

ALEXYS

installation guide

LC connection kit, TCC I



Symbols

The following pictogram is used in this installation guide:



Caution

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.



Use proper eye and skin protection when working with solvents.



Use of this product outside the scope of this guide may present a hazard.

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.

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CHAPTER 1

Installation guide

Introduction

The LC connection kit TCC I (p/n 180.0166) is a tailor-made kit containing all tubing assemblies to make the necessary LC connections in a complete ALEXYS 100 LC-EC system with a DECADE II TCC in an online micro dialysis configuration (parallel or time resolution configuration). This procedure specifically describes the installation of these assemblies. It is assumed that:

Equipment has been unpacked and checklists are verified. The installation procedures of ALEXYS is followed as described in manuals/ installation guides, in the order: OR 100, AC 100, LC 100, DECADE II and OMD valve options.

Follow the installation instructions in the ALEXYS installation checklist and user manuals and keep this document at hand. Sections describing installation of liquid tubing are referring to this document.

Order	Part no.	User manual / installation guide
	185.0010	ALEXYS data system
1	184.0010	OR 100 organizer rack
2	183.0010	AC 100 acquisition controller
3	182.0010	LC 100 pump
5	171.0010	DECADE II
6	250.7008	Installation guide OMD valve option

Before complete installation, it is advised to passivate all metal parts in the HPLC system (for procedure see page 27).

For optimal performance all metal parts in the system should be passivated with a 15% nitric acid solution for 20 minutes.

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 ALEXYS LC connection kit has been inspected and tested to ensure the best possible performance.

Tools

The following tools are necessary for the installation of the LC connection kit:

- 5/16" - 1/4" wrench
- Small flat head screw driver
- PEEK tubing cutter



Figure 1 Photograph of listed tools. From left-to-right: 5/16" - 1/4" wrench, flathead screwdriver and tubing cutter.

Schematic drawing of all ALEXYS LC connections

TCC parallel sampling configuration

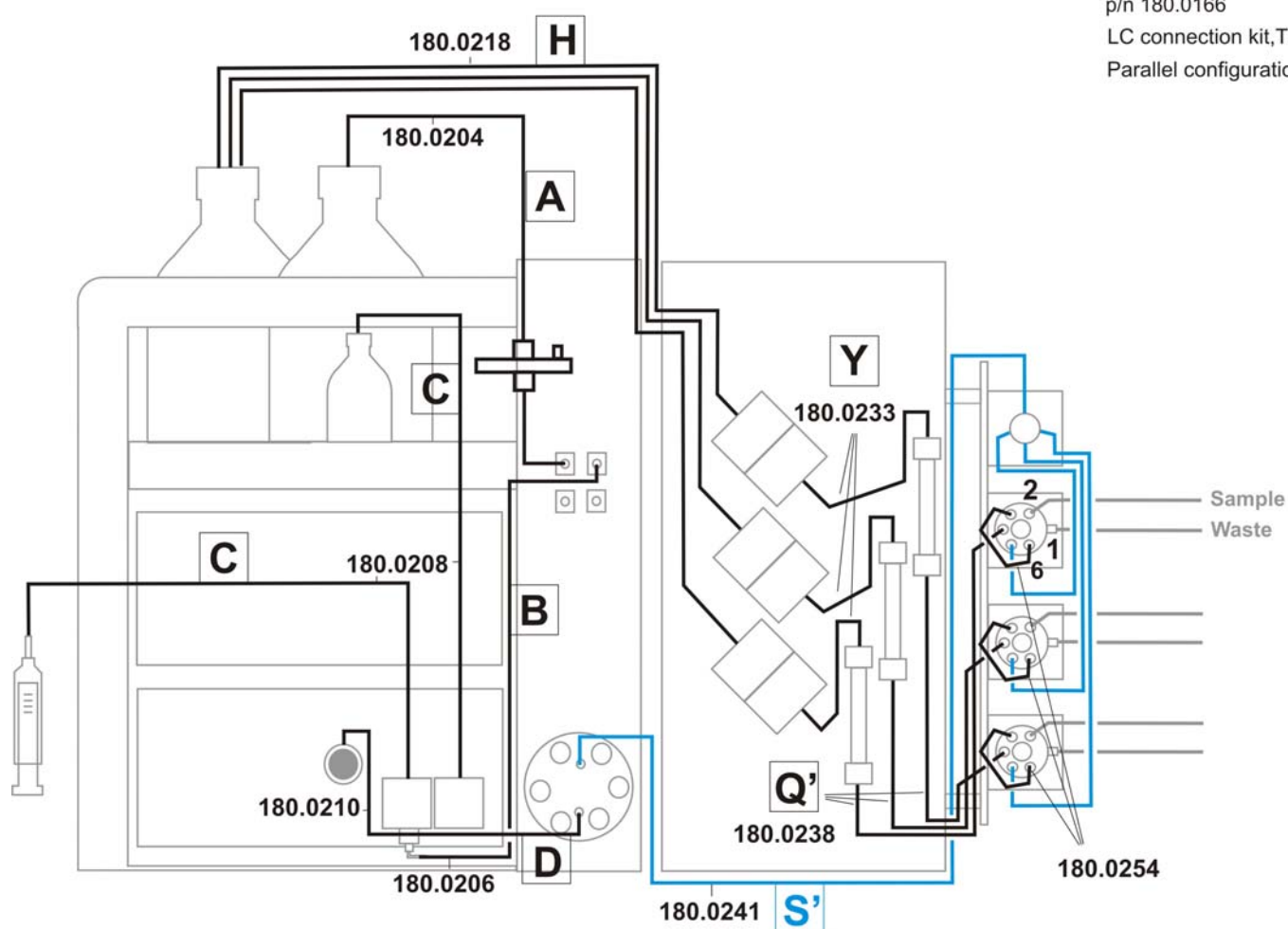


Figure 2. Schematic drawing of all LC connections available in the LC connection kit, TCC I in the parallel sampling configuration [a] The numbers refer to the part numbers on the plastic bags in which the assemblies are shipped. [b] For identification each individual assembly has a vinyl label with letter code (A, B etc.) attached.

