

## INSTALLATION REQUIREMENTS FOR THE ALEXYS® MONOAMINES ANALYZER

This document lists the requirements at the customer's site for a successful installation of the ALEXYS® Monoamines Analyzer (p/n 180.0088C). It also summarizes all relevant conditions, chemicals and consumables that are to run the monoamines application.

The customer must arrange the listed necessities before the start of the installation.

Therefore, this document should be send to the customer well in advance of the installation date in order for them to be able to take the necessary actions.

### **Computer**

The minimal PC hardware and software requirements for the installation of the ALEXYS® system in combination with the Clarity® HPLC data software are listed in document 195.7000.

### **Laboratory facilities**

- For the preparation of the LC solutions and standards, access to the proper facilities is a prerequisite (microbalance, pH meter and relevant pH standards, analytical pipettes, pipette tips, tubes, glassware such as measuring cylinders, etc.).
- An ultra sonic bath is required for degassing the mobile phase, the piston backwash solution and the auto sampler wash liquid before installation in the ALEXYS® system.  
**Do not use vacuum filtering units for degassing** or filtering because it can introduce electrochemically active contaminations. The ALEXYS® system is equipped with in-line filters and degassers.

### **System consumables: flow cell cleaning**

The following items should be available for flow cell cleaning:

- Soft paper tissue (for instance Kleenex facial tissues)
- A squeeze bottle with acetone
- A squeeze bottle with deionised water

## **System consumables: sample plates**

The AS 110 auto sampler has two positions to place standard shaped sample plates. The ALEXYS Analyzer is shipped with transparent sample trays ('AS 110 vial holder 96 low, start-up kit', p/n 191.0600) that consists of two base plates and two 96 positions PCR plates. These plates will fit the 300 µL PP fraction collector vials from Microbiotech (Fig. 1). The plates can be reused, but in case of damage, replacements can be reordered at Greiner bio-one (Fig. 2).



*Fig. 1 Assembly of 96- positions base plate and PCR plate with 5 inserted fraction collector vials.*



*Fig. 2 Left: transparent 96-positions plate (reordering info: Greiner bio-one, pn. 652280), and right: 96-positions flat bottom plate holder (reordering info: Greiner bio-one, pn. 655101).*

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## System consumables: sample vials

To prevent an autosampler needle crash, the needle height needs to be checked during an installation. Needle height adjustment is especially important when using alternative vials. How to do this is explained in the Appendix of document 'Installation LC connections' (p/n 180.7001).

With each ALEXYS Analyzer the following parts are delivered to handle samples:

1. AS 110 sample vials PP, start-up kit (p/n 250.0602), containing:
  - Polypropylene 300 µL fraction collector vials (± 200 pcs)
  - aluminum crimp seals with PTFE septa (± 200 pcs)
2. Crimper tool 8 mm (p/n 250.1300)



Fig. 3. Crimper tool, caps and vials shipped with each ALEXYS neurotransmitter analyzer.

Table 1. Recommended autosampler vials and caps for small volume microdialysate samples. Any real equivalent from another supplier can be used.

For sample tray	Type	Supplier	p/n
96 positions	Sample Vials polypropylene 300 uL	Microbiotech	4001048
96 positions	8 mm Crimp cap with PTFE seal for single use	Chromacol	8-ACT
96 positions	Blue snapcap with precut FEP/Silicone inlay (alternative for the aluminum crimp caps: requires no crimper tool, but more expensive)	MicroLiter Analytical Supplies, Inc.	07-0020 B



NOTE: There are subtly different shapes of fraction collector vials on the market. The types that fit best are depicted on the left side in Fig. 4. The types depicted on the right are slightly too wide to fit well in the 96 position tray, but such vials can be sampled by placing them in adaptors that fit in the 48 positions tray. Adaptors can be purchased at Antec Leyden (pn. 181.0726; Microdialysis coll. vial adaptors, 100pcs)

Fig. 4. Picture of two slightly different fraction collector vials.

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## CHEMICALS

### General

- All relevant chemicals should be available at the lab at the moment of installation to make mobile phase, standards, reagent etc.
- For LC-ECD only chemicals of sufficient specific quality should be used to be able to set up an optimal system with good performance.

Note: chemicals that are highly purified for application with UV detection may contain electrochemically active impurities! Therefore, HPLC grade water that was tested for UV-active impurities is not recommended for use with EC detection. Instead, use **deionised water with a resistivity of at least 18 MOhm-cm.**

- See the appendix for more detailed descriptions of the chemicals that have been used in the Antec R&D laboratory.

