Antec Scientific
Hoorn 131
2404 HH Alphen a/d Rijn
The Netherlands

LC connections guide

180.7001W, Edition 8, 2025

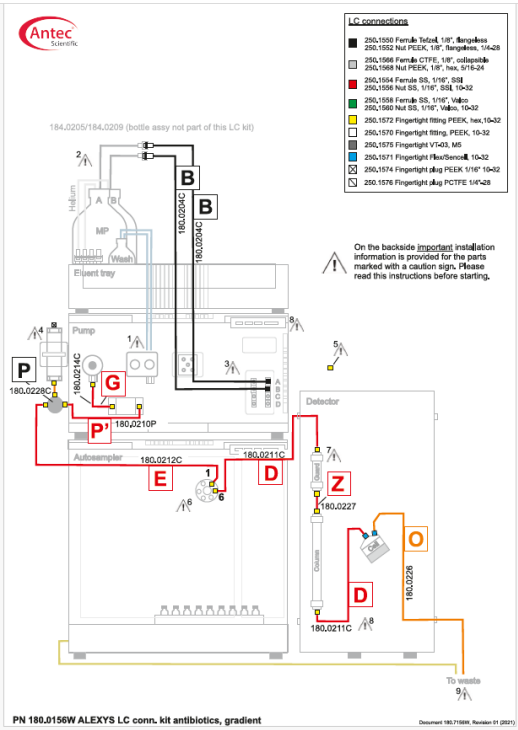


Table of contents

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.

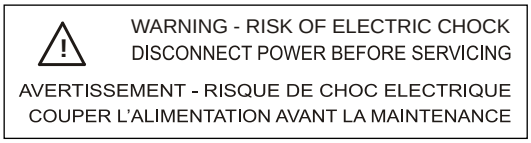
The note sign signals additional information. It provides advice or a suggestion that may support you in using the equipment.

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 a ROXY or ALEXYS system.

Preparation

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

- Equipment is unpacked and checklists are verified.
- 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.
- Not all paragraphs are relevant to each system; make sure to select the correct paragraph for your reference.

Required tools

The following tools are/may be required for the installation of the LC flow path.

- 3/16", 5/16" and/or 1/4" wrench (Figure 1)
- 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). 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). This item is supplied with a few specific LC connection kits where it is relevant.

CHAPTER 2

The LC connections kit

Every ROXY Exceed system and 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 the flow path, and the specific schematic installation drawing shows where each part should be installed.

Details of the schematic LC installation drawing

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

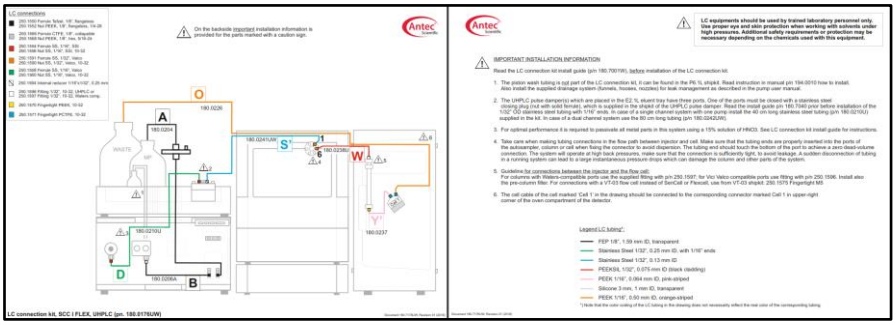


Figure 4. Example of LC connections drawing, front side (l) and backside (r).

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

Precautions

General precautions

A good order how to build up an LC flow path is to connect the parts in the direction of the flow and use water as a first test solution for leak-tightness.

Take into account the following general precautions:

- Use the correct connector to attach tubing to the different port types. (check the legend in the schematic installation drawing).
- Make sure to orient the ferrules in the correct direction, as instructed in this manual: flat bottomed ports and tapered ports require a different orientation.
- 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).
- Push the tubing into the port while tightening the nut to prevent it (partly) slipping out and making a dead volume.
- Always disconnect a new connection to inspect if the ferrule was fixed correctly to the tubing tip
- When cutting tubing always use the supplied tubing cutter (p/n 250.1020) to assure straight and neat tubing cuts.

