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LC connections installation guide

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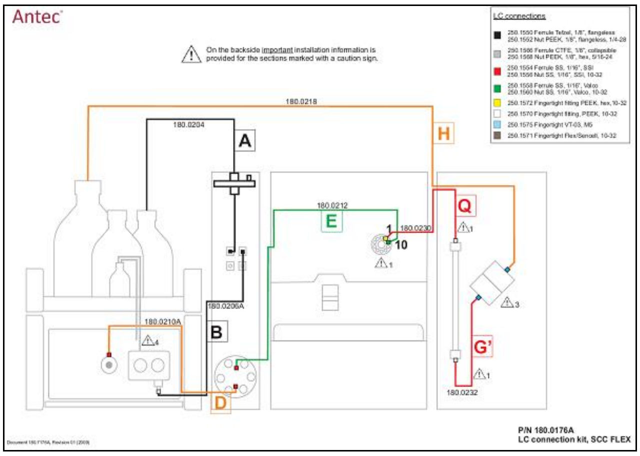

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Symbols

The following pictogram is used in this installation guide:

Caution, calls attention to a procedure, which, if not correctly executed, could result in damage to the equipment or personal injury. Do not proceed beyond a "CAUTION" sign until the indicated conditions are fully understood and met.

General precautions

- Execute periodic leak checks on LC tubing and connections.
- Do not allow flammable and/or toxic solvents to accumulate.
- Do not close or block drains.
- Follow a regulated, approved waste disposal program. Never dispose of such products through the municipal sewage system.

LC equipments should be used by trained laboratory personnel only. 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.

Use of this product outside the scope of this guide may present a hazard and can lead to personal injury.

Spare parts and service availability

Manufacturer provides operational spare parts of instruments and current accessories for a period of five years after shipment of the final production run of the instrument. Spare parts will be available after this five years period on an ‘as available’ basis.
Manufacturer provides a variety of services to support her customers after warranty expiration. Repair service can be provided on a time and material basis. Contact your local supplier for servicing. Technical support and training can be provided by qualified chemists on both contractual or as-needed basis.

CHAPTER 1

Introduction

Every ALEXYS LC-ECD system is delivered with a connection kit consisting of tubing and connectors. For some ALEXYS systems, the connection kit contains parts, tubing and connectors that have to be cut and assembled during installation (e.g. LC connection kit, universal; pn. 190.0150(A) – not for micro-bore applications). Other ALEXYS systems come with dedicated LC connections kits that contain pre-cut pieces of tubing with identification tags and an additional connections drawing.

This document explains how to make LC connections, gives general guidelines about installation of the LC flow path of an ALEXYS LC-ECD system, explains the passivation step, and describes some particular procedures that are system specific.

Before complete installation, **passivation of all metal parts** in the HPLC system (with 15% HNO_3) is strongly advised in case the system is going to be used for trace analysis. The passivation procedure is explained in Chapter 2 of this manual.

Before installing the LC flow path it is assumed that:

- Equipment is unpacked and checklists are verified.
- Installation checklist is used as the master document during installation, and installation sections in the different manuals are noted for details. Sections about installation of liquid connections are referring to this document.
- Installation procedure of the ALEXYS analyzer is followed as described in manuals/installation guides, in the order: OR 110, LC 110, AS 100/AS110, DECADE II and CS valve option.
- The content of this manual is read 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 she may not accept any responsibility. Keep the transport box as it is designed for optimum protection during transport and it may be needed again. Carefully unpack the instrument and inspect it for completeness and possible damage. Contact your supplier in case of damage or if not all marked items on the checklist are included. Prior to shipment, your LC connection kit has been inspected and tested to ensure the best possible performance.

Tools

The following tools are/may be required for the installation of the LC connection kit. Verify the LC connection kit checklist to check if these parts will be supplied.

Figure 1. 3/16" , 5/16" and/or 1/4" wrench. Note: the larger size wrenches are part of the tools provided in the LC110S pump ship kit, and the 3/16” wrench is provided only with LC connections kits UHPLC-style.

Figure 2. Tubing cutter. Note: this item is only supplied with a few specific LC connection kits where cutting of tubing is required.

CHAPTER 2

Schematic installation drawing

The universal LC connection kit (p/n 190.0150A) is a basic kit consisting of parts, tubing and connectors to make all necessary LC connections of an ALEXYS® LC-EC system with standard bore LC flow path. Cutting the tubing to the correct length is done during installation by the installer.

The application specific LC connection kits contain assemblies of the right connectors and precut tubing with identifier tags.

In case the system is going to be used for trace analysis, **passivation of all metal parts** in the HPLC system (with 15% HNO_3) is strongly advised. Before complete installation of the LC flow path, make the partial connections according to the schematic drawing in Chapter 4, and after passivation continue to connect the full flow path.

Explanation of the LC-connections drawing

Both types of connection kits contain a laminated schematic installation drawing showing all LC connections of the ALEXYS analyzer. The following features are present on the drawing:

- A caution sign in combination with an identification number (encircled in black in Figure 3) refers to important installation information provided on the back side of the schematic drawing.
- The backside (Figure 4) contains the legend indentifying the type of tubing in the drawing (material, OD, ID and appearance).

For the application specific LC connection kits, additional info is

- A number (encircled in red in Figure 3) that refers to the part number on the plastic bags in which the tubing assemblies are shipped.
- A letter (encircled in red in Figure 3) on a vinyl label for identification of the precut tubing.

Install all parts as depicted in the schematic installation drawing and take notice of the remarks on the backside of the drawing.

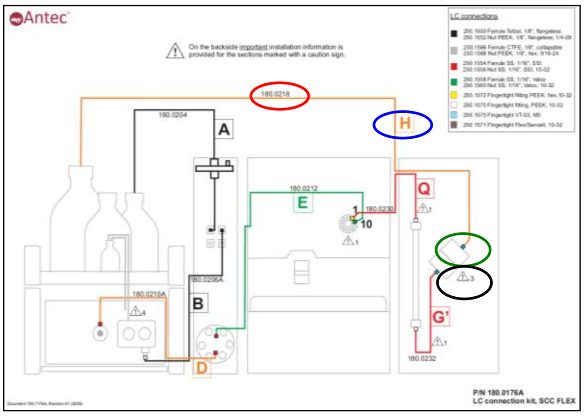
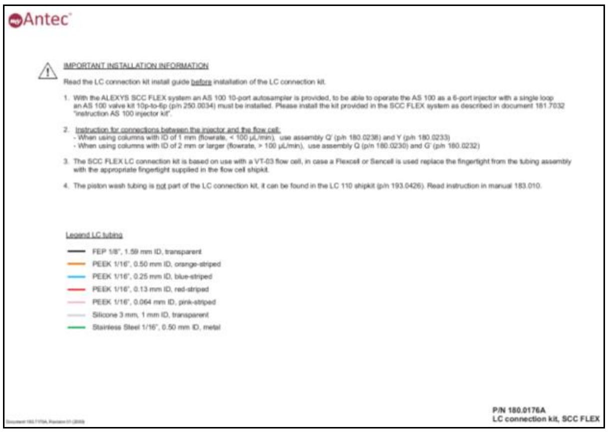


Figure 3. Example of front side of an LC connections schematic drawing with colored circles highlighting specific info (see text).



