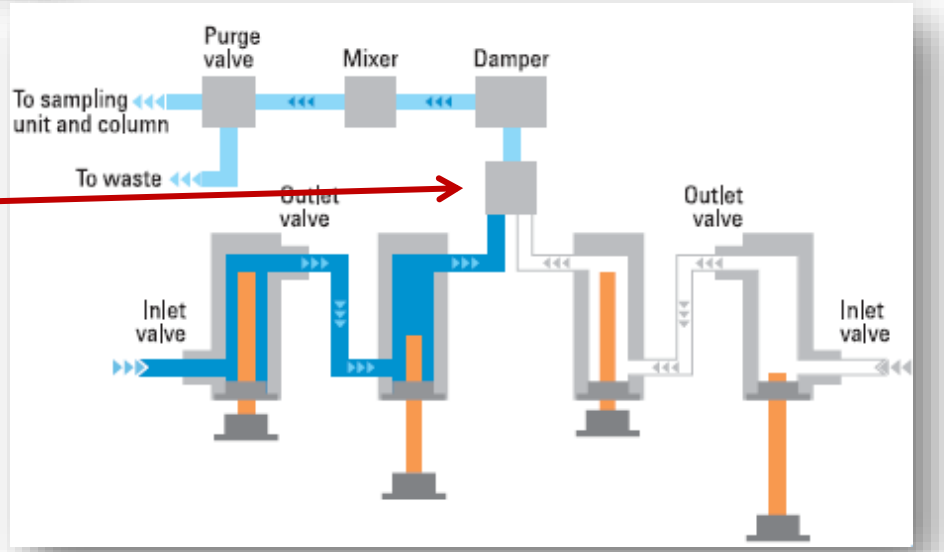



# High Pressure Mixing vs Low Pressure Mixing

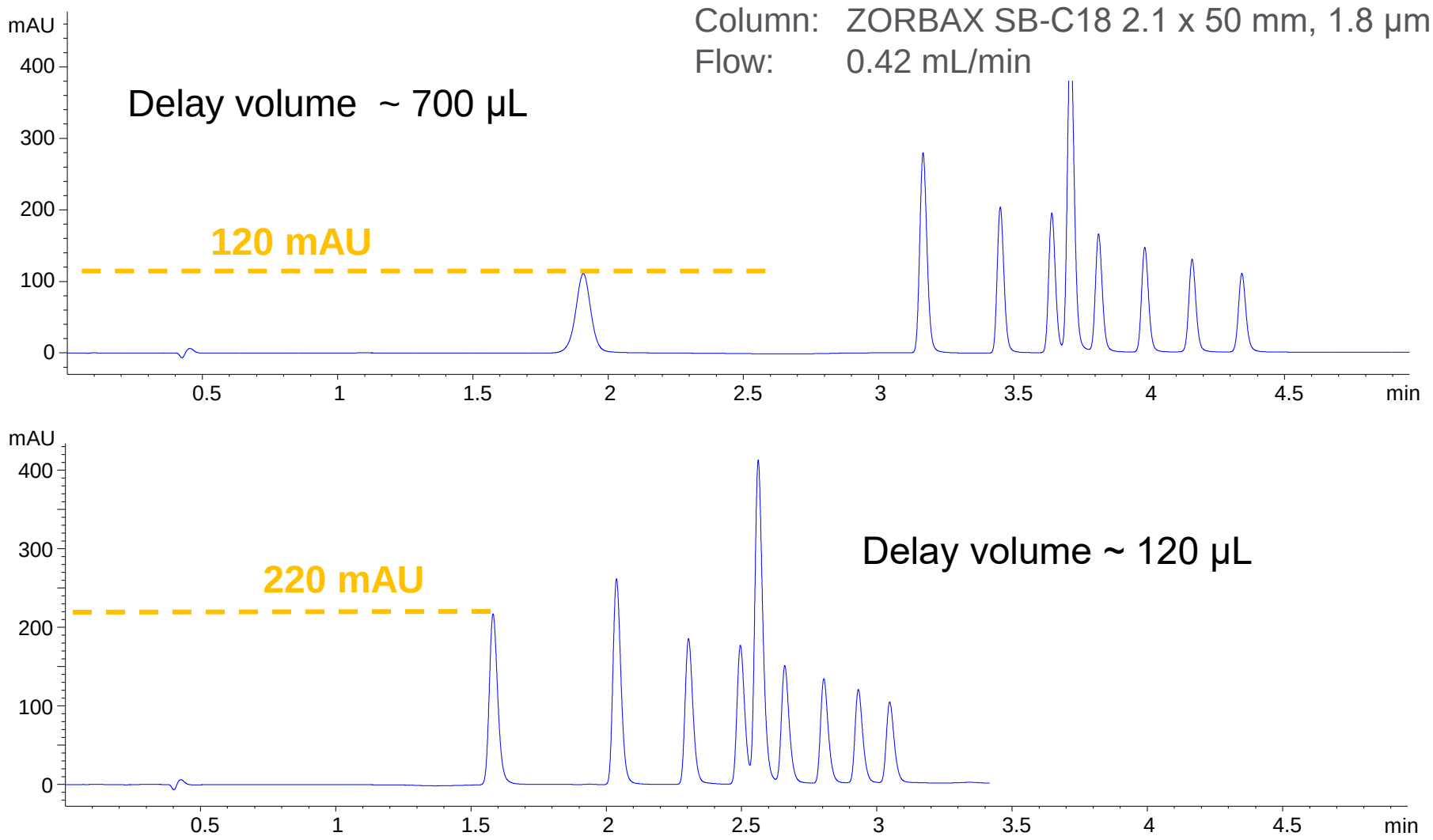



- Point of mobile phase mixing **before** pump head
- Quaternary Pumps
- Flexible Pumps

- Point of mobile phase mixing **after** pump head
- Binary Pumps
- High Speed Pumps



# Delay Volume



| Column Type<br>Particle | **Traditional HPLC**<br>3–5 µm | **UHPLC**<br>2.7 µm (SPP) / < 2 µm (FPP) | | | | **Low Dispersion UHPLC**<br>< 2 µm | | | |
|---|---|---|---|---|---|---|---|---|---|
| Recommended product<br>(Max pressure/bar) | **1. 4 µm Poroshell (600)**<br>2. 3.5 and 5 µm ZORBAX (400)<br>3. 3 and 5 µm Pursuit, Polaris (400) | **1. 2.7 µm Poroshell (600)**<br>2. 1.8 µm ZORBAX RRHT (600) | | | | **1. 1.9 µm Poroshell (1300)**<br>2. 1.8 µm ZORBAX RRHD (1200) | | | |
| Column length (mm) | 50–300 | Short: 30–50 | | Long: 100–150 | | Short: 30–50 | | Long: 100–150 | |
| Column id (mm) | 3.0–4.6 | 2.1 | 3.0–4.6 | 2.1 | 3.0–4.6 | 2.1 | 3.0 | 2.1 | 3.0 |
| **1300 bar Low Dispersion UHPLC – High Speed Pump (1290 Infinity II/III)** | H/I | | | | | | | | |
| **1300 bar Low Dispersion UHPLC – Flexible Pump (1290 Infinity II/III)** | H/I | | | | | V | | | |
| **800 bar UHPLC – Quaternary Pump (1260 Infinity II/III Prime)** | H/I | | | | | V | | P | P |
| **600 bar UHPLC – Binary Pump (1260 Infinity II)** | | V | | | | V+P | V+P | V+P | P |
| **600 bar UHPLC – Quaternary Pump (1260/1220 Infinity II)** | | V | V | V | | V+P | V+P | V+P | V+P |
| **400 bar HPLC (1100, 1260/1220 Infinity II VL)** | | V | V | V+P | P | V+P | V+P | V+P | V+P |

**400 bar =** 6000 psi
**600 bar =** 9000 psi
**1200 bar =** 17000 psi
**1300 bar =** 19000 psi

**Limitations**
**V** – System volume (dispersion/delay)
**P** – Pressure limits
**V+P** – System volume and pressure
**H/I** – if instrument is used for HPLC methods / ISET emulation

**Recommended**
Acceptable
Limited Configurations
Not Recommended

# Solvent Bottles and Solvent Inlet Filters

### InfinityLab LC Filtration

- 5191-6776: InfinityLab Solvent Filtration Assembly, Including Glass Funnel, 250 mL, Membrane Holder Glass Base, Glass Flask, 1 L, and Aluminum Clamp
- 5191-4340: InfinityLab Filter Membrane, Regenerated Cellulose 47 mm, Pore Size 0.2 µm, 100/pk

## Filter buffer and salt solutions

- Filter porosity: 0.45 or 0.2 µm
- Make sure the filter material is compatible

## Inlet filters – not a replacement for good mobile phase hygiene

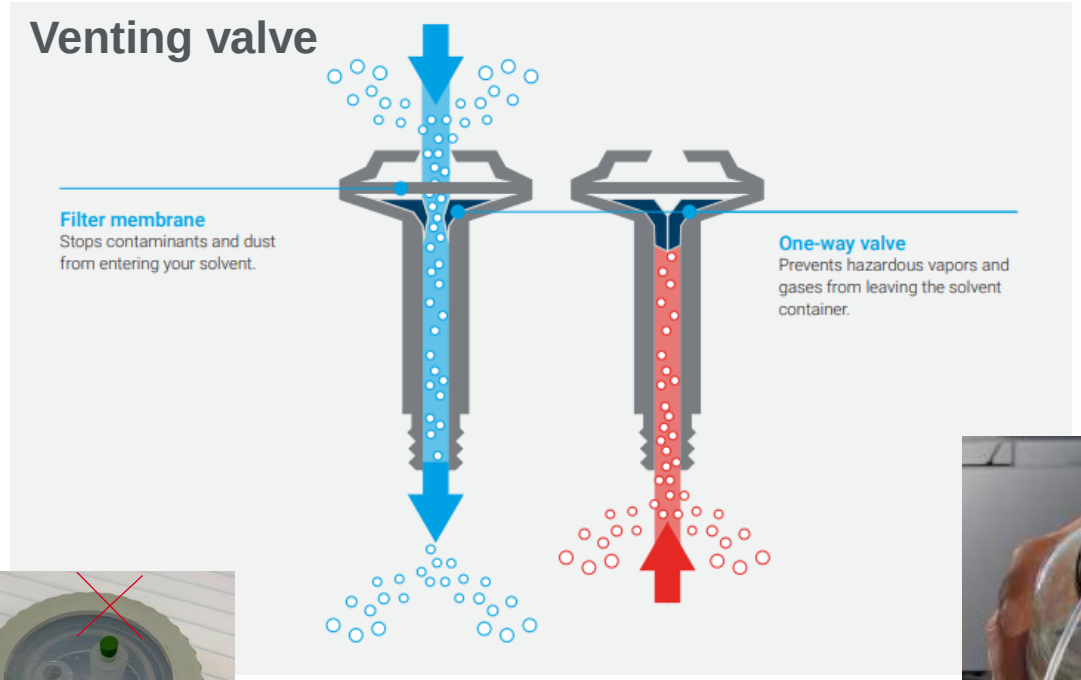
- Glass solvent inlet filter (20 µm), 5041-2168
- Stainless steel solvent inlet filter (recommended for LC/MS), 01018-60025

## Lack of maintenance can lead to problems

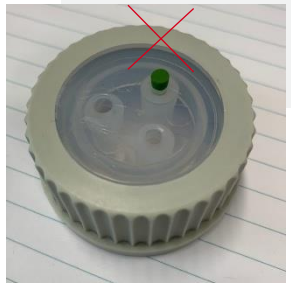
- A dirty solvent inlet frit can cause cavitation. The “bubbles” formed can lead to pump issues.
- Mobile phase with microbial growth can cause high pressure and ghost peaks.
- Replace glass filter or clean steel filter semi-annually.

# InfinityLab Stay Safe Caps

- Prevent harmful vapors from leaving the solvent bottle.
- Reduce evaporation and mobile phase changes.
- Time strip is a reminder to replace the venting valve.







1 port: 5043-1217
2 port: 5043-1218

Stay Safe Caps guide: 5994-1798EN

# Degasser Care



- Check for bubbles in outlet lines
- Flush out buffer salts with water when changing mobile phases
- When switching solvents, make sure they are miscible
- Do not leave aqueous mobile phase in the degasser for an extended time - avoid microbial growth
- Unused channels should be flushed with water to remove any buffers, leave in water/isopropanol

# Housekeeping

**Best Practices for Handling Mobile Phases**

- Use clean borosilicate glass bottles only.
- Rinse bottle with desired solvent before refilling it.
- Bottles can get contaminated with detergents from a dishwasher.
- Exchange water-based solvents daily.
- Prepare solvent volume to be consumed within 1 to 2 days.
- Use only HPLC-grade solvents and water.
- Prepared organic solvents and aqueous buffers must be 0.2 µm filtered.
- Label bottles correctly with bottle content (filling date and expiry date).
- Reduce risk of algae growth:
  - Brown bottles for aqueous solvents.
  - Avoid direct sunlight.
  - Wrap the bottles in aluminum foil.

Extra measures with acetonitrile (ACN):

- ACN and other organic solvents can be filtered using a 0.2 µm PTFE filter membrane (e.g. 5191-4339).
- Use brown bottles and fill with only what will be used within 1 to 2 days to reduce degradation.

# Daily and Weekly Tasks

## Daily Tasks

- Replace solvents and solvent bottles for mobile phases based on water/buffer.
- Replace solvents and solvent bottles for organic mobile phase every second day.
- Check seal wash solvent.
- Purge each channel with fresh solvent at 2.5 – 3 mL/min for 5 min before operation.