TCC time resolution configuration

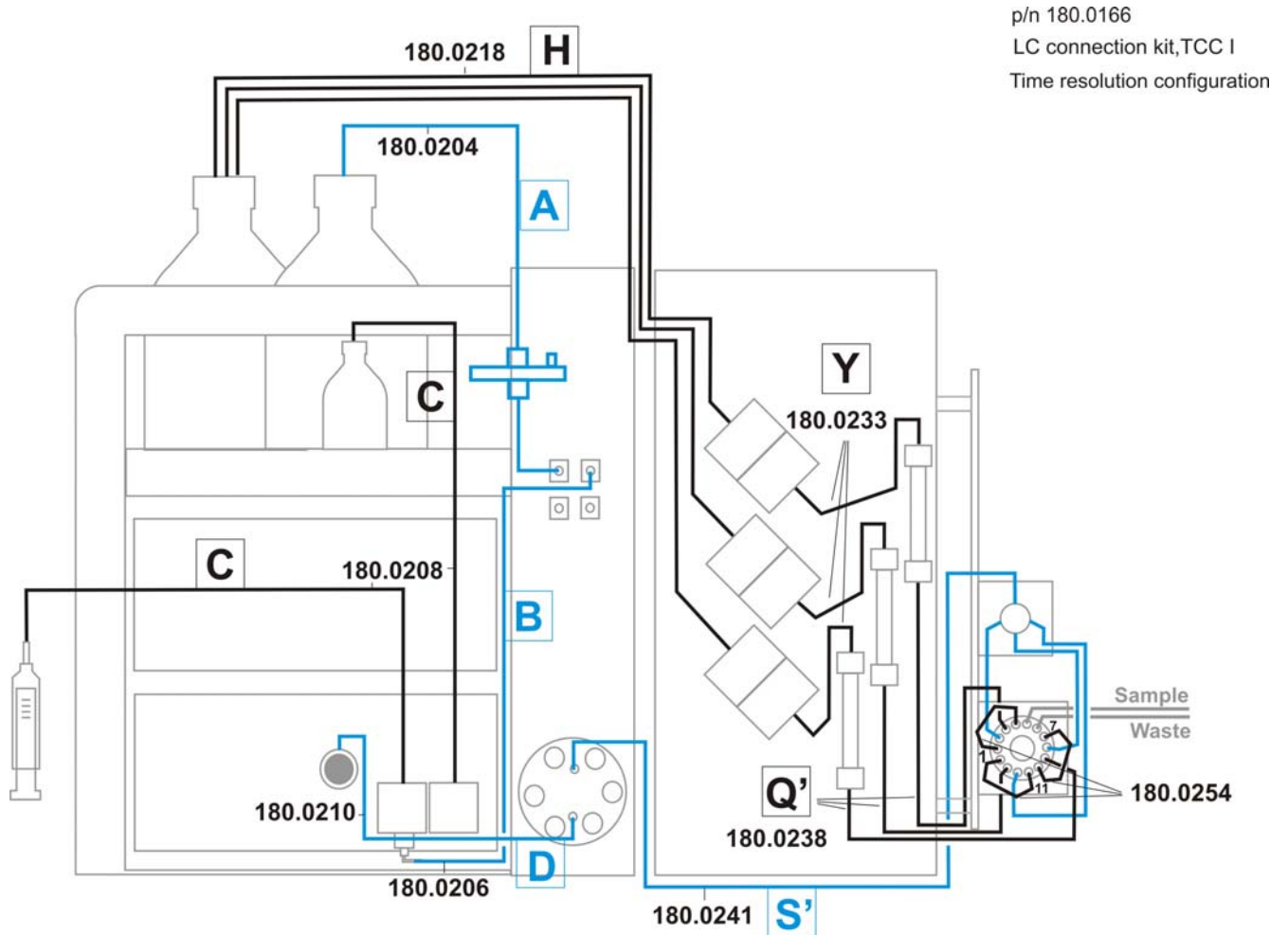


Figure 3. Schematic drawing of all LC connections available in the LC connection kit, TCC I in the time resolution configuration [a] The numbers refer to the part numbers on the plastic bags in which the assemblies are shipped. [b] For identification each individual assembly has a vinyl label with letter code (A, B etc.) attached.

Important installation information

For the installation of the ALEXYS TCC online systems it is necessary to prepare loops using the supplied red-striped PEEK tubing in the loop tubing assembly (p/n 180.0254).



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 tubing assemblies

The installation procedure of the LC connections consist of the following steps:

1. **Assemblies A**, OR 100 degasser inlet assembly (p/n 180.0204):
Place the end of the FEP tubing A which is cut under an edge in the mobile phase bottle as depicted in figure 4.

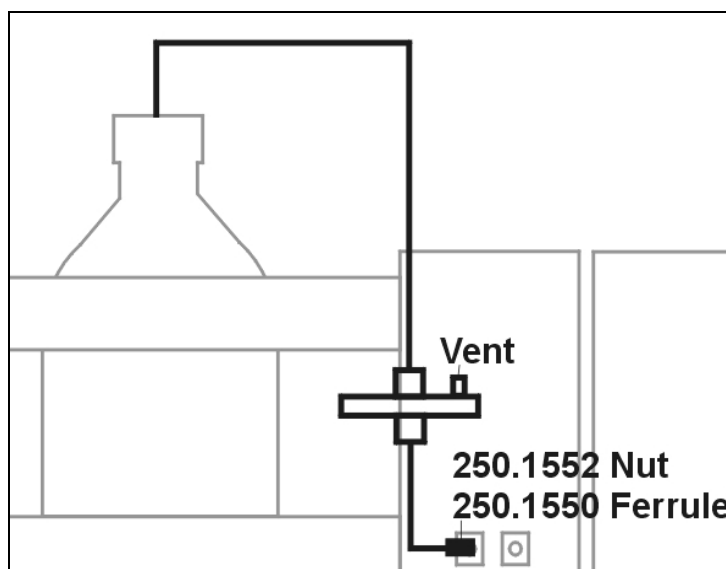


Figure 4. Assembly A: Connection between bottle - degasser.

Make sure that the vent of the in-line filter is facing towards the solvent bottle. Prepare a solution of 200 mL 5% v/v Methanol/Water to initially flush the system. Open the filter (vent) and draw the flushing solution through the tubing using a syringe until the top is completely wetted and the upper compartment is air bubble free. Close the vent.

Connect the syringe to the outlet tubing and gently draw the plunger to fill the outlet tubing with solvent. Remove the syringe and hold the outlet tubing well below the liquid level in the flushing solution bottle. The outlet tubing should spontaneously siphon solvent. If this is not the case consult the trouble-shooting section in chapter 3.



For detailed operating instructions of the Whatman IFD in-line filters see chapter 3 in this installation guide.

When all tubing is filled with mobile phase connect the outlet to the inlet of the degasser using the supplied nut and ferrule as depicted in figure 5.

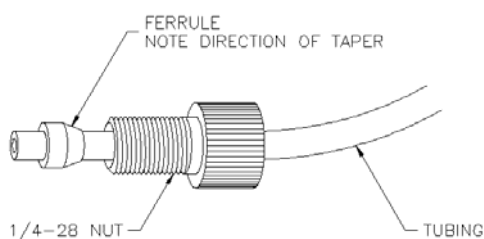


Figure 5. Configuration of 1/4-28 Nut, Ferrule and Tubing.

2. **Assemblies B**, OR 100 degasser outlet assembly (p/n 180.0206):
For the LC connections between the degasser and the pump the assembly B is provided.

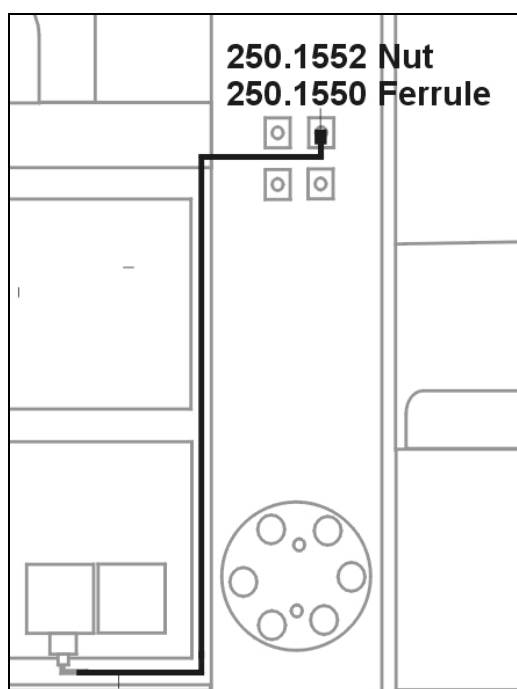


Figure 6. B: Connection between degasser – pump.

