

# ALEXYS

## installation guide

### LC connection kit, post-column II





## 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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## C H A P T E R 1

## Installation guide

### Introduction

The LC connection kit aminoglycosides (p/n 180.0150) is a tailor-made kit containing all tubing assemblies to make the necessary LC connections in a complete ALEXYS 100 LC-EC glycosides system. This procedure specifically describes the installation of these assemblies. It is assumed that:

1. Equipment has been unpacked and checklists are verified
2. 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.**

*Table I. ALEXYS 100 LC-EC system user manuals.*

Order	Part no.	User manual
1	185.0010	ALEXYS data system
2	184.0010	OR 100 organizer rack
3	183.0010	AC 100 acquisition controller
4	182.0010	LC 100 pump
5	181.0010	AS 100 auto sampler
	171.0010	DECADE II

Before complete installation, it is advised to passivate all metal parts in the HPLC system (for procedure see page 16).

**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

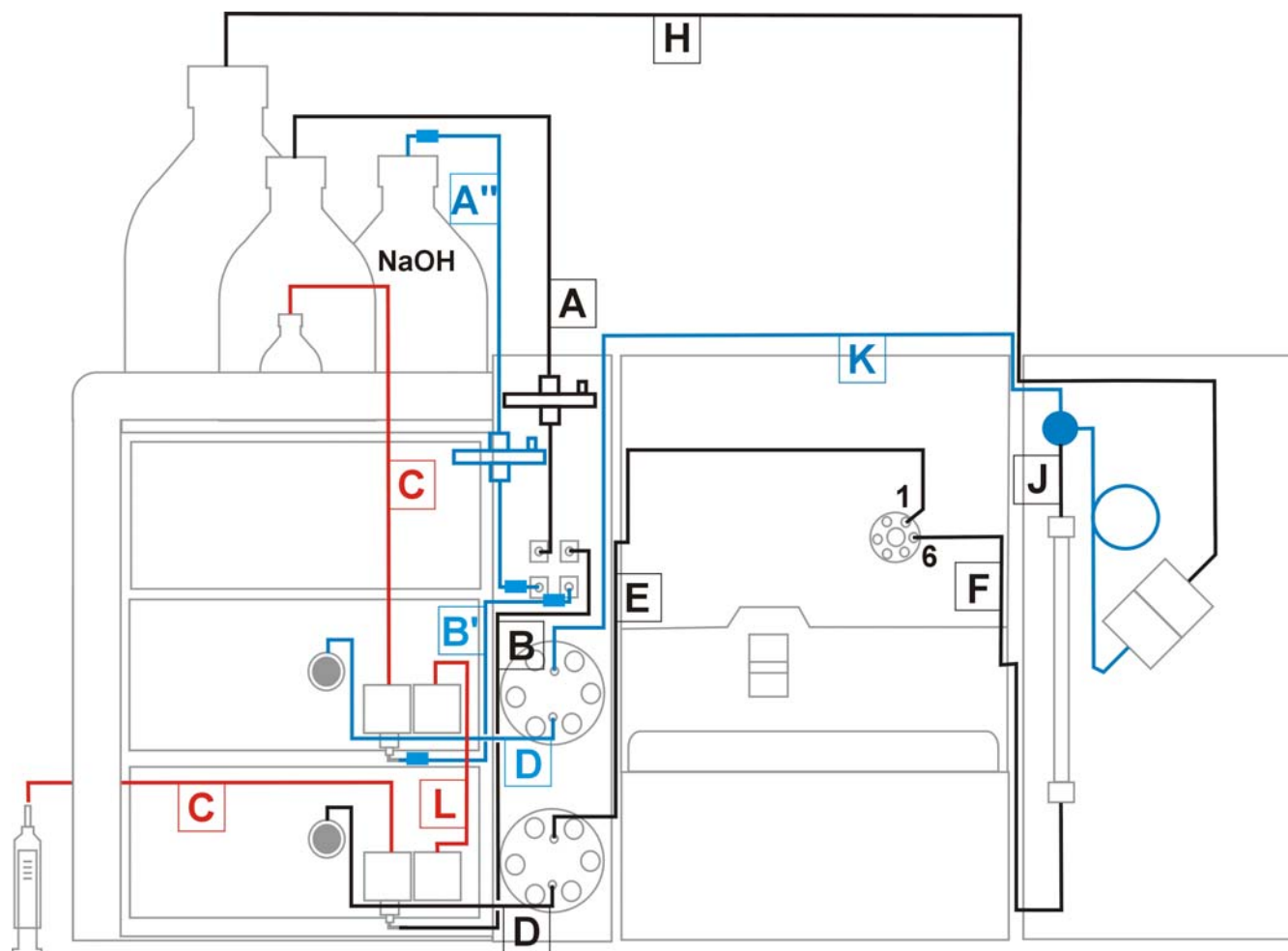
The following tool is necessary for the installation of the LC connection kit:



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



## Schematic drawing of all ALEXYS LC connections

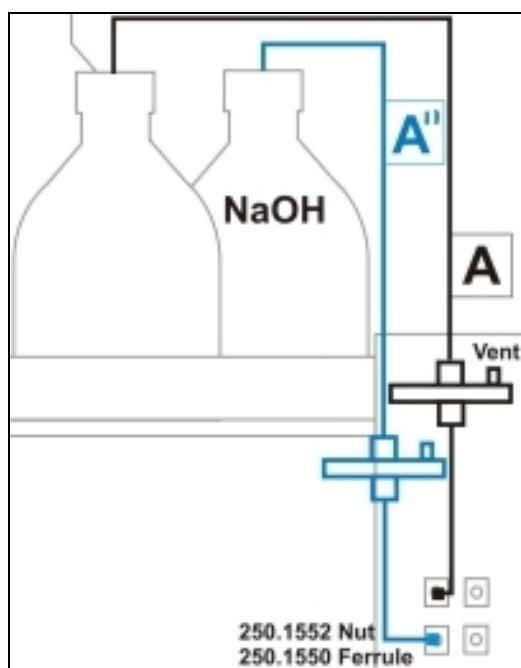


**Figure 2.** Schematic drawing of ALEXYS 100 LC-EC glycosides system with all LC connections. For identification each individual assembly has a vinyl label with letter code (A - L) attached.

## Installation procedure

The installation procedure consists of the following steps:

1. **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  $\mu\text{m}$  IFD in-line filter. Place the end of the FEP tubing A in the mobile phase bottle as depicted in figure 3.



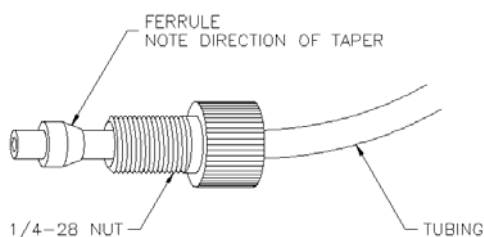
**Figure 3.** Assemblies A and A'': Connection between bottle - degasser.

Make sure that the vent of the in-line filter is facing towards the solvent bottle. Open the filter (vent) and draw the mobile phase through the tubing using a syringe until the filter 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 mobile phase bottle. The outlet tubing should spontaneously siphon solvent. If this is not the case consult the trouble-shooting section in chapter 3. Assembly A'' has two blue labels for identification purposes. Assembly A'' should be used for the delivery of the NaOH solution (pump 2).



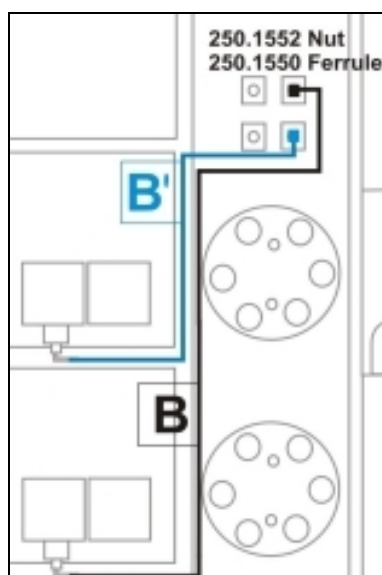
**For detailed operating instructions of the Whatman IFD in-line filters see chapter 3 in this installation guide.**

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 4.