## Weekly Tasks

- Change seal wash solvent (10 % isopropanol in water) and bottle.
- If applications with salts were used, flush with water.
- Inspect solvent filters for dirt or blockages.
- Exchange if no flow is coming out of the solvent line when disconnected at the degasser inlet.

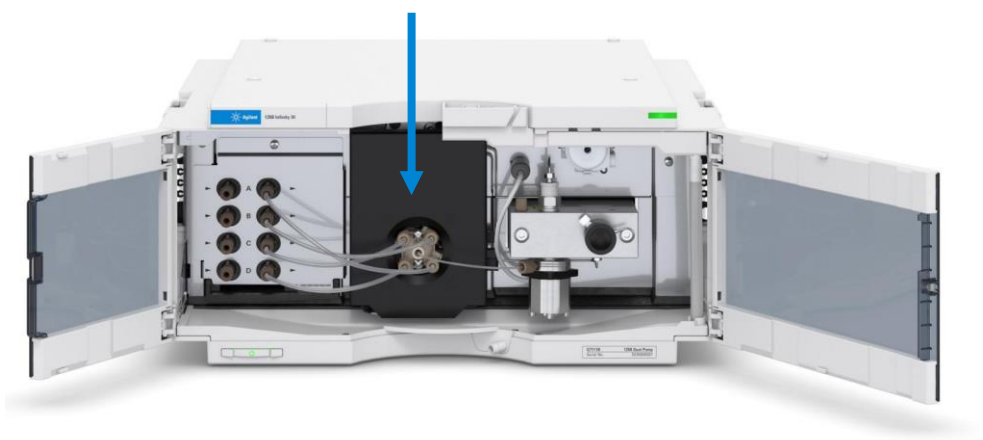
5067-6131

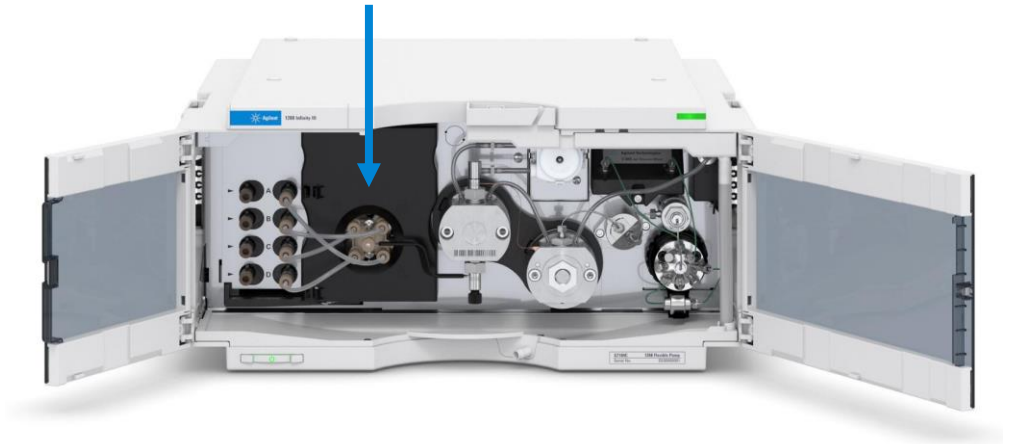
# Caring for the Gradient Valve

Agilent InfinityLab

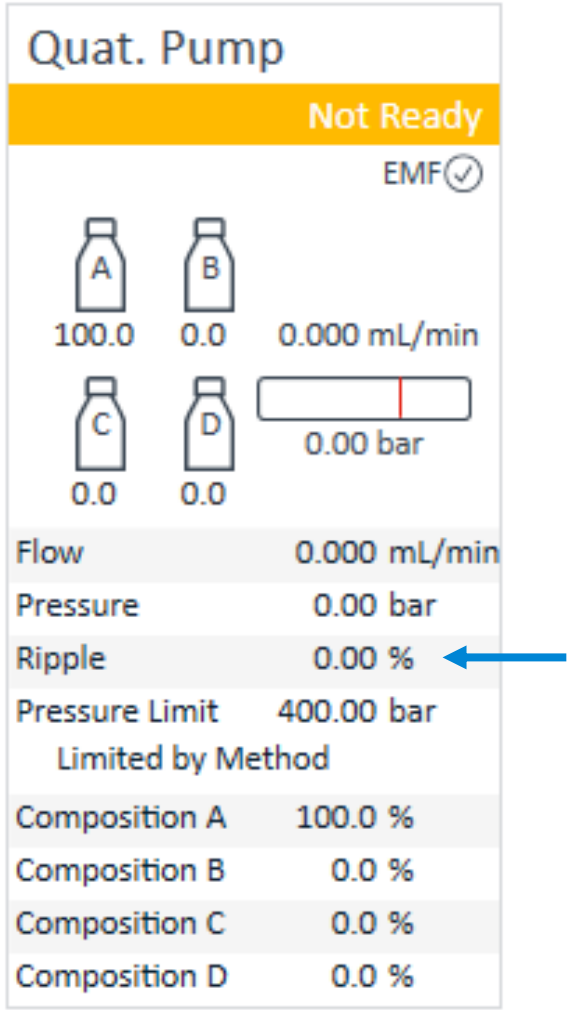
Recommendations for Multi Channel Gradient Valve (MCGV).

- Use lower channels (A and/or D) for buffer solutions.
- To remove possible salt deposits, flush with water at 5 mL/min for at least 10 min per channel.
- Check compatibility of buffers and organic solvents to avoid precipitation in the MCGV mixing chamber.





# Ripple



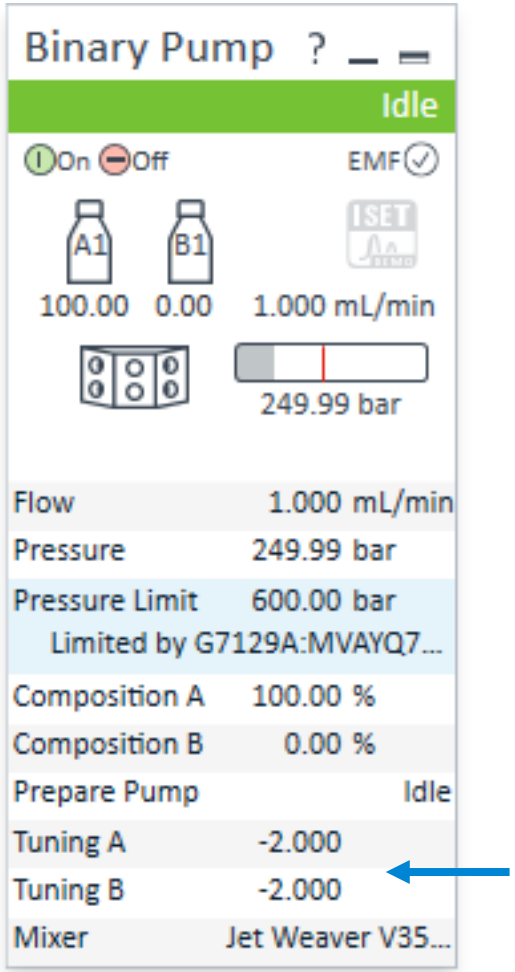

Always keep an eye on your system pressure

- 1100, 1200, 1260 models have Ripple value
- Pressure ripple (fluctuations or delta) is a measure of pump performance
- Usually less than 2% (often much better) when the system is equilibrated.

Excessive ripple is indicative of a problem.

- Leaks in the system allowing air
- Solvent compressibility problems
- Immiscible solvents

# Tuning



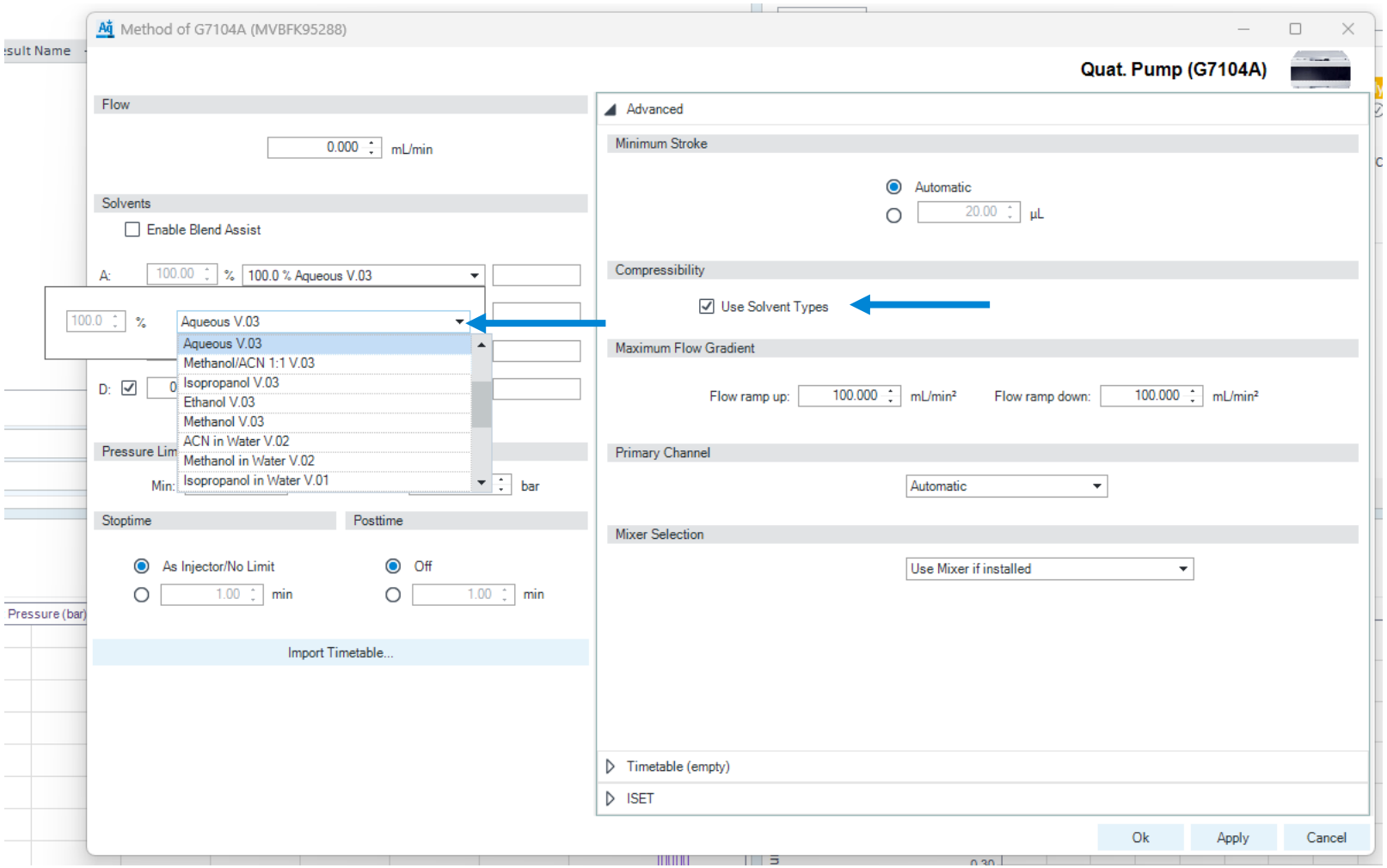

For certain pumps (1290), these have a Tuning value

- For pumps operating as expected, value should be –1 to +1.
- The full scale is –2 to +2.
- Ideal performance is at 0.