Antec

IMPORTANT INSTALLATION INFORMATION

Read the LC connection kit install guide before installation of the LC connection kit.

1. With the ALEXYS SCC FLEX system an AS 100 10-port autosampler is provided, to be able to operate the AS 100 as a 6-port injector with a single loop an AS 100 valve kit 10p-to-6p (p/n 250.0034) must be installed. Please install the kit provided in the SCC FLEX system as described in document 181.7032 "instruction AS 100 injector kit".
2. Instruction for connections between the injector and the flow cell:
   - When using columns with ID of 1 mm (flowrate, < 100 µL/min), use assembly Q' (p/n 180.0238) and Y (p/n 180.0233)
   - When using columns with ID of 2 mm or larger (flowrate, > 100 µL/min), use assembly Q (p/n 180.0230) and G' (p/n 180.0232)
3. The SCC FLEX LC connection kit is based on use with a VT-03 flow cell, in case a Flexcell or Sencell is used replace the fingertight from the tubing assembly with the appropriate fingertight supplied in the flow cell shipkit.
4. The piston wash tubing is not part of the LC connection kit, it can be found in the LC 110 shipkit (p/n 193.0426). Read instruction in manual 183.010.

Legend LC tubing

- FEP 1/8", 1.59 mm ID, transparent
- PEEK 1/16", 0.50 mm ID, orange-striped
- PEEK 1/16", 0.25 mm ID, blue-striped
- PEEK 1/16", 0.13 mm ID, red-striped
- PEEK 1/16", 0.064 mm ID, pink-striped
- Silicone 3 mm, 1 mm ID, transparent
- Stainless Steel 1/16", 0.50 mm ID, metal

P/N 180.0176A
LC connection kit, SCC FLEX

Figure 4. Example of back side of an LC connections schematic drawing with additional application specific info (see text).

C H A P T E R 3

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).

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 nut/ferrule or one-piece connector to connect tubing to the different port types. Check the schematic installation drawing.

⚠ **Connecting tubing with the wrong type of connector can damage the port and result in leakage!**

- Make sure that the tubing is fixed correctly into the port and avoid making a dead volume at the connection (Figure 5).
- When making LC connections using the universal LC connection kit:
  - 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.

⚠ **Avoid introduction of dead volumes in your LC system, especially between injector and flow cell. Dead volumes may lead to poor peak shapes and therefore affect separation and detection sensitivity.**

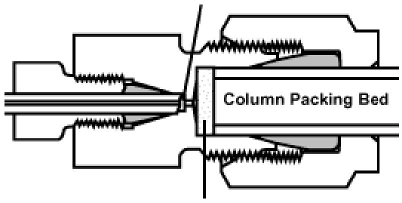


Figure 5. If the capillary tubing does not "bottom-out" inside the union, or if it does not have a smooth, flat end, an unacceptable amount of dead volume may be added to the system.

Connecting the low pressure LC lines

Applies to the tubing connections between the following parts:

1. Mobile phase bottle → in-line filter → Degasser
2. Degasser → LC 110S

The installation of the tubing to the in-line filter and the degasser can temporarily be skipped in case passivation has to be performed (see Chapter 4) until after the passivation procedure

Instructions

- In case of using the universal LC connection kit:
    - Use the 1/8" OD 1.59 mm ID FEP tubing (p/n 250.0910) in the flow path up to the LC 110S pump
    - Install the Whatman inline filter to the tubing according to the detailed instructions in Chapter 5.
    - Make the line between degasser and pump long enough to be able to move the pump tray in and out of the pump rack.
- For connecting the tubing to the degasser ports, assemble the nut and ferrule according to Figure 6.

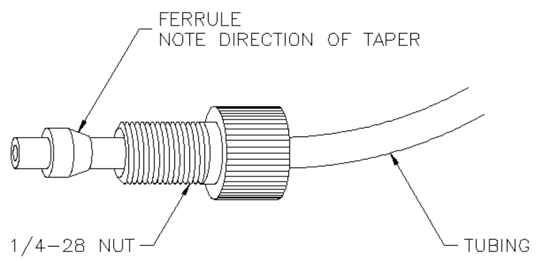


Figure 6. Assembly of ¼-28 nut, ferrule and tubing for connection to degasser ports.

- Connect the FEP tubing from the degasser to the inlet of the LC 110S pump with the appropriate nut and ferrule, see drawing.

Arrangement of low pressure LC lines

- The tubing between in-line filter and degasser can be folded into the gap next to the OR110 (Figure 7).
- Make sure the in-line filter hangs above the solvent rack, so in case of leakage, no solution will drip on any electronics (Figure 7).

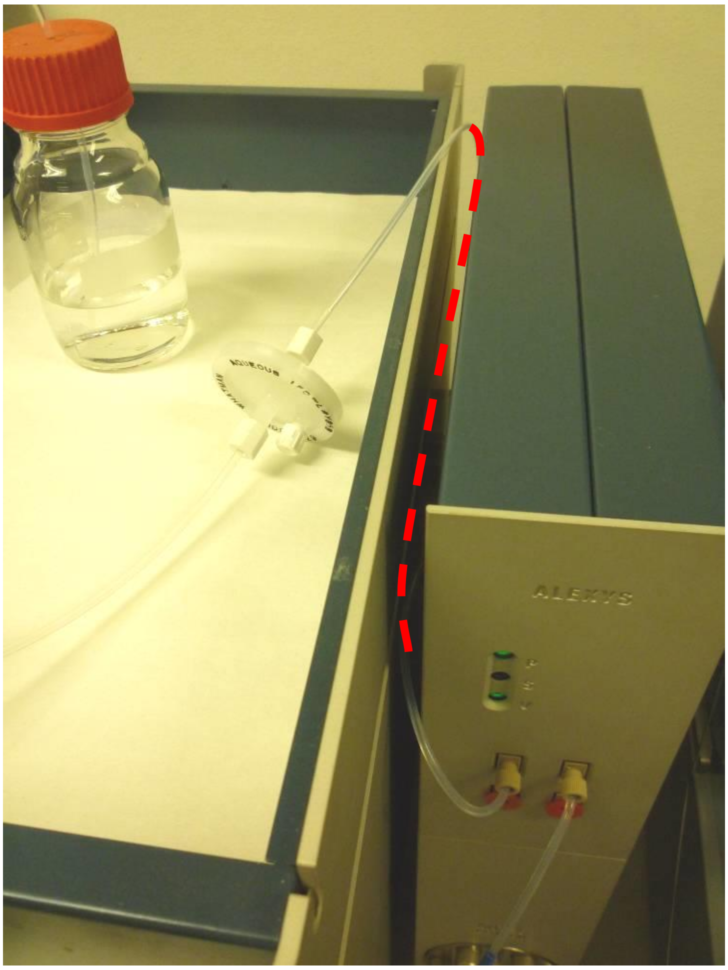


Figure 7. The tubing between filter and degasser port is folded into the gap between the racks (dashed red line). The in-line filter is hanged in the solvent rack so in case of leakage, the solution will not drip onto any electronics. This picture also shows a paper filter on the bottom of the solvent rack (not provided with the system) for protection against scratches.