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

2. **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 5.** B and B': Connection between degasser – pump.

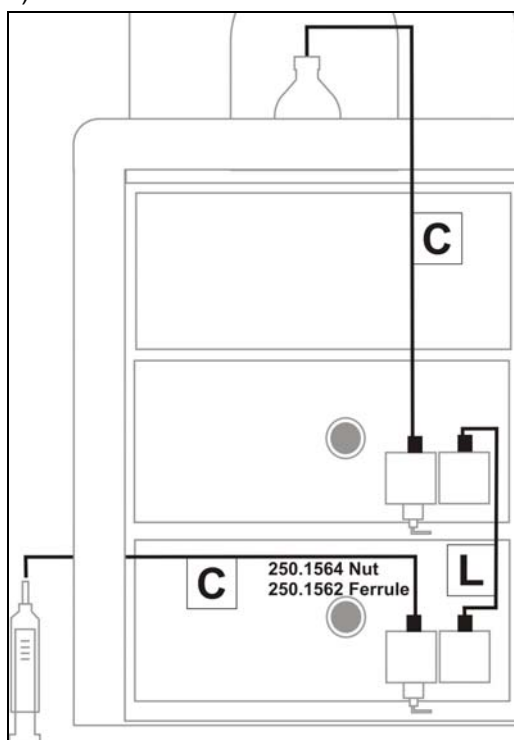
Connect the end of the FEP tubing without label ("LC 100 side") to the degasser output using the supplied nut and ferrule (Fig. 4 p/n 250.1552 and 250.1550). Connect the other end of the tubing to the

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.**

3. **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).

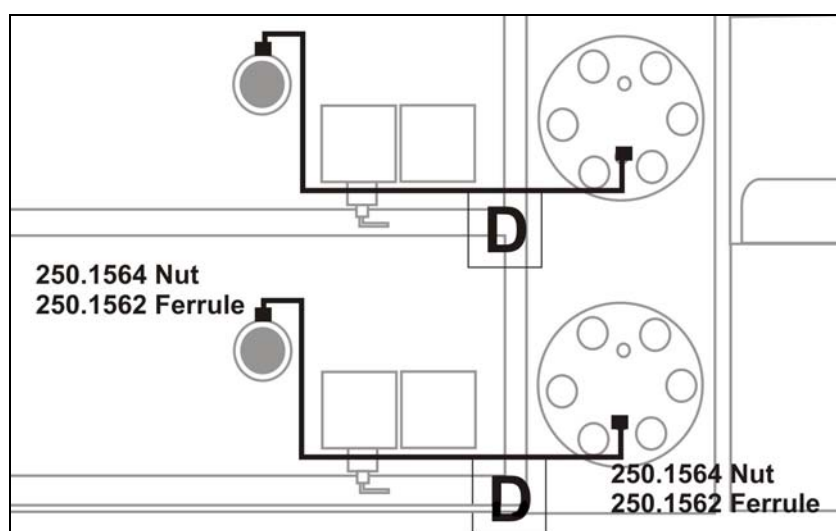


**Figure 6.** Assemblies C & L: Connection of piston wash of the two LC 100 pumps.

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

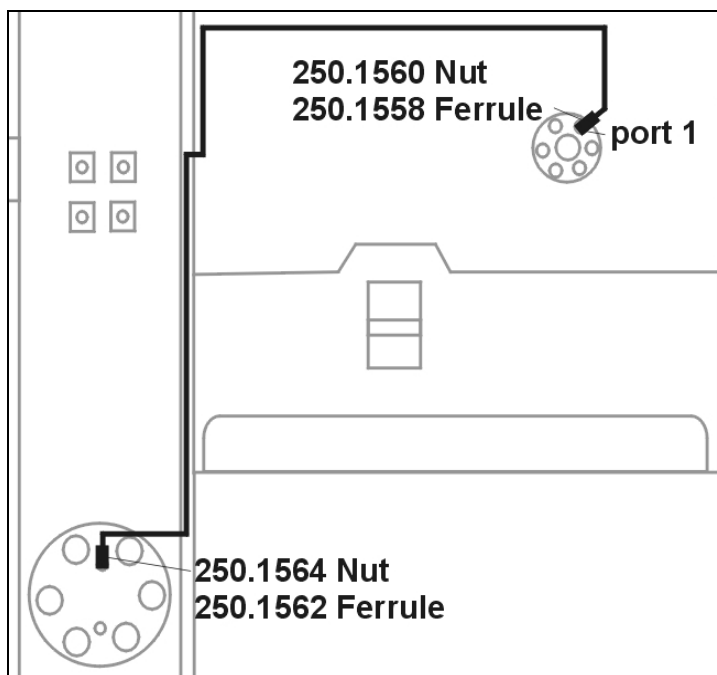
ship kit to the other tubing. Install the interconnection tubing (L) and fill the piston wash with wash solvent by withdrawal of the syringe.

