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Instructions for use

GABA ELISA

REF **IB89150R**

2°C – 8°C

Σ 96

RUO

For research use only – Not for use in diagnostic procedures

Table of contents

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of gamma-aminobutyric acid (GABA) in urine, to evaluate GABA homeostasis.
After extraction and derivatization gamma-aminobutyric acid (GABA) is quantitatively determined by ELISA.
The subsequent competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized analyte concentrations of the standards, controls and samples compete with the solid phase bound analytes for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate resulting in a colour reaction. The reaction is monitored at a wavelength of 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations. Manual processing of the ELISA is recommended. The use of automatic laboratory equipment is the responsibility of the user.
This product is not intended to clinical diagnoses.

1.2 Background

GABA (γ-aminobutyric acid) is one of the most important inhibitory neurotransmitter in the central nervous system (CNS). GABA operates through interneurones by the inhibition of the release of excitatory neurotransmitters. In the CNS GABA is synthesized from L-glutamic acid which is an excitatory neurotransmitter.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, guidelines and warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and must be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) The principles of Good Laboratory Practice (GLP) must be followed.
(3) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(4) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water. Avoid repeated freezing and thawing of reagents and specimens.
(5) The microplate contains snap-off strips. Unused wells must be stored at 2 – 8 °C in the sealed foil pouch with desiccant and used in the frame provided. Microtiter strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up.
(6) Duplicate determination of sample is highly recommended.
(7) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials, and devices are prepared for use at the appropriate time.
(8) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(9) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(10) A standard curve must be established for each run.
(11) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report provided with the kit.
(12) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(13) Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(14) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Rinse contaminated items before reuse.
(15) For information about hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(16) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

(17) In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components must not be used for a test run. They must be stored properly until the manufacturer decides what to do with them. If it is decided that they are no longer suitable for measurements, they must be disposed of in accordance with national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.

2.2.1 Interfering substances and proper handling of specimens

Urine

Please note the sample preparation stabilization of the urine sample! It cannot be excluded that high acid concentrations lead to incorrect results. Up to 20 µl 6 N HCl per 1 ml urine no influence on the results was observed.

2.2.2 Drug and food interferences

It is recommended to forgo food supplements which might influence GABA levels (lemon balm, velerian, vitamin B6, L-theanine and kava) 24 hours before urine sampling.
Furthermore, additional diets before urine sampling are not described in the literature.
In case of unusual GABA levels it is recommended to check if these are caused by interaction of the above listed substances.

2.2.3 High-Dose-Hook effect

No hook effect was observed in this test.

3. Storage and stability

Store kit and reagents at 2 – 8 °C until expiration date. Do not use kit and components beyond the expiry date indicated on the kit labels. Once opened, the reagents are stable for 2 months when stored at 2 – 8 °C. Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquots for max. 2 months at < -15 °C and may be thawed only once. Once the resealable pouch of the ELISA plate has been opened, care should be taken to close it tightly again including the desiccant.

4. Materials

4.1 Contents of the kit

BA D-0033	**48**	**Macrotiter Plate** – ready to use
Content:	2 x 48 well plate, empty in a resealable pouch	
BA D-0090	**FOILS**	**Adhesive Foil** – ready to use
Content:	Adhesive foils in a resealable pouch	
Number:	3 x 4 foils	
BA E-0030	**WASH-CONC** **50x**	**Wash Buffer Concentrate** – concentrated 50x
Content:	Buffer with a non-ionic detergent and physiological pH	
Volume:	1 x 20 ml/vial, purple cap	
BA E-0040	**CONJUGATE**	**Enzyme Conjugate** – ready to use
Content:	Goat anti-rabbit immunoglobulins conjugated with peroxidase	
Volume:	1 x 12 ml/vial, red cap	
Description:	Species is goat	
BA E-0055	**SUBSTRATE**	**Substrate** – ready to use
Content:	Chromogenic substrate containing 3,3',5,5'-tetramethylbenzidine, substrate buffer and hydrogen peroxide	
Volume:	1 x 12 ml/vial, black cap	
BA E-0080	**STOP-SOLN**	**Stop Solution** – ready to use
Content:	0.25 M sulfuric acid	
Volume:	1 x 12 ml/vial, grey cap	