Connect the end of the FEP tubing without label ("LC 100 side") to the degasser output using the supplied nut and ferrule (p/n 250.1552 and 250.1550). Connect the other end of the tubing to the low-

pressure inlet of the pump (bended metal tubing on bottom of pump head). Apply force to push the FEP tubing **all the way up** the bended metal tubing. Otherwise leakage will occur at this point! The second assembly B (depicted in blue) should be used for the delivery of mobile phase to pump 2. For priming instructions of the pump please consult the manual of the LC 100 (p/n 182.0010).



Never push the solvent through the channel of the degasser.

3. **Assembly C**, LC 100 piston back flush assembly (p/n 180.0208)
Connect two pieces of 1/16" FEP tubing to the piston wash connections on the top-side of the pump head. Use the supplied stainless steel nuts and ferrules (p/n 250.1564 and 250.1562). Place one tubing in the bottle with wash solvent. Connect the syringe (p/n 182.0408) supplied in the LC 100 ship kit to the other tubing. Fill the piston wash with wash solvent by withdrawal of the syringe.

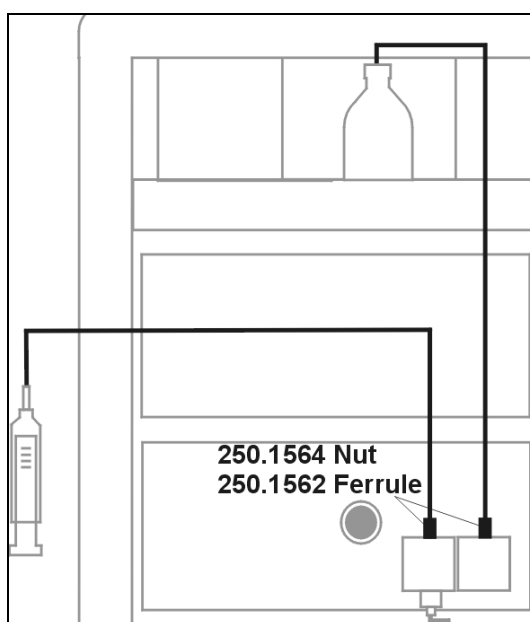


Figure 7. Assembly C: Connection of piston wash of LC 100 pump.

Place one tubing end in the bottle with wash solvent as depicted in figure 7. Connect the syringe (p/n 182.0408) supplied in the LC 100 ship kit to the other tubing. Install the interconnection tubing (L) and fill the piston wash with wash solvent by withdrawal of the syringe.

4. **Assembly D**, OR 100 pulse damper inlet assembly (p/n 180.0210):
For both pumps an assembly D is provided. Connect the pre-shaped stainless steel tubing to the high-pressure outlet of the pump and the inlet of the pulse damper with the supplied nuts and ferrules (p/n 250.1564 and 250.1562).

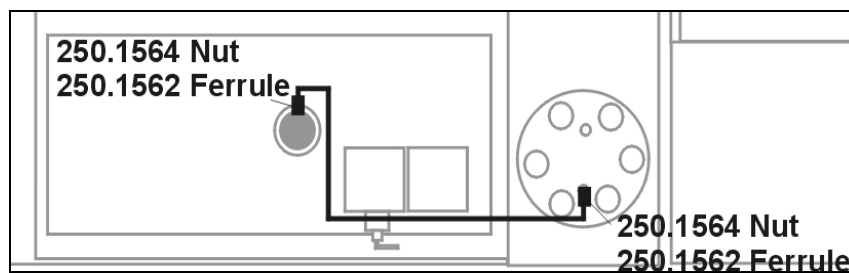


Figure 8. Assembly D: Connection between pump – pulse damper.

5. **Assembly S'**, OR 100 PD outlet assembly TCC (p/n 180.0241):
Assembly S' is provided to connect the OR 100 pulse damper outlet with three inlet ports on the 14-port OMD valve option, or to an inlet port on three 6-port valves in case of the TCC parallel configuration. In assembly S' a 4-port manifold is integrated to divide the solvent stream coming from the pump into three separate flow paths. For the installation of this assembly follow the step-by-step instructions below:
- a. First fix the 4-port manifold plate onto the DECADE II valve mounting panel as depicted in the next figure.

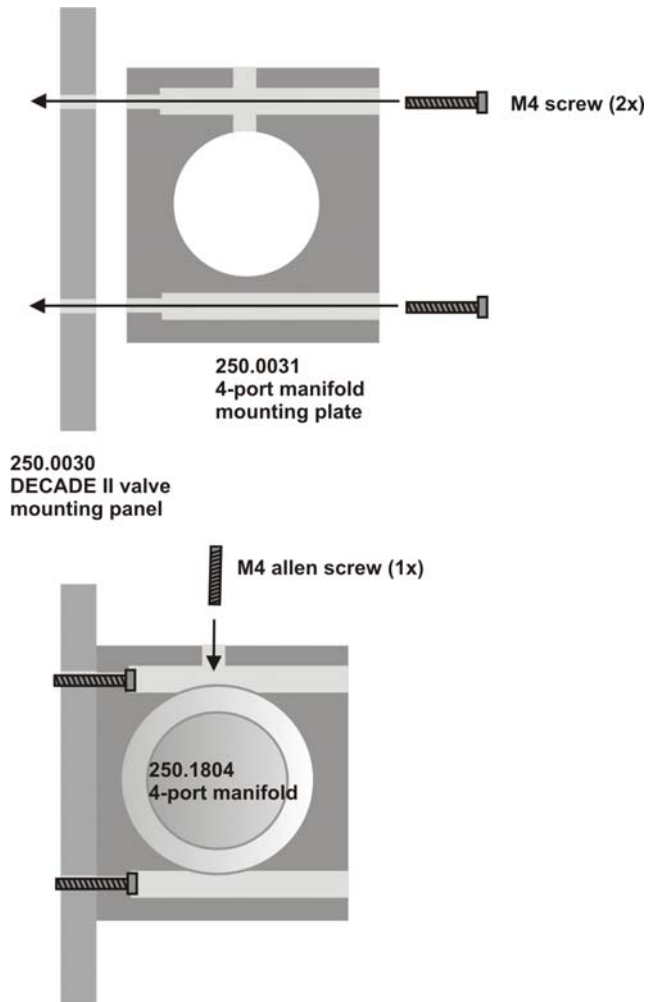


Figure 9. Assembling the 4-port manifold mounting plate.

Fix the plate into the valve panel using the two supplied M4 screws. Subsequently, insert the manifold with the 4-port head facing towards the front and fix the manifold by means of the M4 Allen screw located in the thread hole on the top of the mounting plate.

- b. Mount a guard column holder with the appropriate guard column cartridge into the inlet of the 4-port manifold as shown in the figure below. The guard column holder and cartridges are not included in the LC connection kit itself but are a part of the ALEXYS system solution. See the ALEXYS system solution checklist.



Figure 10. 4-port manifold with Guard column holder.

- c. Connect one end of the 75 cm PEEK tubing to the outlet of the OR 100 pulse damper, using the supplied PEEK fingertight p/n 250.1570 (see figure 11). Guide the tubing under the bottom plate of the DECADE II to the other side, and connect the other end of the tubing to the guard column on the 4-port manifold using a fingertight (p/n 250.1570).
- d. Block the top outlet of the 4-port manifold using a PEEK fingertight plug (p/n 250.1574).
- e. In case of the installation of a TCC parallel configuration, see figure 11 on the next page. Connect the ends of the three 30 cm long PEEK tubing pieces to the three outlet ports of the manifold using fingertights p/n 250.1570. Subsequently connect the opposite tubing ends to port 5 of the three 6-ports valves using the supplied narrow fingertights (p/n 250.1572).