Possible causes for Tuning deviations:

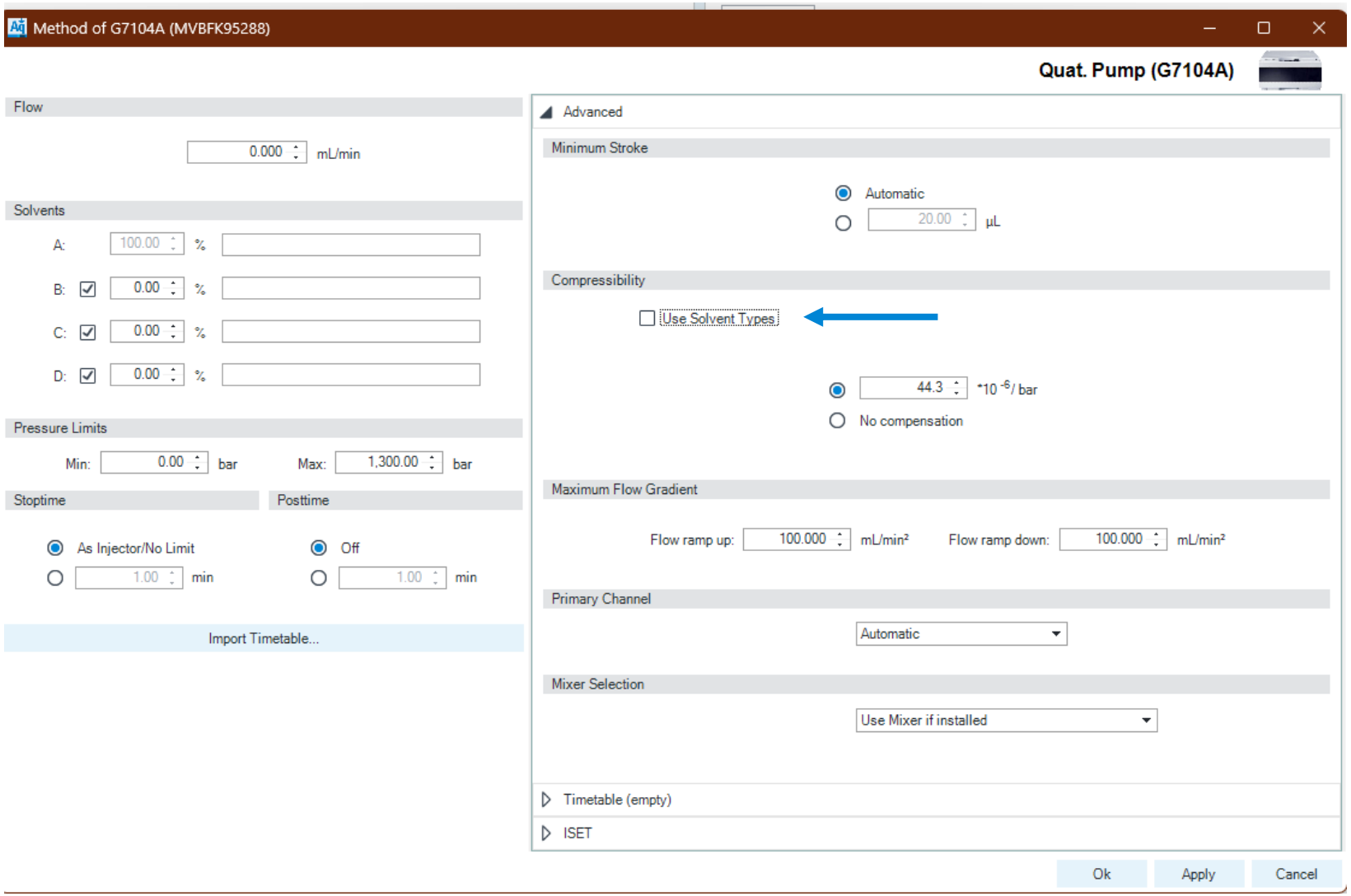
- Gas bubbles
- Suboptimal compressibility settings
- Leaks in seals or fittings
- Problems with the valves
- Blockage of filters or capillaries

# Compressibility




- For best flow accuracy and mixing performance, select the mobile phase being used.
- Select this check box (recommended) to use the automatic compressibility calibration.
- Then select the calibrated solvent from the drop-down list.

# Compressibility

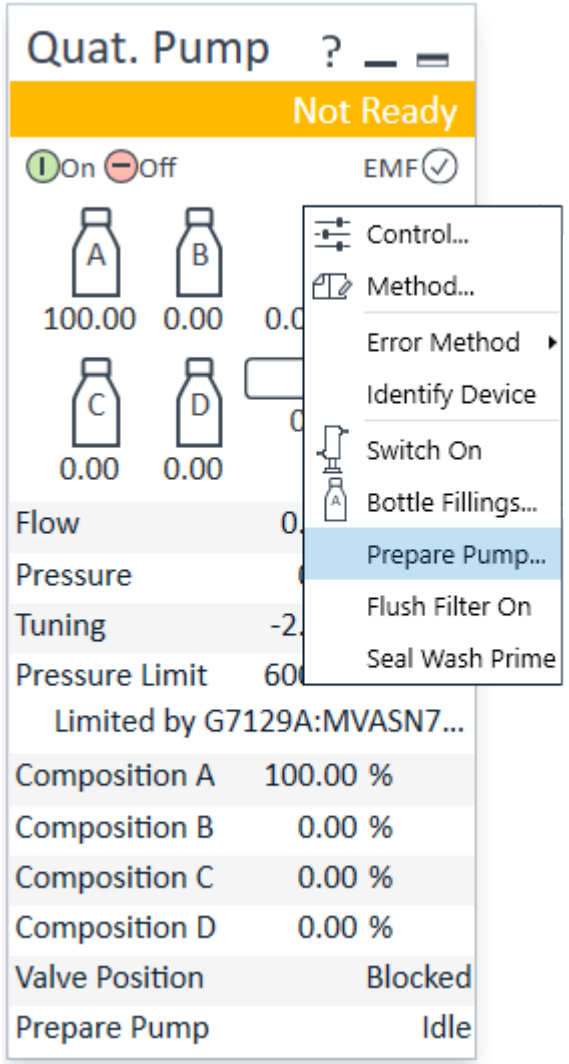



- Clear this check box to enter manual compressibility values.
- This setting is available for method backward compatibility, e.g. from 1260 Infinity pumps.

# Manual Purging

- To install a fresh or different mobile phase
- Open the manual purge valve
- Do not unscrew completely
- Purge each channel with 2.5 to 3 mL/min for ~5 min

# Purge vs Conditioning vs Prime



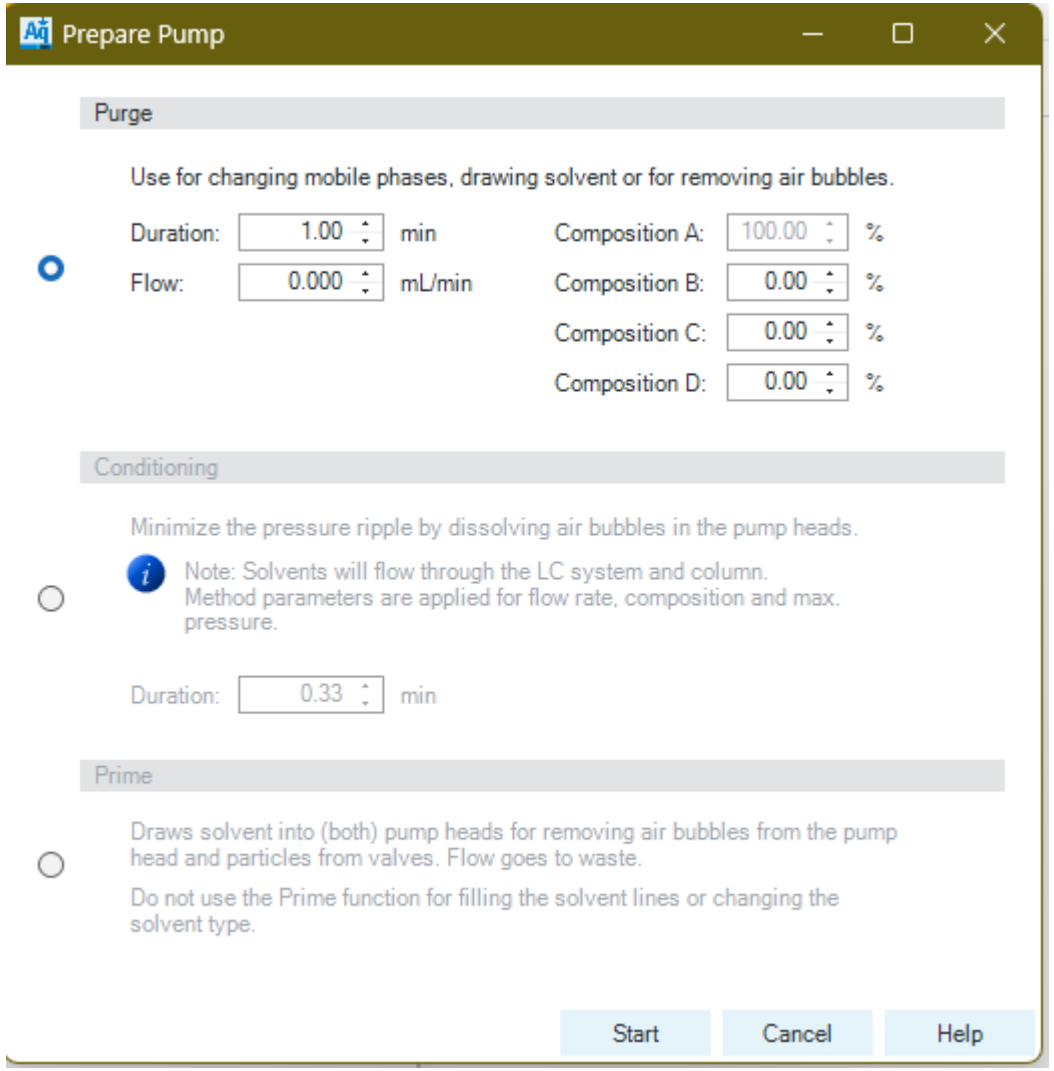

For 1290 Infinity and 1290 Infinity II Pumps

- Agilent 1290 Infinity and 1290 Infinity II Pumps are equipped with automatic purge valves.
- Prepare Pump
- Additional functions not available with manual purge valve.
- Purge, Condition, or Prime with the software

CAUTION when filling empty solvent lines

- Potential for damage to the pump seals
- Use a syringe or the Purge function to fill empty solvent lines.
- Do not use the Prime procedure to fill empty solvent lines.

# Purge vs Conditioning vs Prime

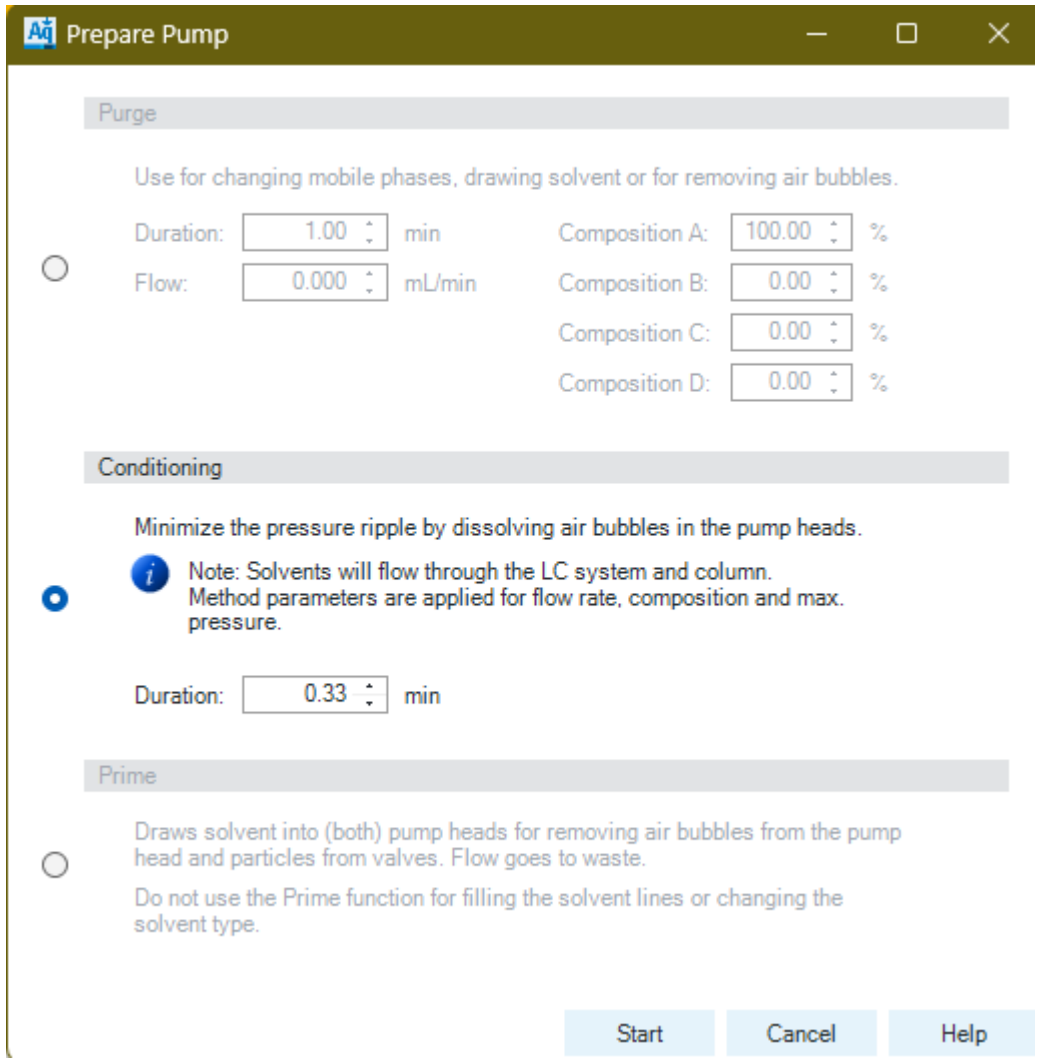



Purge

Use the Purge function to:

- Fill the system with fresh or different solvent.
- Ensure that the new solvent is miscible with the previous solvent.
- Prevent damage to the degasser or pump by using an intermediate step with a commiscible solvent, if necessary.
- Remove air bubbles in tubing and pump heads.
- After the pump has been idle for a few hours or more (air may be in the solvent lines).
- As soon as the purge procedure ends the module automatically switches to analytical conditions again.

# Purge vs Conditioning vs Prime




## Condition

If micro air bubbles persist in the pump head, the overall pump performance may be compromised, and flow accuracy/precision may be negatively affected. This may be indicated by pressure ripple and/or detector baseline ripple.

