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# LC connections guide

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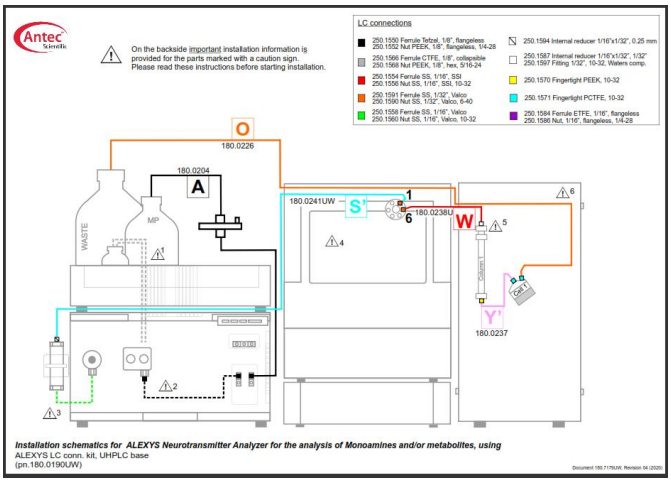

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# Symbols

Explanations of symbols & labels on the device or in user manual:

The following symbols are used in this guide:


The danger sign warns about a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in injury or loss of life.
Do not proceed beyond a danger sign until the indicated conditions are fully understood and met.


The warning sign denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in severe injury or damage or destruction of parts or all of the equipment. Do not proceed beyond a warning sign until the indicated conditions are fully understood and met.


The caution sign denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in damage or destruction of parts or all of the equipment. Do not proceed beyond a cautions sign until the indicated conditions are fully understood and met.


The biohazard sign draws attention to the fact that use of biological materials, viral samples and needles may carry a significant health risk.

The attention sign signals relevant information. Read this information, as it might be helpful.

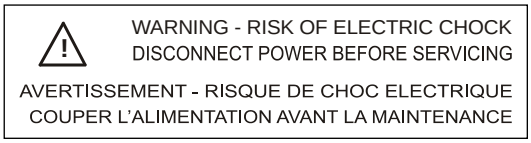
The note sign signals additional information. It provides advice or a suggestion that may support you in using the equipment.

# Safety practices

The ALEXYS installation may only be performed by a qualified service engineer trained by the manufacturer. The following safety practices and protective measures are intended to ensure safe operation of the instrument.


## Electrical hazards

Never open a device when it is connected to an electrical power source! Removal of protective panels on the instrument can result in exposure to potentially dangerous voltages which may lead to **severe injury or loss of life**! The instrument may only be opened by authorized service engineers of the manufacturer or a company authorized by the manufacturer.





## Solvents

Organic solvents are highly flammable. Since capillaries can detach from their screw fittings and allow solvent to escape, it is prohibited to have any open flames near the analytical system.

If a leakage occurs, turn off the power of the instrument and remedy the situation immediately. Regularly check for leaks and clogged LC tubing and connections. Do not close or block drains or outlets. Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of such products through the municipal sewage system.


## Toxicity

Organic solvents are toxic above a certain concentration. Ensure that work areas are always well-ventilated! Wear protective gloves, safety glasses and other relevant protective clothing when working on the device!
Use proper eye and skin protection when working with solvents. Additional safety requirements or protection may be necessary depending on the chemicals used in combination with this equipment. Make sure that you understand the hazards associated with the chemicals used and take appropriate measures with regards to safety and protection.

C H A P T E R 1

# Introduction

This document explains how to make the different LC connections throughout the ALEXYS system and describes some additional procedures that are system-specific.

**Passivation (for stainless steel HPLC system)**

Before completing the installation, **passivation of all metal parts** in the stainless steel HPLC system (with 15% $HNO_3$) is strongly advised, especially when the system will be used for trace analysis. The passivation procedure is explained in the document pn. 180.7070C 'General requirements for installation of ALEXYS systems'.


**Do not passivate metal-free or bioinert LC systems with nitric acid solutions.**

## Preparation

Before installing the LC flow path, it is assumed that:

- Equipment is unpacked and checklists are verified.
- The document 'ALEXYS installation checklist' (pn. 180.0011W) is being used as the master document during installation, and installation sections in the different manuals are noted for details.
- The content of this manual is read and understood before installation of the LC flow path.

## Unpacking

Inspect the *transport box* for possible damage as it arrives. Immediately inform the transport company in case of damage, otherwise they may not accept any responsibility. Inspect the box for completeness and contact your supplier if not all marked items on the checklist are included and in case of damage.

# Tools

The following tools are/may be required for the installation of the LC connection kit.

- 3/16", 5/16" and/or 1/4" wrench
- Tubing cutter for 1/16” and 1/8” OD PEEK/FEP tubing (Figure 2)
- Tightening tool for 1/16” OD hex-head fittings (Figure 3)

*Figure 1. 3/16", 5/16" and 1/4" wrench. Note: the larger size wrenches are part of the tools provided in the pump ship kit, and the 3/16” wrench is provided in combination with UHPLC-style LC connections kits.*

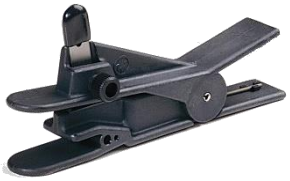


*Figure 2. PTFE tubing cutter (pn. 250.1020). Note: this item is supplied with a few specific LC connection kits where cutting of tubing is required.*

*Figure 3. Tightening tool for PEEK hex-head 1/16" nuts (pn. 250.0094). Note: this item is supplied with a few specific LC connection kits where it is relevant.*

CHAPTER 2

# The LC connections kit

Every ALEXYS LC-ECD system is delivered with a dedicated LC connections kit consisting of this document, a schematic drawing, and a specific set of tools, tubing and connectors.

This document explains how to make the different connections in an ALEXYS® LC-EC system, and the specific schematic installation drawing shows where each part should be installed.

A tubing cutter is provided with kits where cutting of some tubing to a specific length is considered to be part of the installation.