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 7.** Assembly D: Connection between pump – pulse damper.

5. **Assembly E**, OR 100 pulse damper outlet assembly (p/n 180.0212):  
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.

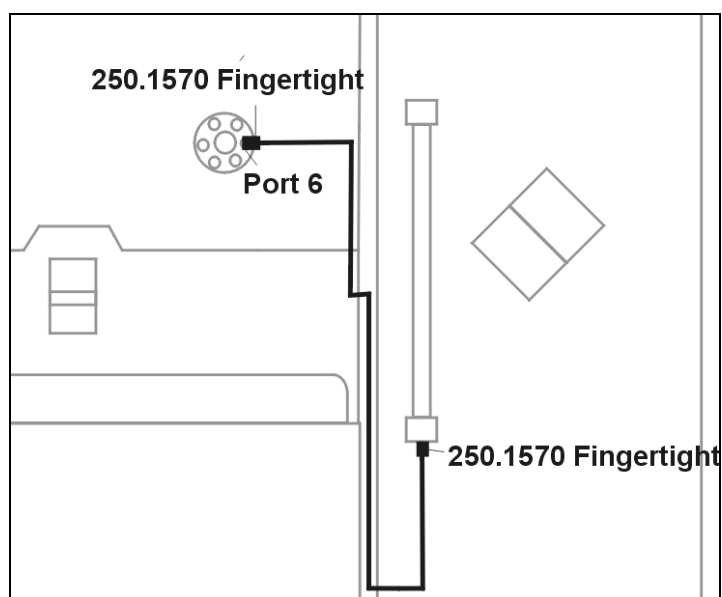


**Figure 8.** Assembly E: Connection between pulse damper – AS 100.



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

6. **Assembly F**, AS 100 outlet assembly (p/n 180.0214): Connect the PEEK tubing to port 6 of the auto sampler using the supplied PEEK finger tights (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 lower tubing hole in the DECADE II. Subsequently connect the tubing to the inlet of the HPLC column using the supplied PEEK finger tights (p/n 250.1570).

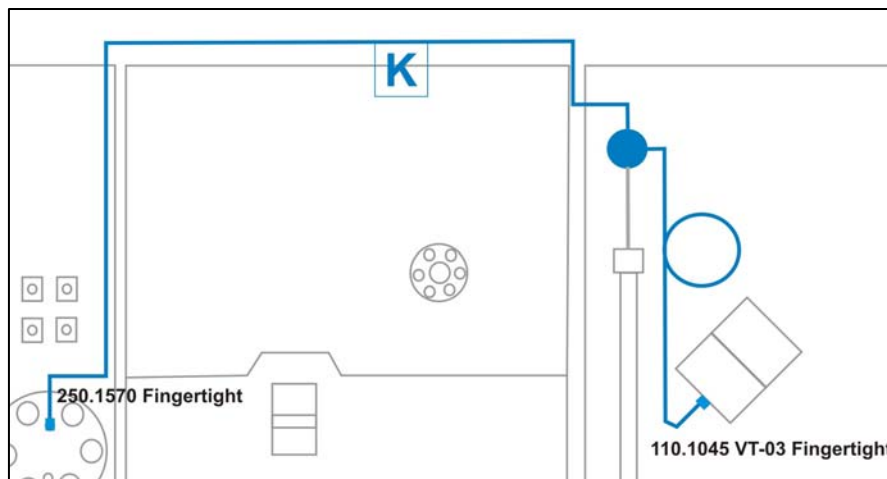


**Figure 9.** Assembly F: Connection between AS 100 – column.



Check that the column is connected to the pump, which delivers the mobile phase, and NOT to the pump delivering NaOH solution. Exposure to concentrated NaOH solution will damage the column.