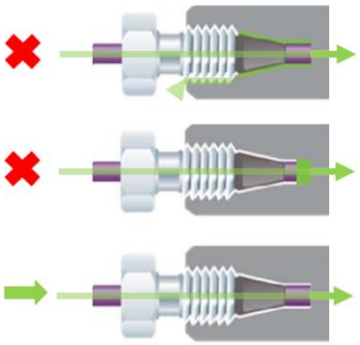


Figure 5. If the capillary tubing is not well 'bottomed' into the receiving port, an unacceptable amount of dead volume may be added to the system (middle figure). Another type of wrong connection can result in leakage (top figure). The correct connection does not leak and does not add dead volume to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

Precautions specific for ALEXYS systems

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 of an ALEXYS system (between injector, column, and flow cell).

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.
- 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.

C H A P T E R 4

Making tubing connections to an ALEXYS pump

Connecting the pump piston backwash lines

An ALEXYS 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 for reversed-phase separations is a mixture of 80/20 v/v% water/isopropanol. For HPAE-PAD applications (carbohydrate analysis) or acetylcholine analysis, no organics should be present in the piston wash: use pure HPLC-grade water as piston wash liquid.

Instructions for connecting the piston backwash lines

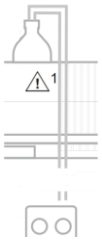


Figure 6. Piston backwash tubing as pictured in the LC connections schematics.

- The bottle kit contains a 100 mL glass bottle or HDPE 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), which should be cut in two pieces with an adequate length. It is recommended to make a V-shaped cut to the end of silicone tubing that is inserted into the bottle, 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.

Connecting low pressure 1/8” OD lines

Applies to: tubing connections to the degasser, SSV ports and the pump inlet.

Instructions for connecting the low pressure LC lines

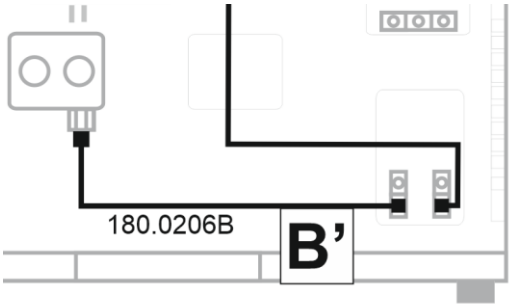


Figure 7. Connections at the degasser and pump inlet as pictured in an LC connections schematics. Note: on a P6.1L pump with integrated degasser, the assembly 180.0206B is already preinstalled.

- 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 (**note the orientation of the ferrule** in Figure 8).

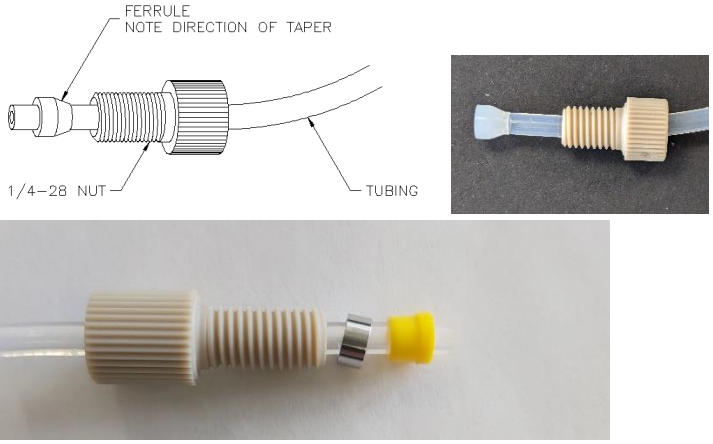


Figure 8. Assembly of 2 different models of ¼-28 nut, ferrule and 1/8” OD tubing for connection to degasser ports. The top photo shows a correctly aligned and fixed ferrule at the tubing tip after installation. The bottom photo shows the correct orientation for the assembly of an alternative connector that uses a locking nut.

- 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.