### Details of the schematic LC installation drawing

Each LC connection kit contains a laminated schematic installation drawing showing all LC connections in the ALEXYS analyzer. This is an example of such a drawing:

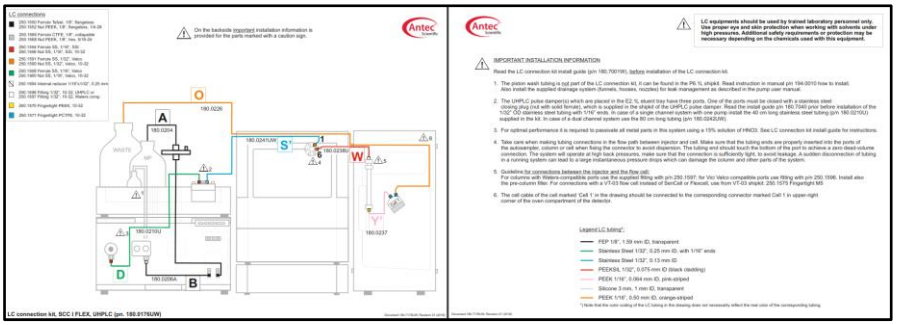


*Figure 4. Example of LC connections drawing, front side (l) and backside (r).*

The following information is shown on the LC connections drawing:

- A caution sign in combination with an identification number refers to important installation information provided on the back side of the schematic drawing.
- The frontside contains the legend identifying the type of connector in the drawing (part number, shape, material, thread/size).
- The backside contains the legend identifying the type of tubing in the drawing (material, OD, ID and appearance).
- The part number of a tubing assembly (where applicable).
- A letter, which corresponds to a vinyl label attached to a specific piece of tubing for identification.

C H A P T E R 3

# Making tubing connections

## General

Tubing connections are a crucial part of an LC system, and influence the performance. This is especially true for the connections in the analytical flow path (between injector, column, and flow cell).

A good order how to build up an LC system is to start with priming the inlet filter and connect the parts in the direction of the flow.

Take into account the following precautions:


- Do not install the column until the lines are purged with the appropriate solvent to prevent pumping air through the column.
- Use the correct connector to attach tubing to the different port types. (Check the schematic installation drawing).
- Make sure that the tubing is fixed correctly into the port and avoid making a dead volume or a leak at the connection (Figure 5).
- When cutting tubing always use the supplied tubing cutter (p/n 250.1020) to assure straight and neat tubing cuts.
- Keep tubing length between injector-to-column and column-to-cell to a minimum (low dead volume).
- PEEKSil tubing should never be cut; it should be used in its precut length because permanent damage will be caused by conventional cutters.

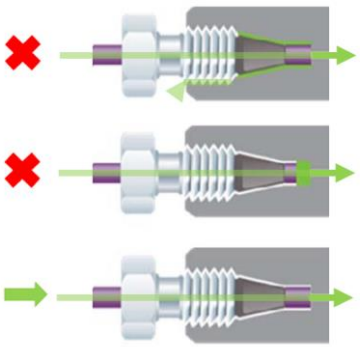


*Figure 5. If the capillary tubing is not well 'bottomed' into the receiving port, an unacceptable amount of dead volume may be added to the system (middle figure). Another type of wrong connection can result in leakage (top figure). The correct connection does not leak and does not add dead volume to the flow path (bottom figure).*

# Connecting the pump piston backwash lines

The pump is equipped with an automatic piston wash. Two pieces of tubing must be installed between the pump head and a bottle with wash solution to recirculate the wash solution.
The typically recommended piston backwash solution a mixture of 80/20 v/v% water/isopropanol. In case that no organics are present in the mobile phase, like in HPAE-PAD applications, use pure HPLC-grade water as an alternative wash liquid.

**Instructions for connecting the piston backwash lines**

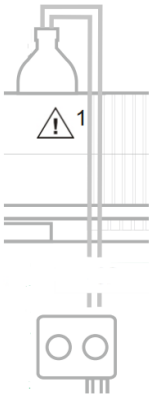



*Figure 6. Piston backwash tubing as pictured in the LC connections schematics.*

- The bottle kit contains a 100 mL glass bottle for the wash solution and a pierced cap (4 holes). Fill the bottle with piston backwash solution.
- The pump ship kit contains silicone tubing (3 mm OD; 1 mm ID) that should be cut in two pieces with an adequate length. It is recommended to make a V-shaped cut to one end of silicone tubing, and keep the other end straight.
- Connect the straight end of one tubing to the inlet point of the piston wash (designated with ▲) and insert the V-shaped end into the bottle with piston backwash solution.
- Connect the straight end of the second piece of tubing to the open end on top of the pump head and attach a syringe to the other tubing end.
- Fill the piston wash tubing with wash solution by drawing some liquid.
- Disconnect the syringe and insert the open V-shaped end of the tubing in the bottle with wash solvent.

# Priming the inlet tubing (with Whatman in-line filter disk)

Applies to the mobile phase inlet tubing inserted with one end into the mobile phase bottle:

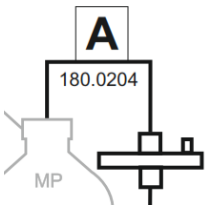



*Figure 7. Tubing inlet assembly with Whatman in-line filter disk as pictured in the LC connections schematics.*

**Instructions**

- Pre-wetting: open the vent of the Whatman in-line filter and insert a few drops of ethanol until the filter is completely wetted.
- Insert the tip of a syringe (part of the pump ship kit) into the filter vent opening.
- Place the inlet of the FEP tubing in a bottle with water (with resistivity >18MOhm.cm, and TOC<5ppb).
- Close the outlet of the tubing with the tip of a gloved finger while drawing the water into the Whatman filter. Keep drawing until the inlet compartment of the filter is flushed and air-free.
- Remove the syringe, close the vent and prevent the solution from running back into the bottle (by keeping the filter below the solutions level in the connected bottle while removing the syringe).
- Let gravity spontaneously siphon the solution through the filter until it fills up the back part of the Whatman filter and the line is completely flushed/air-free. If the solution is not siphoning spontaneously, consult the troubleshooting section in Appendix I.
- Connect the line to the inlet port of the degasser (details on next page).

