

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

Installation requirements

for ALEXYS® systems

180.7070C, Edition 12, 2024





Warning Symbol



The warning sign denotes a warning. It calls attention to a procedure or practice which, if not adhered to, could result in costs, damage or destruction of parts or all of the equipment. Do not proceed beyond a warning sign until the indicated conditions are fully understood and met.

For research purposes only. The ALEXYS system is <u>not</u> tested by the manufacturer to comply with the In Vitro Diagnostics Directive.

Observe safety

Operation of an electrochemical detector can involve the use of hazardous materials including corrosive fluids and flammable liquids. The instrument should only be operated by users with the following expertise:

- Completed degree as chemical laboratory technician or comparable vocational training
- Fundamental knowledge of liquid chromatography
- Knowledge and experience in the safe handling of toxic and corrosive chemicals and knowledge of the application safety measures prescribed for laboratories.
- Participation in an end-user training (daily use of system and chromatography software) performed by the manufacturer or a company authorized by the manufacturer.



Unskilled, improper, or careless use of the instrument and the related chemicals can create fire hazards, or other hazards which can cause death, serious injury to personnel, or severe damage to equipment and property.

Observe all relevant safety practices at all times.

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Introduction

Thank you for ordering an ALEXYS LC-ECD system. For a successful on-site installation of the system, please arrange the following requirements at your location in advance:

- a computer (see document 195.7000 for the PC requirements)
- laboratory conditions and facilities
- consumables
- chemicals



Arrange these requirements well in advance before the installation to prevent (costly) delays.

This document lists the general laboratory requirements, chemicals and consumables that are necessary during and after the installation of ALEXYS systems:

The application specific requirements, chemicals and consumables are listed in a separate application-specific document or have to be arranged based on other information.

Laboratory requirements

Laboratory environment

□ Environmental requirements for the ALEXYS Analyzer:

Parameter	Requirement
Operating temperature	+22 °C (± 3 °C)
Operating humidity	20 – 80%, non-condensing

Laboratory equipment

- Water purification apparatus
 For a fresh supply of high-quality deionised water with resistivity of >18
 MOhm.cm and low TOC level (<10 ppb). Bottled water is not recommended.
- Ultra-sonic bath for degassing purpose*
 Should fit at least 1 L bottles
- Analytical balance
- □ pH meter and relevant pH standards
- □ analytical pipettes, pipette tips, tubes
- □ glassware such as measuring cylinders
- \Box etc.

*The ALEXYS[®] system is equipped with inline filters and an in-line degasser for the mobile phase, but it is strongly advised to sonicate the mobile phase before use for additional degassing. Do not use reusable vacuum filtering units as an alternative for degassing purposes, as these units can be a source of contamination.

Bench

- □ Stabile, clean, flat and smooth surface
- \Box Enough mechanical strength to hold > 90 kg (198 lbs).
- □ Free space for an ALEXYS system (about 110 cm wide, 75 cm deep and 100 cm high or 43.5" wide, 30" deep and 43.5" high).
- □ Additional space is necessary for the PC, and around the system to prevent obstruction of the fans.

Power

- The number of free power sockets needed for a system depends on the number of instruments in the system. Each instrument needs its own power socket. Usually, the system consists of 1 detector, 1 injector, 1 pump, 1 dedicated LAN network switch, 1 computer, 1 monitor and 1 printer (7 sockets needed). Every additional instrument (2nd pump, additional column thermostat, USB-LAN converter or additional detector) needs their own additional power socket.
- □ The total maximum power consumption of an ALEXYS system is in the range of 500-800W, excluding the computer. See table below for details of instruments.

Instrument options	Туре	Max. power consumption (W)
EC detector	DECADE Elite/Lite	260
_		
Pump	P 6.1L	100
Autosampler	AS110/AS 6.1L	200
Column thermostat	CT 2.1L	100
UV detector	UVD 2.1L	100
MW detector	MWD 2.1L	65
LAN switch box	undefined	± 3
Computer	undefined	± 100
Monitor	undefined	± 80

Consumables

Flow cell cleaning:

- □ Soft paper tissue (for instance Kleenex facial tissues)
- A drip-lock squeeze bottle filled with acetone
- A squeeze bottle filled with (deionized) water

Standard sample vials (in case the ALEXYS is equipped with an autosampler)

The AS110 and AS 6.1L autosamplers of an ALEXYS system are standard delivered with a set of 48-positions sample trays for use with

- □ 11.6 mm OD autosampler sample vials
 - For carbohydrate analysis, do not choose glass vials: plastic vials are recommended

Sample vials for small samples (<20 µL) - ALEXYS system with autosampler

The ALEXYS[®] Neurotransmitters system (180.0091U & -92U) is delivered with an additional set of special 96-positions sample trays that fit narrow fraction collector vials. A start-up kit containing a sample set of about 200 vials and caps is part of these ALEXYS systems.

For additional vials and caps the reordering information is (supplier and pn):

- Sample Vials polypropylene 300 uL (Microbiotech, pn. 4001048 see also Figure 1.)
- □ Snap caps 8 mm with slit (J.G. Finneran, pn. 5870-08)
- Alternative for the snap caps: 8 mm aluminium crimp cap with PTFE seal (Chromacol, pn. 8-ACT) and a cap crimper tool

Any real equivalent from other suppliers may be used.





NOTE: There are subtly different shapes of fraction collector vials on the market. The types that fit best are the ones from Microbiotech, depicted on the left side in Figure 1. The types depicted on the right are slightly too wide to fit in the 96-position tray and cannot be used without a suitable adaptor.

Figure 1. Two slightly differently shaped fraction collector vials.

Chemicals and solutions

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Have these chemicals and solutions ready at the start of the installation.