Connecting low pressure 1/16” OD lines

Applies to dedicated tubing set for GABA-Glu analysis with only 1 pump:

Instructions for connecting the low-pressure LC lines

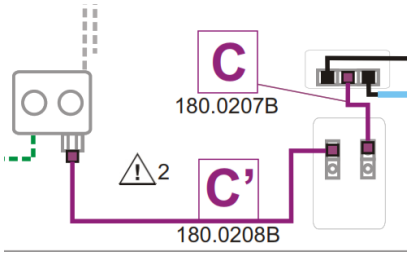


Figure 9. Connections between the Solvent Switch Valve (SSV), degasser and pump inlet as pictured in the LC connections schematics.

- Visually confirm a straight cut of the 1/16” OD tubing end.
- Slide a nut and a ferrule in the correct direction onto the tube end (**note the orientation of the ferrule** in Figure 10Figure 8).

Figure 10. Assembly of ¼-28 nut, ferrule (for connection in a flat bottom port), and 1/16” OD tubing for connection to SSV and degasser ports. Make sure to align the tubing end with the ferrule bottom (it should not protrude out).

- Tighten the nut firmly with fingers.
- Make sure the tubing is not inserted too deep into the ports, as this will cause blockage. The tip of the tubing should not protrude past the ferrule bottom, as this will lead to blockage of the port.
- If bubbles are introduced at one of the ports during pump purging, then this nut should be tightened a bit more.

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

- There are tubing guides behind the (pull off) front panel of both the eluent tray and the pump (Figure 11).

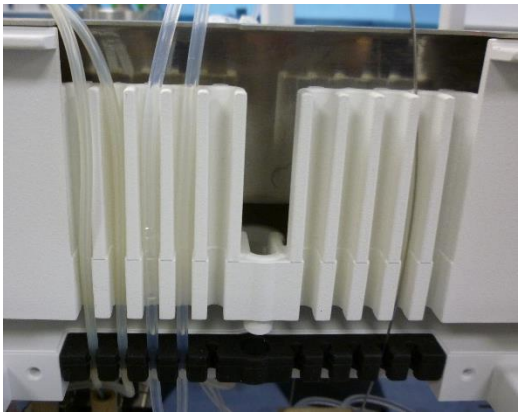


Figure 11. The tubing that is connected to the pump can be neatly organised behind the front panels of the pump and solvent tray.

For more detailed information about how and where to attach the pump drain lines and funnel, see the pump user manual.

C H A P T E R 5

Making high-pressure resistant 1/16” OD PEEK tubing connections in an ALEXYS system

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.

Connecting PEEK 1/16” OD lines with metal nuts and ferrules

Instructions for connecting PEEK LC lines with metal nuts and ferrules

Make sure to make the connection correct the first time, as the ferrule will be fixed to the tubing and the position cannot be corrected (it will have to be cut off and replaced by an unused one).

- Slide a nut and a ferrule in the correct direction onto the tube end (as in Figure 12).

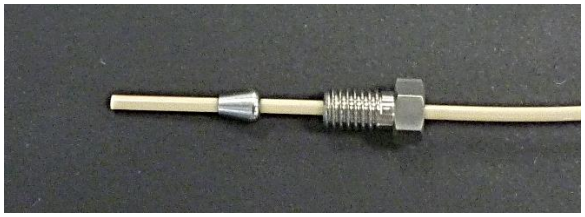


Figure 12. Assembly of stainless-steel nut and ferrule onto 1/16” OD PEEK tubing.

- 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.

Connecting PEEK 1/16” OD lines with a PEEK connector

Instructions for connecting PEEK LC lines with a PEEK connector

Be aware that there are different types of connectors (Figure 13). Always use the correct connector in the designated inlet/outlet port.

- Slide the connector over the tube end (as in Figure 13).

