INSTALLATION REQUIREMENTS FOR THE ALEXYS® GABA/Glu ANALYZER

In this document the requirements for the installation of the ALEXYS® GABA/Glu analyzer (p/n 180.0070A) 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 GABA/Glu 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 arranged 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 auto sampler wash liquid before use in ALEXYS® system. Do not use vacuum filtering units because it can introduce electrochemical contaminations.

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

We recommend the following vials in combination with air-caps, which are available at Grace Alltech. Any equivalent from an other supplier can also be used.

Table 1. Recommended autosampler vials. These are available at Grace Alltech.

Sample	Segment	Vial type & dimensions	p/n (Grace Alltech)
Standards and reagents	A	1.5 mL Snap Ring Vials, 32 x 11.6mm, with airtight caps	98030
Reagents	C	5 mL Crimp Neck Vial, 38x22 mm, with airtight caps	AV208011
Small volumes (microdialysates)	A	Topsert (tm) TPX-short thread Vial, 32 x 11.6mm with 2 mL glass micro inserts	AV061890

Note that the ALEXYS monoamines analyzer comes with a set of aluminium adapters for fraction collector vials. In that case fraction collector vials (35 x 5.5 mm OD) can be directly transferred to the autosampler without loss of sample. Do not use fraction collector vial caps that are deeper than 4 mm (see vials and caps on the right-side on the photos shown below), these caps cannot be penetrated by the AS 100 pre-puncturing needle.

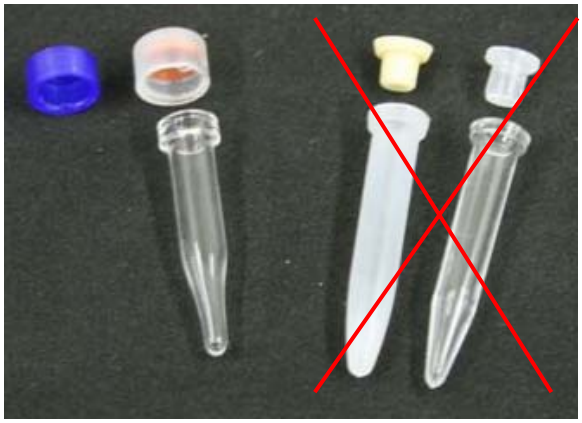

Table 2. Recommended fraction collector vial and caps. These are available at VWR-Omnilabo. See vial and caps on the lefft-side on the photos shown above.

Part	P/N (VWR-Omnilabo)
Vial conic glass (0.3 mL)	548-0078
Cap blue	548-0810
Cap re-pierceable	548-0374

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 the use with UV detection may contain electrochemically active impurities. For example, HPLC grade water (tested for UV-active impurities) is not recommended for 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 are use at 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).

- Iso-propanol
- Demineralised water with a resistivity of at least 18 MOhm-cm
- Acetone
- Methanol
- 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.

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.

- About 50 mL of 15% HNO_3 is needed once during the installation for passivation of the metal parts of the ALEXYS system.
- 500 mL 20% (v/v) MeOH in demineralised water, degassed
- 250 mL 20% (v/v) iso-propanol in demineralised water, degassed
- 1 L demineralised water, degassed

MOBILE PHASE AND REAGENT FOR THE ANALYSIS OF GABA/GLU

Mobile phase

Table 3. Mobile phase composition for the analysis of GABA and glutamate.

Mobile phase composition	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 3:

- 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.

OPA reagent

Table 4. OPA reagent composition for the analysis of GABA and glutamate.

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

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 4:

- 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 aluminium 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 GABA/Glu 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 5. Brands and purities of chemicals used in the Antec R&D lab during application development.

Component	Purity	Brand
Acetone	General purpose grade	Fisher
Citric acid, monohydrate	p.a.	Acros
Ethylenediaminetetraacetic acid (EDTA)	99%	Acros
Isopropanol	pure (>99.99%)	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	De-ionized to resistivity >18 MOhm-cm with Elga UHQ apparatus	
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