## Connecting the low pressure 1/8” OD lines

Applies to all the tubing connections at the degasser and the pump inlet:

**Instructions for connecting the low pressure LC lines**

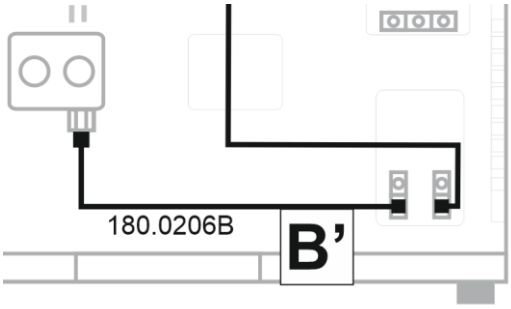



*Figure 8. Connections at the degasser and pump inlet as pictured in the LC connections schematics. Note: on a P6.1L pump with integrated degasser, the assembly 180.0206B is already preinstalled.*

- Visually confirm a straight cut of the 1/8” OD FEP tubing end.
- Slide a nut and a ferrule in the correct direction onto the tube end (as in Figure 9).

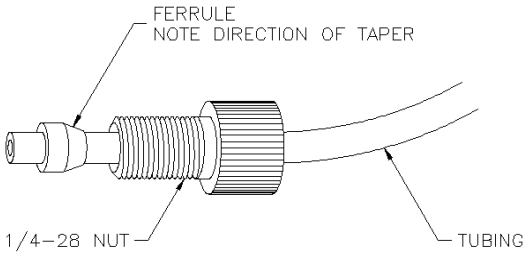



*Figure 9. Assembly of ¼-28 nut, ferrule and tubing for connection to degasser ports.*

- Tighten the nut firmly with fingers.

- If air bubbles are introduced at the degasser port during pump purging, then the nuts at the degasser should be tightened a bit more.

## Arrangement of all the LC lines that are connected to the pump

- There are tubing guides behind the front panel (pull off) of both solvent tray and the pump (Figure 10).
- Make sure the Whatman in-line filter hangs above the solvent tray, so in case of leakage, no solution will drip on any electronics

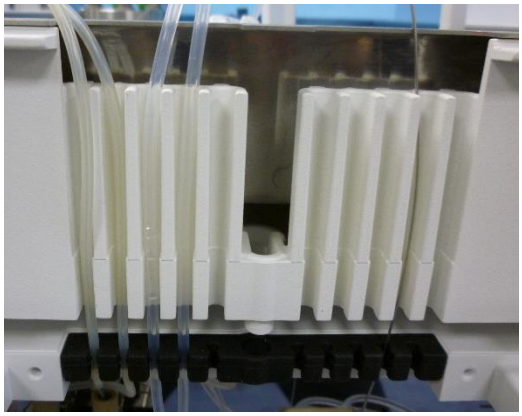


*Figure 10. The tubing that is connected to the pump can be neatly organised behind the front panels of the pump and solvent tray.*

For more detailed information about the pump see the pump user manual.

## Connecting the PEEK high pressure 1/16” OD lines

Applies to the tubing connections between the following parts in a regular HPLC system (PEEK tubing) and after the column in UHPLC systems:

1. Pump purge valve → pulse damper
2. Pulse damper → injector
3. Injector → column
4. Column → flow cell

- Double check the schematic drawing to identify which connector and tubing have to be used as different combinations are needed.
- To access the valve of the AS110 autosampler more easily, the autosampler top cover can be temporarily slid backwards
  Make sure to turn off the power first and disconnect the power cord from the instrument! Then unscrew the two retaining screws on both front sides (see also autosampler manual).
- If the system needs to be passivated, do not connect the column yet, but direct the tubing from the injector into a waste bottle and connect the column only after flushing the lines with water and the appropriate solvent that is compatible with the column (see column instruction manual).

### Instructions for connecting PEEK LC lines with metal nuts and ferrules

Be aware that there are different shapes of nuts and ferrules (SSI, Valco), which are not interchangeable. Always use the correct nut and ferrule in the designated inlet/outlet port.

- Slide a nut and a ferrule in the correct direction onto the tube end (as in Figure 11).

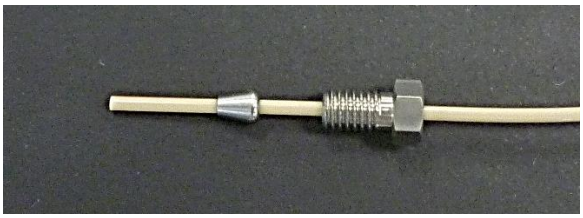


*Figure 11. Assembly of stainless-steel nut and ferrule onto 1/16" OD PEEK tubing.*

- Insert the tip of the tubing into the receiving port.
- While holding the tubing in place, tighten the nut with a ¼ wrench.
- Disconnect the tubing and double check if the ferrule has swaged on the tubing a few mm away from the tip. Reconnect.

### Instructions for connecting PEEK LC lines with a PEEK connector

Be aware that there are different shapes of connectors (Figure 12). Always use the correct nut and ferrule in the designated inlet/outlet port.

- Slide the connector over the tube end (as in Figure 12).

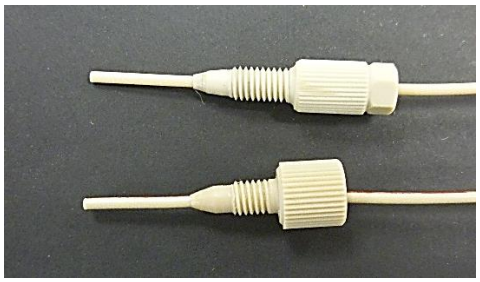


*Figure 12. Assembly of a bulkhead (bottom) and narrow (top) PEEK connector onto a 1/16" OD PEEK tubing.*

- Insert the tip of the tubing into the receiving port.
- While holding the tubing in place, tighten the nut well using fingers. The narrow connector with hex head should be tightened using the (blue) tightening tool for 1/16" OD PEEK tubing connectors.