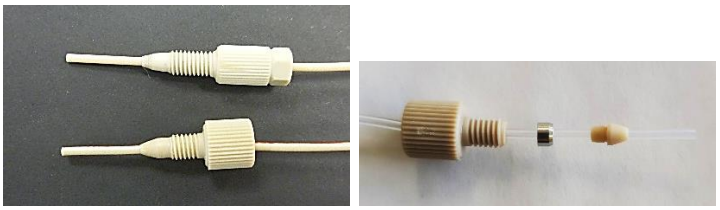


Figure 13. Assembly of a bulkhead (bottom) and narrow (top) PEEK connector onto a 1/16” OD PEEK tubing. The right photo shows the correct orientation of parts for the assembly of an alternative connector that uses a locking nut.

- 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.

Connecting PEEK 1/16” OD lines to a flow cell

For detailed priming instructions and installation of a flow cell, refer to the flow cell user manual.

Instructions for connecting the PEEK lines to a flow cell

Figure 14. Connections at a SenCell (left) or FlexCell (right) as pictured in 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.

Figure 15. Assembly of a dedicated flow cell connector ('Fingertight fitting PCTFE 10-32', pn. 250.5171) 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.

Arrangement of waste line between cell and waste container

Depending of the location of the waste container, the waste line has to be brought up to the eluent tray, or down to a container below the system. These are the advises related to the waste line:

- Make sure that the tubing inside the detector is not positioned in front of the heater fans: this might result in unwanted interferences and baseline noise.
- Lead the outlet tubing from the flow cell through one of the holes in the side panel of the detector. 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).

- In case the autosampler is placed next to the pumps and the waste bottle is placed in the eluent tray, then the waste line can be guided through the autosampler (Figure 16).

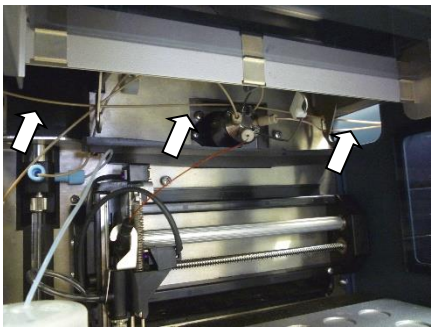


Figure 16. Picture of the autosampler with the waste tubing arranged through the valve compartment towards the waste bottle in the solvent tray.

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).

C H A P T E R 6

Making high-pressure resistant 1/32” OD tubing connections in an ALEXYS system

Applies to the tubing connections between the following parts in a UHPLC system:

1. Pump purge valve → pulse damper
2. Pulse damper → injector

- Double check the schematic drawing to identify which connector and tubing have to be used as different combinations are needed.

Connecting stainless steel 1/32” OD tubing with 16” OD sleeves

Applies to the tubing connections between:

1. Pump purge valve → pulse damper

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

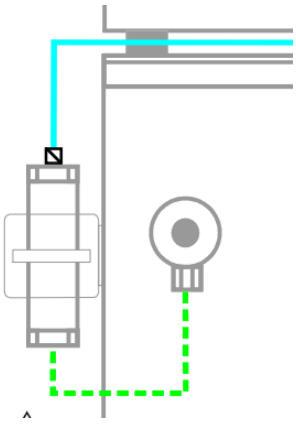


Figure 17. Connections between the pump purge valve and pulse damper as pictured in the LC connections schematics for metal containing ALEXYS systems.

When these sleeved types of tubing are connected while bottomed well into the receiving port, they can become deformed and start leaking (Figure 18). The creation of a small dead volume in the connection prevents deformation during connection (Figure 19).

Figure 18. Sleeved SS tubing leaks and shows deformation after tightening the nut with the tubing end bottomed into the receiving port

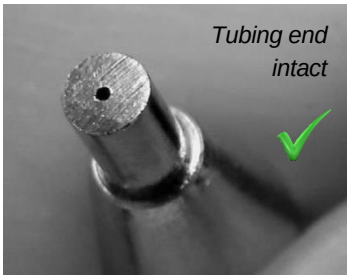
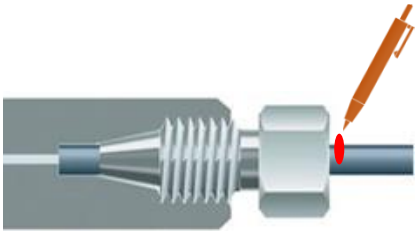


Figure 19. Sleeved SS tubing does not leak after installation with the described procedure.

