

ALEXYS

installation guide

LC connection kit, DCC I-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 (p/n 180.0162) 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 Dual Cell Control configuration in a parallel arrangement. This procedure specifically describes the installation of these assemblies. It is assumed that:

Equipment has been unpacked and checklists are verified
Installation procedure of ALEXYS is followed as described in manuals in order: OR 100, AC 100, LC 100, AS 100 and DECADE II.

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
	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
4	181.0010	AS 100 autosampler
5	171.0010	DECADE II

Before complete installation, passivation of all metal parts in the HPLC system is required. The passivation step is described in step 5 of the installation procedure.

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 tool is necessary for the installation of the LC connection kit:



Figure 1 5/16" - 1/4" wrench.

Schematic drawing of all ALEXYS LC connections

LC connection kit, DCC I-I (p/n 180.0162)

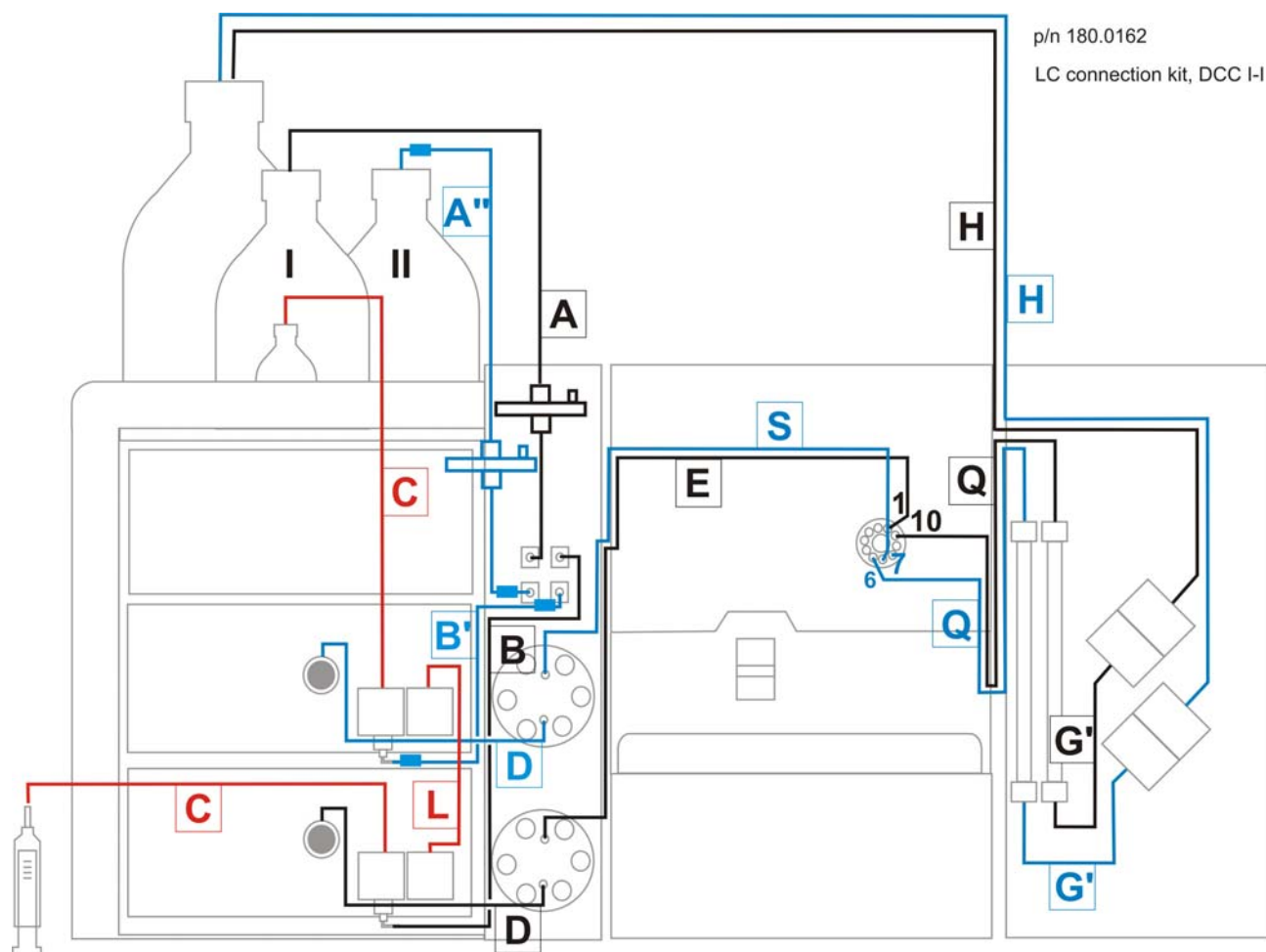


Figure 2a Schematic drawing of all LC connections available in the LC connection kit, DCC I-I. [a] The numbers refer to the part numbers on the plastic bags in which the assemblies are shipped. [b] For identification each individual assembly has a vinyl label with letter code (A, B etc.) attached.

LC connection kit, DCC I-I, micro (p/n 180.0163)

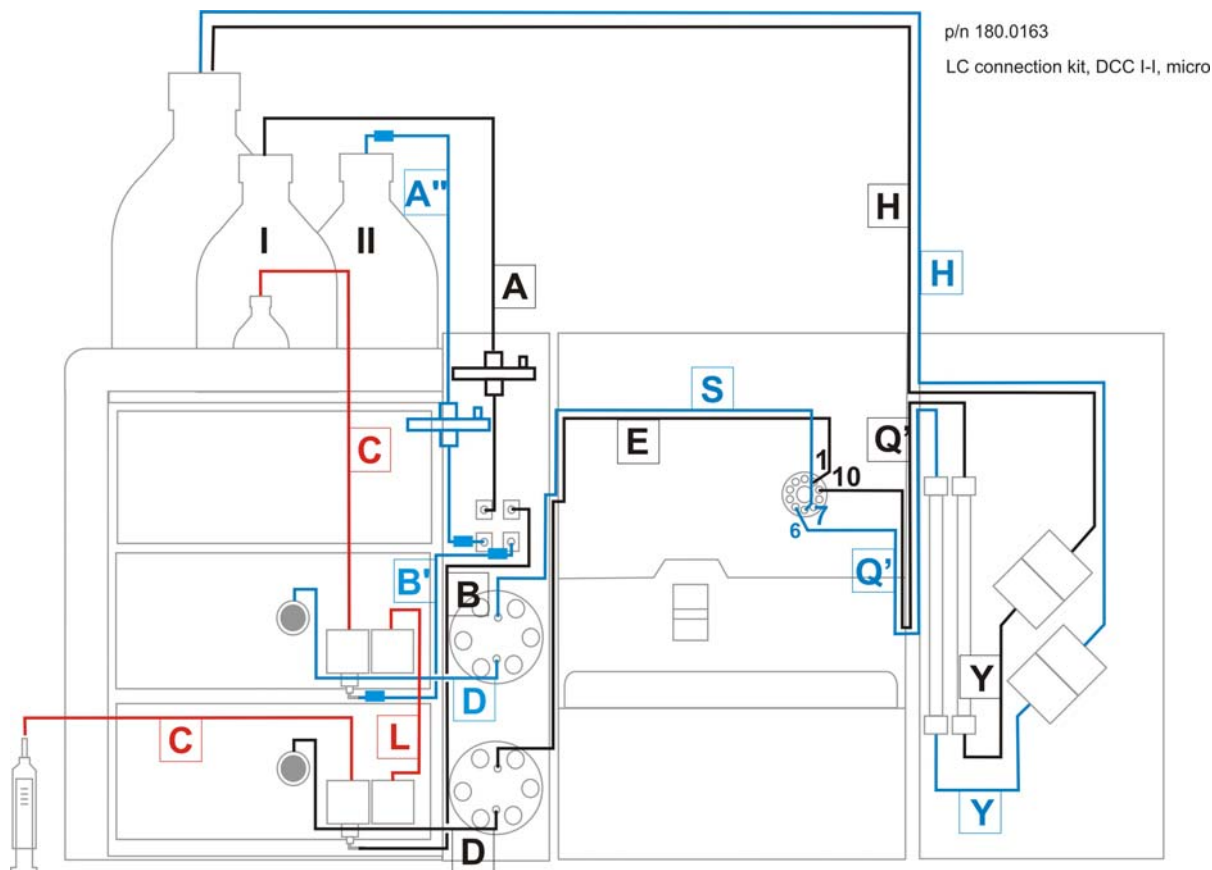


Figure 2b Schematic drawing of all LC connections available in the LC connection kit, DCC I-I, micro [a] The numbers refer to the part numbers on the plastic bags in which the assemblies are shipped. [b] For identification each individual assembly has a vinyl label with letter code (A, B etc.) attached.