### Instructions for connecting the PEEK lines to a flow cell

For detailed priming instructions and installation of a flow cell, refer to the flow cell user manual available on the supplied USB memory stick and the quick start guide that is provided in print with each flow cell ship kit (SenCell only).

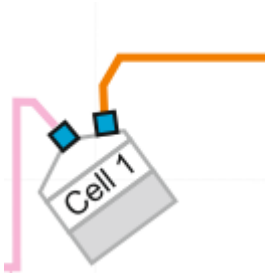



*Figure 13. Connections at the flow cell as pictured in the LC connections schematics.*

- Slide the connector over the tube end. Make sure to use only the 10-32 PCTFE connector provided with the flow cell ship kit. Do not use 10-32 fingertights made of PEEK or harder materials to prevent damage to the inlet/outlet ports of the cell.
- Insert the tip of the tubing into the receiving port.
- While holding the tubing in place, tighten the nut well using fingers.

- Lead the outlet tubing from the flow cell through the left upper hole in the side panel of the detector and through the autosampler (if present, see Figure 14) into the waste bottle.

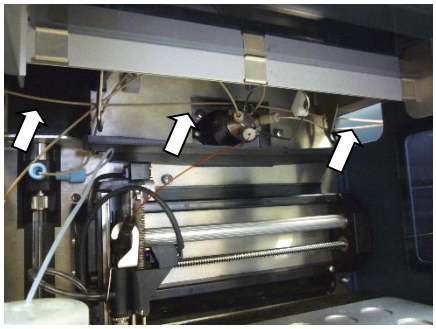


*Figure 14. Picture of the autosampler with the waste tubing arranged through the valve compartment towards the waste bottle in the solvent tray.*

- Make sure that the tubings inside the detector are not positioned in front of the fans of the heater: this might result in unwanted interferences and baseline noise.
- Fix or shorten the excess of tubing that goes to the waste bottle. If it is too long and ‘dangling’, it may pick-up on-air movements or other disturbances, thus resulting in excessive baseline noise.

- Insert the waste line deep enough into the waste bottle to prevent dripping (which can result in spikes on the baseline).

Under certain conditions it might be that, despite the abovementioned pre-cautions, unwanted interference cannot be completely eliminated. In such cases it may help to install a grounded metal union in the waste line. A dedicated grounding kit is available for this purpose (pn 250.0035D).

## Connecting stainless steel 1/16” OD UHPLC-compatible LC lines

Applies to the tubing connections between the following parts in UHPLC systems:

1. Pump purge valve → pulse damper

**Instructions for connecting the flexible 1/32” OD tubing with fused 1/16” end sleeves**

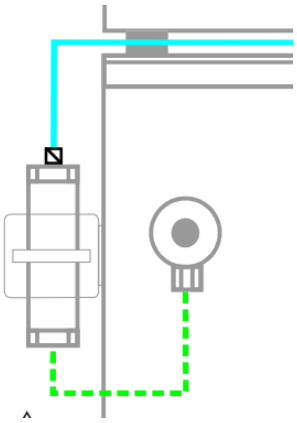


*Figure 15. Connections between the pump purge valve and pulse damper as pictured in the LC connections schematics for UHPLC systems.*


When these sleeved types of tubing are connected while bottomed well into the receiving port, they can become deformed and start leaking (Figure 16). The creation of a small dead volume in the connection prevents deformation during connection (Figure 17).


*Figure 16. Sleeved SS tubing leaks and shows deformation after tightening the nut with the tubing end bottomed into the receiving port*

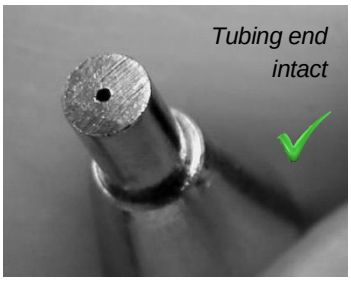
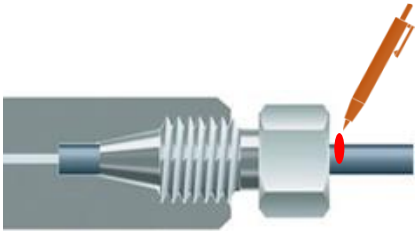



*Figure 17. Sleeved SS tubing does not leak after installation with the described procedure.*

- Mount the nut and ferrule on the tubing end and **bottom the tubing into the receiving port**.
- Tighten the nut with fingers (the tubing should still be able to move)
- 
  With a marker, draw a line at the point where the tubing goes into the nut.
- 
  Retract the tubing **about 0.5 - 1 mm** and tighten the nut with a ¼ wrench.
- Disconnect the tubing, and visually inspect if the ferrule is attached a bit away from the tubing end.
- Make the connection and tighten well.

# Connecting the 1/32” OD UHPLC-compatible LC lines

Applies to the tubing connections between the following parts in UHPLC systems:

1. Pulse damper → injector
2. Injector → column

**Instructions for connecting stainless steel 1/32” OD tubing in a port for 1/16” OD tubing**

The connection of a 1/32” OD tubing into a port designed to receive 1/16” OD tubing requires the use of an internal reducer.

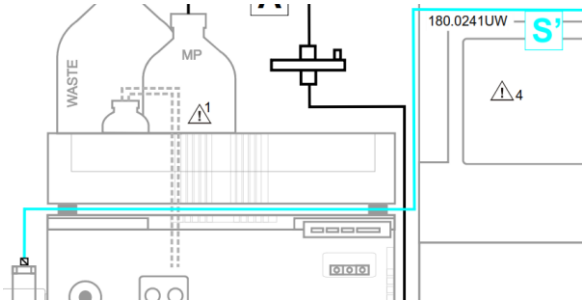



*Figure 18. Connection at the pulse damper as pictured in the LC connections schematics for UHPLC systems.*

- Slide the 1/16” OD nut and ferrule over the filler and insert into the receiving port.
- With the 1/32” OD tubing holding the filler on the bottom of the receiving port, tighten the large nut with fingers and an additional ¼ turn with a ¼ wrench.
- While holding the tubing into place, tighten the 1/32 nut with fingers and then an additional ¼ turn with a 3/16 wrench.