### System chemicals

The following chemicals are necessary for general system performance (piston wash solution of the pump, column cleaning, needle wash solution of the autosampler, column storage, and for flow cell cleaning).

- Demineralised water with a resistivity of at least 18 MOhm-cm
- Acetone
- Methanol
- Iso-propanol (for occasional check valve cleaning)
- Acetonitril (column storage)

## **Application-specific chemicals**

For the analysis of NA, DA, 5-HT and the acidic metabolites DOPAC, HVA and 5-HIAA, the following chemicals are necessary:

- Phosphoric acid (we recommend the commercially available solution of 85% w/v in water)
- Citric acid, monohydrate
- Ethylenediaminetetraacetic acid (EDTA)
- Octane sulfonic acid, sodium salt (OSA)
- Methanol
- Demineralised water with a resistance of at least 18 MOhm-cm
- 50% w/w NaOH in water (commercially available solution)
- Standards of the components of interest in high purity grade
- Perchloric acid (acidifier for standard stock solutions)
- Acetic acid (acidifier for standard dilutions)

## **Solutions**

The following solutions should be available at the start of the first installation day. These solutions should thus be arranged/prepared in advance by the customer.

- A small bottle with about 50 mL of 15% HNO<sub>3</sub> is needed once during the installation for passivation of the metal parts of the ALEXYS system.
- 1 L demineralised water, degassed
- 250 mL 20% (v/v) MeOH in demineralised water, degassed

**Mobile phase for the analysis of the catecholamines and metabolites**

Two different mobile phases are required for the analysis of NA, DA, 5-HT and the metabolites. One mobile phase composition is specific for the analysis of DA and 5-HT. The other mobile phase is specific for the separation of NA and the metabolites.

Table 2. Mobile phase composition and Ecell for the analysis of catecholamines and metabolites.

	Flow path 1	Flow path 2
Analysis	DA and 5-HT	NA and metabolites
Column	NeuroSep 105	NeuroSep 115
Ecell	460 mV	900 mV
Mobile phase composition	50 mM phosphoric acid 0,1 mM EDTA 17,5 % methanol 400 mg/L OSA pH 6.0	50 mM phosphoric acid 50 mM citric acid 0,1 mM EDTA 17.5 % methanol 600 mg/L OSA pH 3.0

**Preparation of 1 L mobile phase**

- Dissolve 0.0292 g EDTA in about 20 mL demineralised water, with 2-3 drops of 50% w/w NaOH solution, in a small glass beaker and a clean stirring bean.
- In a large glass beaker containing about 0.5 L demineralised water, add 3.45 mL 85% w/v phosphoric acid solution. Also dissolve 10.51 g citric acid at this point for preparation of the mobile phase of flow path 2 (see Table 2).
- Transfer the dissolved EDTA solution to the mobile phase.
- Set the pH of the mobile phase to the correct pH (see Table 2) using 50% NaOH solution.
- Add the required volume of methanol (see Table 2).
- Dissolve the required amount of OSA in the mobile phase (see Table 2).
- Fill up to 1 L with demineralised water.
- Degas the mobile phase 15 minutes in a sonic bath.

**APPENDIX**

A list of chemicals is shown below as a guideline for the purchase of chemicals at the customer site. The listed brands/purities are not necessarily the best chemicals, but the application was developed at the Antec R&D laboratory using these specific brands/purities. If for any reason alternative chemicals need to be purchased use the following guidelines:

- The chemicals should have at least the same purity or better than the chemicals listed in the table below
- Do not purchase ultra dry grade or anhydrous chemicals

*Table 3. Brands and purities of chemicals used for application development at Antec.*

Component	Purity	Brand
1-Octane sulphonic acid, sodium salt (OSA)	HPLC grade	Acros
Acetone	General purpose grade	Fisher
Citric acid, monohydrate	p.a.	Acros
Ethylenediamine-tetraacetic acid (EDTA)	99%	Acros
Methanol	HPLC gradient grade	Baker
NaOH, 50% w/v in water	puriss., p.a., for HPLC; 50%	Fluka
Acetic acid	99.8% for analysis	Acros
Perchloric acid, 70% (PCA)	p.a.	Acros
Phosphoric acid, 85% w/v in water	p.a.	Acros
Water	Deionised, resistivity >18 MOhm-cm (Barnstead Easypure II)	

**Manufacturers**

ACROS Organics  
 JT-Baker  
 Fluka  
 Fisher Scientific