- 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 stainless steel 1/32” OD tubing to ports for 16” OD tubing (before injector)

Applies to the tubing connections between:

1. pulse damper → autosampler

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.

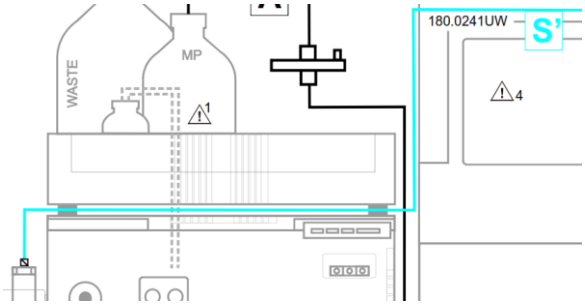


Figure 20. Connection at the pulse damper with a 1/32” OD stainless steel tubing as pictured in the LC connections schematics for UHPLC systems.

- Take the internal reducer apart into its 5 components and identify each part (Figure 21).

Figure 21. Exploded view of internal reducer (1/32” to 1/16”) with the smaller 1/32’ nut and ferrule mounted on the 1/32” OD tubing and the filler in the middle.

- Start with sliding the larger 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.
- Disconnect, inspect and confirm the ferrule to be fixed on the filler. Repeat the previous step if not yet fused. Reconnect and tighten.
- Slide the smaller 1/32” OD nut and ferrule over the tubing (Figure 23).
- While holding the tubing bottomed into place, tighten the 1/32 nut with fingers and then an additional ¼ turn with a 3/16 wrench.

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!

Connecting 1/32” OD tubing to 1/32” ports

With parts being more delicate when they are 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.

Applies to the tubing connections in a UHPLC autosampler with 1/32” ports at the valve

1. pulse damper → autosampler
2. autosampler → column

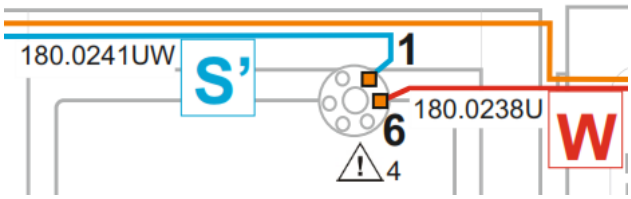


Figure 22. Connection at the injector valve as pictured in the LC connections schematics for UHPLC systems.

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

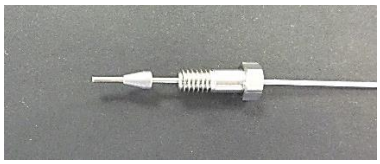


Figure 23. Assembly of 1/32” OD tubing with stainless steel nut and ferrule for 1/32” receiving ports

- 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!

Connecting PEEKSil 1/32” OD tubing to ports for 16” OD tubing (after injector)

Applies to the tubing connection coming from a UHPLC autosampler with 1/32” ports at the valve

1. autosampler → pre-column or column filter

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 24).

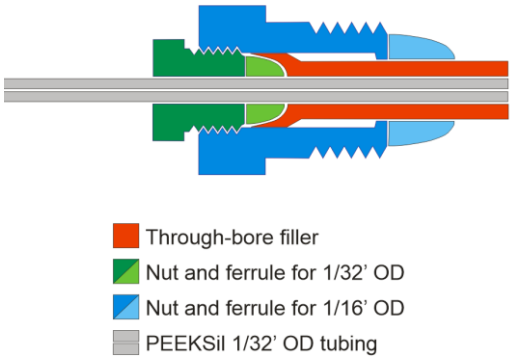


Figure 24. Schematic representation of an assembled Internal ‘through bore’ reducer (1/32” to 1/16”). When it is installed correctly, the end of the PEEKSil tubing aligns with the end of the through-bore filler at the bottom of the receiving port.

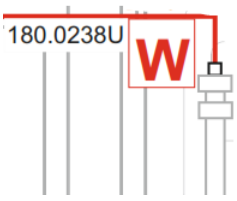


Figure 25. Connection at the column prefilter as pictured in the LC connections schematics for UHPLC systems.