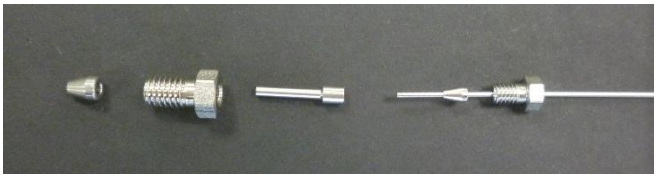


*Figure 19. Exploded view of internal reducer (1/32” to 1/16”) with the 1/32’ nut and ferrule mounted on the 1/32” OD tubing*

- Only in case of leakage is it allowed to tighten each nut an additional bit as the parts are very delicate and can easily break under too much force!

### Instructions for connecting 1/32” OD tubing (PEEKSil or stainless steel)

With parts being more delicate when smaller, it is important not to apply too much force as the nut can easily break! However, when a small leakage occurs a little additional tightening will normally solve this.

To access the valve of the AS110 autosampler more easily, the autosampler top cover can be temporarily slid a bit backwards after unscrewing the two retaining screws on both front sides (see also autosampler manual).

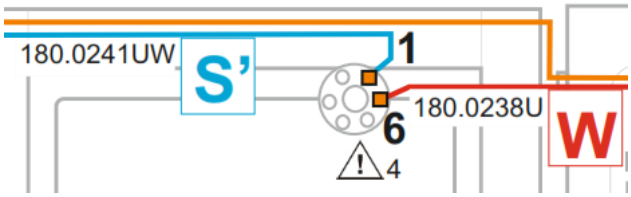



*Figure 20. Connection at the injector valve as pictured in the LC connections schematics for UHPLC systems.*

- Slide a nut and a ferrule in the correct direction onto the tube end (as in Figure 21).

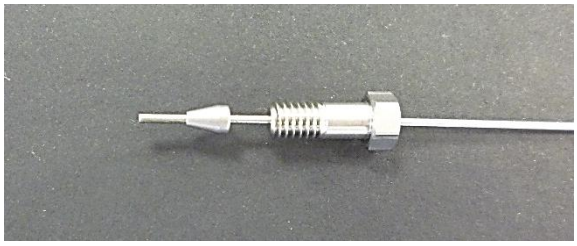


*Figure 21. Assembly of 1/32” OD tubing with stainless steel nut and ferrule for 1/32” receiving ports*

- Tighten the nut with a 3/16 wrench. This does not require a lot of force; too much force can easily break this delicate nut!

**Instructions for connecting PEEKSil 1/32” OD tubing in a port for 1/16” OD tubing (connector type used until 2020)**

The zero-dead connection of a 1/32” OD tubing into a port designed to receive 1/16” OD tubing requires the use of a filler. The PEEKSil tubing is part of the analytical flow path and therefore the use of the internal reducer as mentioned in the previous chapter is not advised.


The connection of this part is based on swaging of the PEEK sleeve, the PEEK outer part of the PEEKSil tubing and the high-pressure ferrule. Slippage of the 1/32” tubing by an improper connection is a real danger and must be prevented as large sudden pressure drops can damage the pulse damper and/or the column. This connection needs quite some force to make!

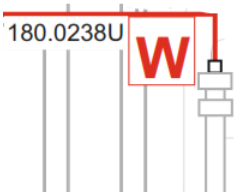



*Figure 22. Connection at the column prefilter as pictured in the LC connections schematics for UHPLC systems.*

- Slide the nut, ferrule and PEEK sleeve in the correct direction onto the tube end (as in Figure 23).

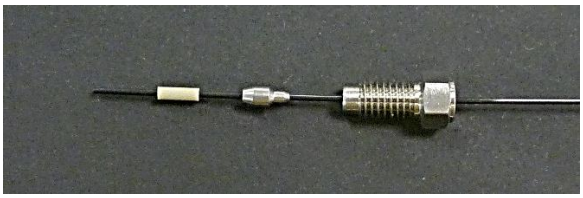


*Figure 23. Assembly of PEEK sleeve, nut and ferrule onto 1/32” OD PEEKSil tubing for connection with a 1/16” receiving port.*

- Insert the tubing end into the receiving port and tighten the nut with a 1/4 wrench until it starts to feel more force is needed.
- Disconnect the fitting from the receiving port and inspect how well the ferrule has attached to the sleeve. The sleeve can probably be moved up and down the tubing at this stage.
- Align the end of the sleeve to the end of the tubing (Figure 24 ).

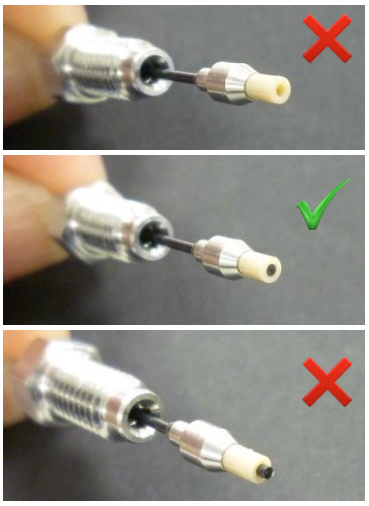


*Figure 24. PEEK sleeve and PEEKSil tubing aligned correctly (middle), or incorrectly (top and bottom).*

- Insert into the receiving port and while manually holding the tubing into the bottom of the receiving port, retighten with more force (Figure 25). At this stage, the PEEK sleeve will start holding onto the PEEKsil tubing.