- Fold the tubing from the degasser to the pump into the slit of the pump rack and in the crevice between the pump and the side of the pump rack (Figure 8).

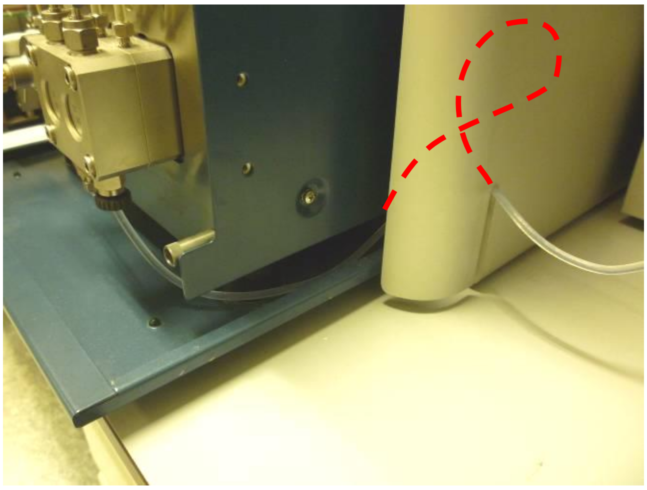


Figure 8. The tubing between degasser ports and pump is manoeuvred into the slit on the side of the pump rack, and folded into the gap between the pump and the pump rack (dashed red line).

Priming the low pressure LC lines

Water (with resistivity >18MOhm.cm, and TOC<5ppb) can be used to pre-flush the system.

Instructions

- 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 LC 110 S ship kit) into the filter vent opening.
- Place the open end of the inlet FEP tubing in the bottle with solution, which is placed on top of the ALEXYS system in the solvent tray.
- Draw the solution through the tubing until the upper compartment of the filter is flushed and air free. Close the vent.
- Open the purge valve of the LC110S pump, and connect the syringe to the purge outlet (using the stainless steel needle and a short piece of silicon tubing; both part of LC 110 S ship kit)

- Gently draw the solution trough the purge outlet until the lines are completely filled with solution.
- Remove the syringe. The purge outlet should spontaneously siphon solvent by gravity. If this is not the case consult the trouble-shooting section in chapter 3.

⚠ **Never push the solvent through the channel of the degasser**

Connecting the LC 110 piston wash

The LC 110S pump is equipped with an automatic piston wash. Two pieces of tubing have to be installed to make a close circuit between the pump head and a bottle with wash solution.
Typically the wash liquid for an ALEXYS analyzer is a mixture of 80/20 v/v% water/methanol. In case that no organics are present in the mobile phase, use pure HPLC-grade water as wash liquid.
For detailed information about tubing connections on the LC 110S pump and priming instructions see the LC 110S user manual.

Instructions

- The bottle kit contains a 100 mL glass bottle for the wash solution and a pierced cap.
- The LC 110S ship kit contains two pieces of silicone tubing (3 mm OD; 1 mm ID).
- Connect one tubing to the inlet point of the piston wash (designated with ▲) and the other to the pump head of the LC 110S.
- The open ends of the tubing should be inserted in the bottle with wash solvent.
- Fill the piston wash tubing with wash solution by drawing some liquid with a syringe from the open end of the piston wash outlet line (which is connected to the pump head).

Arrangement of LC 110 piston wash tubing

- In case of setting up a system configuration where the SR 110 **solvent rack is stacked onto the AS 110 autosampler**
    - lead the piston wash tubing through the upper tubing holder as shown in Figure 9.
    - lead the tubing over the pump to the backside of the rack and up to the wash solvent bottle that is placed in the solvent tray.

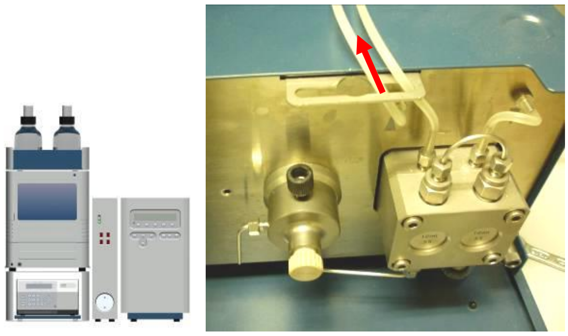


Figure 9. The piston wash inlet and outlet tubing is manoeuvred through the upper tubing holder to secure their position in an open slot when the pump door is closed. The icon display the configuration to which this applies.

- In case of setting up a system configuration where the SR 110 **solvent rack is stacked onto one or two LC 110S pumps**
    - lead the piston wash tubing behind the interconnecting tubing as shown in Figure 10.

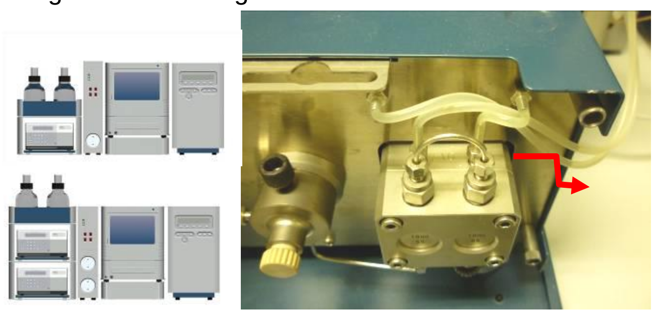


Figure 10. The piston wash inlet and outlet tubing is manoeuvred behind the short interconnecting tubing to secure their position in the open slot when the pump door is closed. The icons display the configuration to which this applies.

- lead the tubing up as shown in Figure 11.

Figure 11. Lead the piston wash up to the solvent tray trough the rim of the pump racks (green arrow).

- The piston wash tubing can be secured to the rim of the SR 110 solvent rack with a clip like displayed in Figure 12.

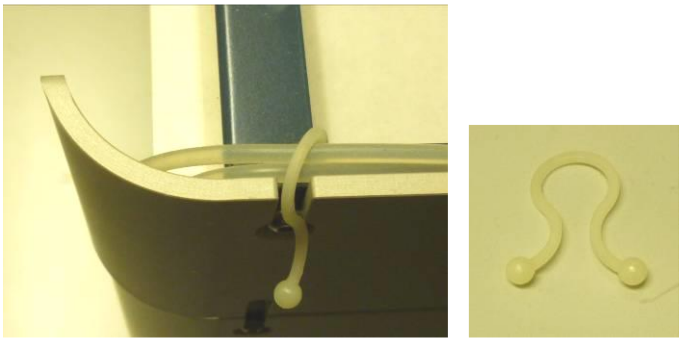


Figure 12. A clip can be used to secure the piston wash tubing to the rim of the solvent rack. The clip that is displayed is standard attached (for packaging) to the DECADE II I/O cable (pn. 250.0130), which can be found in the DECADE II ship kit.

