Antec Scientific
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Requirements

for GABA & glutamate analysis

using the ALEXYS® Neurotransmitters system (180.0091UA) with/without gradient upgrade

180.7052u, Edition 6, 2025

Warning Symbol

The warning sign denotes a warning. It calls attention to a procedure or practice which, if not adhered to, could result in costs, damage or destruction of parts or all of the equipment. Do not proceed beyond a warning sign until the indicated conditions are fully understood and met.

For research purposes only. **The ALEXYS system is not tested by the manufacturer to comply with the In Vitro Diagnostics Directive.**

Observe safety

Operation of an electrochemical detector can involve the use of hazardous materials including corrosive fluids and flammable liquids. The instrument should only be operated by users with the following expertise:

- Completed degree as chemical laboratory technician or comparable vocational training
- Fundamental knowledge of liquid chromatography
- Knowledge and experience in the safe handling of toxic and corrosive chemicals and knowledge of the application safety measures prescribed for laboratories.
- Participation in an end-user training (daily use of system and chromatography software) performed by the manufacturer or a company authorized by the manufacturer.

Unskilled, improper, or careless use of the instrument and the related chemicals can create fire hazards, or other hazards which can cause death, serious injury to personnel, or severe damage to equipment and property.

Observe all relevant safety practices at all times.

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C H A P T E R 1

Introduction

Thank you for ordering an ALEXYS LC-ECD system. For a successful on-site installation of the **GABA-Glu** analysis on the ALEXYS system, please arrange the following requirements at your location in advance:

- a computer (see document 195.7000 ‘PC requirements and settings’)
- general laboratory conditions and facilities, consumables and chemicals for use with the ALEXYS system (see document 180.7070C ‘General requirements for installation of ALEXYS systems’)
- application specific chemicals and consumables (this document)

Arrange these requirements well in advance before the installation to prevent (costly) delays.

This document lists the application specific chemicals for the analysis of GABA and glutamate using the combination of the following hardware:

ALEXYS® Neurotransmitters system (180.0091UA)

SenCell 2 mm GC sb (116.4120)

Add-on parts for GABA-Glu analysis (2-p HPG option) 180.0504UA

or Add-on parts for GABA-Glu analysis, using SSV option (180.0504SA)

CHAPTER 2

Chemicals

For LC-ECD applications, only chemicals of sufficient specific quality should be used to be able to have an optimal system with good performance. The appendix shows detailed descriptions of some of the chemicals that have been used in the Antec R&D laboratory, as an example of what works.

Have the chemicals and solutions ready at the start of the installation.

Standard stock solutions

Chemicals

- Standards of GABA and glutamate in high purity grade
- Water (Resistivity >18MOhm.cm, TOC<10ppb)

Preparation

- 10 mL 1 mM GABA in water
  Store at 4 °C until use (max 1 month)
- 10 mL 1 mM Glutamate in water
  Store at 4 °C until use (max 1 month)

Microdialysis perfusion solution

In case the sample is in a background of perfusion solution, the standards should also be prepared in this solution.

Chemicals

- Chemicals for preparation of microdialysis perfusion fluid
- Water (Resistivity >18MOhm.cm, TOC<10ppb)

Preparation

- □ 100 mL microdialysis perfusion fluid
  Store at 4 °C until use (max 2 month)

  Suggestion for perfusion fluid composition:

Component	Concentration (mM)
NaCl	147
KCl	3
$MgCl_2.6H_2O$	1.2
$CaCl_2$	1.2

Note: sample should not be acidified.

Calibrator solutions

Preparation (on the day of use)

- □ For a 5-point linear calibration in a relevant concentration range mix and dilute the standard stock solutions in a background of perfusion fluid to 1 mL total volume each:
  - Calibrator 0: Blank (microdialysis perfusion fluid)
  - Calibrator 1: 50 nM GABA; 0.5 uM glutamate
  - Calibrator 2: 100 nM GABA; 1 uM glutamate
  - Calibrator 3: 150 nM GABA; 1.5 uM glutamate
  - Calibrator 4: 200 nM GABA; 2 uM glutamate
  - Calibrator 5: 250 nM GABA; 2.5 uM glutamate

Mobile phase

Mobile phase A (Separation)	*Mobile phase B (Clean-up)*
50 mM phosphoric acid 50 mM citric acid 0,1 mM EDTA pH 3.50 2% v/v Acetonitrile	50 mM phosphoric acid 50 mM citric acid 0,1 mM EDTA pH 3.50 50% v/v Acetonitrile

Note: these mobile phase compositions are suggested starting conditions and not guaranteed to give best results for all kinds of unknown samples. Condition optimisation may be necessary.

Chemicals

- □ Phosphoric acid (commercial solution of 85% w/v in water)
- □ Citric acid, monohydrate

- □ Di sodium ethylenediaminetetraacetic acid (EDTA. Na_2)
- □ Acetonitrile
- □ 50% w/w NaOH in water (commercial solution)
- □ Water (Resistivity >18MOhm.cm, TOC<10ppb)

Preparation

Base solution

1. Add about 0.9 Litre de-ionized low-TOC water in a wide, clean glass beaker and add a clean stir bar
2. Add 0.0744 g $Na_2EDTA.2\ H_2O$ and stir till it is completely dissolved
3. Add 6.85 mL 85% w/v phosphoric acid solution
4. Add 21.015 g citric acid and stir till it is completely dissolved.
5. Set the pH of the solution to pH 3.5 using 50% NaOH solution.
6. Add de-ionized low-TOC water to a total volume of 1.0 Litre

This solution can be stored in the fridge at 4°C for about one week

Mobile phase A

1. Pour 250 mL base solution in a clean glass cylinder
2. Add 10 mL Acetonitrile
3. Add de-ionized low-TOC water to a total volume of 500 mL
4. Transfer the mobile phase to a cleaned GL45 glass bottle that is provided with the system
5. Degas the mobile phase in a sonic bath for about 15 minutes.