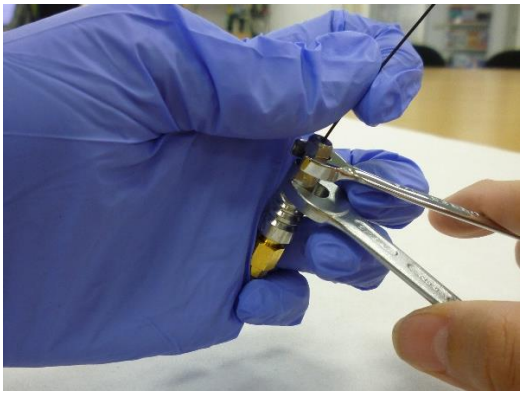


*Figure 25. Connecting the 1/32" OD PEEKSil tubing to a prefilter with 1/16" receiving port requires holding the tubing into the bottom of the port while closing the nut with considerable force.*

- Disconnect the fitting once more and inspect again how well the sleeve has attached to the PEEKSil tubing. The sleeve should be fixed on the tubing end, and the end of sleeve and tubing should be visibly aligned. Correct if necessary.
- Move the tubing end into the detector compartment and connect again to the receiving port, this time tightening with enough force to the stage where a sudden large increase in resistance can be felt while tightening. This is the stage where the fitting is tightened enough; the PEEKSil tubing should not be able to slip out of the PEEK sleeve when pressurized.

**Instructions for connecting PEEKSil 1/32” OD tubing in a port for 1/16” OD tubing**

The zero-dead connection of a 1/32” OD tubing into a port designed to receive 1/16” OD tubing requires the use of the stainless steel ‘through-bore’ internal reducer.

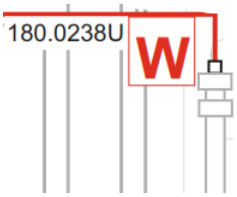



*Figure 26. Connection at the column prefilter as pictured in the LC connections schematics for UHPLC systems.*

*Figure 27. Exploded view of internal reducer (1/32” to 1/16”) with the 1/16’ nut and ferrule to the left, the 1/32’ nut and ferrule mounted on the 1/32” OD PEEKSil tubing and the through-bore filler in the middle.*

- Disassemble the internal reducer.
- Slide the 1/16” OD nut and ferrule over the filler and insert it into the receiving port.
- Push the filler into the bottom of the port with the end of a 1/16” OD tubing (for example the waste line) and hold it there to make sure it is seated (zero dead volume).
- Tighten the nut with fingers and an additional 1/3 turn with a ¼ wrench to lock the ferrule onto the liner at the correct place (Figure 28).

*Figure 28. Filler, nut and ferrule being mounted on a pre-column filter while being held into place with a piece of 1/16’ OD tubing. The second wrench to hold the precolumn filter in place is not shown on the left photo for clarity (the actual photo of how to hold everything is shown on the right – alternatively, do this with two person or in a vice).*

- Remove the 1/16” OD tubing and disconnect the filler/nut assembly to inspect if the ferrule has locked onto the filler.
- Slide the 1/32” OD PEEKSil tubing through the filler/nut assembly to check that it can still go through completely.
- Slide the 1/32” nut and ferrule over the PEEKSil tubing and then the filler/nut assembly.
- While holding the 1/32” OD PEEKSil tubing all the way pushed into the receiving port, tighten the large nut with fingers and then an additional 1/8 turn with a ¼ wrench. Then tighten the small nut first with fingers followed by an additional 1/3 turn with a 3/16 wrench (Figure 29).

*Figure 29. Internal ‘through bore’ reducer (1/32” to 1/16”) being mounted on a pre-column filter. For clarity, the hands are not on the left photo, as the tubing needs to be held firmly bottomed into the port while tightening (the actual photo of how to hold everything is shown on the right – alternatively, do this with two persons or in a vice).*

- Only in case of leakage is it allowed to tighten each nut an additional bit as the parts are very delicate and can easily break under too much force!
- Use only a 1/8 addition turn with a wrench for each nut when reinstalling an already used connector.

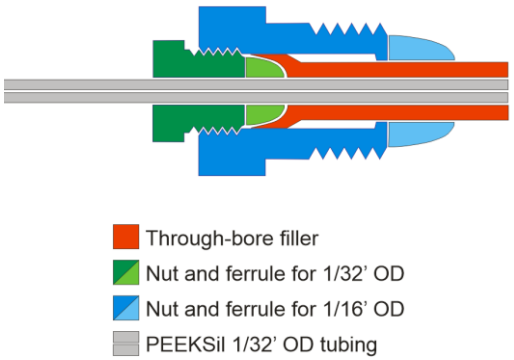



*Figure 30. Schematic representation of an assembled Internal ‘through bore’ reducer (1/32” to 1/16”). When it is installed correctly, the end of the PEEKSil tubing aligns with the end of the through-bore filler at the bottom of the receiving port.*

A P P E N D I X I

# Detailed operating instructions Whatman IDF

## Specifications

The information listed below is compiled from the original Whatman data sheet (reference number 90600A).

### In-line filters AQUEOUS and SOLVENT

The AQUEOUS IFD (product number 6726-5002A) with specifications listed in Table 1 is designed to work with aqueous mobile phases. Whatman recommends the SOLVENT lFD (product number 6725-5002A) for mobile phases with organic modifier concentrations > 30%.

### Operating considerations

Proper operation of the system requires flow rates < 2.5 mL/min. The filter unit should always be changed when changing from one mobile phase to another.