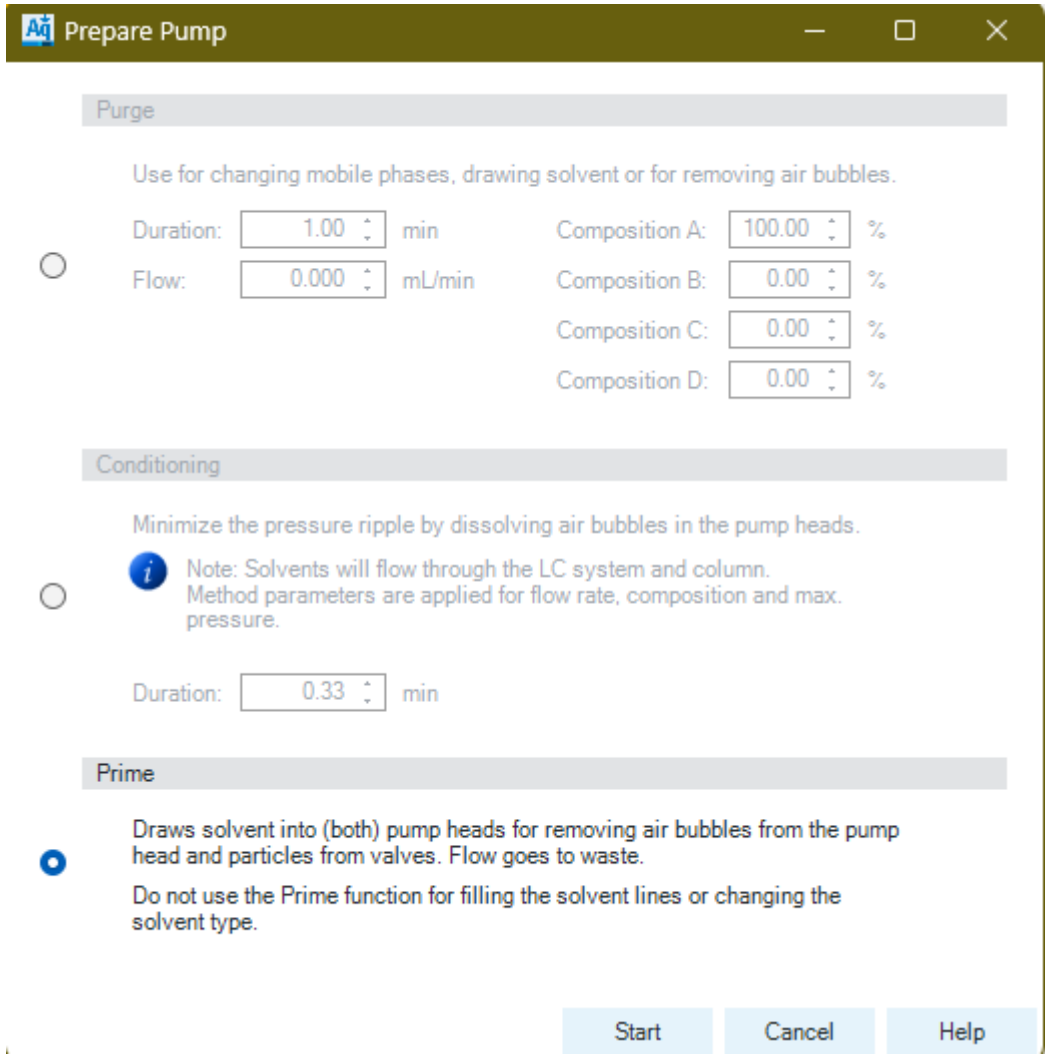
- Often indicated by a negative but slowly increasing tuning signal value (below –1).
- To remove the bubbles, the Condition function can be used.
- During conditioning, the pump is delivering flow into the system (column) and the last used method settings, like flow, composition and max pressure are used.

Use a reasonable flow rate (for example 1.5 mL/min), composition setting (for example A: 50% B: 50%) and backpressure (>200 bar) to ensure efficient air bubble removal from all pump heads.

Conditioning may be necessary:

- After a long period of standby
- After running out of solvent
- After service or repair

# Purge vs Conditioning vs Prime




**Prime**

The Prime function is helpful if:

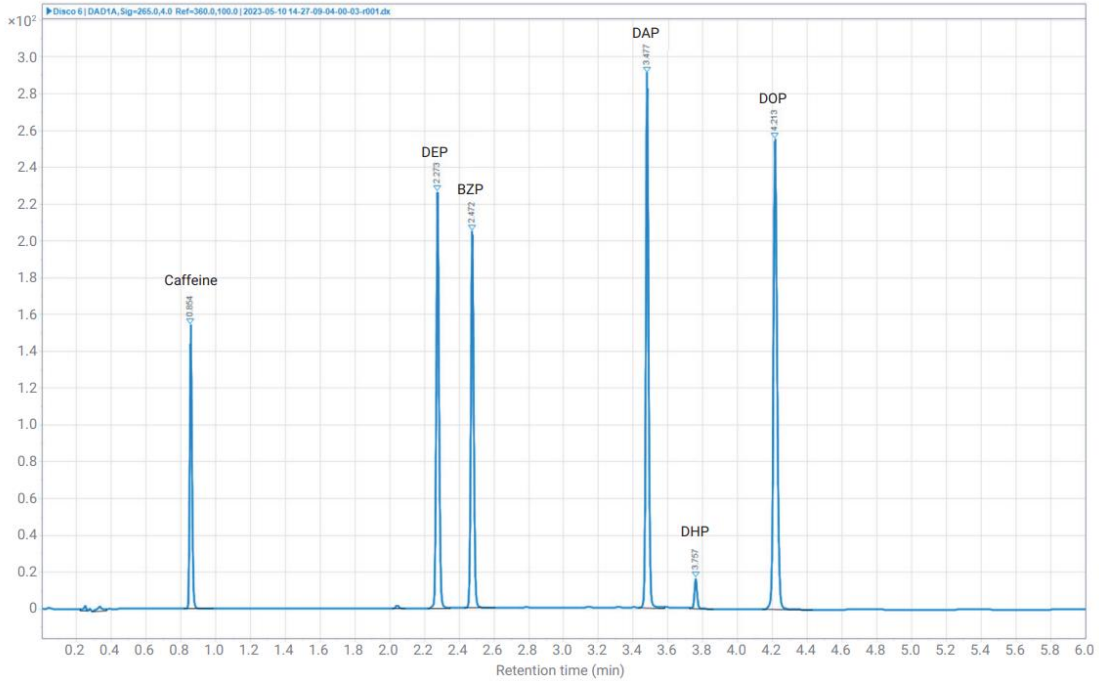
- Air has entered the pump heads.
- Not removed by conditioning for 15 minutes.
- The module draws solvent at a high speed with all pump drives simultaneously and dispenses it into the waste position of the automatic purge valve.
- This is done 20 times (stress on the valve and rotor seal).
- Should be performed only as a last resort.
- Before filling the pump heads with a syringe or attempting to repair the pump heads.
- Can also be used to free a potentially stuck valve.

# Check Your Instrument Pressure

- Before installing your column, test your instrument pressure with no column
- Install a restriction capillary (part number 5022-2159)
- Measure the system pressure under desired conditions
- Check the system pressure using just a union

ZDV universal union, 5022-2184

# InfinityLab Performance Standard

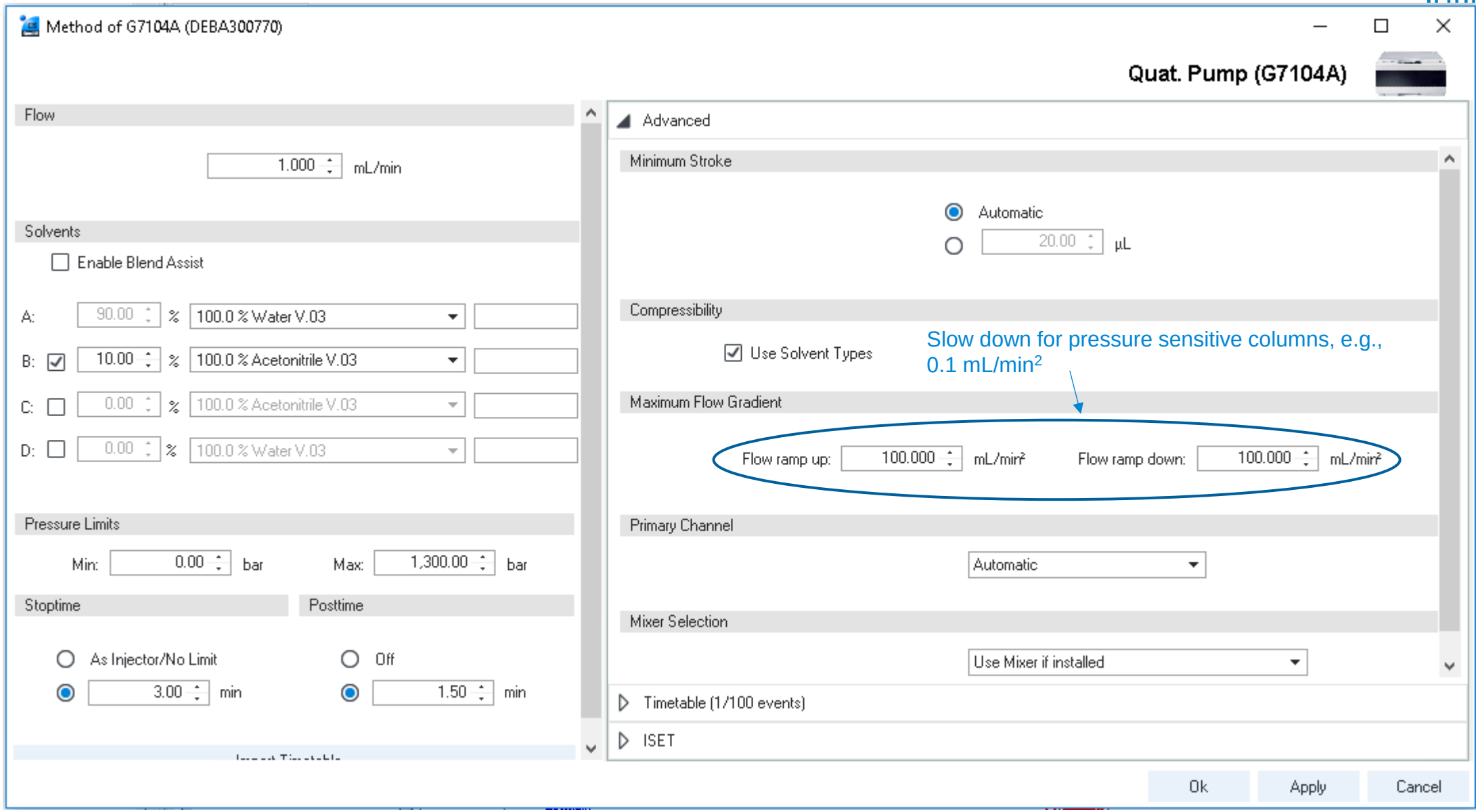




InfinityLab LC Performance Standard, 5191-4547
InfinityLab Poroshell 120 EC-C18 3.0 × 50 mm, 2.7 µm, 699975-302

Flow Rate 1 mL/min
Column Temperature 40 °C
Mobile Phase A: water with 0.1% formic acid
Mobile Phase B1: acetonitrile with 0.1% formic acid
Mobile Phase B2: methanol with 0.1% formic acid
Injection Volume 3 µL
5% B at 0 min
95% B at 3 min
95% B at 4.5 min
5% B at 5 min

5994-6584EN

# Pump Setting for Pressure Sensitive Columns



# Special Considerations for 1290 Infinity Pumps


- Reliable operation during analysis cannot be guaranteed if pressure falls below 20 bar.
- For optimal results, pressure should be at least 50 bar continuously.
- When using columns that create low backpressure (<50 bar, such as SEC or Hi-Plex columns), install a restriction capillary (such as 5022-2159, steel or 5005-0046, MP35N) between the pump and sampler to achieve at least 50 bar.

# Seal Wash

**Seal Wash** helps ensure optimal piston seal life. Highly concentrated buffer solutions can reduce the lifetime of the seals and pistons in your pump.

Use Seal Wash:

- Buffers with elevated salt concentrations (e.g. 0.1 M or higher)
- When using volatile solvents with a non-volatile additive
- If you have Seal Wash installed it is mandatory to use it

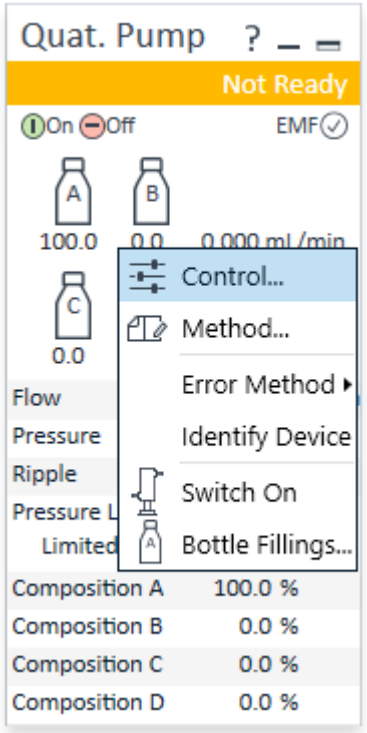
- Flushes the back side of the seal with a wash solvent.
- Uses a wash bottle filled with 90% water/10% isopropanol.
- Above the pump in the solvent cabinet.
- Seal wash waste into the waste bottle underneath.
- Peristaltic pump moves a flow through the pump head.