- Take the internal reducer apart into its 5 components and identify each part (Figure 26)

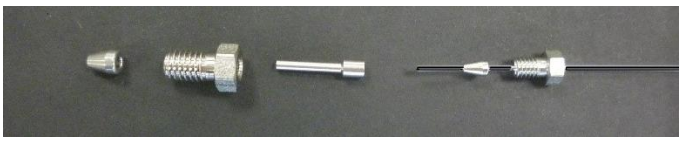


Figure 26. Exploded view of internal reducer (1/32” to 1/16”) with the 1/16’ nut and ferrule to the left, the 1/32’ nut and ferrule mounted on the 1/32” OD PEEKSil tubing and the through-bore filler in the middle.

- Start with sliding the larger 1/16” OD nut and ferrule over the filler and insert 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).

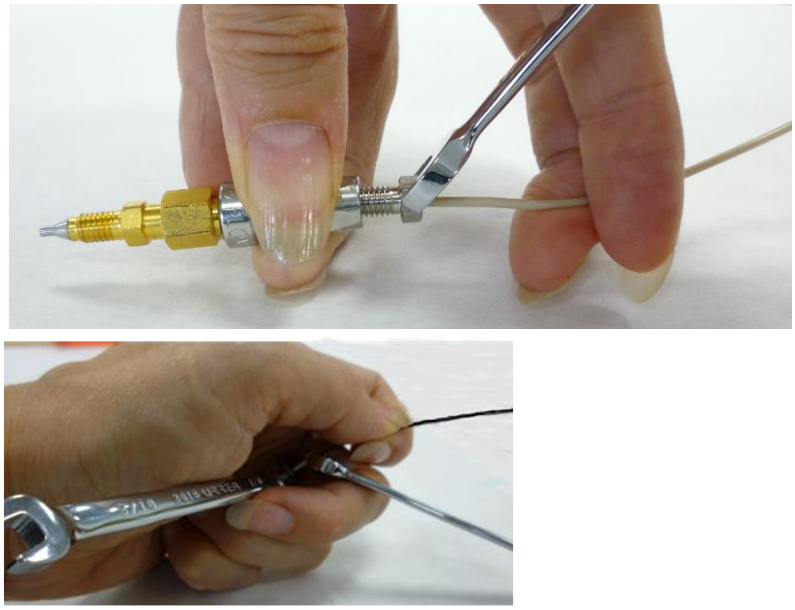


Figure 27. Filler, nut and ferrule being mounted on a pre-column filter while being held into place with a piece of 1/16’ OD tubing. The second wrench to hold the precolumn filter in place is not shown on the top photo for clarity (the actual photo of how to hold everything is shown on the bottom – alternatively, do this with two person or in a vice).

- Tighten the large nut with fingers and an additional 1/3 turn with a ¼ wrench. This requires the assistance of a second person to hold the items, or a special hand-grip as shown in Figure 27.
- 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 smaller 1/32” OD nut and ferrule over the PEEKSil tubing in the correct orientation (Figure 28Figure 23).

Figure 28. 1/32’ nut and ferrule mounted on the 1/32” OD PEEKSil tubing.

- Slide the 1/32” OD PEEKSil tubing all the way into the filler, and while keeping it pushed against the port bottom, 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).

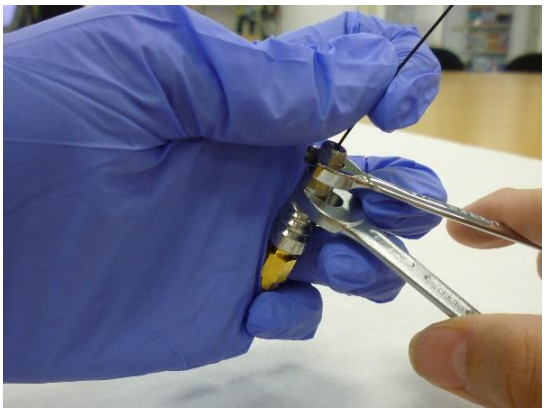


Figure 29. Tighten the small nut into the large nut while holding the PEEKSil tubing bottomed into the receiving port. Do this with the special handgrip or with two persons, or in a vise)

- Use only a 1/8 addition turn with a wrench for each nut when reinstalling an already used connector.