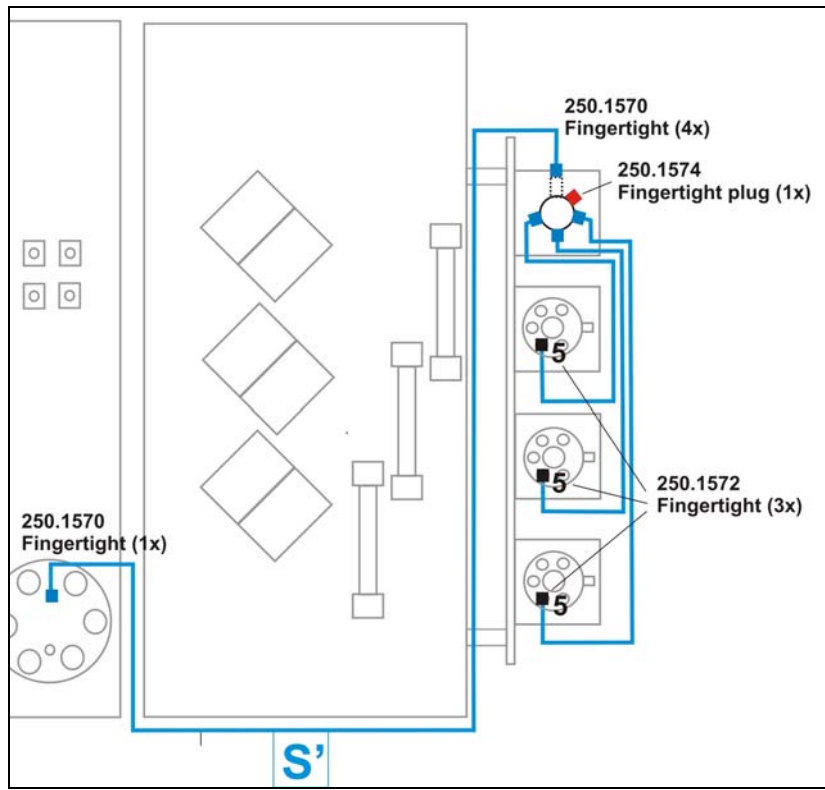


Figure 11. Connection of assembly S', TCC parallel configuration.

- f. For the installation of the TCC time resolution configuration, see figure 12 below.

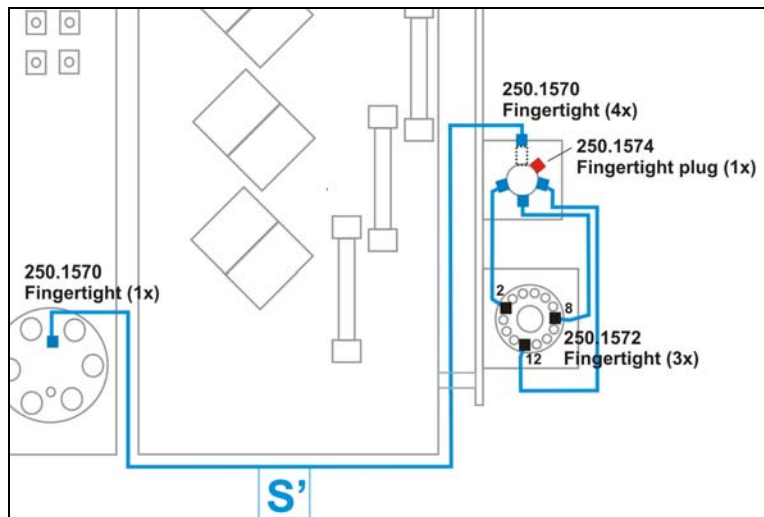


Figure 12. Assembly S', TCC time resolution configuration.

Connect the ends of the three 30 cm long PEEK tubing pieces to the three outlet ports of the manifold using fingertights p/n 250.1570. Subsequently connect the opposite tubing ends to port 2, 8 and 12 of the 14-ports valve using the supplied narrow fingertights (p/n 250.1572).

6. **Loops.** *Loop tubing assembly (p/n 180.0254):* A loop tubing assembly is provided to prepare tailor-made loops, with a volume between 2 – 5 μL , for the TCC online system. 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.

To determine the exact internal diameter of the loop tubing provided following the procedure below:

- Determine the exact length of the provided PEEK tubing using a ruler.
- Install the AC 100 analog input cable for DECADE II (p/n 183.0508). Plug the 9 pins sub-D connector into "Channel 1/2" on the back of the AC 100. Connect the DIN plugs "Analog 1" to pump 1.
- Connect the piece of red-striped PEEK tubing to the outlet of the pulse damper. Place the other end in a waste bottle.
- Prepare a mobile phase consisting of water with 5% methanol.
- Open the system file "LC_100_P-test_xxyy.smt" in the ALEXYS HPLC data system software. This system file can be downloaded from the Antec distributors web site. This system file is used to monitor the back-pressure of the.

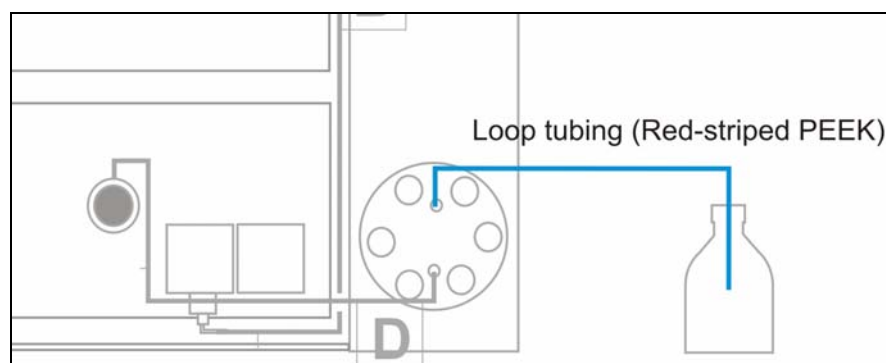


Figure 13. Configuration to determine the back pressure of the piece of PEEK tubing in the loop tubing assembly.

- Open the system file "LC_100_P-test_xxyy.smt" in the ALEXYS HPLC data system software. This system file can be downloaded from the Antec distributors web site. This system file is used to monitor the back-pressure of the PEEK tubing.
- Record a pressure traces of pump 1 for two minutes with the flow of the pump switched-off to determine the zero-point. After 2 minutes start pump 1 manually at a flow rate of 800 uL/min.
- Record the pressure profile for at least 20 minutes at the relevant flow rate (see example trace in figure 14). Check after 20 minutes if the back pressure is stabile (pressure line running horizontally). Continue recording the pressure signal in case equilibrium is not yet reached.

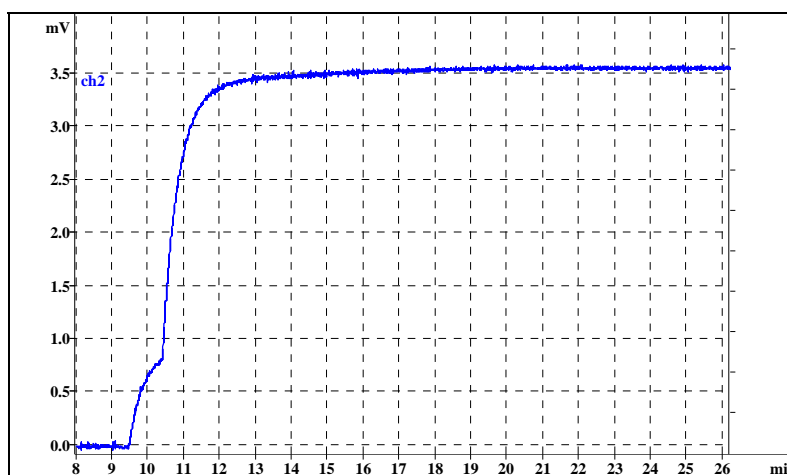


Figure 14. Example of a pressure trace recorded with ALEXYS data system software.