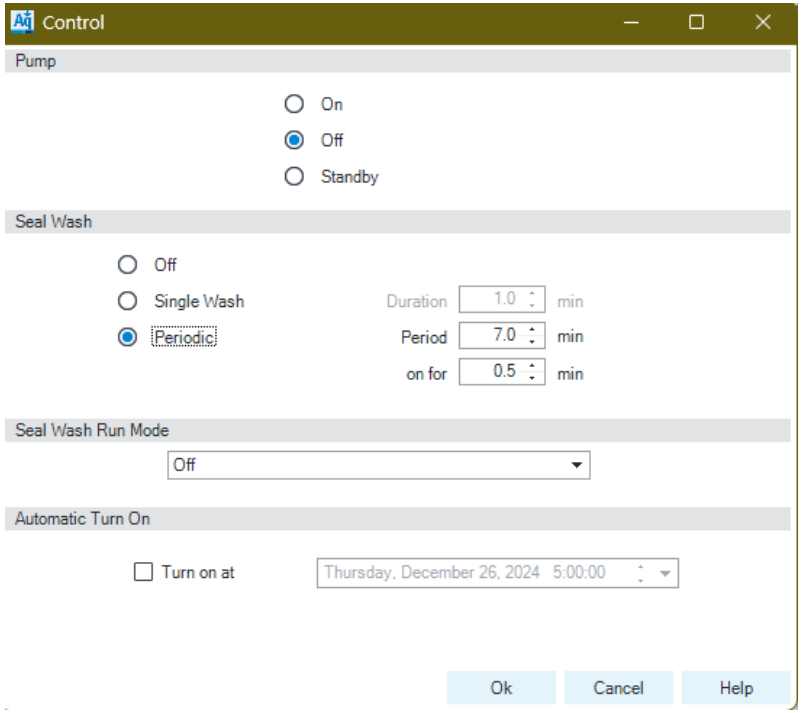
**Caution:**

- Do not recycle seal wash solvent (damage to piston seals).
- Exchange seal wash solvent weekly.
- Typical solvent consumption: 0.5 L per week.
- Use of Seal Wash Bottle Head Kit (5067-6131) is strongly recommended.

5067-6131

# Seal Wash

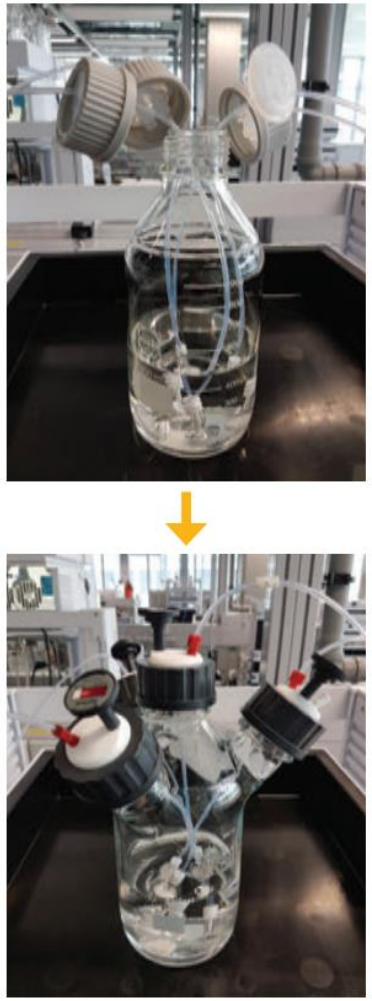



**Seal Wash operation:**

- In the Pump Control menu option.
- Recommended interval is 30 seconds on, every 7 min.
- Typical solvent usage is about 0.5 L per week.
- Use 10% isopropanol in water.
- Position wash solvent bottle above and waste bottle below instrument.
- Do not refill the seal wash solvent, always use properly cleaned, fresh bottles.
- Do not recycle seal wash solvent bottles.

# InfinityLab Stay Safe Purging Bottle



Bottle 5043-1339
Or kit with caps 5043-1340

| | Traditional purging process | Purging with InfinityLab Stay Safe purging bottle |
|---|---|---|
| Avoid harmful solvent vapor | Solvent vapors can leach into laboratory air because the solvent bottle is either open or just covered with parafilm or aluminum foil. | No leaching of harmful solvent vapors into the air.<br>Closing caps seal off mobile phase bottles during purging. |
| Ease-of-handling | All solvent channels with bulky solvent filters run through the narrow GL45 bottle opening. Solvent filters can fall off when pulling the solvent tubing out of the bottle. | Solvent lines can be easily put into or pulled out of the bottle, with no risk of losing solvent filters. |
| Prevent air in LC lines | Solvent lines and bottle caps hang loosely. Filters can slide out of the solvent leading to suction of air into the LC. | Solvent lines are securely attached, preventing suction of air into the LC. |

# Flushing

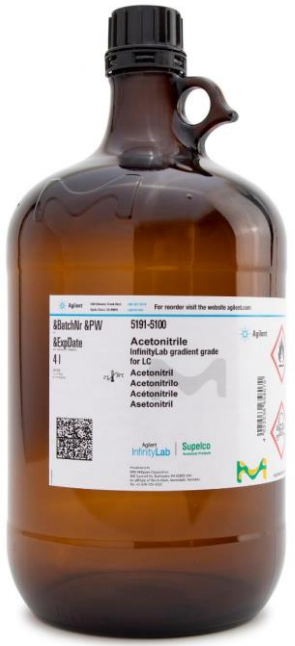
When using salt-containing mobile phases:

- Perform at least once a week, or prior a long standby or off time.
- When switching from salt-containing mobile phase to reversed phase or high organic.
- Flush the column with recommended storage solvent.
- Replace the column with a union.
- Replace the salt-containing solvent bottle with a new bottle of HPLC-grade water.
- Clean the bottle head assembly using lint-free wipes.
- Purge each pump channel for at least 10 min at 5 mL/min.
- Flush the entire system flow path with water for at least 10 min at 2 mL/min.
- During this step, switch the injection valve and the column selection valve (if installed) position every 1 min, and repeat this until every position has been selected for at least 5 times.
- Replace water with fresh solvent bottles to minimize salt carryover.

# Working with Acetonitrile

Acetonitrile (ACN) is a solvent that is frequently used in reversed-phase chromatography but can be a source of issues if not handled correctly. As acetonitrile ages/polymerizes, residues can get stuck on internal pump surfaces causing issues with valve performance (affecting retention time precision).
When using acetonitrile:

- Use high-quality solvents
- Use fresh solvents and filter them (not older than 48 to 72 h).
- Filter ACN using a 0.45 or 0.2 µm nylon filter depending on initial solvent quality (unless you use High Sensitivity LC/MS).
- Minimize exposure to light and air/oxygen.
- Choose the appropriate bottle size (amber).
- Use the amount that will be needed, including in-between-run time, plus a 20 to 30% allowance
- Acids accelerate solvent aging (refresh solvents more frequently.)
- Pure acetonitrile ages faster.
- If the application allows, mix with 5 to 10% HPLC grade water.
- Do not leave an unused system in acetonitrile.
- At least once per month, perform a warm water (60 to 70 °C, HPLC grade) flush at 2 mL/min for 20 to 30 min, to get rid of ACN reaction products.




LC-MS: 5191-5101
LC-UV: 5191-5100

Agilent InfinityLab solvents guide: 5994-6607EN

# Normal Phase and GPC

- Normal phase applications on Agilent Infinity pumps require yellow PE piston seals.
- By default, 1290 Infinity, 1290 Infinity II/III, and 1260 Infinity II/III Flexible Pumps use ceramic pistons and yellow PE seals.
- But 1260 Infinity I/II/III pumps use sapphire pistons and black PTFE piston seals by default.
- Black PTFE seals with normal phase applications will wear faster and generate small particles.
- For normal phase applications (hexane/heptane) on 1260 Infinity I/II/III pumps, use yellow PE seals.
- GPC applications using THF should use the black reversed phase seals and standard inlet and outlet valves.

**Recommended valves for normal phase applications (type N)**

| | Binary or High-Speed Pumps (G4220A/B, G7120A) | Quaternary or Flexible Pumps (G4204A, G7104A/C) | 1100/1200/1260 (400 and 600 bar) |
|---|---|---|---|
| Inlet Valve | G4220-60122 | G4204-60122 | G1312-60166 |
| Outlet Valve | G4220-60128 | G4220-60128 | G1312-60167 |

**Caution**:

- Normal phase valves corrode quickly in aqueous solutions and acids (at or below pH 7).
- Do not use normal phase valves in methods with aqueous mobile phases.

# Using Normal Phase and Unstable Solvents

- Whenever possible, use stabilizers, e.g. butylated hydroxytoluene (BHT) for ethyl ether and THF.
- Use isopropanol (IPA) to flush out the previous solvent when converting a system.  from normal phase to reverse phase, or vice versa.
- Store all unused channels in IPA.
- Don’t turn off the pump while it is filled with solvents with low boiling points.
- Leave a low flow throughout the used channels when the pump is not in use.


9301-6525

# Shutting Down the Pump

- Install a union or a restriction capillary and flush the system extensively with water, especially after using buffers.
- Flush with isopropanol if using normal phase solvents.
- Flush and store the system in 50% methanol or 50% isopropanol in water, without additives.

# Instrument Supplies Model Numbers

G#####A/B/C/D

# Pump Supplies

## Maintenance points on pumps

- PTFE frits that trap pump seal wear (semiannually)
- Pump seals (annually)
- Inlet valve
- Outlet valve

## Lack of maintenance can lead to:

- Pressure fluctuations
- Shifting retention time
- UV baseline problems

PTFE frits

Part number: 01018-22707

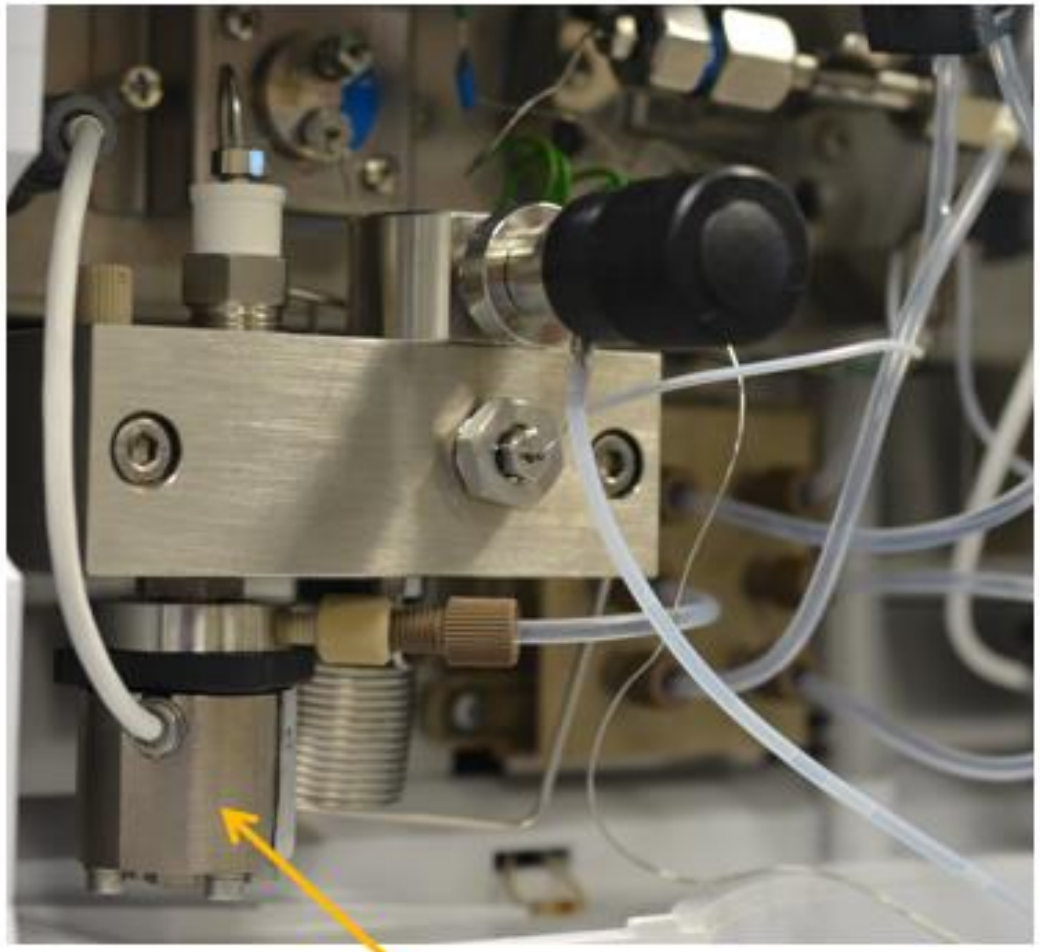
Outlet valve

Passive inlet valve: Better for retention time reproducibility

or

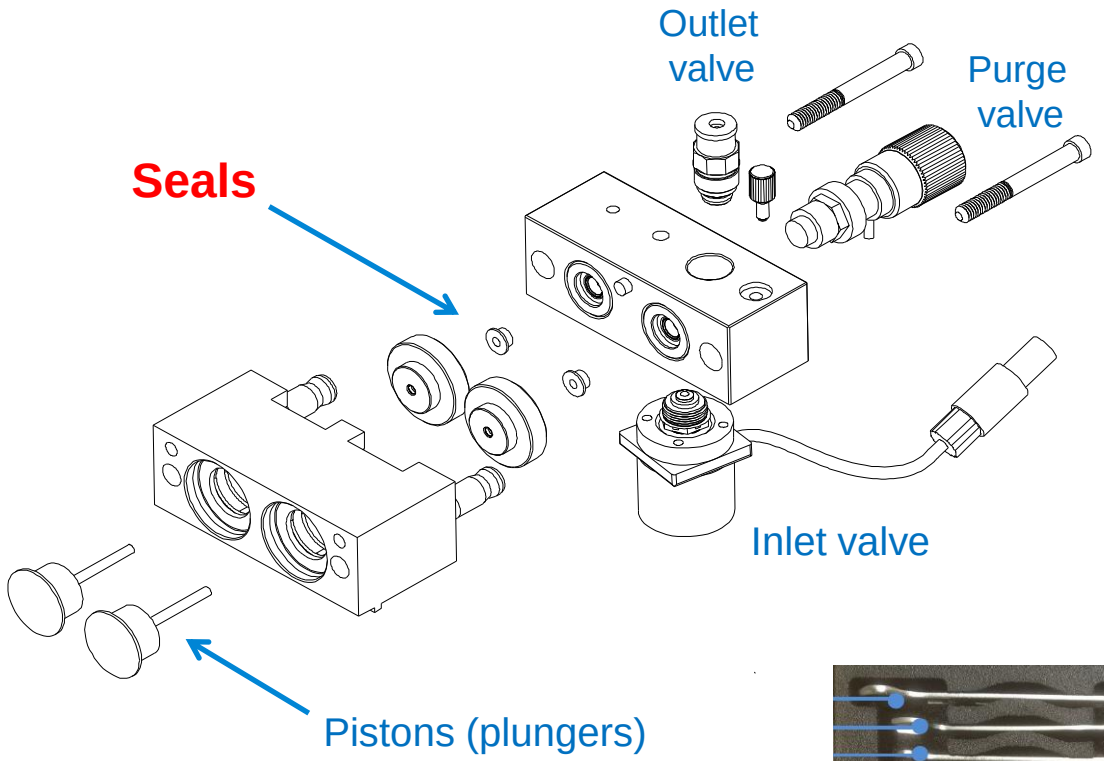
Active inlet valve: Better for robustness with buffered mobile phases