Connecting the high pressure LC lines

Applies to the tubing connections between the following parts:

1. Pump outlet → pulse damper
2. Pulse damper → injector
3. Injector → column

Instructions

- Check the schematic drawing to identify which connector and which tubing have to be used.
- In case of using the universal LC connection kit:
    - Make the line between pump and pulse dampener long enough (>40 cm) to be able to slide the pump in and out of the pump rack.
- To access the valve of the AS110 autosampler more easily, the autosampler top cover can be temporarily slit backwards after unscrewing the two retaining screws on both front sides (see also autosampler manual). *Be careful in case of a solvent tray on top!*
- If the system needs to be passivated, do not connect the column to the tubing at this point, but direct the tubing into a waste bottle and continue after passivation.

⚠ **Do not connect the column before flushing the lines with water and then the appropriate column flush solvent (see instruction sheet of the column for appropriate solvent composition).**

- Connect the tubing to the column (and guard column if applicable).

Arrangement of high pressure LC lines tubing

- The tubing that is connected to the purge valve (going to the pulse dampener) can be directed trough the interconnecting tubing on top of the pump head to secure its position (Figure 7).

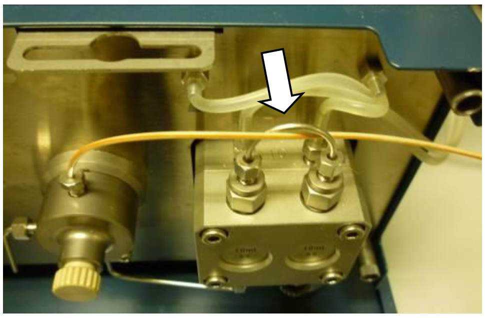


Figure 13. Lead the PEEK tubing from the purge valve through the tubing on top of the pump head to secure its position.

- The autosampler has special openings and internal clips to arrange incoming and out going tubing.
- Lead the tubing from the injector through one of the holes on the side of the DECADE II to the column.

Connection of LC lines to the flow cell

Applies to the tubing connections between the following parts:

1. Column → flow cell
2. Flow cell → waste bottle

Instructions

- Check the schematic drawing to identify which connector and which tubing has to be used.
- For detailed priming instructions and installation of a flow cell, refer to the flow cell user manual and DECADE II user manual.

Arrangement of tubing from and to the flow cell

- Lead the out-going tubing from the flow cell through the holes in the side panel of the DECADE II and into the waste bottle.
    - In case of an ALEXYS with autosampler AS110, lead the waste tubing out of the DECADE II through the upper left hole of the oven compartment. Lead the waste tubing through the valve compartment of the autosampler and into the waste bottle (see Figure 14).

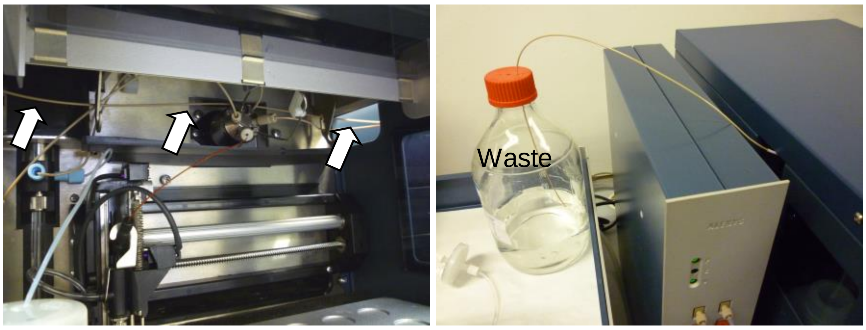


Figure 14. Picture of the autosampler with the waste tubing arranged through the valve compartment and into the waste bottle.

- Arrange the tubing inside the oven so that it is not in front of one of the vents.
- 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).

C H A P T E R 4

Passivation of metal parts

For optimal performance, all metal parts in the system should be passivated. Metal parts that are in contact with the mobile phase are:

- the pump head
- metal tubing on the pump
- the pulse dampener
- valve
- metal sample loops

In principle, the procedure consists of running (preferably) a 15% nitric acid solution trough the metal parts for 20 minutes.

Make sure that all non-metallic parts that are not acid-resistant (nylon Whatmann in-let filters, degasser channels, column and flow cell) are not connected during this procedure.

Connection instructions

- Make the tubing connections as depicted in Figure 15 or Figure 16 by attaching/detaching the appropriate parts. If all the tubing in the system was already connected, make sure to disconnect the tubing between the degasser and pump at the degasser side.
- For the connection between injector and waste bottle, the tubing of the eventual application that is between cell and waste bottle can be used. Connect it to the valve with a PEEK finger-tight connector
    - *In case of having an AS 110 autosampler with valve ports for 1/32“ OD tubing:* attach the tubing that will connect the column, and insert the end into a waste bottle close to the autosampler.
- Insert the inlet of the tubing connected to the pump inlet into a bottle with water (due to the length of the tubing, the bottle may not be able to stand inside the solvent tray on top of the system).

Procedure

- Purge the flow path with water to remove air.
- Insert the tubing into a bottle with 15% nitric acid solution.
- Set the flow rate of the pump to a reasonable setting, check there is enough solution in the bottle, and run the acid trough the system for

20 minutes. **Regularly switch the valve of the injector** to assure that both Load and the Inject positions will be passivated!

- Flush the system thoroughly with water after running the acid through the system, with the valves regularly switching between Inject and Load positions.
- Check the pH of the waste solution with pH paper to confirm that all nitric acid has been flushed out before continuing with the installation.

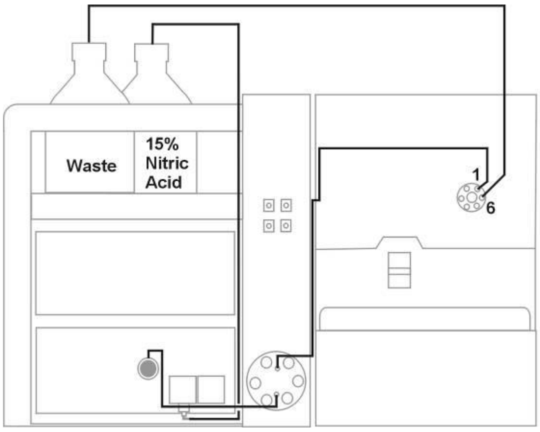


*Figure 15. Flow path configuration principle for passivation of the metal parts in a **single channel** ALEXYS system.*

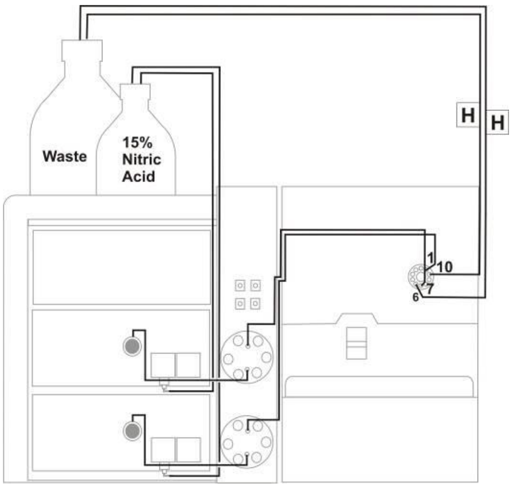


*Figure 16. Flow path configuration principle for passivation of the metal parts in a **dual channel** ALEXYS system.*

CHAPTER 5

Operating instructions Whatman IFD

Specifications

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

Table III. 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 + 1/18" O.D. Ferrule Nuts
Biosafe	All Materials Pass USP Class VI

Table III. Chemical compatibility summary.*

Classes of Substances 20°C	PolypropylenelNylon 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 inhouse 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.