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

C H A P T E R 7

Making tubing connections at a ROXY system

Connecting 1/16” OD lines to a Luer-lock connector

Applies to the tubing connections between the syringe and a cell in a ROXY system:

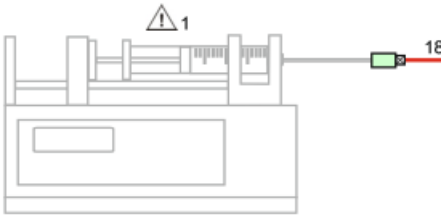


Figure 30. Connection of a syringe to the tubing as pictured in the LC connections schematics.

- Visually confirm a straight cut of the 1/16” OD tubing end.
- Slide a nut and a ferrule in the correct direction onto the tube end (**note the orientation of the ferrule** in Figure 31).

Figure 31. Assembly of ¼-28 nut and dedicated ferrule for connection of 1/16” OD tubing in a flat bottom for 1/8” OD port.

- Tighten the nut firmly with fingers.

Connecting 1/16” OD lines to a µPrepCell 2.0/SS

For detailed priming instructions and installation of a ROXY flow cell, refer to the flow cell user manual.

Instructions for connecting PEEK LC lines to a µPrepCell 2.0/SS

Applies to the tubing connections between the syringe and cell in a ROXY system:

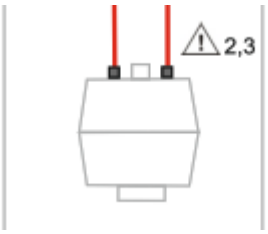


Figure 32. Tubing connections at a µPrepCell 2.0/SS as pictured in the LC connections schematics.

- Slide the connector over the tube end (as in Figure 33Figure 13).

Figure 33. 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 connector with hex head should be tightened using the (blue) tightening tool for 1/16” OD PEEK tubing connectors to make a high pressure resistant leak-tight connection.

Connecting 1/16” OD lines to a ReactorCell

For detailed priming instructions and installation of a ROXY flow cell, refer to the flow cell user manual.

Instructions for connecting PEEK LC lines to a ReactorCell

Applies to the tubing connections between the syringe and ReactorCell in a ROXY system:

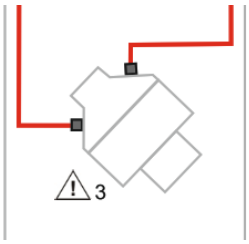


Figure 34. Tubing connections at a µPrepCell 2.0/SS as pictured in the LC connections schematics.

- 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.

Figure 35. Assembly of a dedicated flow cell connector (‘Fingertight fitting PCTFE 10-32’, pn. 250.5171) 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.

A P P E N D I X 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 received by sending an request to Antec Scientific by e-mail.

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.

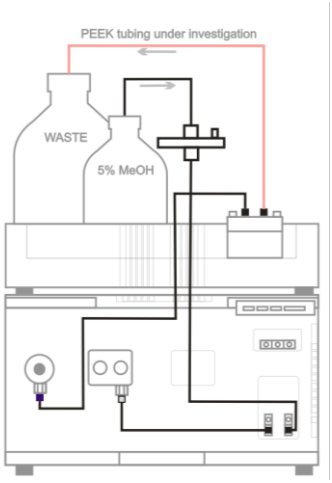


Figure 36. Configuration to determine the internal diameter of a piece of tubing by measuring the backpressure generated at a certain flow rate.

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

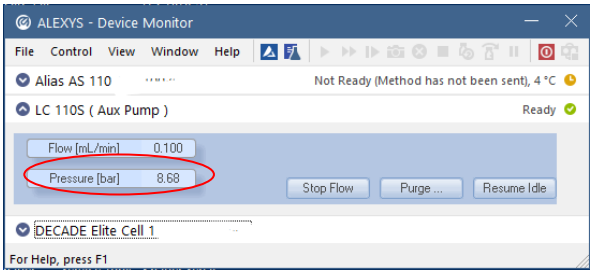


Figure 37. The Device Monitor window in Clarity software gives an accurate pressure reading.