# Pump – Inlet Valve




14 mm wrench

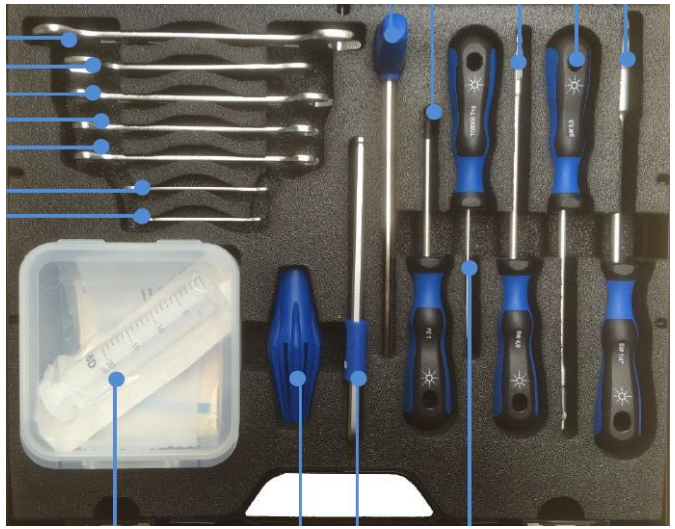
# Pump Head




5063-6589, PTFE (reversed-phase)

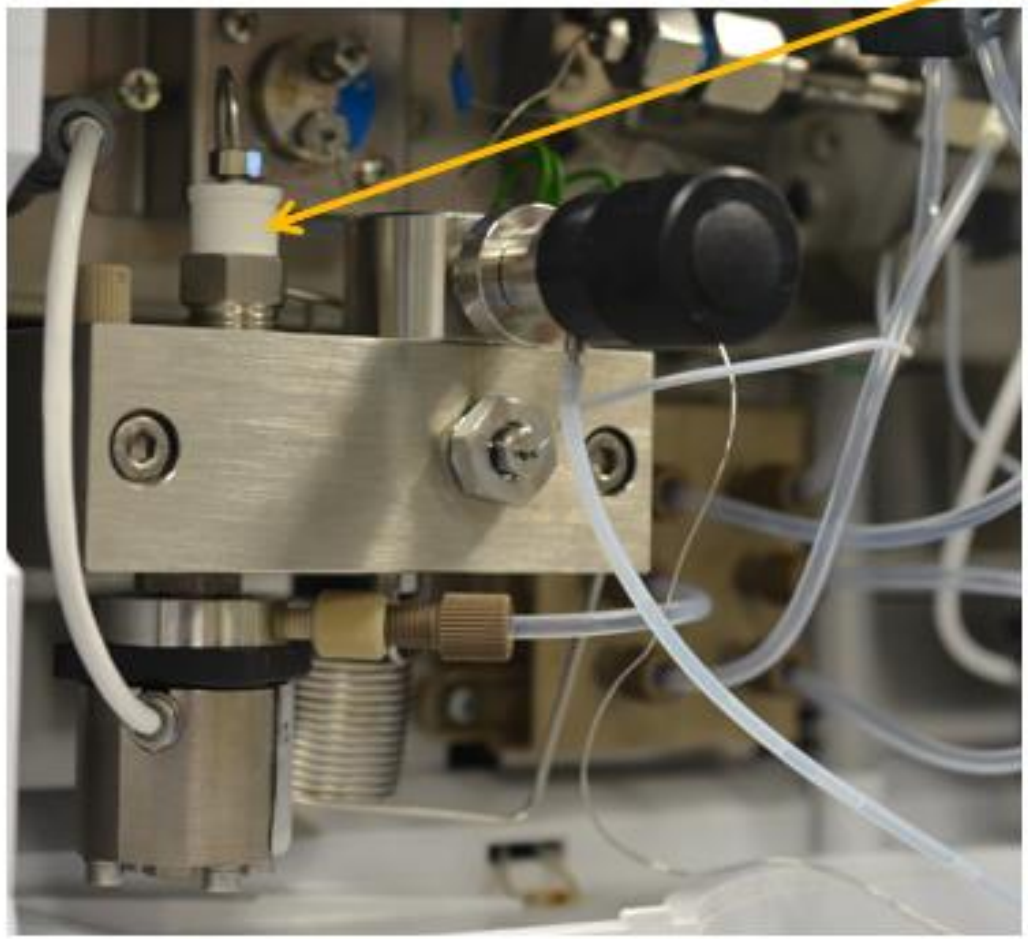
0905-1420, polyethylene (normal phase)

0905-1719, 1290/high speed/flexible pump



Tool kit
G7120-68708

# Pump – Outlet Valve



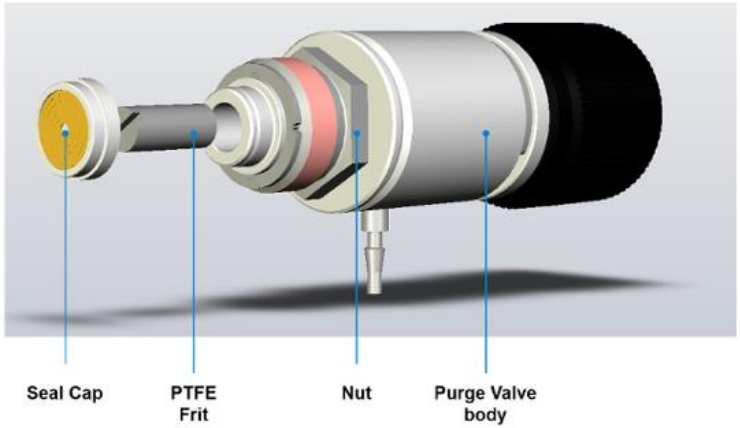
14 mm wrench

8710-1924

# Pump – Purge Valve

14 mm wrench

- A dirty frit is a source of high pressure
- A pressure drop of >10 bar across the frit (5 mL/min water with the purge valve open) could indicate a blockage
- Change it after changing pump seals



