

INSTALLATION REQUIREMENTS FOR THE ALEXYS® MONOAMINES ANALYZER

In this document the requirements for the installation of the ALEXYS® Monoamines analyzer (p/n 180.0088B) are listed. It summarizes all relevant conditions, chemicals and consumables that are required at the customer site for a successful installation and to run the monoamines application. This document should be send to the customer well in advance of the installation date in order to be able to take the necessary actions. The customer must arrange these necessities before the start of the installation.

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 of the mobile phase and the auto sampler wash liquid before use in ALEXYS® system. Do not use vacuum filtering units because it can introduce electrochemically active contaminations.

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 vials

The autosampler can hold two sample trays. The ALEXYS system comes with two sets of different trays. One set of trays consists of a holder and a 96 positions plate intended to fit slim fraction collector vials (Fig. 1).

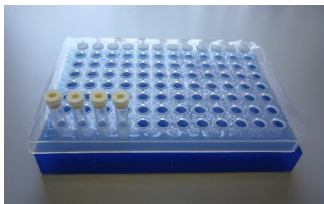


Fig. 1 Assembly of 96 positions plate and holder with 4 microvials capped with soft rubber caps.

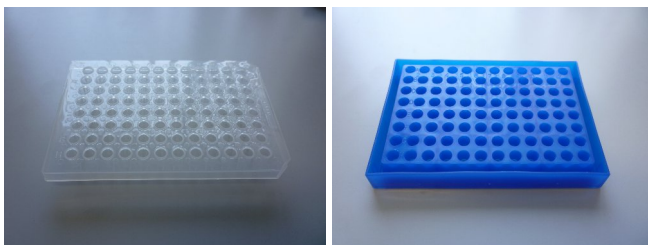


Fig. 2 Transparent 96 positions plate (reordering info: Greiner bio-one, pn. 652280) and blue plate holder (reordering info: Greiner bio-one, pn. 880070).

The other set of trays are stainless steel plates that can each hold 48 vials with an outer diameter (OD) of 11.6 mm

There are subtly different shapes of fraction collector vials on the market. The types that fit best are depicted on the right side in Fig. 3. The types depicted on the left 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 adapters, 100pcs)



Fig. 3. Picture of two slightly different fraction collector vials. The subtly slimmer type on the right side will fit in the 96 positions tray of the autosampler. For the slightly wider type on the left, adapters are necessary, which fit in the 48 positions tray.

We recommend using vials in combination with airtight caps. Do not use hard plastic caps, as these can result in a broken autosampler sampling needle.



Fig. 4. Typical fraction collector caps, recloseable. Do not use the hard plastic type on the left, as this may damage the needle of the autosampler. The soft rubber type on the right can be used.

Table 1. Recommended autosampler vials and caps. Any equivalent from another supplier can be used.

| Sample | For sample tray | Type & dimensions | Supplier | p/n |
|---------------------------------|-----------------|---|---------------|----------|
| Standards | 48 positions | 1.5 mL Snap Ring Vials, 32 x 11.6mm, clear | Grace Alltech | 98030 |
| Small volumes | 48 positions | Topsert (tm) TPX-short thread Vial, 32 x 11.6mm with 0.2 mL glass micro inserts | Grace Alltech | AV061890 |
| Small volumes (microdialysates) | 96 positions | Sample Vials polypropylene 300 uL | Microbiotech | 4001048 |
| Small volumes (microdialysates) | 96 positions | Caps re-sealing | Microbiotech | 400 1030 |

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-EC only chemicals of sufficient specific quality should be used to be able to set up an optimal system with good performance. Note that chemicals that are highly purified for application with UV detection may contain electrochemically active impurities! Therefore, HPLC grade water (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
- 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

At the start of the installation the following solutions are necessary on day 1. These solutions should be arranged/prepared in advance by the customer. The customer will also be asked to prepare mobile phase on day 1.

- About 50 mL of 15% HNO₃ 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 for the analysis of catecholamines and metabolites.

| | Flow path 1 | Flow path 2 |
|--------------------------|--|---|
| Analysis | DA and 5-HT | NA and metabolites |
| Column | ALF-105 | ALF-115 |
| Mobile phase composition | 50 mM phosphoric acid 0,1 mM EDTA 12,5% methanol 500 mg/L OSA pH 6.0 | 50 mM phosphoric acid 50 mM citric acid 0,1 mM EDTA 10 % methanol 500 mg/L OSA pH 3.25 |

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.
- 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 0.500 g OSA in the mobile phase
- Fill up to 1 L with demineralised water.
- Degas the mobile phase

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 Monoamines 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 then 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