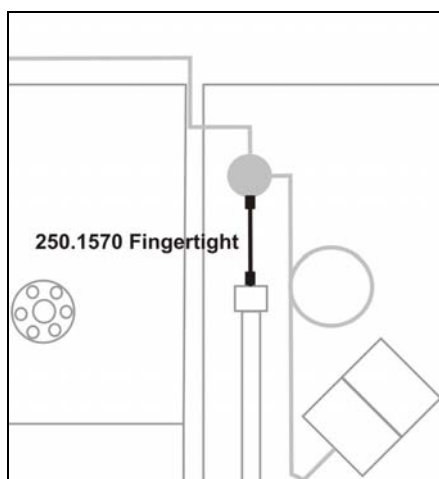
7. **Assembly K**, *DII post-column mixer assembly (p/n 180.0220)*:  
Connect the PEEK tubing to the outlet of the pulse dampener using the supplied PEEK finger tight (p/n 250.1570). Connect the PEEK coil end to the flow cell using the VT-03 finger tight (p/n 110.1045). The PEEK coil is the reeled tubing with the orange stripe on its surface.



**Figure 10.** Assembly K: Connection between pulse damper - cell.

Detailed information about the installation of the flow cell can be found in the manual of the DECADE II (p/n 171.0010).

8. **Assembly J**, *DECADE II column- to-splitter assembly (p/n 180.0217)*: Connect one end of the PEEK tubing to the outlet of the HPLC column using the PEEK finger tight (p/n 250.1570).

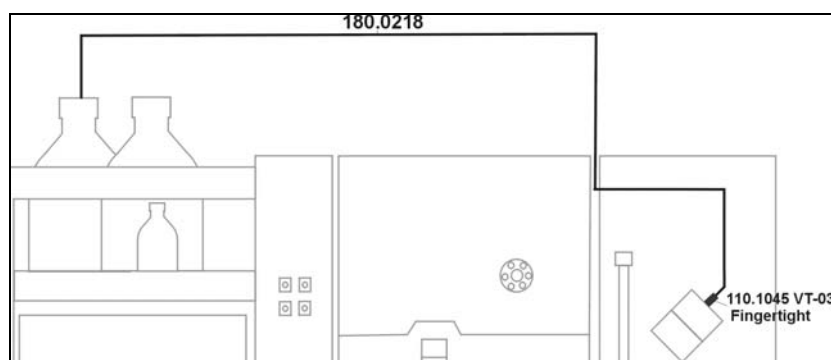


**Figure 11.** Assembly J: Connection between column - splitter.



Connect the other end of the tubing to the splitter of the post column mixer assembly using a PEEK finger tight (p/n 250.1570).

9. **Assembly H, DECADE II outlet assembly (180.0218):** Connect the PEEK tubing to the outlet of the VT-03 flow cell using the VT-03 finger tight. Guide the tubing through the tubing hole in the DECADE II into the waste bottle.



**Figure 12.** Assembly H: Connection between cell – waste bottle (p/n 180.0218).

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 II. Tubing dimensions and materials.*

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
F	PEEK (Blue)	0.01	1/16
H	PEEK (Orange)	0.02	1/16
J	PEEK (Blue)	0.01	1/16
K	PEEK (Red)	0.005	1/16
	FEP	0.02	1/16
L	FEP	0.03	1/16