- Determine the back pressure (with two decimals) of the PEEK tubing using the recorded pressure traces (1 mV = 1 MPa):

$$P_{\text{PEEK tubing}} = P_{\text{at 800 uL/min}} - P_{\text{at 0 mL/min}}$$
- Open the Excel spreadsheet "Antec Calculator" which can be downloaded from the Antec distributors web site. 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 flowrate (uL/min)

- The spreadsheet will calculate the necessary tubing length for the required loop volume.
- Cut three pieces of PEEK tubing of exactly the calculated length using the supplied tubing cutter.

Parallel configuration.

In case of the parallel configuration connect the loops as depicted in figure 15. Connect the loop to port 3 and 6 of the 6-port valve using the supplied stainless steel nut and ferrule (p/n 250.1560 and 250.1558 respectively).

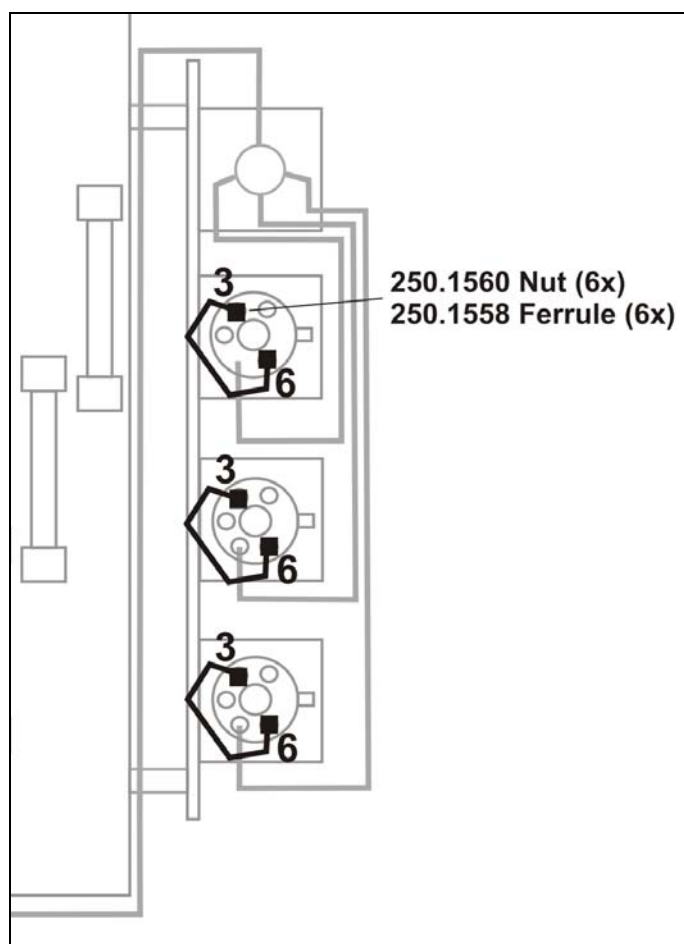


Figure 15. Installation of the tailor-made loops in the case of the TCC parallel configuration.

Time resolution configuration.

In case of the time resolution configuration connect the loops as depicted in figure 16. Connect the loops 1, 2 and 3 to port 1/4, 7/10 and 11/14 of the 14-port valve using the supplied stainless steel nut and ferrule (p/n 250.1560 and 250.1558 respectively).

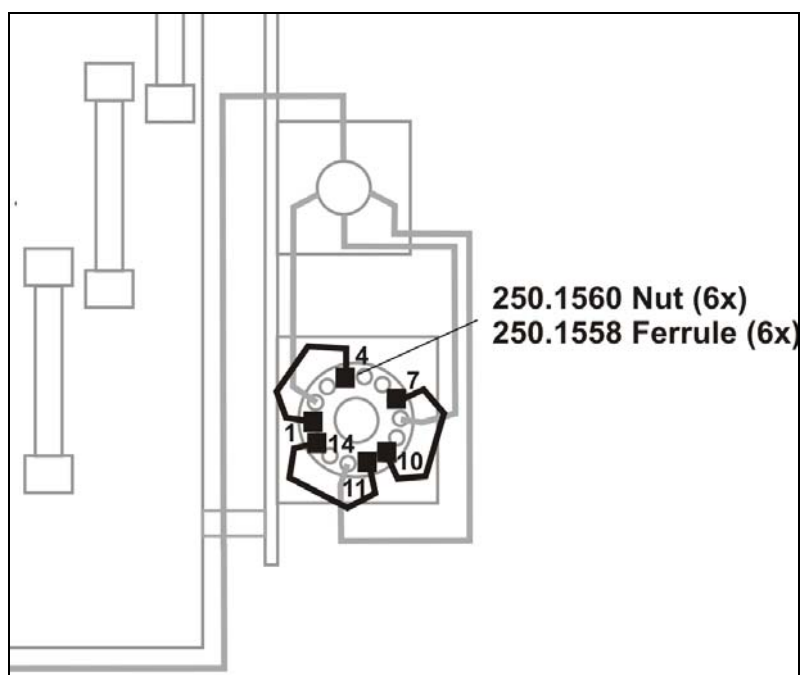


Figure 16. Installation of the tailor-made loops in the case of the TCC time resolution configuration.

7. **Assembly Q'**, AS 100 outlet assembly -2, micro (p/n 180.0238): three assembly Q' are provided to connect the OMD valve(s) to the three columns in the system.

TCC parallel configuration:

Connect one end of the PEEK tubing pieces to port 4 of the three 6-port valves as depicted in the figure below, using the supplied narrow fingertights (p/n 250.1572).

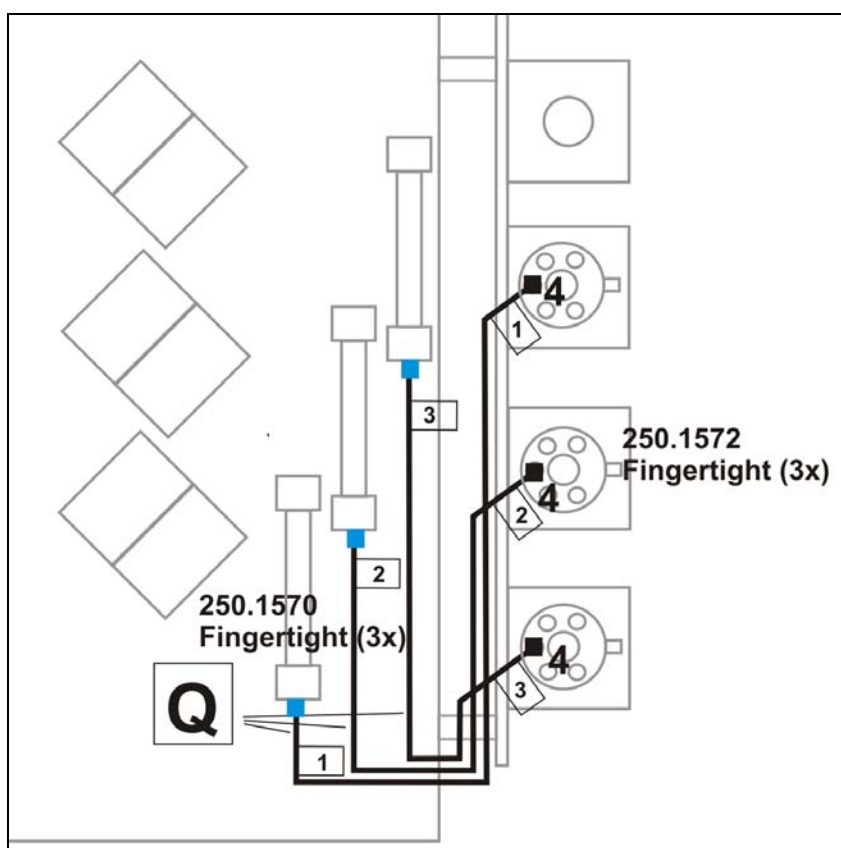


Figure 17. Assembly Q', TCC parallel configuration.