For LC-ECD applications, only chemicals of sufficient specific quality should be used to be able to have an optimal system with good performance. The appendix shows detailed descriptions of some of the chemicals that have been used in the Antec Scientific R&D laboratory, as an example of what works.

The general list below has to be further supplemented with the application specific chemicals. Depending on the application, we have documents with additional specifications available.

General solution for use during installation

Water
 A fresh supply of high-quality deionised water with resistivity of >18
 MOhm.cm and low TOC level (<10 ppb) from a water purification apparatus

Rinsing / passivation solutions for use during installation

- □ For a system containing stainless steel parts in the flow path:
 50 mL 15% HNO₃ in water, in a small, glass bottle
- For a metal-free system containing polymer tubing, dedicated to analysis of carbohydrates
 2 M NaOH in water, in a plastic bottle

Specific solutions (degassed by sonication) for different ALEXYS Analyzers

ALEXYS system	Piston back wash (1L)	Needle wash (250 mL)
ALEXYS Carbohydrate Analyzer	water	water
ALEXYS Neurotransmitter Analyzer	20% isopropanol	water
ALEXYS Clinical Analyzer	20% isopropanol	5% MeOH in water

Passivation of new metal-containing systems

For optimal performance of the ALEXYS Analyzer, some parts of the LC system should be rinsed with an appropriate solution before the system is used.

The systems that contain metal parts in the flow path are the ALEXYS Neurotransmitter Analyzer and the ALEXYS Clinical Analyzer.

All new metal parts in the system should be passivated before use. 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 through the metal parts for 20 minutes and rinsing it clean afterward with water.



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

(Dis)connection instructions

- First make sure that all the connections are leak-tight by pumping water through the lines and inspecting each connection.
- If already attached, disconnect the tubing between the injector and (pre)column at the (pre)column side and lead the tubing outlet into a dedicated waste bottle.
- Disconnect the 1/8" OD outlet tubing from the degasser, slide the connector away from the tubing end (if already attached), and place the open end in a small bottle with about 50 mL of 15% nitric acid.
- A schematic picture of the connected tubing is given in Figure 2.

Procedure

 Set the flow rate of the pump to a reasonable setting (resulting in a backpressure between 5-50 bar), check if there is enough solution in the bottle to run 20 min at the chosen flow rate and run the acid through the system for 20 minutes.

- **Regularly switch** the valve of the injector to assure that both Load and Inject positions will be passivated!
- After running the acid through the system, flush the system thoroughly with water, with the injector **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 of the system. The pH should be around 7 (water) before continuing with the installation.

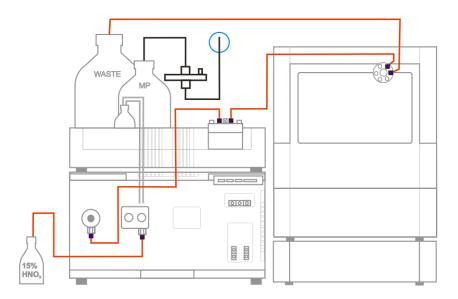


Figure 2. Flow path configuration schematics for passivation of the metal parts in an ALEXYS system. Note that the nitric acid is not going through the Whatman in-line filter, the detector, nor the degasser channels. In case of a dual channel system or gradient set-up the same principle is applied.

Rinsing procedure for new metal-free systems

A new ALEXYS Carbohydrate Analyzer should be flushed with a high concentration of NaOH before installing a column. The column and cell can be installed after the rinse.

Procedure

- First make sure that all the connections are leak-tight by pumping water through the lines and inspecting each connection.
- Connect one of the eluent lines from the degasser channel to the bottle filled with 2 M NaOH solution.
- Set the flow rate of the pump to a reasonable setting (resulting in a backpressure between 5-50 bar), check if there is enough solution in the bottle to run 20 min at the chosen flow rate and run 2 M NaOH through the system for 20 minutes.
- Make sure to flush the high concentration of NaOH away with 0.1 M NaOH solution (check the column specification sheet for the flushing instructions) before continuing with the installation and connecting the column.



Do not let the system stand still for a prolonged period after using NaOH as the mobile phase to avoid the formation of NaOH salts and system blockage.

APPENDIX

A list of general use chemicals with purity and purchase details is shown below as a guideline. The listed brands/purities are not necessarily the best chemicals, but these have been giving good results at the Antec Scientific R&D laboratory.

If for any reason alternative chemicals need to be purchased, be aware that chemicals that have a specification of high purity may have been tested for UV-active impurities, which can mean that they may still contain electrochemically active impurities. This is one of the reasons why 'HPLC grade' water is not recommended for use with EC detection:

- choose chemicals with the same purity or better
- do not choose ultra dry grade or anhydrous chemicals

Component	Purity	Brand	Order no:	Mw	kg/L
HNO ₃	65% solution	Fluka	84380	63.01	D:1.40
NaOH	50% w/w solution, certified grade	Fisher	SS254500	40.00	D:1.56
	50% in water, Pro analyse, carbonate free	Boom	80011912	40.00	D:1.57
	50-52%, eluent for IC	Sigma Aldrich	72064	40.00	D:1.53
Acetone	General purpose grade	Fisher	A/0520/17	58.08	D:0.79
Water	TOC <10ppb and deionised, r	esistivity >1	8 MOhm-cm (Ba	arnstead Eas	sypure II)

Table 1. Brands and purities of chemicals used for application development at Antec Scientific.

Manufacturers/vendors

Sigma-Aldrich	http://www.sigmaaldrich.com
Fluka	http://www.sigmaaldrich.com
Fisher Scientific	http://www.fishersci.com
Barnstead	http://www.thermoscientific.com