Note that in case of an LC connection kit, DCC I-I -micro, the tubing assemblies from the injector- to - column (assembly Q') and the column - to - cell (assembly Y) differ from that of the LC connection kit, DCC I-I displayed on the previous page. The tubing ID of assembly Q' and Y is 64 μ m (0.0025"), the ID of assembly Q and G' are 130 μ m (0.005").

Installation procedure

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

1. **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).

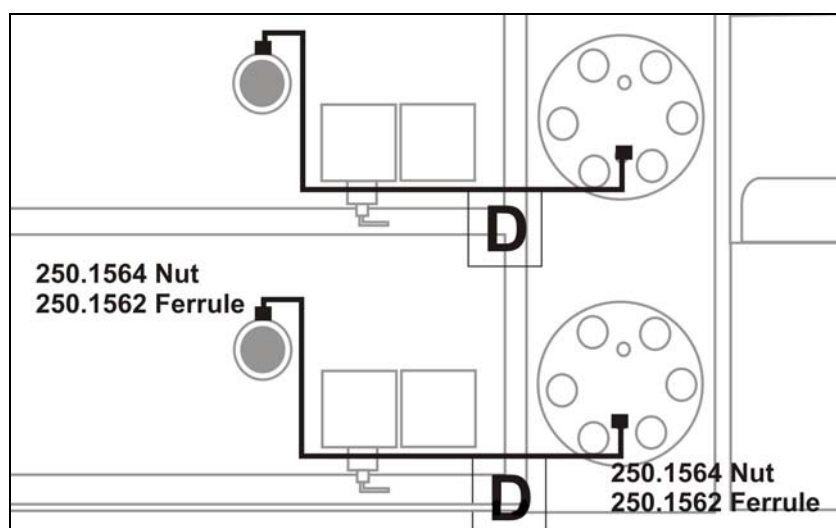


Figure 3. Assembly D: Connection between pump – pulse damper.

2. **Assembly E, OR 100 pulse damper outlet assembly (p/n 180.0212):**
Assembly E is supplied to connect system 1 to the autosampler. Connect the pre-shaped stainless steel tubing to the outlet of the lower pulse damper in the OR 100 and port 1 of the AS 100 injection valve. The supplied nuts and ferrules for the connection of tubing to the pulse damper and auto-sampler are not the same. The auto sampler valve has Valco-type ports the pulse damper SSI-type. Use parts 250.1562 and 250.1564 to connect the outlet of the pulse damper. For the connection on the injector on the auto sampler use parts 250.1558 and 250.1560.

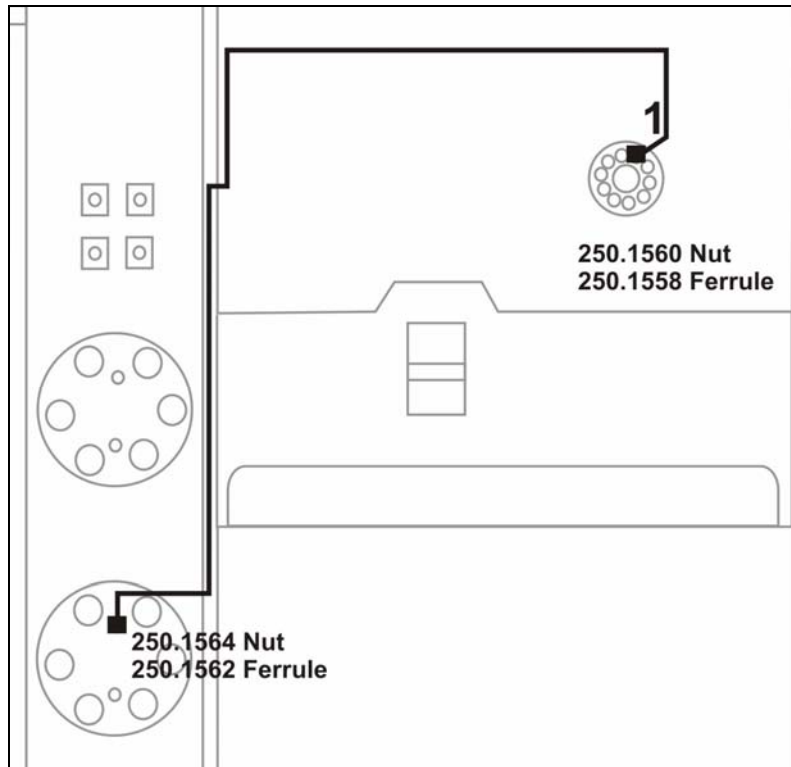


Figure 4. Assembly E: Connection between pulse damper 1–AS100.