Fix the corresponding labels "1", "2" and "3" from the TCC label set (p/n 802.0406) on the tubing for identification as depicted in figure x on the previous page.

Guide the three tubing ends through the lower-right tubing hole in the DECADE II. **Switch on the flow of pump 1 and make a "wet"**

connection (dripping tubing) with the column to prevent the introduction of air bubbles into the column. Connect the tubing ends to the inlet of the HPLC column using the supplied PEEK fingertights (p/n 250.1570).

TCC time resolution configuration:

Connect one end of the PEEK tubing pieces to port 3, 9 and 13 of the 14-port valve as depicted in the figure below, using the supplied narrow fingertights (p/n 250.1572).

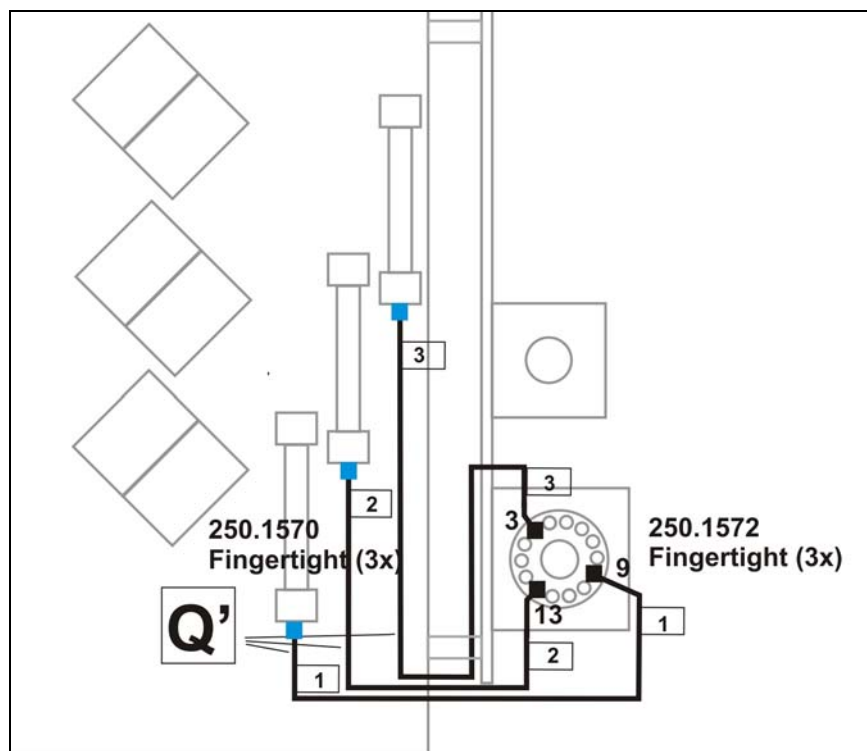


Figure 18. Assembly Q', TCC time resolution configuration.

Fix the corresponding labels "1", "2" and "3" from the TCC label set (p/n 802.0406) on the tubing for identification as depicted in figure x on the previous page.

Guide the three tubing ends through the lower-right tubing hole in the DECADE II. **Switch on the flow of pump 1 and make a "wet" connection (dripping tubing) with the column to prevent the**

introduction of air bubbles into the column. Connect the tubing ends to the inlet of the HPLC column using the supplied PEEK fingertights (p/n 250.1570).

8. **Assembly Y, DECADE II inlet assembly 2, micro (p/n 180.0233):** Three DECADE II inlet assemblies are provided in the ship kit to connect the columns to the corresponding flow cells. Connect one end of the PEEK tubing to the outlet of the HPLC column using the supplied PEEK finger tight (p/n 250.1570). Connect the other end of the tubing to the inlet of VT-03 flow cell using the KEL-F VT-03 fingertight (p/n 110.1045). Do the same for the second and third column and cell. Installation of assembly Y for the parallel configuration is shown in figure 18.



Make sure that the columns and cells are connected in exactly the same order as depicted in figure 19 or 20.

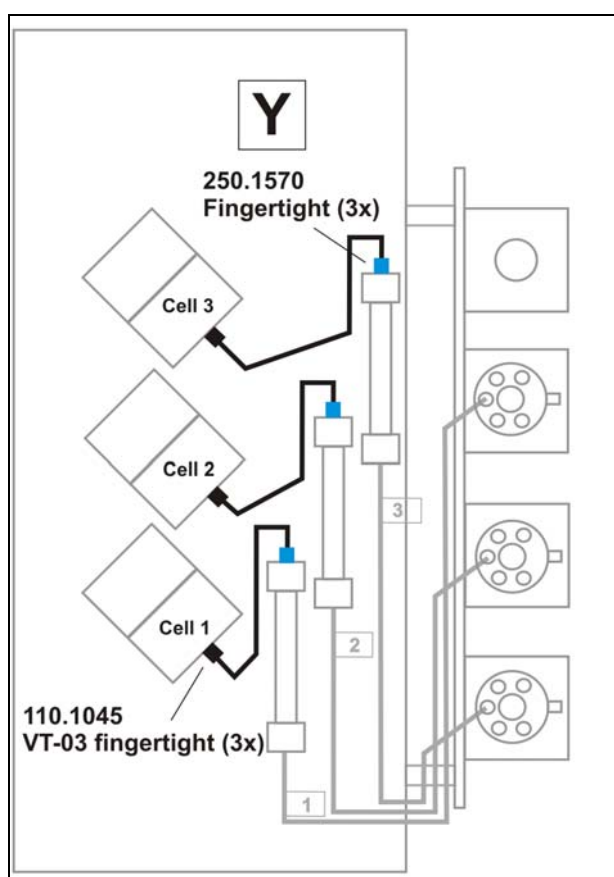


Figure 19. TCC Parallel configuration: Assembly Y: connection between column - flow cell.

Installation of assembly Y for the time resolution configuration is shown in figure 20.