The AQUEOUS IFD, product number 6726-5002A, is designed to work with aqueous mobile phases. Whatman recommends the SOLVENT IFD product number 6725-5002A for organically based mobile phases (organic modifier concentrations > 30%).

Operating instructions

Safety: Considering the special factors of your application 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. Care should be taken not to exceed the recommended pressures. Hold the filter to the syringe when pressure is applied to prevent disengaging the filter from the syringe. This could occur if excessive pressure is applied.**

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 flow rates and 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.

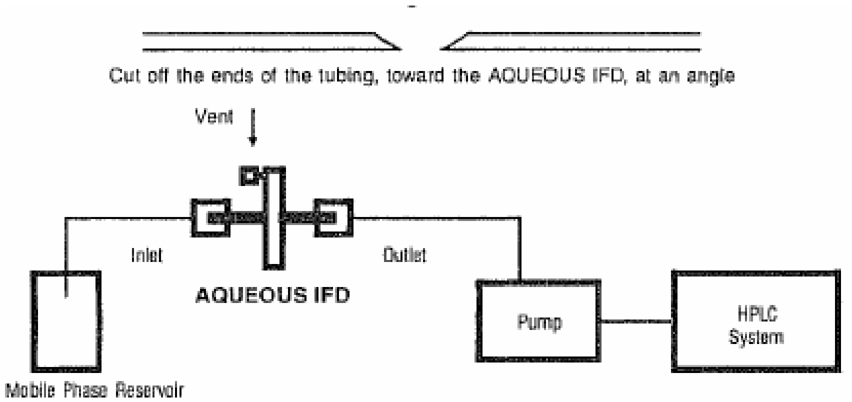


Figure 11. *Schematic drawing of filter Installation in HPLC set-up.*

Filter installation and priming

1. Establish continuous, bubble free flow from mobile phase reservoir to pump by aspirating with a syringe through the pump bleed valve.
2. Cut, at an angle, an appropriate length of 118" O.D. tubing to reach comfortably from the mobile phase reservoir to the inlet of the AQUEOUS IFD. Slide a ferrule nut over the cut tubing. Connect tubing to the inlet of the AQUEOUS IFD, angled end toward the AQUEOUS IFD, by tightening the nut firmly.

Gently insert cutted tubing ends into filter to prevent damage of the filter media.

3. Plug the inlet tubing, or seal the end by attaching and clamping off a short length of flexible tubing.
4. Fill a 10 ml syringe with the mobile phase, remove vent cap and secure the syringe to the vent.
5. With outlet pointed up SLOWLY push the syringe plunger completely wetting out the filter media and filling the AQUEOUS IFD housing.
6. Connect outlet to 1/18" 0.D. pump inlet tubing (cut at an angle) with a ferrule nut, as in step 2.
7. Unplug or unclamp AQUEOUS IFD inlet tubing and place it in the mobile phase reservoir.
8. Making sure the vent is on the upper side of the AQUEOUS IFD, fill the tubing leading to the mobile phase reservoir by pushing slowly on the syringe plunger.
9. Slowly pull on the syringe plunger to withdraw a few ml of the mobile phase into the syringe. Note: This should remove any remaining entrapped air from the inlet side of the AQUEOUS IFD housing.
10. Maintaining the AQUEOUS IFD at the same level as the mobile phase in the reservoir, remove the syringe and replace the vent cap on the vent.
11. Pump mobile phase through system, bypassing the column, for 15 minutes to purge any remaining entrapped air in the tubing between the AQUEOUS IFD and the pump.

Trouble shooting

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 steps 7 through 10 exactly. Check for mobile phase leaks and or air leaks (bubbles), step 1 of Trouble Shooting section.

No flow immediately after Installation:

a. Check for air blocking the inlet side of the AQUEOUS IFD by repeating steps 7 through 10.
b. To determine if the mobile phase is flowing from the mobile phase reservoir to the inlet side of the AQUEOUS IFD; secure a syringe filled with mobile phase to the vent and push the syringe plunger. Mobile phase should flow back from the AQUEOUS IFD to the mobile phase reservoir with a small amount of pressure on the syringe plunger.
c. To determine if the mobile phase is flowing through the AQUEOUS IFD to the pump; plug the tubing to the mobile phase reservoir, secure a syringe filled with mobile phase to the vent and push the syringe plunger. Mobile phase should flow easily through the AQUEOUS IFD and the tubing to the pump.

Slow or no flow after use:
Check for air blockage and clear any entrapped air by following steps 7 through 10. If problem persists, the AQUEOUS IFD is probably 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. 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.

Check the AQUEOUS IFD connections for air tightness:
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.

Integrity Testing, Bubble Point (BP) Test:

Flush filter with 10 ml or more of an appropriate solution. After the media is completely wet, with outlet pointed upward, apply air under controlled pressure to the inlet until air breaks through the media and bubbles from the outlet. The pressure where air begins to pass through the media is the BP.

APPENDIX I

Column Switching (DCC I-I, CS)

This appendix contains additional installation information for the LC connection kit DCC I-I CS (p/n 180.0164A). This installation section describes an efficient pressure adjustment procedure.

For good system stability and to prevent damage to column or pulse damper it is absolutely necessary to match the pressures of the different LC channels during the installation of an ALEXYS DCC I-I analyzer (column switch configuration). The balancing of the system pressures is realised by cutting the supplied restriction capillaries to the correct lengths.

Two restriction capillary assemblies are provided in the kit:

Part no	assembly	Description
180.0242	T	OR 100 pd outlet assembly, DCC, CS -R
180.0250	X	CS valve-to-cell connection -R
250.0901*		Tubing PEEK 1/16", 0.0025" ID, 1 m

*) 2 spare pieces of restriction capillary (p/n 250.0901) are supplied in the kit.

Back pressure adjustment procedure

⚠

Please follow the steps in this pressure adjustment procedure carefully to prevent damage to columns and dampers.

Do not switch the CS valve in a running LC system, before the pressure adjustment procedure is completed. In an unadjusted system, stop the pumps and wait for the pressure to come down to ambient before switching the valve to the other position.

Please ensure that all PEEK fingertights of the LC connections are securely tightened when pressurising the system.

Perform the pressure adjustment procedure using a mobile phase with exactly the same composition as used for the analysis. Prior to the adjustment procedure equilibrate the system with the proper mobile phase.

System preparations

Perform the following steps prior to starting the back pressure adjustment procedure:

- Measure and write down the length of the two pieces of restrictor tubing (assembly X and T, material: pink-striped PEEK) before their installation.
- Check that the manual control box is connected to the CS valve and set the valve to position A (make sure the system pressure is zero at this point!).
- All the hardware elements of the system should have been installed at this point (including flow cells and all electrical cables).
- Flush the pumps with mobile phase and run for at least 30 min at 100 µL/min (both pumps) to equilibrate the system with mobile phase.
- Open the zip-file 'Pressure-monitor.zip' and install the Clarity method files to the appropriate locations in the Clarity folder. The zip-file can be found on the Clarity-CD using Explorer.

