

INSTALLATION REQUIREMENTS FOR THE ALEXYS® OMD ANALYZERS

This document lists the requirements for the installation of the ALEXYS[®] OMD time resolution system (p/n 180.0083C) and the ALEXYS[®] OMD high throughput system (p/n 180.0082C). It also summarizes all relevant conditions, chemicals and consumables to run the OMD time resolution or high throughput applications.

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 preparation of LC solutions and standards
 - □ Microbalance
 - D pH meter and relevant pH standards
 - □ analytical pipettes, pipette tips, tubes
 - glassware such as measuring cylinders
 - □ etc.
- Ultra sonic bath

□ The mobile phase, piston backwash solution and auto sampler needle wash solution require degassing before use in the ALEXYS® system.





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• Water apparatus

For supply of fresh high quality deionised water with resistivity of >18
MOhm.cm and low TOC level (<10 ppb)

System consumables

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





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 and TOC<10ppb.

• See the appendix for more detailed descriptions of the chemicals that have been used in the Antec R&D laboratory, as an example.

System chemicals

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

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





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Application-specific chemicals

For the analysis of NA and DA or DA and 5-HT, the following chemicals are necessary:

- Phosphoric acid (we recommend the commercially available solution of 85% w/v in water)
- Di sodium ethylenediaminetetraacetic acid (EDTA. Na2)
- 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
- Appropriate chemicals to prepare standard stock solutions
- Perchloric acid (acidifier for standard stock solutions)
- Acetic acid (acidifier for standard dilutions)

Standard stock solutions

• 10 mL 1 mM standard in 0.1 M perchloric acid. Store at 4 °C until use (max 1 month)

Solutions

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

 A small bottle with about 50 mL of 15% HNO₃ is needed once during the installation for passivation of the metal parts of the ALEXYS system.

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- 1 L demineralised water, degassed
- 250 mL 20% (v/v) MeOH in demineralised water, degassed





Mobile phase for the analysis of monoamines

Two different mobile phases are available for analysis. The choice of mobile phase is dependent on the need to analyse the combination of DA and 5-HT or the combination of NA and DA. Note that for these different sets of components, also different columns are necessary. The standard columns that come with the systems are the NeuroSep-105. Please check that additional/alternative columns were ordered with the system if necessary.

| Table 1. Mobile phase composition for the analysis of DA and 5-HT or NA | and DA |
|---|--------|
|---|--------|

| Analysis | DA and 5-HT | NA and DA |
|--------------|-----------------------|-----------------------|
| Columns | NeuroSep-105 | NeuroSep-115 |
| Mobile phase | 50 mM phosphoric acid | 50 mM phosphoric acid |
| composition | 0,1 mM EDTA | 0,1 mM EDTA |
| | 17,5 % methanol | 12,5% (v/v) methanol |
| | 400 mg/L OSA | 500 mg/L OSA |
| | рН 6.0 | рН 6.0 |

Preparation of 1 L mobile phase

- Add 0.8 litre water and a clean stir bar to a clean glass beaker and let it stir on a stirring plate.
- Add 0.0372 g Na2EDTA.2 H2O
- Let it stir until completely dissolved before adding the next component.
- Add 3.45 mL 85% w/v phosphoric acid solution.
- Set the pH of the mobile phase to 6.0 using 50% NaOH solution.
- Add the required volume of methanol (175 or 125 mL, see Table 1).

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- Dissolve the required amount of OSA in the mobile phase (see Table 1).
- Fill up to 1 L with demineralised water.
- Degas the mobile phase for 15 min in a sonic bath. By any means do <u>not</u> filtrate the mobile phase as this can be a source of electrochemical contamination.





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 then the chemicals listed in the table below
- Do not purchase ultra dry grade or anhydrous chemicals as these contain electrochemical contaminants from the drying process

| Component | Purity | Brand | Order no: | Mw | Kg/L | |
|--|------------------------------|-------|-----------|--------|--------|--|
| Ortho-Phosphoric acid, 85% w/v in water | p.a. | Fluka | 79620 | 98.00 | D:1.68 | |
| Na₂EDTA. 2 H₂O | SigmaUltra, 99% | Acros | 147855000 | 372.23 | | |
| 1-Octane sulphonic acid, sodium salt (OSA) | HPLC grade | Acros | 384771000 | 216.28 | | |
| Methanol | HPLC gradient grade | | | | | |
| NaOH, 50% w/v in water | puriss., p.a., for HPLC; 50% | Fluka | 71686 | 40.00 | D:1.54 | |
| Perchloric acid | p.a. | Acros | 223310010 | 100.46 | 1.66 | |
| Acetic acid | 99.8%, for analysis | Acros | 222140010 | 60.05 | 1.048 | |
| Water TOC <10 ppb and deionised, resistivity >18 MOhm-cm (Barnstead Easypure II) | | | | | | |

Table 2. Brands and purities of chemicals used for application development at Antec.

Manufacturers

Sigma-Aldrich

http://www.sigmaaldrich.com

Fluka

http://www.sigmaaldrich.com



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Barnstead

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