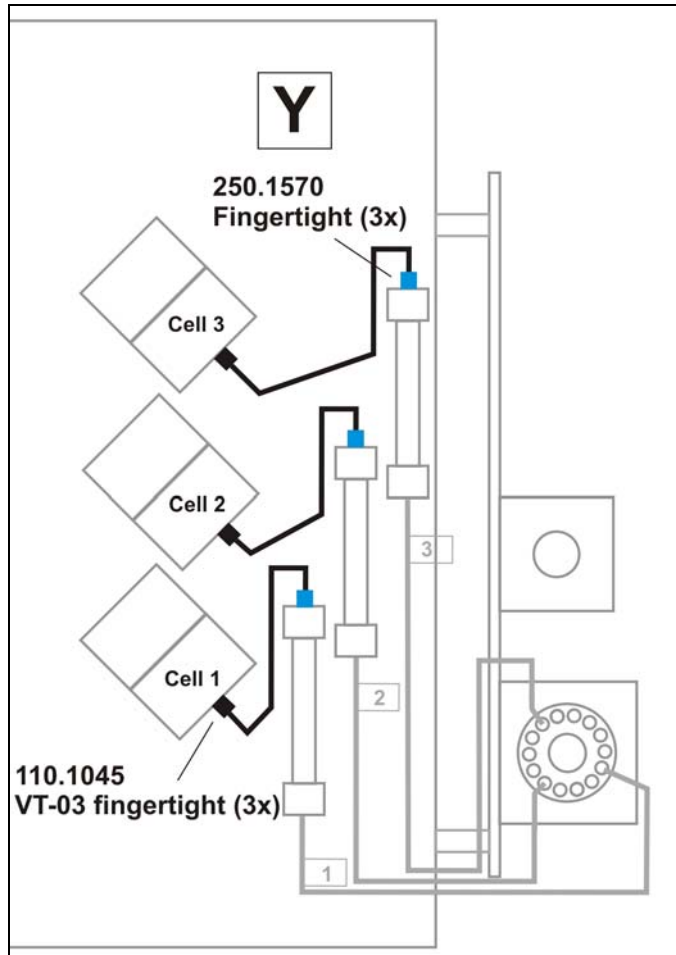


Figure 20. *TCC time resolution configuration: Assembly Y: connection between column - flow cell.*

Detailed information about the installation of the flow cell can be found in the manual of the DECADE II (p/n 171.0010). Make sure that the flow cell 1, 2 and 3 are electrically connected to the corresponding connectors marked "cell 1", "cell 2" and "cell 3", respectively.

9. **Assembly H** *DECADE II outlet assembly (180.0218)*: Three assemblies H are provided to connect the flow cells to the waste bottle(s). Connect the PEEK tubing to the outlet of the VT-03 flow cell using the KEL-F fingertight. Guide the tubing through the tubing hole in the DECADE II into the waste bottle as depicted in figure 21.

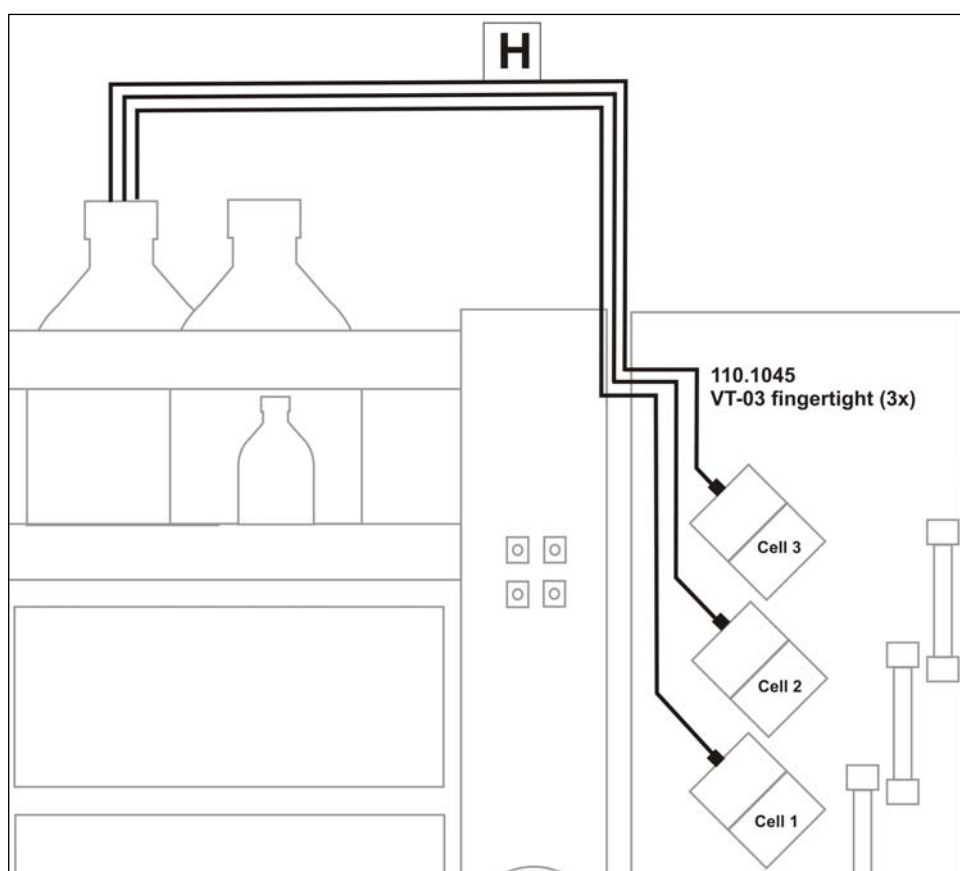


Figure 21. *Assembly H: Connection between cell – waste bottle.*

This completes the installation of all tubing and connectors of the ALEXYS 100 LC-EC kit. After successful installation a number of parts in the individual ship kits will be superfluous.

For reference all tubing dimensions of the assemblies in the LC connection kit are listed in the table below:

Table I. Tubing dimensions and material.

assembly	Material	I.D. (Inch)	O.D (Inch)
A	FEP	1/16	1/8
B	FEP	1/16	1/8
C	FEP	0.03	1/16
D	Stainless steel	0.02	1/16
Q'	PEEK (Pink)	0.0025	1/16
Y	PEEK (Pink)	0.0025	1/16
S'	PEEK (Blue)	0.01	1/16
loops	PEEK (Red)	0.005	1/16
H	PEEK (Orange)	0.02	1/16

Passivation procedure

For optimal performance all metal parts in the system should preferably be passivated with a 15% nitric acid solution for 20 minutes.



Make sure that all parts that are not acid-resistant such as: nylon inlet filters, column and flow cell are not connected during this step.

Make the connections as depicted in figure 22 for the parallel configuration. In case of the time resolution configuration see figure 23. Use the Teflon tubing (p/n 182.0400) supplied in the ship kit of the pump to deliver the nitric acid solution to the pump. The acid should be flushed through the pump, the pump tubing, the dampener, the valves(s) and to waste. **Flush the injector in both the load and the inject position!** After flushing with nitric acid, the system must be thoroughly flushed with demi-water. Make sure that no traces of nitric acid are left in the tubing or pulse dampener (check with pH paper).