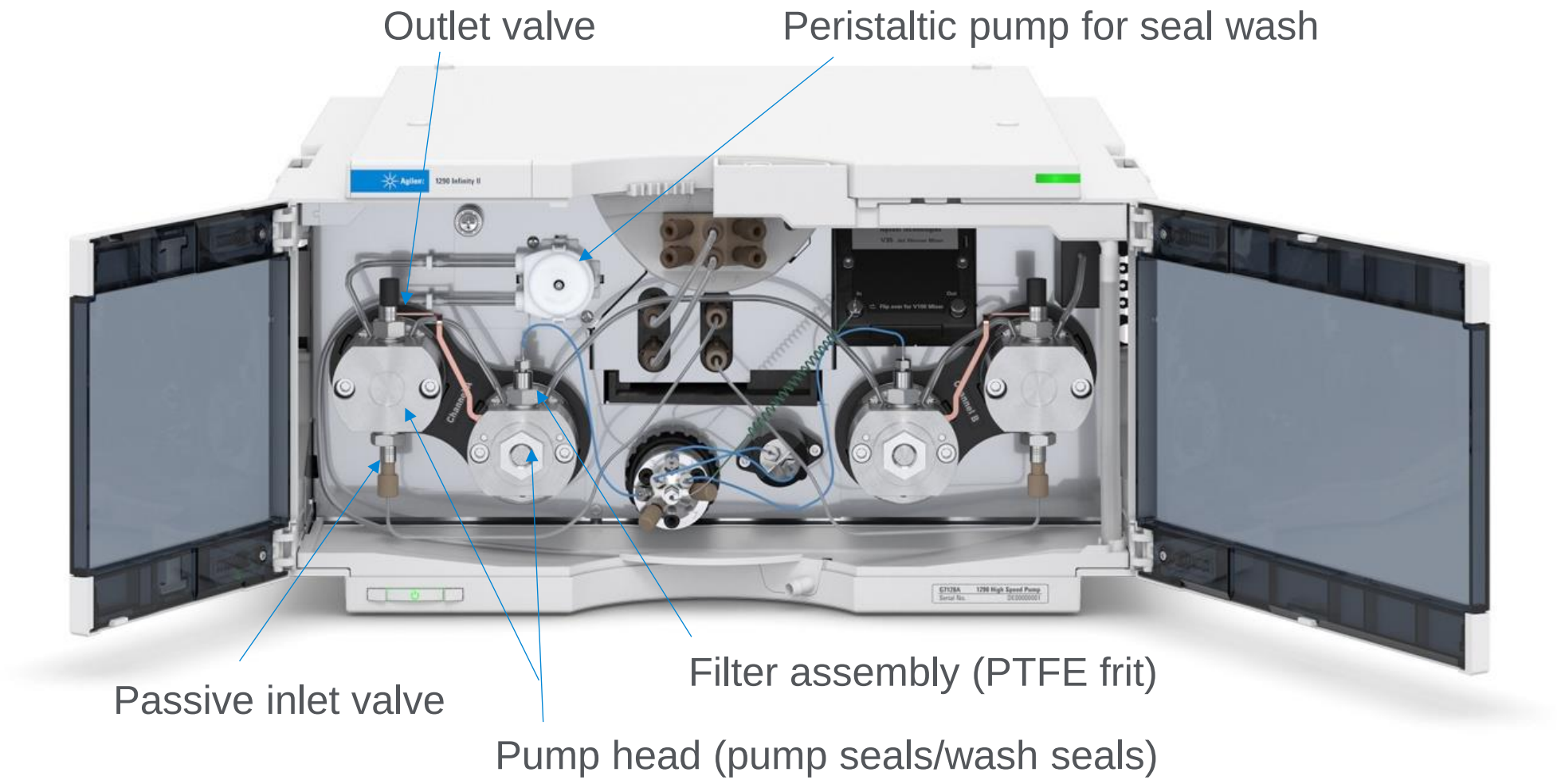

Seal cap

5067-4728

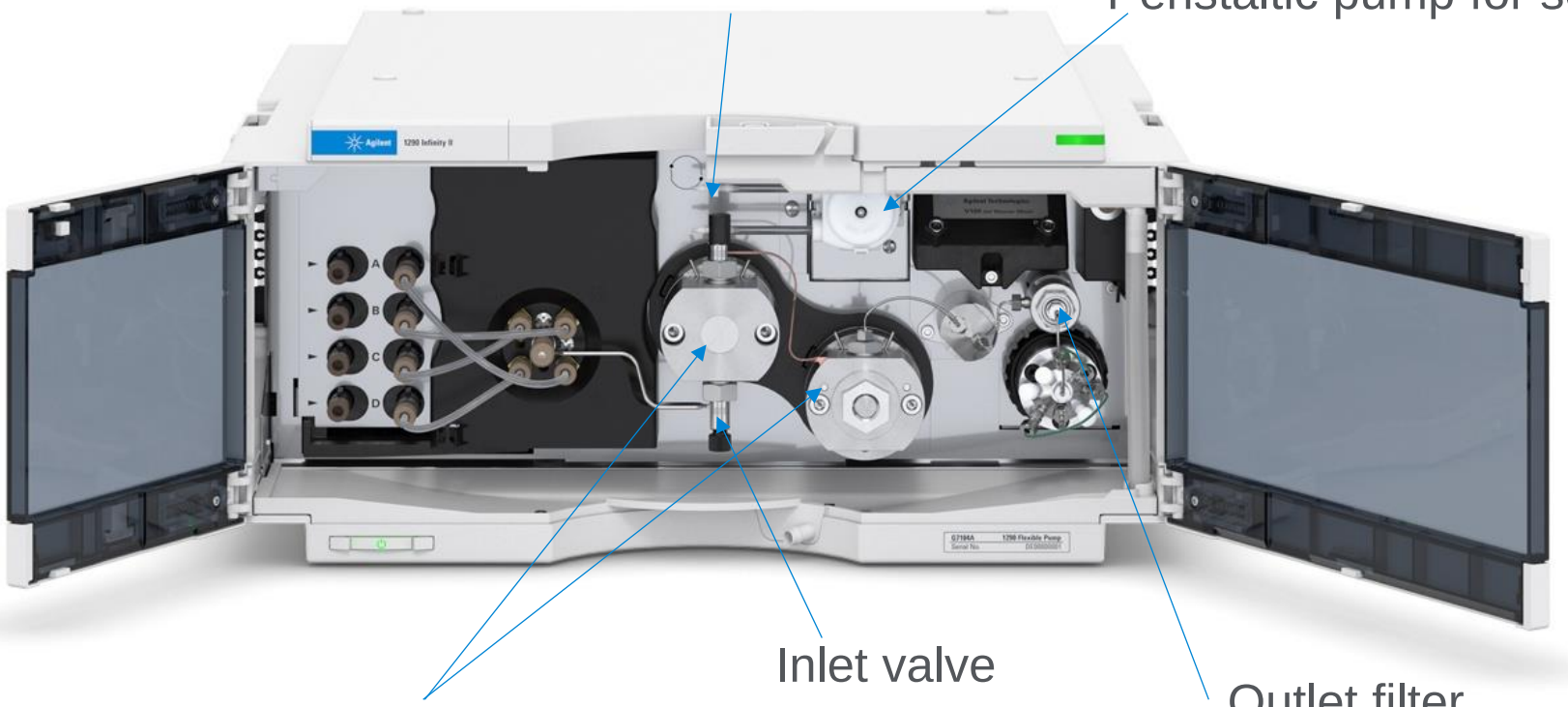
PTFE frits

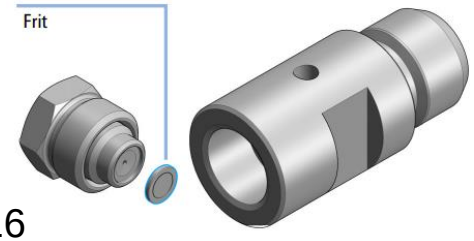
01018-22707

# 1290 Infinity II High Speed Pump



# 1290 Infinity II Flexible Pump



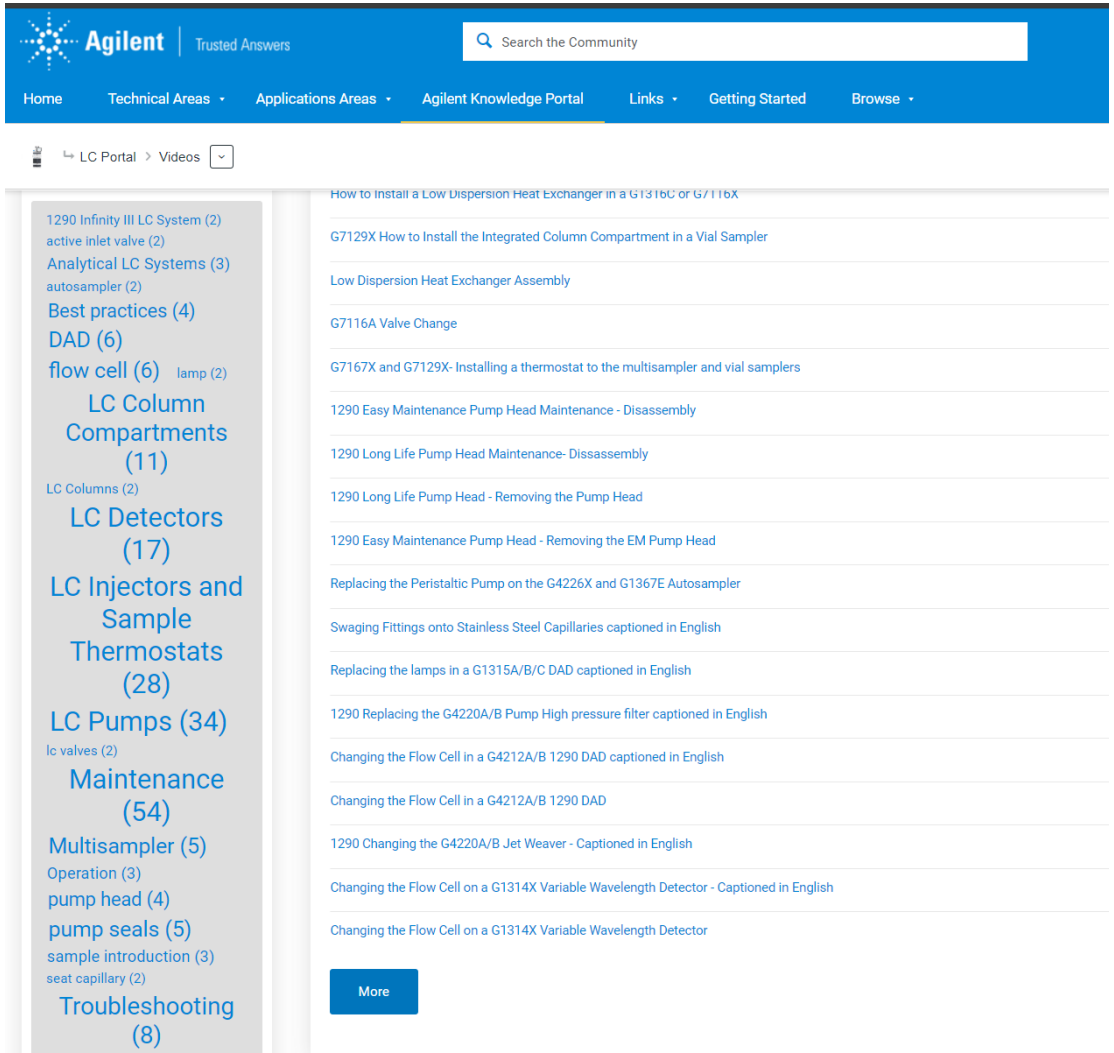



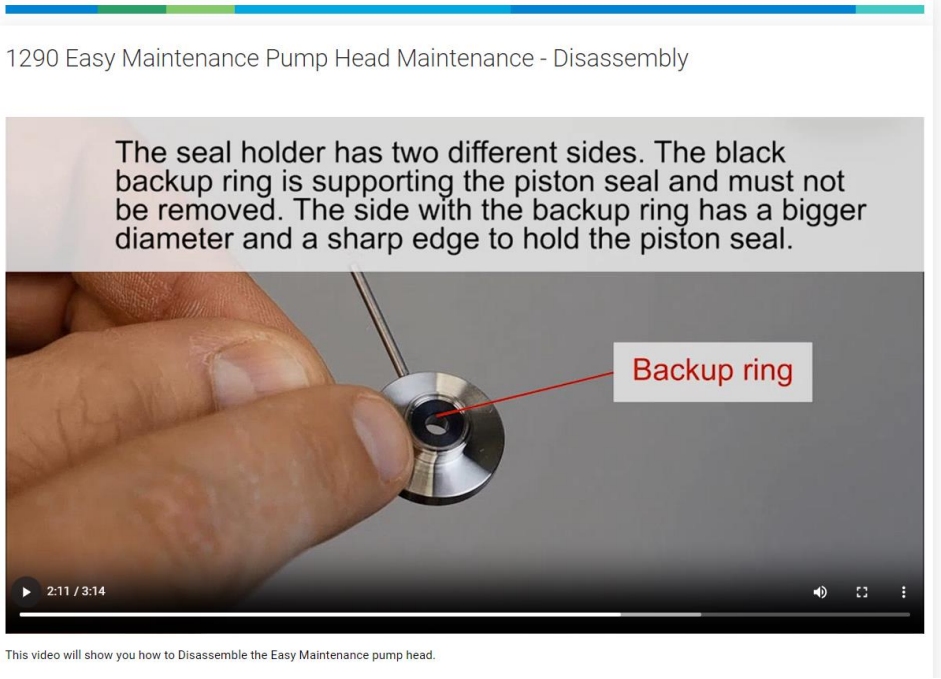

5067-5716

# Maintenance Videos

Agilent InfinityLab

## Community.Agilent.com

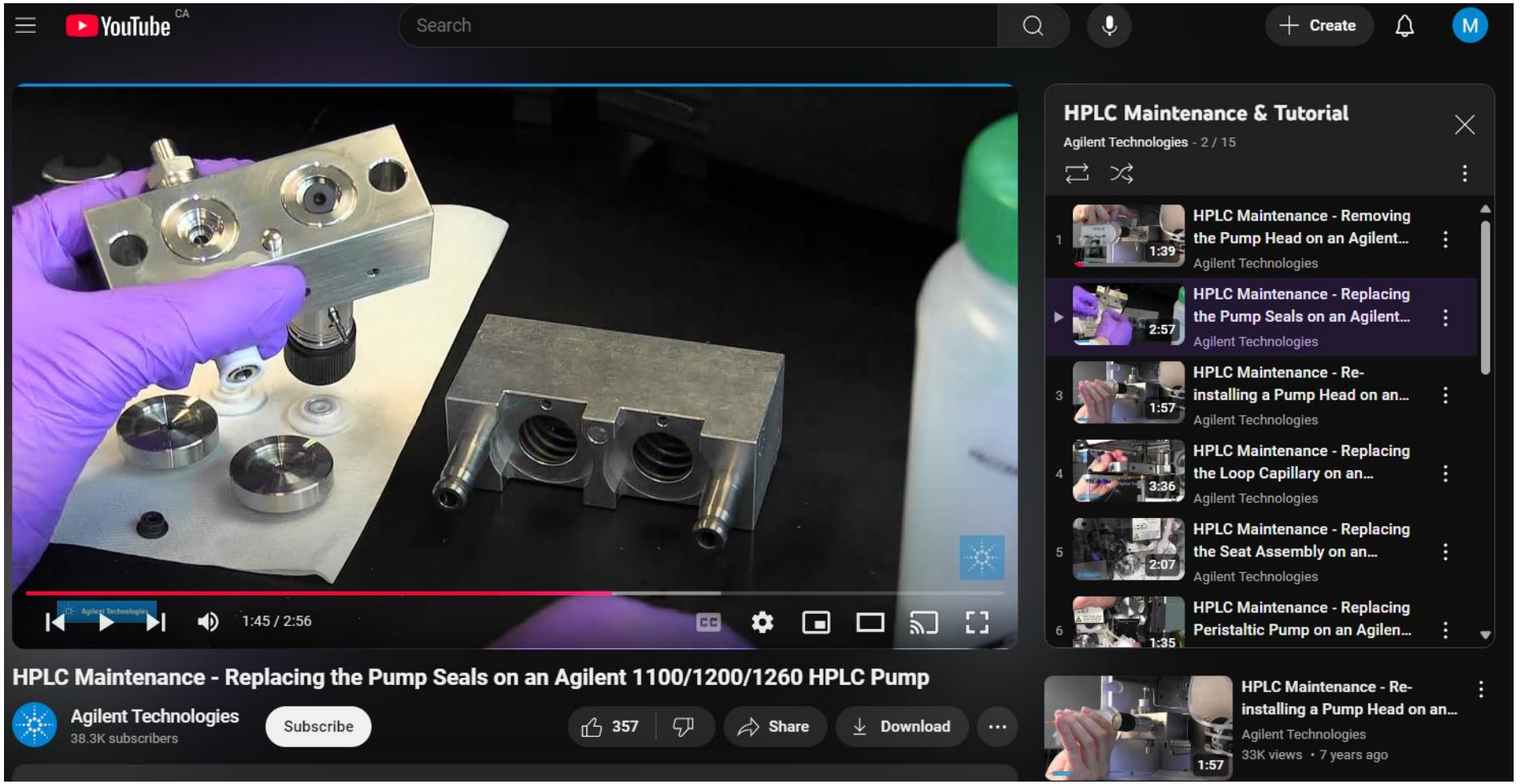


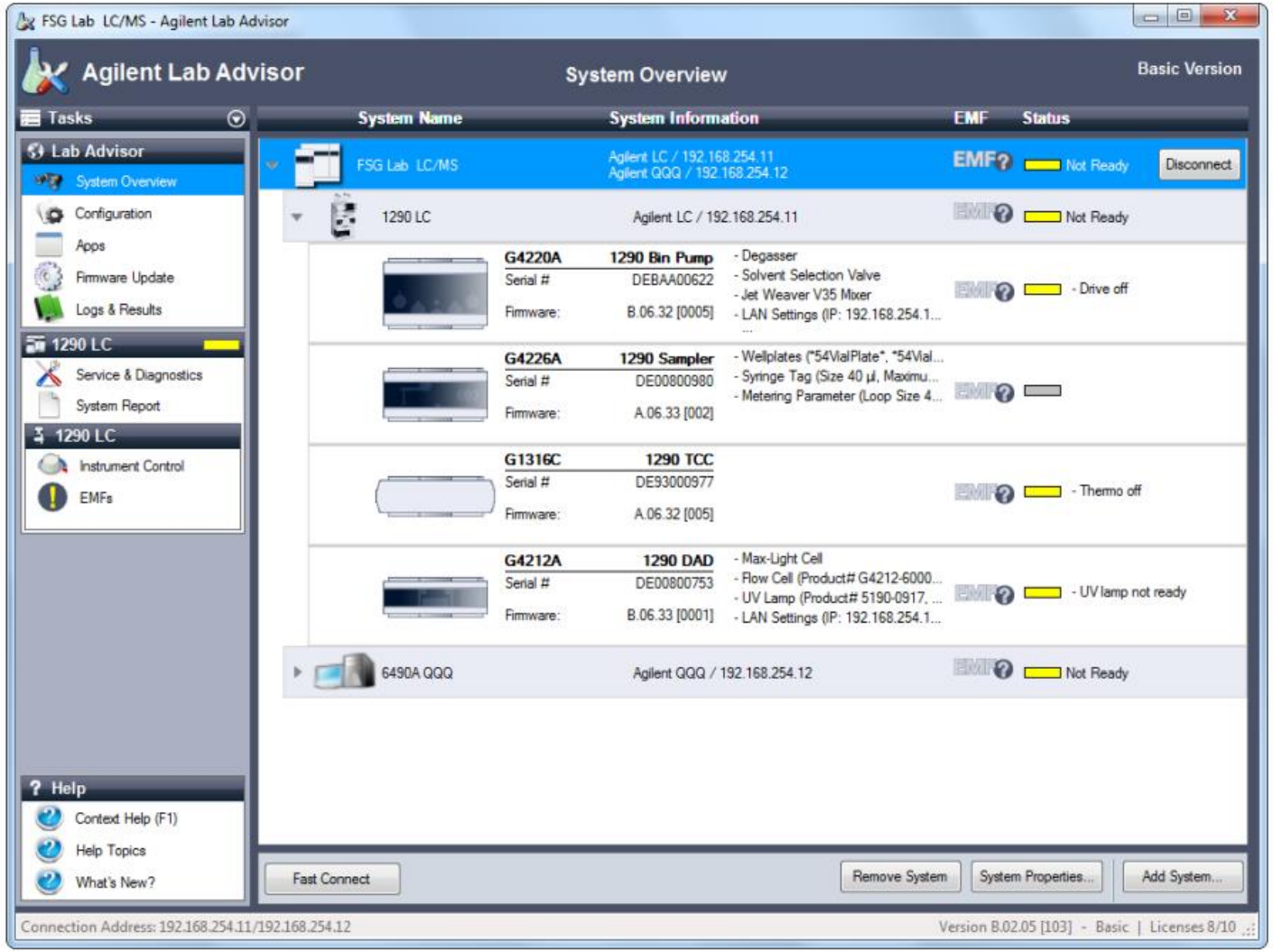
# Maintenance Videos

Agilent InfinityLab

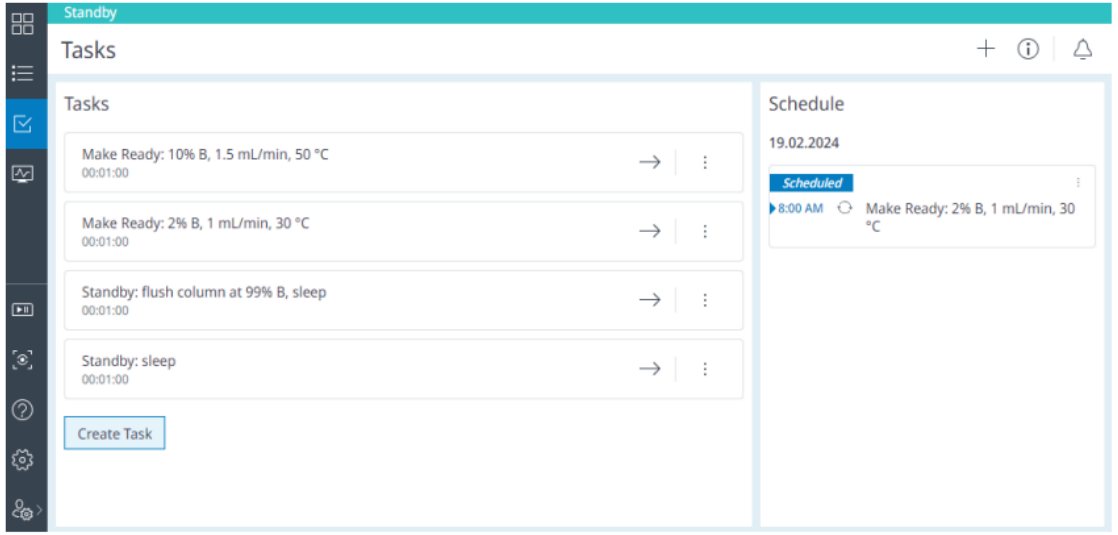
## Agilent YouTube Channel

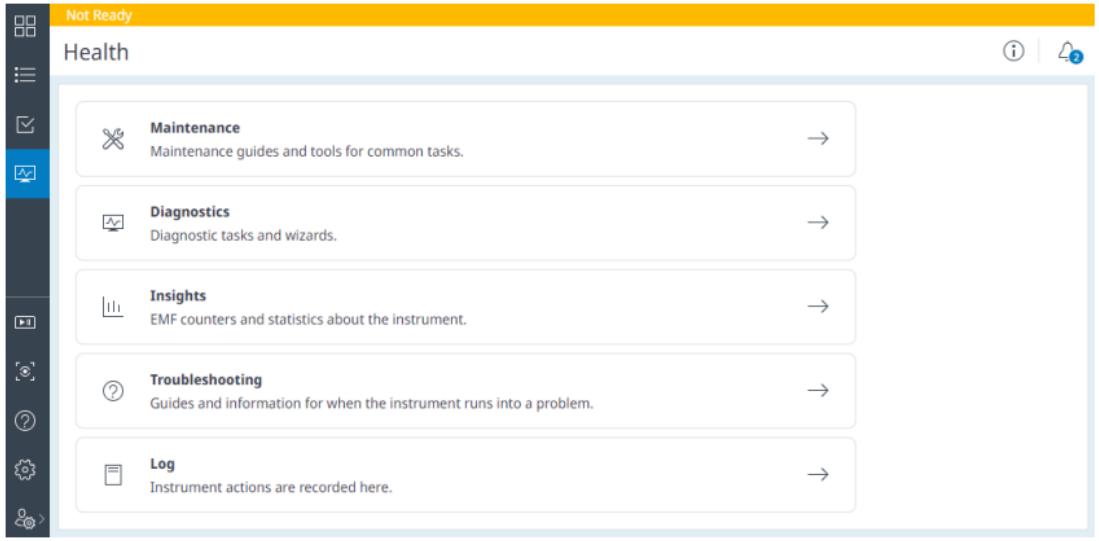


# Lab Advisor

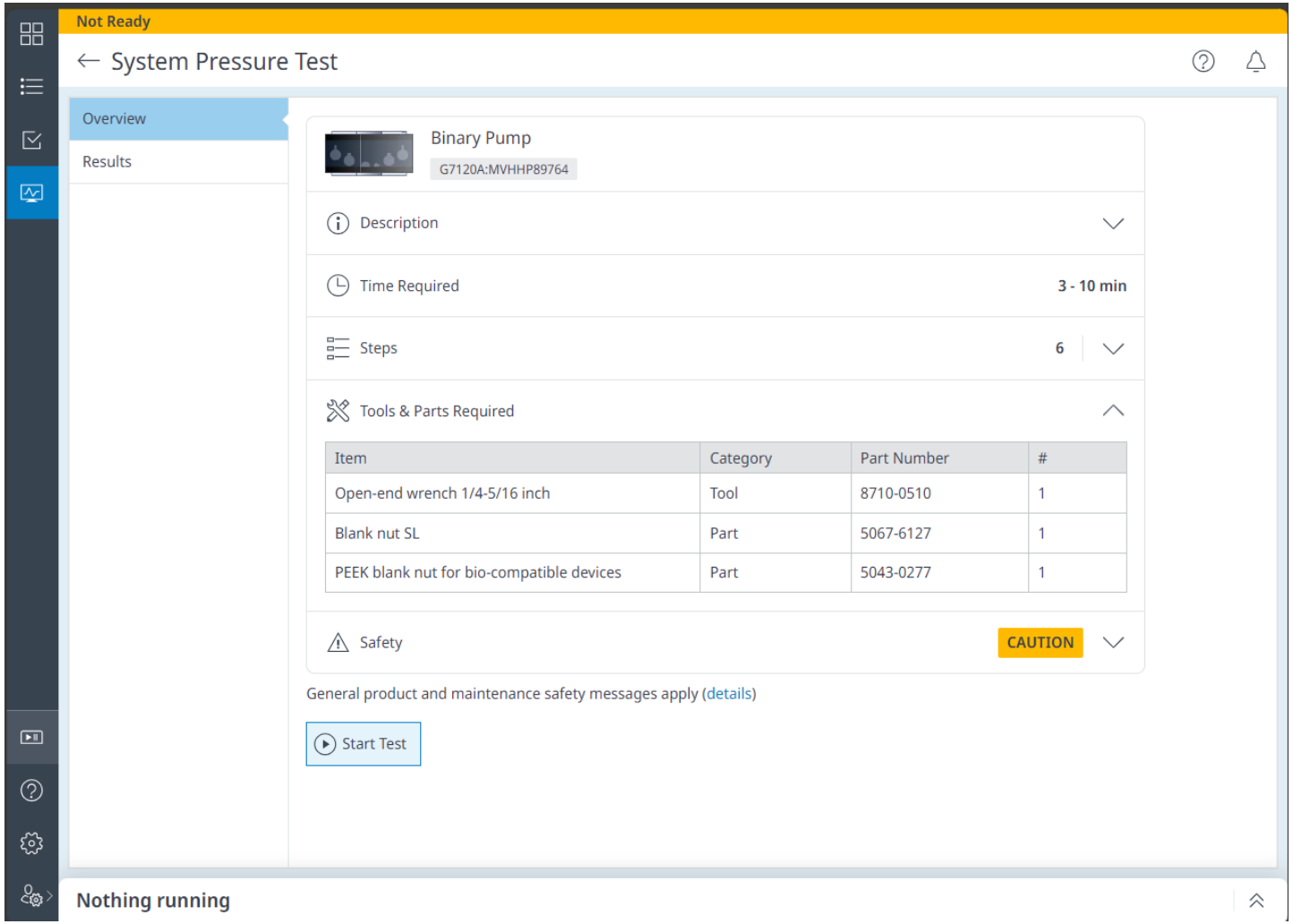

FSG Lab LC/MS - Agilent Lab Advisor
Agilent Lab Advisor
System Overview
Basic Version
Tasks
Lab Advisor
System Overview
Configuration
Apps
Firmware Update
Logs & Results
1290 LC
Service & Diagnostics
System Report
1290 LC
Instrument Control
EMFs
Help
Context Help (F1)
Help Topics
What's New?
System Name
System Information
EMF
Status
FSG Lab LC/MS
Agilent LC / 192.168.254.11
Agilent QQQ / 192.168.254.12
Not Ready
Disconnect
1290 LC
Agilent LC / 192.168.254.11
Not Ready
G4220A
1290 Bin Pump
Serial #
DEBAA00622
Firmware:
B.06.32 [0005]
- Degasser