The mobile phase A should be refreshed at least every 3 days when it is kept at room temperature.

Mobile phase B

1. Use a cleaned 1L GL45 glass bottle that is provided with the system
2. Pour 250 mL of the base solution in the 1L bottle
3. Add 250 mL Acetonitrile and stir
4. Degas the mobile phase in a sonic bath for about 15 minutes.

The mobile phase B should be refreshed at least every week when it is kept at room temperature on the system.

Check the mobile phases daily for microbial growth. Refresh mobile phase A **at least once every 3 days** or more often if bacterial growth is observed earlier!

Do not filter the solutions by any means; the 0.2 µm inline Whatman filters present in the low pressure solvent lines will take care of filtering.

0.1 M borate buffer, pH 10.4 (reagent component)

Chemicals

- □ Boric acid
- □ 50% w/w NaOH in water (commercial solution)
- □ Water (Resistivity >18MOhm.cm, TOC<10ppb)

Preparation

1. Add about 90 mL de-ionized low-TOC water in an empty and clean glass beaker and add a clean stir bar
2. Add 0.618 g boric acid and stir till completely dissolved
3. Set the pH of the solution to pH 10.4 using 50% NaOH solution
4. Transfer to a measuring cylinder
5. Add de-ionized low-TOC water to a total volume of 100 mL

This solution can be stored in the fridge at 4°C for about one month

1 M sulfite solution (reagent component)

Chemicals

- □ sodium sulfite, anhydrous
- □ Water (Resistivity >18MOhm.cm, TOC<10ppb)

Preparation

1. Weigh 126 mg sodium sulfite and add to an Eppendorf vial
2. Add 1 mL de-ionized low-TOC water
3. Shake vigorously until fully dissolved

This solution cannot be stored and has to be prepared shortly before preparing the OPA reagent.

OPA reagent

The derivatization 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 1.

Table 1. 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

Chemicals

- Methanol
- 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 Pickering only.

Preparation

1. Weight 25 mg Ortho-Phthalaldehyde and transfer to a small glass beaker.
2. Add 250 uL methanol and shake gently until crystals are dissolved
3. Add 250 µL 1M sodium sulfite solution.
   The solution will turn turbid/white.
4. Add 4.5 mL 0.1 M borate buffer, pH 10.4
   The reagent will turn clear again.
5. For the method that uses 9 uL of sample, transfer 150 uL reagent to a microdialysis/autosampler vial, cap and place in the sample tray compartment.
   For the method that uses only 5 uL of sample, the reagent has to be diluted 1:1 with water before placing in the sample tray.

The OPA reagent is light sensitive. The remaining batch can be covered with aluminum foil and stored for about 2 days in the fridge at 4°C.

[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

System wash solutions

Chemicals

- □ Isopropanol
- □ Acetonitrile
- □ Water (Resistivity >18MOhm.cm, TOC<10ppb)

Preparation

- □ 250 mL water, degassed (autosampler needle wash solution)
  Cap and store at room temperature until use (max 1 day)
- □ 1 L 20% isopropanol in water, degassed (piston wash)
  Cap and store at room temperature (max 1 month). Refresh the solution that is connected with the pump every week.
- □ 250 mL 20% acetonitrile, degassed (column flushing)
  Cap and store at room temperature until use (max 1 month)
- □ 250 mL 50% acetonitrile, degassed (column flushing/storage)
  Cap and store at room temperature until use (max 1 month)

A P P E N D I X

A list of the application specific chemicals with purity and purchase details is shown below as a guideline. The listed brands/purities are not necessarily the best chemicals, but these have been giving good results at the Antec R&D laboratory.

If for any reason alternative chemicals need to be purchased, be aware that chemicals that have a specification of high purity may have been tested for UV-active impurities, which can mean that they may still contain electrochemically active impurities. This is one of the reasons why general 'HPLC grade' water is not suitable for use with EC detection:

- choose chemicals with the same purity or better
- do not choose ultra dry grade chemicals

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

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
Citric acid, monohydrate	p.a.	Acros	124910010	210.14	
$Na_2EDTA.\ 2\ H_2O$	SigmaUltra, 99%	Acros	147855000	372.23	
Acetonitrile	HPLC grade, 99.9%	Acros	268260025	41.05	D:0.781
NaOH, 50% w/v in water	puriss., p.a., for HPLC; 50%	Fluka	71686	40.00	D:1.54
GABA (gamma-aminobutyric acid)	> 99 % (A2129)	Sigma	A2129	103.12	
Glutamate (l-glutamic acid)	> 99% (G1251)	Sigma	49449-100G	147.13	
Ortho-phthalaldehyde (OPA)	97%	Aldrich	P3,940-0	134.13	
OPA (from Pickering)	Chromatographic grade	LCTech	120	134.13	
sodium sulphite, anhydrous	>98%	Sigma	S,0505	126.0	
boric acid	p.a. (>99.8%)	Merck	1650500	61.83	
Water	TOC <10ppb and deionised, resistivity >18 MOhm-cm (Barnstead Easypure II)				

Manufacturers/vendors

JT-Baker	http://www.avantormaterials.com
Sigma-Aldrich	http://www.sigmaaldrich.com
Fluka	http://www.sigmaaldrich.com
Fisher Scientific	http://www.fishersci.com
Barnstead	http://www.thermoscientific.com
LC Tech	http://www.lctech.de