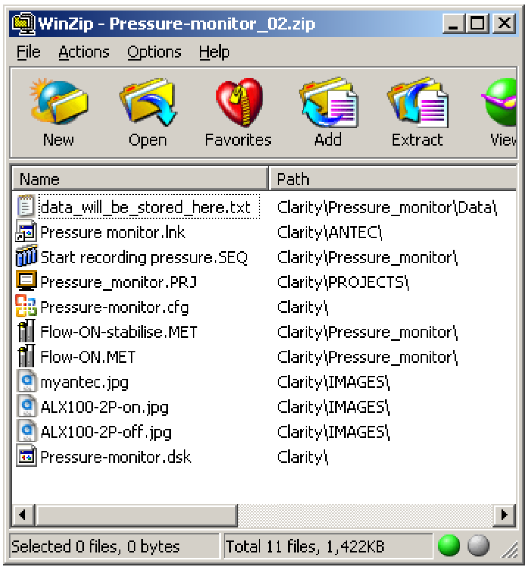


Figure 12. *Contents of the Zipped file 'Pressure-monitor' and the directories for the different files.*

- Activate Clarity using the ‘Pressure-monitor’ ‘shortcut, which can be found in the folder C:\Clarity\Antec (and make a copy of this shortcut to the desktop for your convenience).

- Check that the temperature of the DECADE II oven is set to the same value as used with the analysis (check this in the DECADE II device driver in the Clarity configuration window – default is 35°C).

- Check that in the Clarity method files ‘Flow-ON’ and ‘Flow-ON-stabilise’ the flow rates of both pumps are identical and set to the appropriate value used for the analysis (default is 200 µL/min).

- Click the ‘Apply’ button to start stabilising the system at the correct flow rate (this will also turn on the flow cells automatically).

- Open the ‘Data acquisition’ window and the ‘Device Monitor’ window in Clarity.

The pump pressure displayed in the Device monitor is more accurate than the value given on the pump display: use the device monitor during the procedure to read the system pressures at both pumps.

- Open the sequence file ‘Start pressure recording’.

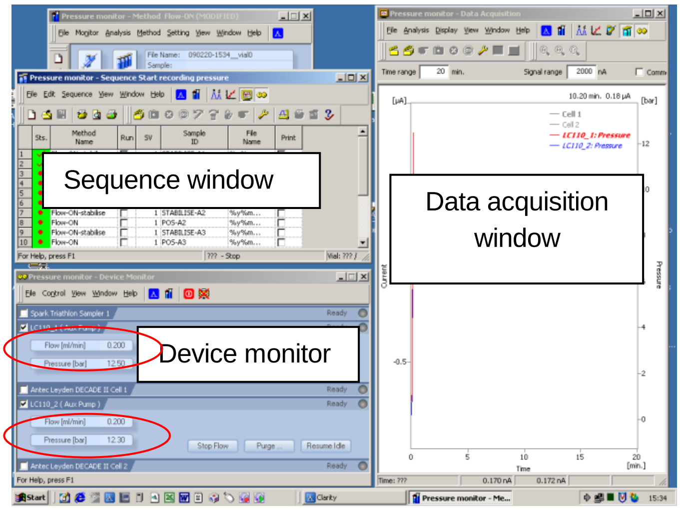


Figure 13. *Screen-dump of Clarity windows necessary for the pressure balance procedure. Read off and write down the pressure from the Device monitor.*

Brief outline of back pressure adjustment procedure

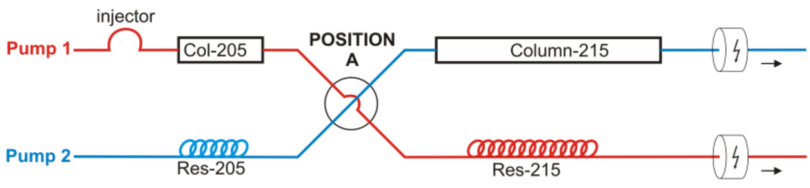


Figure 14. *Schematic representation of the system with column switch valve in Position A: Column 1 (5 cm) and column 2 (15 cm) in parallel. Total pressure in both flow paths is equal ($P_{red} = P_{blue}$).*

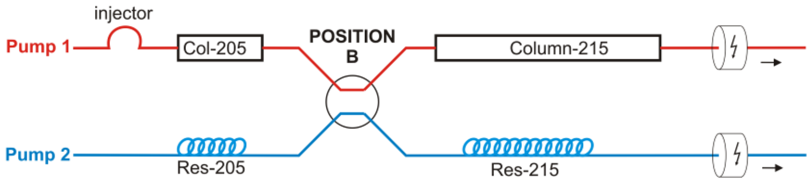


Figure 15. *Schematic representation of the system with column switch valve in Position A: Column 1 (5 cm) and column 2 (15 cm) in series. Total pressure in both flow paths is equal ($P_{red} = P_{blue}$).*

It is necessary to balance the back pressure of the different LC channels during column switching, in order to prevent damage to column, pulse damper and to ensure good detection stability: the backpressures of both restrictors need to match the backpressure of both analytical columns (Figure 14 and 15). To achieve equal pressure levels, the length of the supplied restriction capillaries (restrictor 205 and restrictor 215) needs to be adjusted in a controlled way. The back pressure adjustment procedure consist in principle of the following steps:

1. Measurement of the length of each restrictor tubing (Res-205 and Res-215).
2. Stabilisation of the system with the CS valve in position A.
3. Measurement of the backpressure at both pumps in position A
4. Stabilisation of the system with the CS valve in position B.
5. Measurement of the backpressure at both pumps in position B
6. Calculation of the length of tubing that has to be removed from Res-205 and Res-215 to balance the backpressures of both LC channels.
7. Check the residual backpressure difference after cutting the restrictors, and repeat steps 1-7 to iterate to an optimum.

Detailed steps for back pressure adjustment procedure

An example of a convenient scheme to fill in the different values necessary to calculate the correct lengths of the restrictor tubing is given hereunder.

Tubing length restrictors		Flow off	Flow on	
Res-205 (cm)	…			
Res-215 (cm)	…	Zero offset	Position A	Position B
Pressure @ Pump 1 (bar)		…	… A1: col-res	… B1: col-col
Pressure @ Pump 2 (bar)		…	… A2: res-col	… B2: res-res

Note: In the ALEXYS system pump 1 is at the bottom position and pump 2 at the top position.

1. *Pressure measurement with CS valve in position A ('Flow on').*

   The Clarity sequence 'Start pressure recording' has 2 different method files that are comparable, except for the run duration: 'Flow-ON-stabilise' has a run time of 5 min, and 'Flow-ON' 30 min.

   - After having performed the system preparations (page 26), run the 'Start pressure recording' sequence.

   - Let the running system stabilise for 25 min. Read the pressure values of both pumps from the Clarity Device monitor and write them down. Check the pressure values again after 30 min to check if the system pressure is stabile (pressure difference < 0.2 bar compared to P reading at t=25 min).