- Solvent Selection Valve
- Jet Weaver V35 Mixer
- LAN Settings (IP: 192.168.254.1...
- Drive off
G4226A
1290 Sampler
Serial #
DE00800980
Firmware:
A.06.33 [002]
- Wellplates ("54VialPlate", "54Vial...
- Syringe Tag (Size 40 µl, Maximu...
- Metering Parameter (Loop Size 4...
G1316C
1290 TCC
Serial #
DE93000977
Firmware:
A.06.32 [005]
- Thermo off
G4212A
1290 DAD
Serial #
DE00800753
Firmware:
B.06.33 [0001]
- Max-Light Cell
- Flow Cell (Product# G4212-6000...
- UV Lamp (Product# 5190-0917, ...
- LAN Settings (IP: 192.168.254.1...
- UV lamp not ready
6490A QQQ
Agilent QQQ / 192.168.254.12
Not Ready
Fast Connect
Remove System
System Properties...
Add System...
Connection Address: 192.168.254.11/192.168.254.12
Version B.02.05 [103] - Basic | Licenses 8/10

# InfinityLab Assist

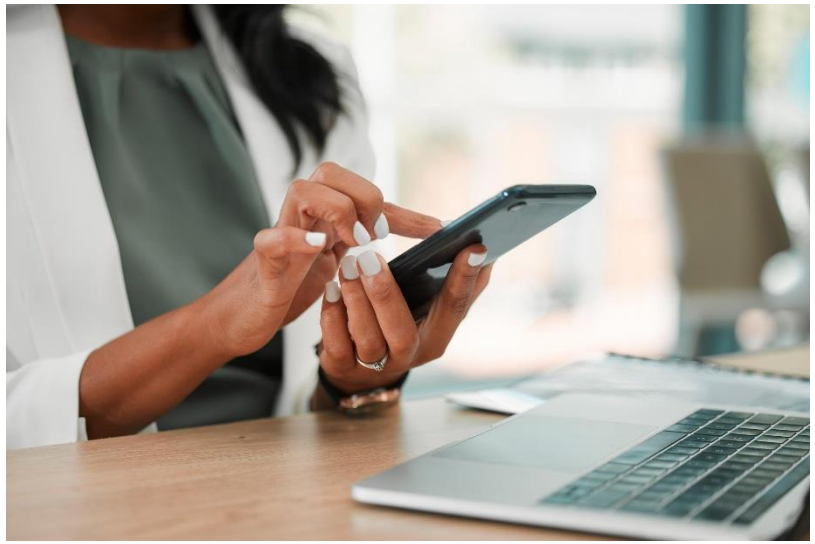


# InfinityLab Assist



# Contact Agilent Chemistries and Supplies Technical Support



Available in the U.S. and Canada

1-800-227-9770 option 3, option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

gc-column-support@agilent.com

lc-column-support@agilent.com

spp-support@agilent.com

spectro-supplies-support@agilent.com

chem-standards-support@agilent.com

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