*Table 1 Specifications of Whatman AQUEOUS IFD Disposable Filters.*

| Parameter | Specification |
|---|---|
| Dimensions | 53 mm (2.1 in.) x 44.5mm (1.75 in.) |
| Weight | 11.5 grams (20 grams with ferrule nuts) |
| Filtration Area | 16 cm2 |
| Maximum Pressure | |
| Housing Burst | 4.1 bar (60 psi) |
| Operating | 2.1 bar (30 psi) |
| Housing | Polypropylene |
| Vent | On Inlet with Luer Lock Cap |
| Volume "Hold Up" | Full housing 1.0 mL, with Air Purge < 0.1 mL |
| Filter Media | Nylon |
| Flow Direction | Flow should enter from the inlet |
| Operating Flow Rate | < 2.5 mL/min |
| Connectors | 5/16-24 threads + 1/8" O.D. tubing |
| Biosafe | All Materials Pass USP Class VI |

*Table 2. Chemical compatibility summary*.*

| Classes of Substances 20°C | Polypropylene Nylon Guide for use |
|---|---|
| Acids, dilute | Usable |
| Acids, concentrated | Not usable |
| Alcohols (selected) | Usable |
| Aldehydes | Not usable |
| Bases | Usable |
| Esters | Short term use |
| Hydrocarbons, aromatic | Not usable |
| Hydrocarbons, halogenated (selected) | Short term use |
| Ketones | Not usable |

*) This chemical compatibility chart is intended as a general guide only. This guide has been compiled from results of in-house studies, material supplier studies and currently available technical literature. Because of solvent condition variability's, which may exist from lab to lab, component compatibility cannot be guaranteed. In order to verify chemical compatibility, studies on individual chemicals of interest should be undertaken.

## Operating instructions

### Safety

Consider the special factors of your application and consult the table of Technical Data to determine the correctness of use. Do not exceed the pressure, temperature or chemical compatibility recommendations.


High pressures are easily obtained when using syringes. Do not push solvents through the filter, but only use the vent port to pull solutions through.

### Filter Media Considerations

The 0.2 μm nylon membrane filter media provides an excellent means of filtering aqueous based HPLC mobile phases. It provides high throughput. For specific solutions see the Chemical Compatibility Summary. "Wetted" media will not allow gas to easily pass through the media. The pressure required for gas to pass through wetted media (bubble point) is dependent on the media's pore size. Air entrained on the upstream side of wetted media blocks the flow path and reduces or stops flow.

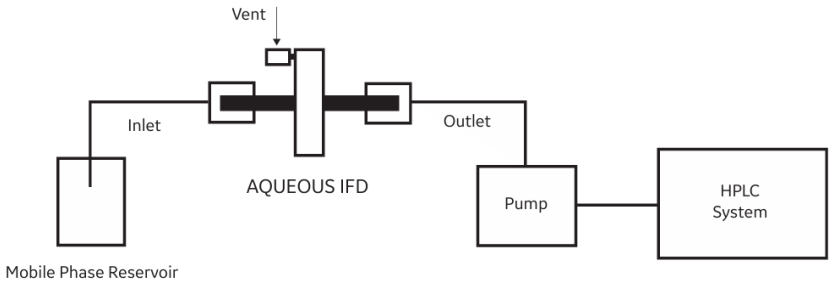



*Figure 31. Schematic drawing of filter Installation in HPLC set-up. Do not insert the tubing too deep into the filter, as it could pierce through the membrane and damage the filter.*

## Filter priming after installation or replacement

- Open the vent of the dry Whatman in-line filter and insert a few drops of ethanol until the filter is completely wetted.
- Attach the tip of a syringe into the opened filter vent port.
- Close the outlet of the Whatman filter with the tip of a gloved finger while drawing solution into the Whatman filter. Keep drawing until the inlet compartment of the filter is flushed and air free.
- Remove the syringe, close the vent and prevent the solution from running back into the bottle (by keeping the filter below the solutions level in the connected bottle while removing the syringe).
- Let gravity spontaneously siphon the solution through the filter until it fills up the back part of the Whatman filter.
- Reconnect the outlet tubing and make sure not to insert it too deep into the receiving port; the tubing should not touch the membrane inside the filter!
- Purge the pump and the lines up to the pump to remove any remaining entrapped air in the tubing between the Whatman filter and the pump.

## Troubleshooting

**Air present in the inlet side of the AQUEOUS IFD during operation:**
The air may be evacuated by holding the AQUEOUS IFD level with the mobile phase in the mobile phase reservoir, removing the vent cap, securing an empty syringe to the vent and pulling back on the syringe plunger. Then remove the syringe and replace the vent cap. Normally a small bubble of air will remain in the vent. This will not interfere with mobile phase flow.

**Trouble with priming:**
Follow the priming instructions exactly. When it is not possible to draw solution through the vent this usually means that the membrane has collapsed onto the vent (make sure to have a closed outlet while trying to

draw solution). The flow between tubing inlet and vent port can be restored in this case by connecting the tip of the syringe to the outlet of the filter housing and slowly drawing a small under-pressure.

**No flow immediately after Installation:**

a. Check for air blocking the inlet side of the AQUEOUS IFD and remove it if present.
b. To determine if the mobile phase is flowing through the AQUEOUS IFD towards the pump, disconnect the tubing at the pump inlet and check if the solution syphons through by gravitational force. It should start dripping readily.

**Slow or no flow after use:**

Check for air blockage and clear any entrapped air. If problem persists, the AQUEOUS IFD was not wetted before installation or it is clogged with particulates and should be replaced.

**Air appears to be passing through the AQUEOUS IFD:**

Perform air tightness check. If no air leaks are observed on the outlet side, replace the AQUEOUS IFD: the media may have ruptured.

**Air tightness check:**

Plug the tubing at the mobile phase reservoir. Remove vent cap and secure an empty syringe to the vent. Pull back on the plunger. If there are any air leaks, air bubbles will be observed (check both sides of the filter).

A P P E N D I X I I

# Preparation of custom-size sample loops

This appendix contains information and instructions on the preparation of custom-sizes sample loops. This is particularly relevant for the on-line microdialysis ALEXYS applications.

## Calculating the right sample loop size for on-line applications

For the installation of the ALEXYS online systems it is necessary to prepare sample loops with an appropriate injection volume (normally in the range of 2 – 10 µL). The optimal injection volume is dependent of the application run time, microdialysis pump speed and number of serially mounted injection loops. Choose the sample loop volumes with the following formula:

$$\text{Sample loop vol (µL)} = \frac{\text{Total analysis time (min) - overfill margin (e.g. 1 min)}}{\text{nr. of serial loops x dialysis pump flow rate (µL/min)}}$$

For example, in case of a 14-port valve with 3 sample loops in series, an analysis of 10 minutes, a microdialysis pump running at 0.9 µL/min, and 1 min of overfill margin (sample going to waste), the optimal volume of each of the 3 sample loops would be (10 - 1) / (3 x 0.9) = 3.3 µL.