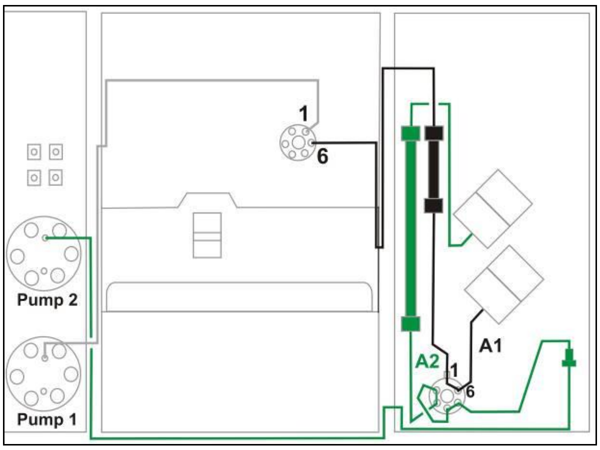
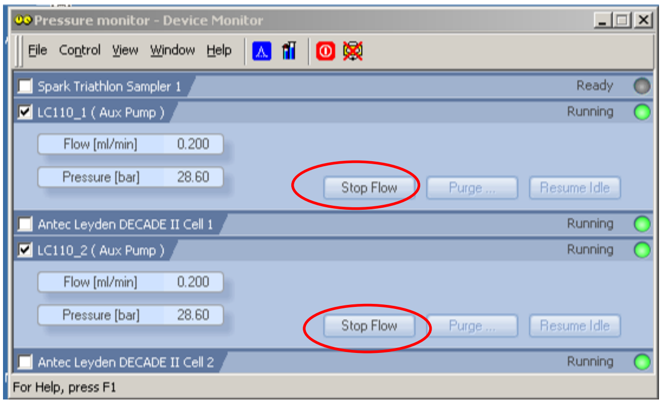


Figure 16. *Schematic drawing of the actual flow paths with CS valve in position A.*

2. *Measurement of the pump P zero offset ('flow off')*

The zero-offset of the pumps has to be recorded to be able to calculate the real pressure in the system.

- Stop the flow in the system with the 'Stop flow' buttons in the Device monitor window of Clarity.



- When the pressure has dropped to less than 3 bar, slowly open the purge valve of both pumps to depressurize the system completely.

- Read and write down the pressure of both pumps from the Clarity Device monitor.

3. *Pressure measurement with CS valve in position B ('flow on')*

The same procedure as for the pressure measurement with the valve in position A has to be repeated with the CS valve in position B.

- With the system pressure at 0 bar, switch the CS valve to position B with the manual control box.

- Prepare the next two sets of runs in the Clarity sequence 'Start pressure recording' by checking their lines.

- With the 'Resume' button, start the next set of lines in the 'Start pressure recording' sequence.

- Let the running system stabilise for 25 min. Read the pressure values of both pumps from the Clarity Device monitor and write them down. Check the pressure values again after 30 min to check if the system pressure is stabile (pressure difference < 0.2 bar compared to P reading at t=25 min).

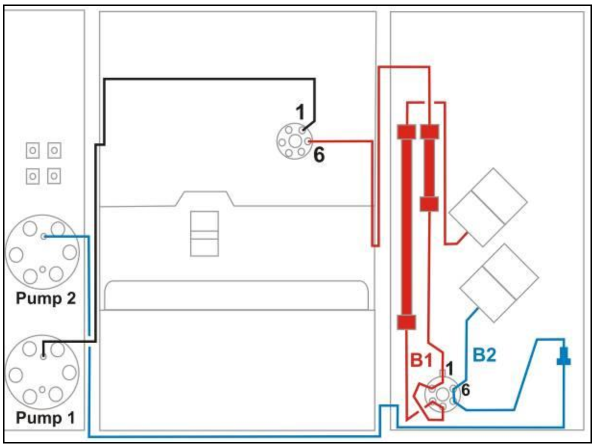


Figure 17. *Schematic drawing of the actual flow paths with CS valve in position B.*

4. <u>*Optimising restrictor lengths ('balancing the back pressure')*</u>

The length of tubing that has to be removed from both restrictors can now be calculated on the basis of the restrictor lengths and the pressure measurements (step 1 – 3).

- Open the Excel spreadsheet 'CS_rev01.xls'. It can be found in the Folder 'Clarity \Antec\Excel'. Fill in the required parameters in the designated cells marked **xxx** (in blue):

    1. Length of Res-205 and Res-215
    2. Zero-offset values of both pumps
    3. Pressure at pump 1 and pump 2 with valve in position A.
    4. Pressure at pump 1 and pump 2 with valve in position B.
    5. Use a safety margin of 20% to prevent cutting the tubing too short

- Stop the flow of both pumps and wait till the pressure drops to ambient before detaching the restrictors from the system to cut them.

- Remove exactly the reported lengths of tubing from the restrictors, using the supplied PEEK tubing cutter. (For reference, the calculation of the cutback lengths is described at the end of this appendix).

Some warning messages can appear in the Excel window were the advised cutback is displayed:

<u>'System was not stabile':</u> In this case an internal calculations detected a too large difference between the pressure recorded in position A and B. The most likely reasons are:

1. The system needed more time to stabilise: allow the system at least 30 min to stabilise. Do not open the oven door during the stabilisation time.
2. A leakage in the system (check all connections).

Despite this warning, it is safe to cut the advised length of tubing (it comes up with a conservative advise), but do increase the safety margin to 30%. The procedure has to be performed again, but the time is not wasted, as after cutting, the optimum is closer.

<u>'One of the restrictors appears to be cut too short':</u> In this case the internal calculations are highlighted in red for your convenience, and you can read how much too short the restrictor was cut. Replace the too small restrictor with the spare restrictor in this case, and repeat the procedure.

5. *<u>Pressure check of restrictors ('iteration towards optimum')</u>*

   The residual pressure imbalance is checked, and the restrictors have to be cut again to come closer to their optimum length.

   - Repeat steps 1 to 4 and fill in the new values for tubing length and system pressures in the Excel sheet.
   - Reduce the safety margin to 10%.
   - Stop the flow of both pumps and wait till the pressure drops to ambient before detaching the restrictors from the system to cut them.
   - Remove the calculated lengths of tubing from the restrictors, using the supplied PEEK tubing cutter.

6. *Pressure check with nearly optimised restrictors*

When the restrictors are so far optimised that the calculated cut-back length of both restrictors is less than 2 cm, the flow does not need to be stopped when switching between position A and B.

- Start the pumps and let the system stabilise again (about 30 min).
- Prepare a run of 'Flow-ON' in the Clarity sequence 'Start pressure recording' by checking its line.
- With the 'Resume' button, start the run in the 'Start pressure recording' sequence.
- After 5 min of running the system, write down the pressure of both pumps from the Clarity Device monitor. Switch the CS valve with the manual control box, and write down the pressure again after 5 more minutes.
- Fill in the new values for tubing length and system pressures in the Excel sheet.
- Stop the pumps, let the system pressure come down to ambient and remove the calculated lengths of tubing from the restrictors, using the supplied PEEK tubing cutter.
- Repeat step 6 to check the final pressure and adjust again if necessary.
- When the pressure difference between pos A and B for the pressures at pump 1 and 2 are both <1 bar, the system is ready for use!