Making a tubing connection with the wrong type of nut and ferrule can damage the port or result in large dead volumes



Making a tubing connection with the wrong type of nut and ferrule can damage the port or result in large dead volumes

3. **Assembly S**, OR 100 pulse damper outlet assembly DCC (p/n 180.0240): Assembly S is supplied to connect system 1 to the autosampler. Connect the pre-shaped stainless steel tubing to the outlet of the upper pulse damper in the OR 100 and port 7 of the 10-ports AS 100 injection valve. The supplied nuts and ferrules for the connection of tubing to the pulse damper and auto-sampler are not the same. The auto sampler valve has Valco-type ports the pulse damper SSI-type. Use parts 250.1562 and 250.1564 to connect the outlet of the pulse damper. For the connection on the injector on the auto sampler use parts 250.1558 and 250.1560.

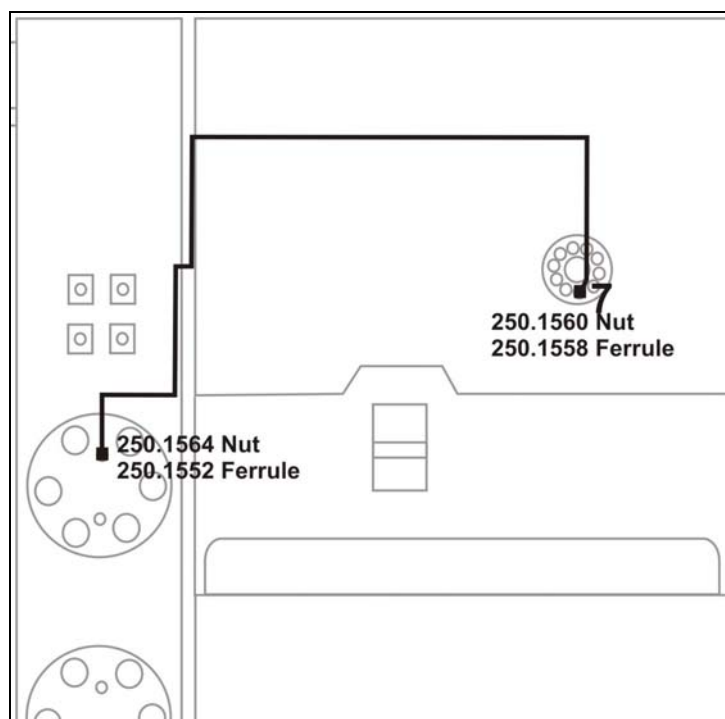


Figure 5. Assembly S: Connection between pulse damper 2 – AS100.

4. **Assembly C**, LC 100 piston back flush assembly (p/n 180.0208) and **Assembly L**, Piston back flush interconnection (p/n 180.0209): Connect the two pieces of 1/16" FEP tubing (C) to the piston wash connections on top of the pump heads on the left side. Use the supplied stainless steel nuts and ferrules (p/n 250.1564 and 250.1562).