- 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 38).

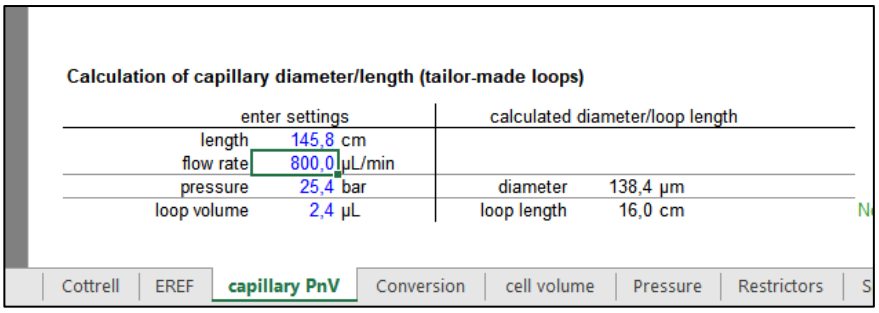


Figure 38. Example of tubing loop calculation with the Excel template ‘AntecCalculator’.

A P P E N D I X I I

Legacy connectors

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!

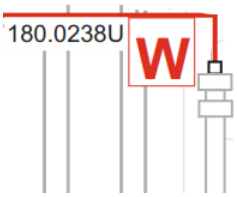


Figure 39. Connection at the column prefilter as pictured in the LC connections schematics for UHPLC systems.

- Slide the nut, ferrule and PEEK sleeve in the correct direction onto the tube end (as in Figure 40).

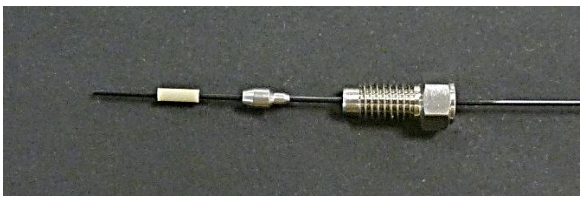


Figure 40. Assembly of PEEK sleeve, nut and ferrule onto 1/32” OD PEEKSil tubing for connection with a 1/16” receiving port.

- 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 41).

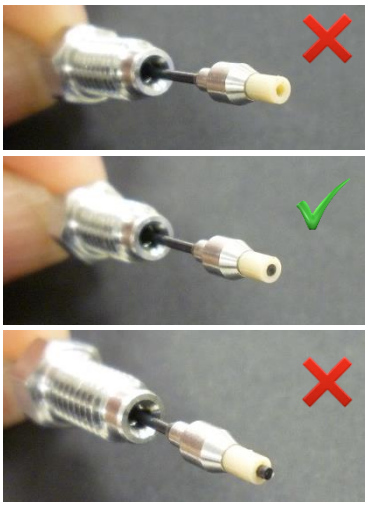


Figure 41. PEEK sleeve and PEEKSil tubing aligned correctly (middle), or incorrectly (top and bottom).

- Insert into the receiving port and while manually holding the tubing into the bottom of the receiving port, retighten with more force (Figure 42). At this stage, the PEEK sleeve will start holding onto the PEEKsil tubing.

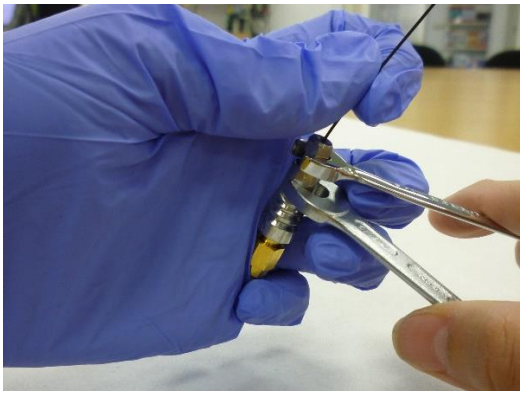


Figure 42. Connecting the 1/32" OD PEEKSil tubing to a prefilter with 1/16" receiving port requires holding the tubing into the bottom of the port while closing the nut with considerable force.

- 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.