

## INSTALLATION REQUIREMENTS FOR THE ALEXYS® GABA/Glu ANALYZER

In this document the requirements for the installation of the ALEXYS® GABA/Glu analyzer (p/n 180.0070B) based on the AS 110 micro auto sampler 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 sent 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

### System consumables: sample vials

The AS 110 auto sampler has two positions for well plates or vial adapters. The ALEXYS GABA-GLU analyzer is shipped with part 191.0600 'AS 110 vial holder 96 low, start-up kit'. It consists of a base plate and 96 positions PCR plate in which 300 µL PP fraction collector vials of Microbiotech fit (Fig. 1).



Fig. 1 Assembly of 96-positions PCR plate with 5 fraction collector vials.



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

With the ALEXYS analyzer the following parts are delivered:

1. Crimper tool 8 mm (part number 250.1300)
2. AS 110 sample vials PP, start-up kit (part number 250.0602)

The sample kit start-up kit consists of 200 Polypropylene 300 µL fraction collector vials and aluminium crimp seals with PTFE septa.



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

Re-ordering information can be found below:

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

Sample	For sample tray	Type & dimensions	Supplier	p/n
Small volumes (microdialysates)	96 positions	Sample Vials polypropylene 300 uL	Microbiotech	4001048
Small volumes (microdialysates)	96 positions	8 mm Crimp cap with PTFE septa (requires crimp tool)	Chromacol	8-ACT
Small volumes* (microdialysates)	96 positions	Blue snapcap with precut FEP/Silicone inlay (requires no tool)	MicroLiter Analytical Supplies, Inc.	07-0020 B

\*) alternative for the alumina crimp caps with PTFE septa

## 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<sub>3</sub> 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 2. 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
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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.

**OPA reagent**

Table 3. 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
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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:

- Dissolve 25 mg OPA in 250  $\mu$ L 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  $\mu$ L 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.**

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- [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 than the chemicals listed in the table below
- Do not purchase ultra dry grade or anhydrous chemicals

Table 4. 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 MΩ·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