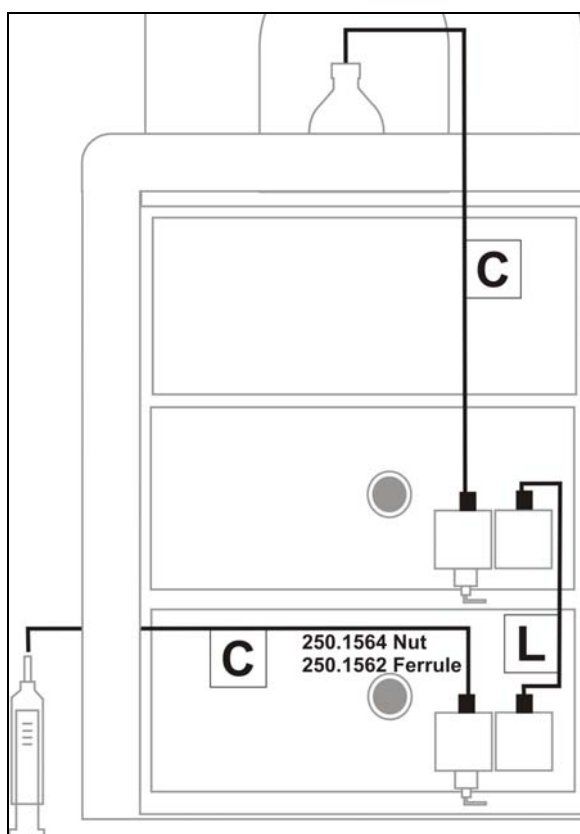


Figure 6. Assemblies C & L: Connection of piston wash of the two LC 100 pumps.

Place one tubing end in the bottle with wash solvent as depicted in figure 6. 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.

5. Passivation of metal parts

For optimal performance always passivate all metal parts in the system 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 7. 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. Temporary use the two tubing assembly H, DECADE II outlet assembly (180.0218), to flush the acid from port 6 and 10 of the AS 100 to waste.

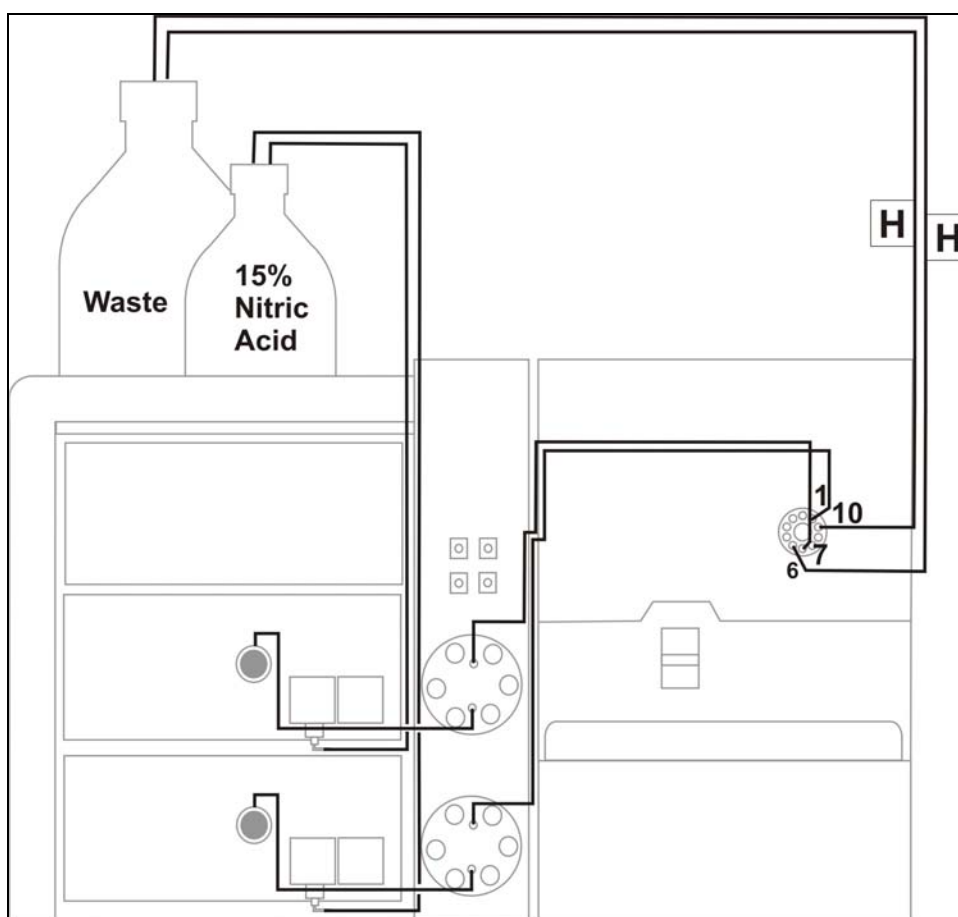


Figure 7. System passivation.