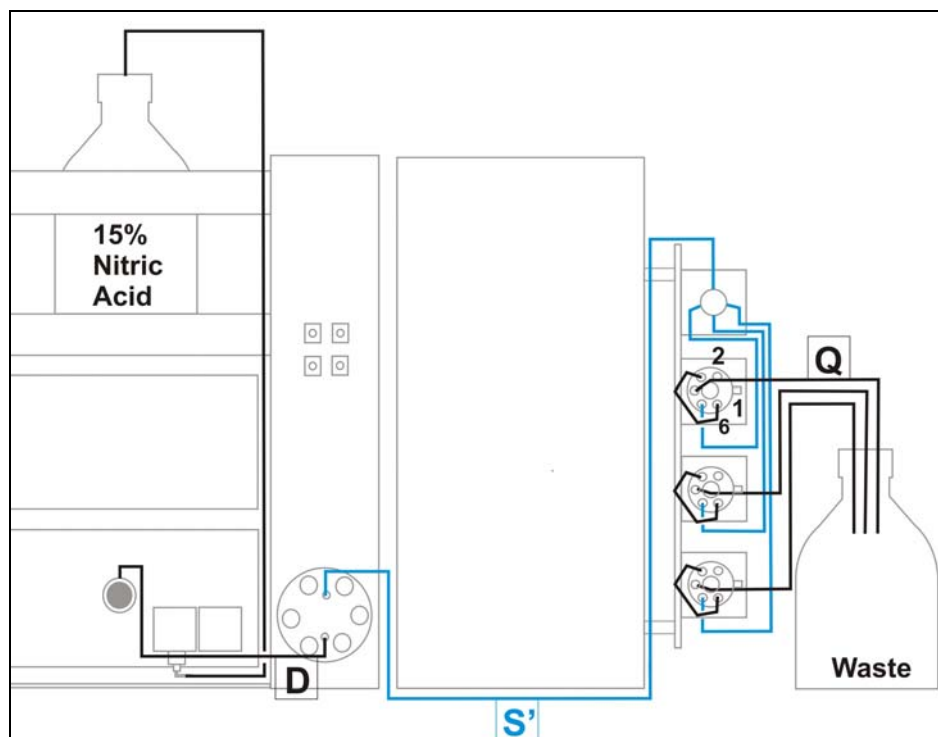


Figure 22. System passivation in case of the parallel configuration.

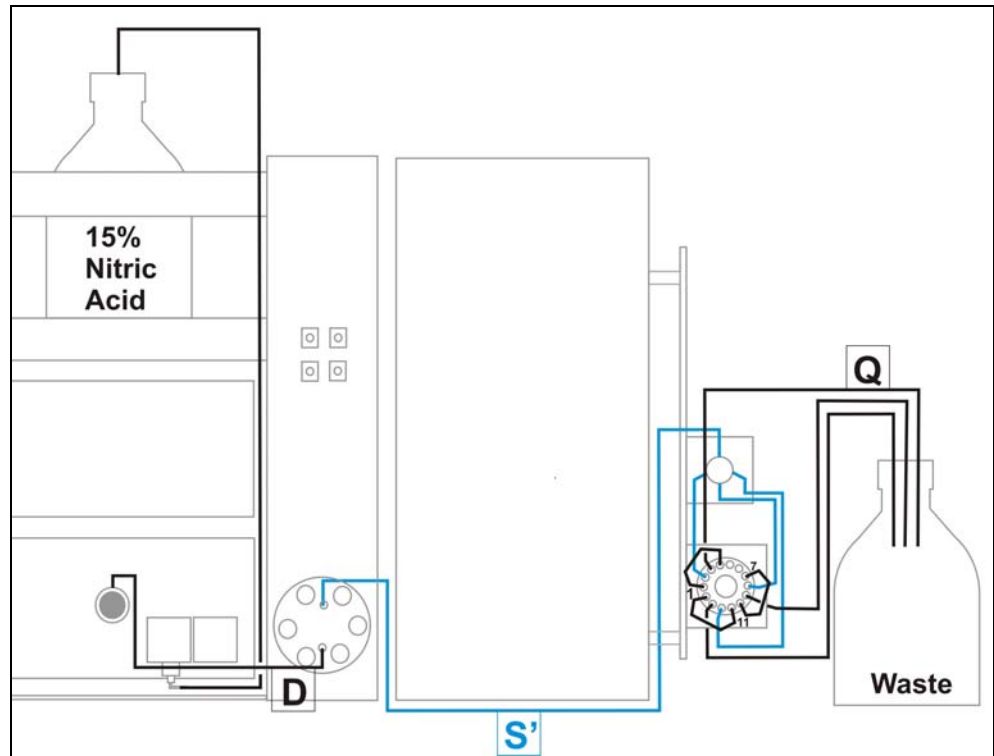


Figure 23. System passivation in case of the time resolution configuration.

CHAPTER 2

Accessories

The LC connection kit is shipped with a number of parts. The listing in the table below may not be complete, see checklist of delivery for complete listing.

Table II. Accessories LC connection kit.

Part no	assembly	Description
180.0204	A	OR 100 degasser inlet assembly
180.0206	B	OR 100 degasser outlet assembly
180.0208	C	LC 100 piston back flush assembly
180.0210	D	OR 100 pulse damper inlet assembly
180.0218	H	DECADE II outlet assembly
180.0233	Y	DECADE II inlet assembly -2 (micro)
180.0238	Q'	AS 100 outlet assembly -2 (micro)
180.0241	S'	OR 100 pd outlet assembly, TCC
180.0254		Loop tubing assembly
802.0406		Label set TCC

CHAPTER 3

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 cm ²
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	Polypropylene Nylon Guide for use
Acids, dilute	Usable
Acids, concentrated	Not usable
Alcohols (selected)	Usable
Aldehydes	Not usable
Bases	Usable
Esters	Short term use
Hydrocarbons, aromatic	Not usable
Hydrocarbons, halogenated (selected)	Short term use
Ketones	Not usable

*) This chemical compatibility chart is intended as a general guide only. This guide has been compiled from results of inhouse studies, material supplier

studies and currently available technical literature. Because of solvent condition variabilities, 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.

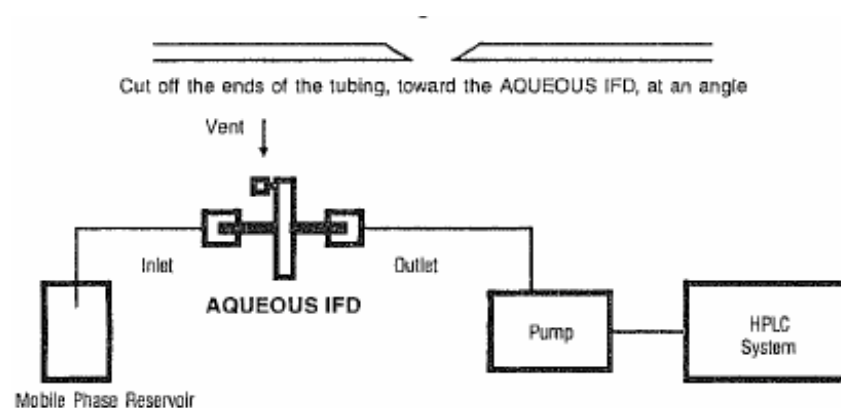


Figure 24. Schematic drawing of filter Installation in HPLC set-up.

Filter installation and priming

- Establish continuous, bubble free flow from mobile phase reservoir to pump by aspirating with a syringe through the pump bleed valve.
- Cut, at an angle, an appropriate length of 1/8" 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.

- Plug the inlet tubing, or seal the end by attaching and clamping off a short length of flexible tubing.
- Fill a 10 ml syringe with the mobile phase, remove vent cap and secure the syringe to the vent.
- With outlet pointed up SLOWLY push the syringe plunger completely wetting out the filter media and filling the AQUEOUS IFD housing.
- Connect outlet to 1/18" O.D. pump inlet tubing (cut at an angle) with a ferrule nut, as in step 2.
- Unplug or unclamp AQUEOUS IFD inlet tubing and place it in the mobile phase reservoir.
- 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.
- 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.
- 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.
- 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

- To 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.
- 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:
 - Check for air blocking the inlet side of the AQUEOUS IFD by repeating steps 7 through 10.
 - 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.
 - 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: Check for air leaks by following Trouble Shooting step 1. 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 of < 2.5 ml/min. The filter unit should

always be changed when changing from one mobile phase to another.

- 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.