Note that for the High Throughput there will be 3 or 4 sample loops, but these are mounted in parallel (‘nr. of serial loops’ is 1).

Use the sample loop assembly kit (p/n 180.0254) from the LC connection kit to make the sample loops. The kit consists of approximately 150 cm red-striped PEEK tubing and 10 sets of stainless-steel nuts and ferrules (p/n 250.1560 and 250.1558 respectively) to fix the loops to the OMD valves. See the procedure below to cut the tubing to the right length (volume).

## Preparing a sample loop with a custom-fit volume

PEEK tubing is ideal to make custom-size sample loops, as it is high-pressure resistant and can be cut easily with a cubing cutter. As the length is (linearly) related with the internal diameter of a tube, the correct length for a certain volume can be calculated with the formula for cylinders.

Due to the relatively large tolerance that manufacturers of PEEK tubing give out for internal diameter (ID), the actual ID must first be measured for the piece of tubing that is going to be used to make loops. The ID of a tubing can be calculated with the formula for flow resistance in capillaries, based on the backpressure measured at a certain flow rate. The Excel spreadsheet

"AntecCalculator" contains these formulas in a convenient template format which can be used to calculate the actual ID of a tubing. A copy of this calculator can be downloaded from the 'Software' section of the Antec Scientific website.

The calculation of ID requires enough accuracy of the backpressure value. The pump front panel does not display the pressure values with enough accuracy. When connected to Clarity software, the pressure can be noted with higher accuracy (two digits) in the Device Monitor of Clarity.

**Procedure for making accurate custom-size sample loops**

- Prepare a solution of water with 5% (v/v) methanol (degassed for 15 min in a sonic bath) and connect it to the pump. Purge the lines up to the pulse damper with this solution.
- Measure the exact length of the PEEK tubing with a ruler.
- Connect the PEEK tubing to the outlet of the pulse damper and place the open end in a waste bottle.

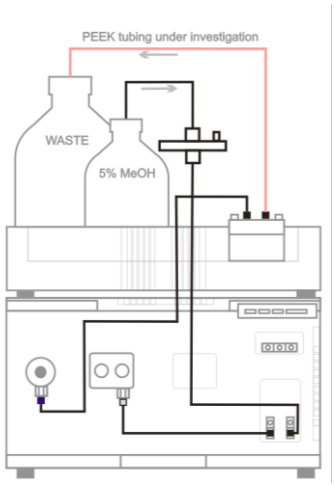



*Figure 32. Configuration to determine the internal diameter of a piece of tubing by measuring the backpressure generated at a certain flow rate.*

- Start up the Clarity software and activate the Device Monitor.

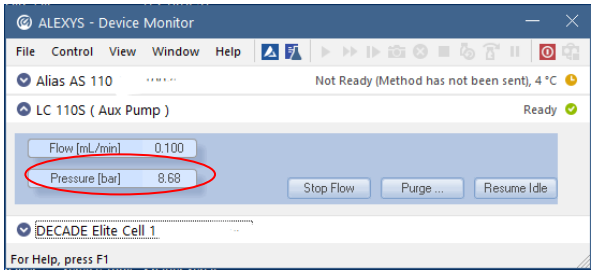



*Figure 33. The Device Monitor window in Clarity software gives an accurate pressure reading.*

- Open the purge valve and determine the zero-pressure value of the system. WARNING: Do not open the purge valve when the system is still under pressure (>3 bar).
- For testing the backpressure of tubing with a length of about 1.5 m and ID of around 125 um (red striped PEEK), set the flow rate to 0.8 mL/min and let the pressure stabilise for 25 min. The expected pressure is between 10 – 70 bar (dependent on the actual ID). For testing other lengths or tubing ID, adjust the flow rate and prevent a system overpressure.
- Write down the pump backpressure after 25 and 30 min. The pressure change must not be larger than 0.1 bar; wait longer in case equilibrium is not yet reached.
- Determine the pump back pressure (with two decimals) of the PEEK tubing, taking into account the zero-offset:
  $P_{PEEK\ tubing} = P_{at\ 800\ uL/min} - P_{at\ 0\ mL/min}$ .
- Open the tab “Capillary PnV” of the Excel spreadsheet “AntecCalculator” and fill in the following parameters in the section “Calculation of capillary diameter/length (tailor-made loops)”:

  1. Length of the PEEK tubing (cm)
  2. Back pressure of the PEEK tubing (bar).
  3. The required loop volume (2 – 10 µL).
  4. The flow rate used during the test (800 µL/min)

  The spreadsheet will calculate the actual internal diameter of the tubing and give the required length to generate the specified sample loop volume (Figure 34).

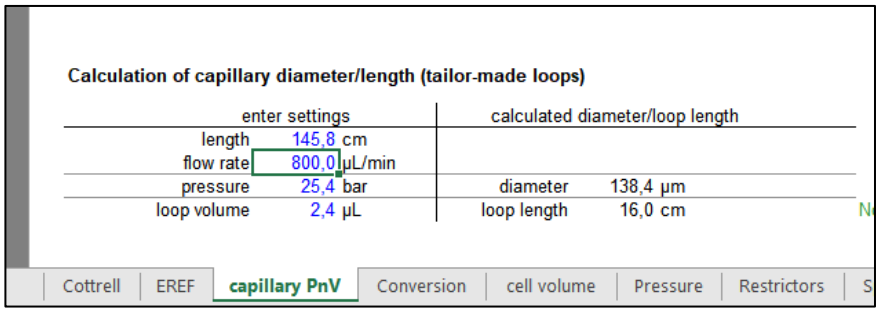



*Figure 34. Example of tubing loop calculation with the Excel template ‘AntecCalculator’.*