The acid should be flushed through the pumps, the pump tubings, the pulse dampers, the injector and to waste. **Flush the injector in both the load and the inject position! This assures that both loops and all injector ports will be passivated.** 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).

6. **Assemblies A and A''**, OR 100 degasser inlet assembly (p/n 180.0204 and 180.0203 and respectively): Two assemblies A and A'' are provided consisting of two pieces of FEP tubing and a 0.2 μm IFD in-line filter. Place the end of the FEP tubing A in the mobile phase bottle as depicted in figure 3.

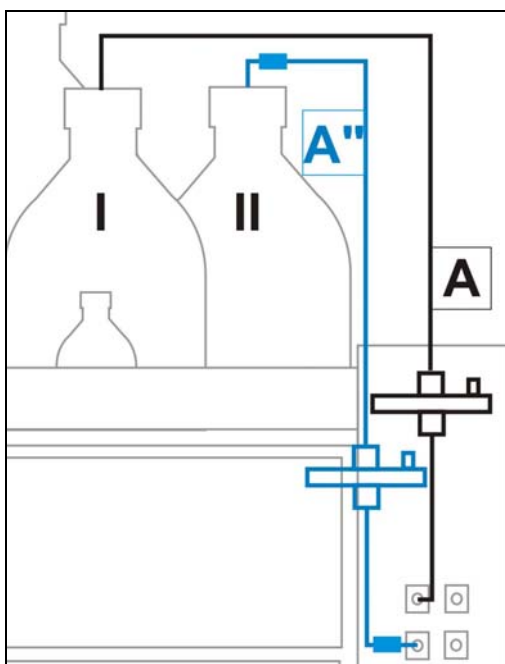


Figure 8. Assemblies A and A'': Connection between bottle - degasser.

Make sure that the vent of the in-line filter is facing towards the solvent bottle. Prepare a solution of 200 mL 10% 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. After the flushing procedure is finished the inlets of both assemblies should be placed in different mobile phase bottles as depicted in figure 8.



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 9.

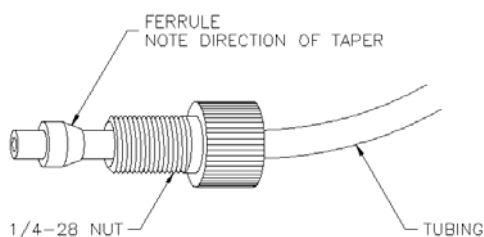


Figure 9. Configuration of 1/4-28 Nut, Ferrule and Tubing.

7. **Assemblies B and B'**, OR 100 degasser outlet assembly (p/n 180.0206 and 180.0207, respectively): For the LC connections between the degasser and the pumps the assemblies B and B' are provided.

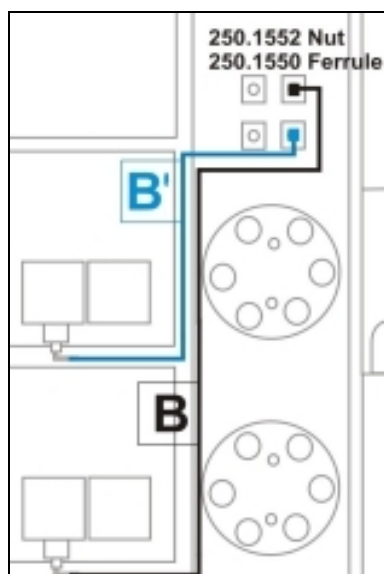


Figure 10. B and B': Connection between degasser – pump.

Connect the end of the FEP tubing without label ("LC 100 side") to the degasser output using the supplied nut and ferrule (Fig. 9, 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! Assembly B' has two blue labels for identification purposes. Assembly B" should be used for the delivery of the NaOH solution to pump 2. For priming instructions of the pump please consult the manual of the LC 100 (p/n 182.0010).



1. **Never push the solvent through the channel of the degasser**
2. **Connect the tubing supplying the NaOH eluent to the top pump.**

8. **Assembly Q or Q'**, AS 100 outlet assembly micro (p/n 180.0230 or 180.0238):

Two assemblies Q or Q' (dependent on kit) are provided to connect the 10-port valve to both columns. System 1: Connect the PEEK tubing to port 10 of the injection valve of the autosampler using the supplied PEEK fingertights (p/n 250.1570). Guide the tubing via the metal tubing holder of the AS 100 to the DECADE II. Guide the tubing through the upper tubing hole in the DECADE II. Subsequently connect the tubing to the inlet of the HPLC column using the supplied PEEK fingertights (p/n 250.1570). System 2: Take the second assembly Q. Connect the PEEK tubing to port 6 of the 10-port valve of the autosampler using the supplied PEEK fingertights (p/n 250.1570). Subsequently, connect the other end of the tubing to the second column following the same procedure as with system 1.