In case one of the restrictors wase cut too short (resulting in a pressure difference of more than 1 bar) repeat the procedure using the spare restrictor tubing supplied in the kit (p/n 250.0901).

Calculations Pressure Adjustment Procedure

The restrictor backpressure per length unit (bar/cm) can be calculated in the following way:

$$C = \frac{P_{B2} - P_{zero\text{-}offset\ pump\ 2}}{(L_{res\text{-}205} + L_{res\text{-}215})}$$

The length of tubing (cm) to be remove from restrictor 205 can be calculated in two ways:

$$L_{cut\text{-}back\ I,\ res\text{-}205} = \frac{(P_{A2} - P_{zero\text{-}offset\ pump\ 2}) - (P_{B1} - P_{zero\text{-}offset\ pump\ 1})}{C}$$

$$L_{cut\text{-}back\ II,\ res\text{-}205} = \frac{(P_{B2} - P_{zero\text{-}offset\ pump\ 2}) - (P_{A1} - P_{zero\text{-}offset\ pump\ 1})}{C}$$

The length of tubing (cm) to be remove from restrictor 215 can be calculated in two ways:

$$L_{cut\text{-}back\ I,\ res\text{-}215} = \frac{(P_{A1} - P_{zero\text{-}offset\ pump\ 1}) - (P_{B1} - P_{zero\text{-}offset\ pump\ 1})}{C}$$

$$L_{cut\text{-}back\ II,\ res\text{-}215} = \underline{(P_{B2} - P_{zero\text{-}offset\ pump\ 2}) - (P_{A2} - P_{zero\text{-}offset\ pump\ 2})}$$

APPENDIX II

Online Microdialysis with three parallel lines

This appendix contains additional installation information for the LC connection kit p/n 180.0166A LC connection kit TCC I.

Please follow the specific steps of the loop adjustment procedure very carefully to ensure proper operation of the ALEXYS TCC online system.

NOTE: In this kit only the LC connections necessary for the LC system are provided. Accessories for the micro dialysis part of the system such as, probes, syringes, syringe pumps and tubing have to be ordered separately.

A label set TCC (p/n 802.0406) is provided to identify the three different flow paths in the system for your convenience. Follow the labelling instructions in this document carefully.

Installation of guard column for filtering

To filter the mobile phase stream coming from the LC 110 pump, a guard column has to be mounted on the inlet of the 4-port manifold. See figure 18, be aware that the actual manifold supplied with the system (p/n 250.1805) is already prefixed on a mounting bracket. The filter consists of a column guard holder 1 mm (p/n 250.1504) with ALF 3um drop-in cartridge 10x1.0mm (p/n 250.1520). The guard column and cartridge are supplied with the ALEXYS system (not included in the LC connection kit itself).

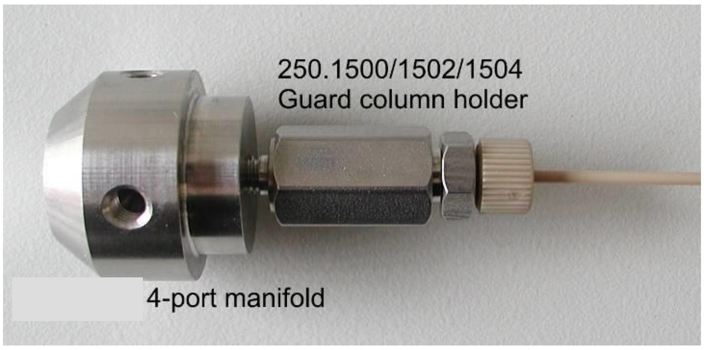


Figure 18. *4-port manifold with guard column holder. Notice that the manifold supplied with the system is already prefixed on a mounting bracket*

Loop adjustment procedure

For the installation of the ALEXYS TCC online systems it is necessary to prepare tailor-made sample loops with a volume of about 2 – 5 µL. The chosen sample loop volumes are dependent of the application run time, microdialysis pump speed and necessary time resolution. Choose the sample loop volumes with the following formula:

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

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), ideally the 3 sample loops would have a volume of (10 - 1) / (3 x 0.9) = 3.3 µL.

To make sample loops with a designated volume, a sample loop assembly kit (p/n 180.0254) is provided in the LC connection kit. 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 into the OMD valves.

Before cutting the tubing to a certain length (linearly related with volume), the exact internal diameter of the red-striped PEEK tubing has to be measured with the procedure below:

- Prepare a solution of water with 5% methanol (degassed for 15 min in a sonic bath) and connect to the pump.
- Measure the exact length of the provided PEEK tubing with a ruler.
- Connect the PEEK tubing to the outlet of the pulse damper. Place the other end in a waste bottle.

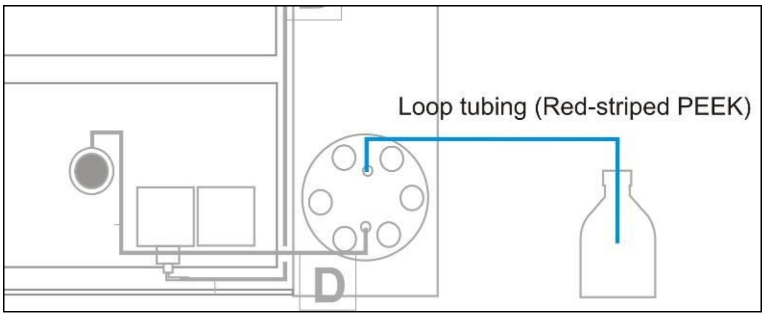


Figure 19. *Configuration to determine the back pressure of the piece of PEEK tubing in the loop tubing assembly.*

- Start the Clarity program and activate the 'Device monitor'.

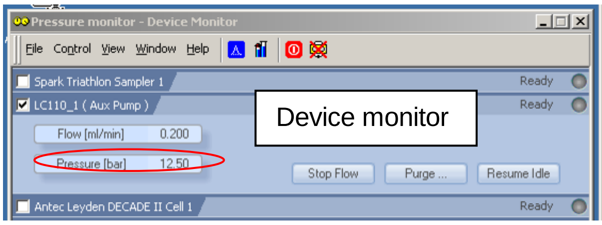


Figure 20. *Screen dump of Clarity Device Monitor window with accurate pressure read out.*

- With the system at ambient pressure (opened purge valve) determine the zero-offset pressure value from the Device monitor. WARNING: Do <u>not</u> open the purge valve when the system is still under pressure (>3 bar).
- Set a flow rate of 0.8 mL/min and let the pressure stabilise for 25 min and determine the pressure. At t= 30 min determine the pressure again to check if the pressure is stabile. The pressure difference between P at t= 25 min and 30 min must not be larger than 0.1 bar. In case equilibrium is not yet reached, wait longer.
- Determine the 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 Excel spreadsheet "Antec Calculator" (it can be down loaded from the Distributors section of the Antec support site if you need a copy). Open the tab "Capillary PnV" 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 µL – 5 µL).
  4. The flow rate used during the test (800 µL/min)

- The spreadsheet will calculate the required tubing length for the specified loop volume.
- Cut three pieces of PEEK tubing of exactly the calculated length using the supplied tubing cutter.
- Connect the sample loops to port 4-7, port 8-11 and port 1-12.