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INSTALLATION REQUIREMENTS FOR THE ALEXYS® GABA/Glu ANALYZER

This document lists the requirements at the customer's site for a successful the installation of the ALEXYS® GABA/Glu analyzer (p/n 180.0070C). It also summarizes all relevant conditions, chemicals and consumables to run the GABA/Glu 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

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System consumables: sample plates

The AS 110 auto sampler has two positions to place sample plates. The ALEXYS Analyzer is shipped with two different sets of sample tray holders. One set contains 48-positions plates that fit vials with an outer diameter of 11.6 mm. The second set ('AS 110 vial holder 96 low, start-up kit', p/n 191.0600) contains two base plates and two 96 positions PCR plates, which fit the 300 µL PP fraction collector vials from Microbiotech (Fig. 1). These plates can be reused, but in case of damage, a replacement 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 crimp 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 adapters, 100pcs).

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

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 GABA and glutamate, 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)
- 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
- Sodium sulfite, anhydrous
- Boric acid
- Ortho-Phthalaldehyde (OPA). Note: the amount and composition of impurities in OPA varies from brand to brand. It is strongly recommended to use OPA from Aldrich only.

Start-up 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% HNO_3 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

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MOBILE PHASE AND REAGENT FOR THE ANALYSIS OF GABA/Glu

Mobile phase

Table 2. Mobile phase composition for the analysis of GABA and glutamate.

50 mM phosphoric acid
50 mM citric acid
0,5 mM EDTA
pH 3.50
5% methanol

Preparation of 2 L of mobile phase with the final composition as given in Table 2:

- Dissolve 0.2922 g EDTA in about 20 mL demineralised water, with 2-3 drops of 50%
- w/w NaOH solution, in a small glass beaker,
- In a large glass beaker containing about 1.7 L demineralised water, dissolve 21.015 g citric acid (monohydrate) and add 6.70 mL 85% w/v phosphoric acid solution.
- Transfer the dissolved EDTA solution to the mobile phase.
- Adjust the pH of the mobile phase to pH 3.5 using a 50% NaOH solution.
- Add 100 mL methanol.
- Fill up to 2 L with demineralised water.
- Degas the mobile phase in a sonic bath.

Refresh the running mobile phase **at least once every 3 days** or more often if bacterial growth is observed earlier!

OPA reagent

The derivatisation procedure and composition of the OPA reagent was modified from Smith & Sharpe (1994) and Beverly *et al* (2001) resulting in the composition given in Table 3.

Table 3. OPA reagent composition for the analysis of GABA and glutamate.

37 mM OPA
50 mM sodium sulphite
90 mM boric acid, pH 10.4
5% methanol

- Dissolve 25 mg OPA in 250 uL methanol in a 5 mL glass autosampler vial.
- Prepare 1 mL of 1M sodium sulphite solution in an Eppendorf vial (do not prepare a stock solution for storage; prepare shortly before adding it to the reagent).
- Add 250 µL 1M sodium sulphite to the OPA-MeOH solution in the autosampler vial.
- The solution will turn turbid/white.
- Add 4.5 mL 0.1 M tetraborate buffer (adjusted to pH 10.4 with 14 M sodium hydroxide), which will turn the reagent clear again.

The tetraborate buffer can be made in advance in a larger quantity and stored in the fridge until use. The OPA-sulphite reagent should be prepared fresh each day.

The OPA reagent is light sensitive. Please cover the vial (except the top part) with aluminum foil to minimize degradation due to sunlight.

[1] S. Smith, T. Sharpe (1994) *Journal of Chromatography B*, **652**: 228-233

[2] L. Beverly, M. G. de Vries, S. D. Bouman, L. M. Arseneau (2001) *Am J Physiol Regulatory Integrative Comp Physiol*, **280**:R563-R569

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

Table 4. Brands and purities of chemicals used for application development at Antec.

Component	Purity	Brand
Phosphoric acid, 85% w/v in water	p.a.	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
Phosphoric acid, 85% w/v in water	p.a.	Acros
Water, deionised with an Easypure II	Resistivity >18 MOhm-cm	Barnstead
GABA (gamma-aminobutyric acid)	> 99 % (A2129)	Sigma
Glutamate (l-glutamic acid)	> 99% (G1251)	Sigma
OPA	97%	Aldrich
sodium sulphite, anhydrous	>98%	Sigma
boric acid	p.a. (>99.8%)	Merck

Manufacturers

ACROS Organics
JT-Baker
Fluka
Fisher Scientific
Sigma-Aldrich