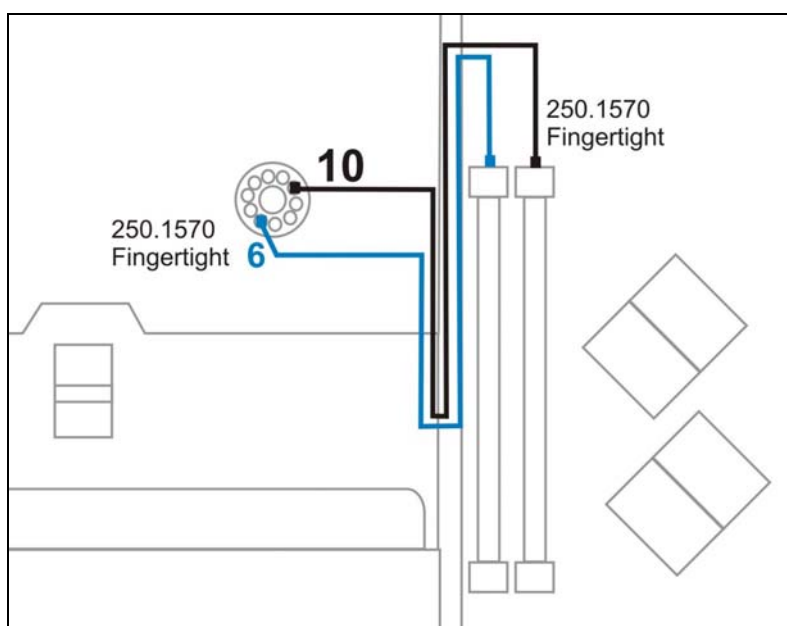


Figure 11 Assembly Q or Q': Connection between AS 100 – column.

9. **Assembly G' or Y** DECADE II inlet assembly micro (p/n 180.0232 or 180.0233): Two DECADE II inlet assemblies G' or Y (dependent on kit) 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 using the KEL-F VT-03 fingertight (p/n 110.1045). Do the same for the second system.

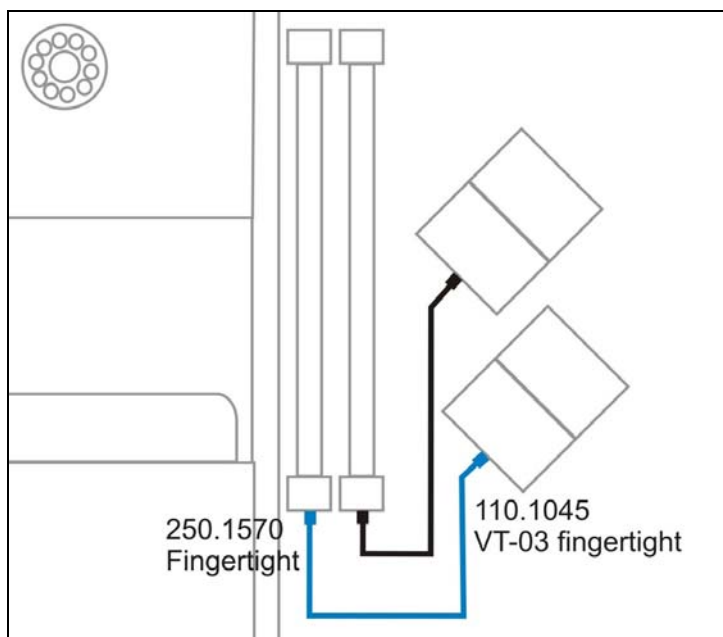


Figure 12. Assembly G' or Y: Connection between column - flow cell.

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 connect to system 1 is electrically connected to the connector marked "cell 1" on the left side of the oven compartment.

10. **Assembly H** *DECADE II outlet assembly (180.0218)*: Two 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 bottles depicted in figure 12.