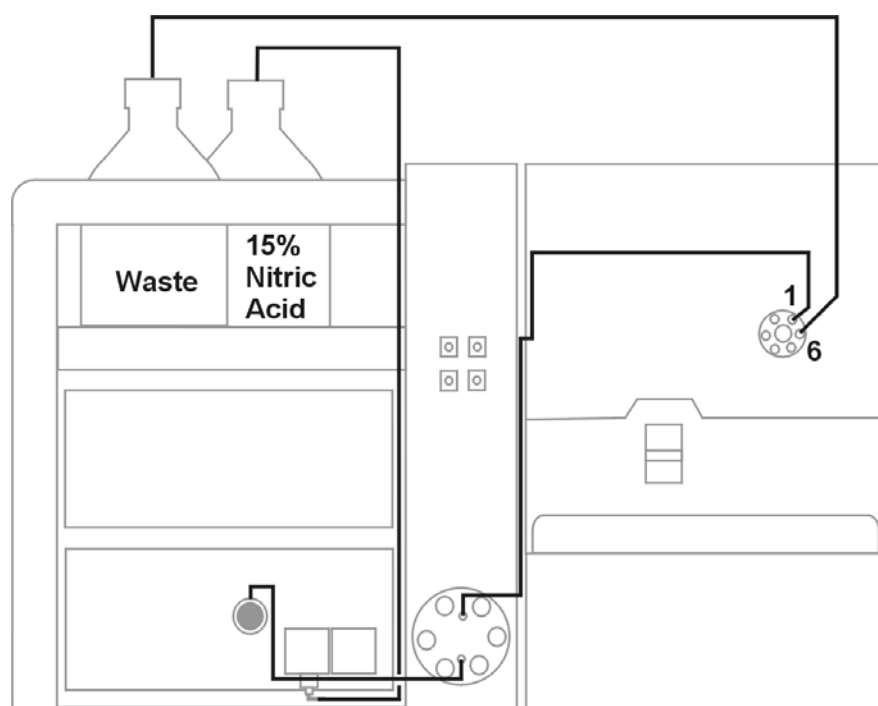
## Passivation procedure

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 12. Note that the connections are shown for only one pump in the set-up. The acid should be flushed through the pump, the pump tubing, the dampener, the injector (in load and inject position) and to waste. 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 12.** System passivation.

## 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 III. Accessories LC connection kit.*

Part no	Description	Assy
180.0203	OR 100 degasser inlet assembly - pump 2	A"
180.0204	OR 100 degasser inlet assembly	A
180.0206	OR 100 degasser outlet assembly	B
180.0207	OR 100 degasser outlet assembly - pump 2	B'
180.0208	LC 100 piston back flush assembly	C
180.0209	LC 100 piston back flush interconnection	L
180.0210	OR 100 pulse damper inlet assembly	D
180.0212	OR 100 pulse damper outlet assembly	E
180.0214	AS 100 outlet assembly	F
180.0217	DECADE II column to splitter assembly	J
180.0218	DECADE II outlet assembly	H
180.0220	DECADE II post-column mixer assembly	K



## C H A P T E R 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 <sup>2</sup>
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 in-house studies, material supplier

studies and currently available technical literature. Because of solvent condition variability's, which may exist from lab to lab, component compatibility cannot be guaranteed. In order to verify chemical compatibility, studies on individual chemicals of interest should be undertaken.

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%).

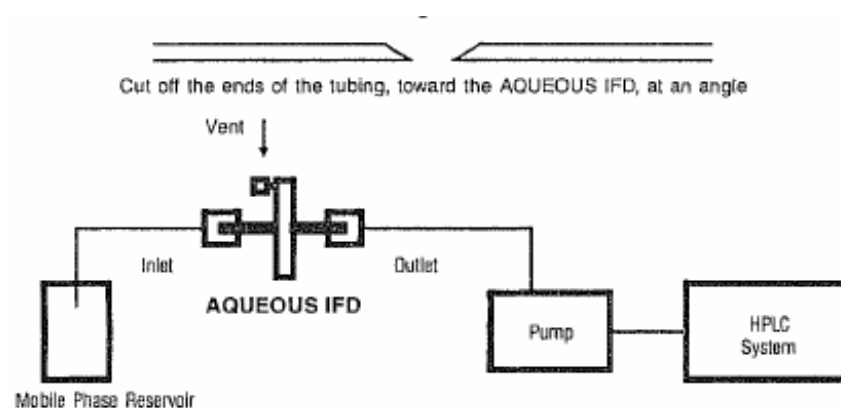
## Operating instructions

**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  $\mu\text{m}$  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 cut 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.