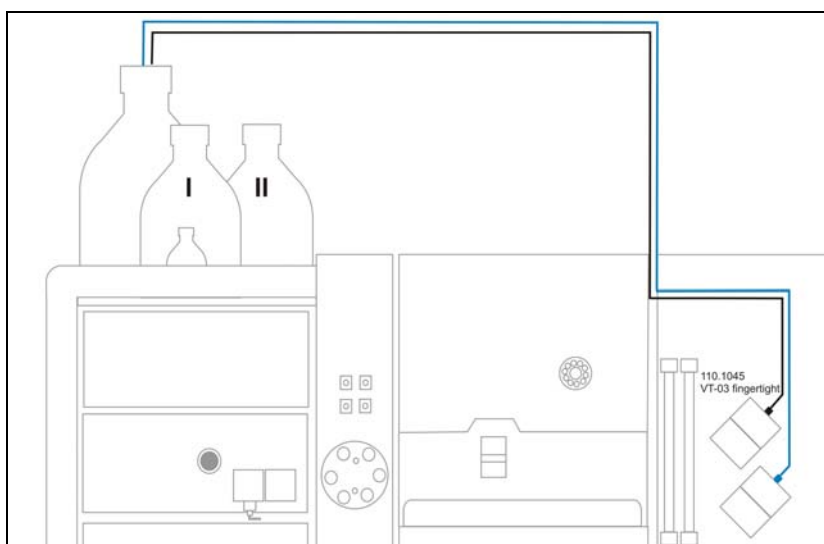


Figure 13. *Assembly H: Connection between cell – waste bottle.*

This completes the installation of all tubing and connectors of the ALEXYS 100 LC-EC 100 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
E	Stainless steel	0.02	1/16
Q	PEEK (Red)	0.005	1/16
Q'	PEEK (Pink)	0.0025	1/16*
G'	PEEK (Red)	0.005	1/16
Y	PEEK (Pink)	0.0025	1/16*
H	PEEK (Orange)	0.02	1/16

* Used in LC connection kit DCC I-I, micro

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 DCC I-I, p/n 180.0162

Part no	Description
180.0204	OR 100 degasser inlet assembly
180.0206	OR 100 degasser outlet assembly
180.0208	LC 100 piston back flush assembly
180.0210	OR 100 pulse damper inlet assembly
180.0212	OR 100 pulse damper outlet assembly
180.0230	AS 100 outlet assembly (micro)
180.0232	DECADE II inlet assembly (micro)
180.0218	DECADE II outlet assembly

Table III. Accessories LC connection kit DCC I-I -micro, p/n 180.0163

Part no	Description
180.0204	OR 100 degasser inlet assembly
180.0206	OR 100 degasser outlet assembly
180.0208	LC 100 piston back flush assembly
180.0210	OR 100 pulse damper inlet assembly
180.0212	OR 100 pulse damper outlet assembly
180.0238	AS 100 outlet assembly -2 (micro)
180.0233	DECADE II inlet assembly -2 (micro)
180.0218	DECADE II outlet assembly

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.

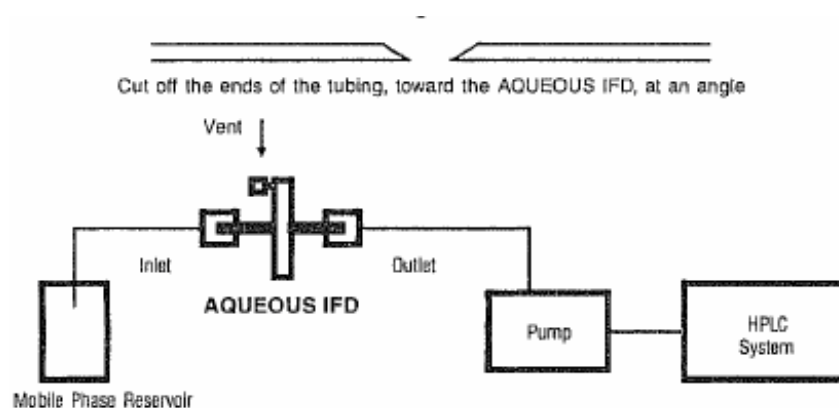


Figure 14 Schematic drawing of filter Installation in HPLC set-up.

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 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.

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 1/8" O.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

1. 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.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.

7. 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.