BA E-2413	**ASSAY-BUFF**	**Assay Buffer** – ready to use
Content:	Buffer with alkaline pH	
Volume:	1 x 20 ml/vial, yellow cap	
Hazard pictograms:	 GHS08 GHS07	
Signal word:	Danger	
Hazardous ingredients:	Boric acid	
Hazard statements:	H360FD May damage fertility. Suspected of damaging the unborn child.	
Precautionary statements:	P201 Obtain special instructions before use. P280 Wear protective gloves, protective clothing, eye protection, face protection. P308+P313 IF exposed or concerned: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	
Additional statements:	Restricted to professional users.	
BA E-2428	**EQUA-REAG**	**Equalizing Reagent** – lyophilized
Content:	Lyophilized protein	
Volume:	1 vial, brown cap	
BA E-2442	**EXTRACT-PLATE** **48**	**Extraction Plate** – ready to use
Content:	2 x 48 well plate, precoated with cation exchanger in a resealable pouch	
BA E-2446	**D-REAGENT**	**D-Reagent** – ready to use
Content:	Crosslinking agent in dimethylsulfoxide	
Volume:	1 x 3 ml/vial, white cap	
Hazard pictograms:	 GHS07	
Signal word:	Warning	
Hazardous ingredients:	Glutaraldehyde	
Hazard statements:	H317 May cause an allergic skin reaction.	
Precautionary statements:	P261 Avoid breathing mist/vapours/spray. P280 Wear protective gloves. P333+P313 If skin irritation or rash occurs: Get medical advice/attention. P501 Dispose of contents/container to an authorised waste collection point.	
BA E-2458	**Q-BUFFER**	**Q-Buffer** – ready to use
Content:	TRIS buffer	
Volume:	1 x 20 ml/vial, white cap	
BA E-2510	**AS** **GABA**	**GABA Antiserum** – ready to use
Content:	Rabbit anti-GABA antibody in buffer with proteins and non-mercury preservative, blue coloured	
Volume:	1 x 6 ml/vial, blue cap	
Description:	Species of antibody is rabbit, species of protein in buffer is bovine	
BA E-2531	**MTP** **GABA**	**GABA Microtiter Strips** – ready to use
Content:	1 x 96 wells (12x8) antigen precoated microwell plate in a resealable foil pouch with desiccant	

BA E-2541	**ELUTION-BUFF**	**Elution Buffer** – ready to use
Content:	Buffer with citric acid	
Volume:	1 x 20 ml/vial, green cap	
BA E-2560	**DILUENT**	**Diluent** – ready to use
Content:	Buffer with acidic pH	
Volume:	2 x 20 ml/vial, blue cap	
Hazard pictograms:	 GHS07	
Signal word:	Warning	
BA E-2561	**I-BUFFER**	**I-Buffer** – concentrated
Content:	Buffer with non-ionic detergent and non-mercury preservative	
Volume:	1 x 4 ml/vial, red cap	
BA E-2787	**NAOH**	**NaOH** – ready to use
Content:	Sodium hydroxide solution	
Volume:	1 x 2 ml/vial, purple cap	
Hazard pictograms:	 GHS07	
Signal word:	Warning	

4.2 Calibration and Controls

Standards and Controls – ready to use

Cat. no.	Component	Colour/Cap	Concentration [ng/ml]	Concentration [nmol/l]	Volume/ Vial
BA E-2501	**STANDARD A**	white	0	0	4 ml
BA E-2502	**STANDARD B**	yellow	75	727	4 ml
BA E-2503	**STANDARD C**	orange	250	2,425	4 ml
BA E-2504	**STANDARD D**	blue	750	7,275	4 ml
BA E-2505	**STANDARD E**	grey	2,500	24,250	4 ml
BA E-2506	**STANDARD F**	black	7,500	72,750	4 ml
BA E-2551	**CONTROL 1**	green	Refer to QC-Report for expected value and acceptable range.		4 ml
BA E-2552	**CONTROL 2**	red			4 ml

Conversion: GABA [ng/ml] x 9.7 = GABA [nmol/l]

Content: Acidic buffer with non-mercury preservative, spiked with defined quantity of GABA

4.3 Additional materials required but not provided in the kit

- Water (deionized, distilled, or ultra-pure)
- Absorbent material (paper towel)

4.4 Additional equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 – 500 µl; 12.5 ml
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 – 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Vortex mixer

5. Sample collection, handling and storage

Urine

Spontaneous urine (second morning urine) stabilized with 10 µl 6 M HCl per 1 ml of urine sample can be used. The measurement results are related to the creatinine content of the sample.
Storage: up to 6 hours (18 – 25 °C); up to 14 days (2 – 8 °C); up to 6 months (< -15 °C).
Repeated freezing and thawing should be avoided. Avoid exposure to direct sunlight.

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Number the Extraction Plate, Macrotiter Plate and microwell plates (Microtiter Strips which are removed from the frame for usage should be marked accordingly to avoid any mix-up). Duplicate determinations are recommended.
The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent. The higher the temperature, the higher the absorption values will be. Varying incubation times will have similar influences on the absorbance. The optimal temperature during the enzyme immunoassay is between 20 – 25 °C.
If the product is prepared in parts, unused wells in Extraction and Macrotiter Plates should be covered to avoid contamination. After preparation, the used wells must be labelled to prevent double use.
During the overnight incubation at 2 – 8 °C with the antiserum, the temperature should be uniform all over the ELISA plate to avoid any drift and edge-effect.

⚠ *The use of a microtiter plate shaker with the following specifications is mandatory: shaking amplitude 3 mm; approx. 600 rpm. Shaking with differing settings might influence the results.*

6.1 Preparation of reagents and further notes

Wash Buffer

Dilute the 20 ml Wash Buffer Concentrate **WASH-CONC** **50X** with water to a final volume of 1000 ml.
Storage: 2 months at 2 – 8 °C

Equalizing Reagent

Reconstitute the **EQUA-REAG** with **12.5 ml** of **ASSAY-BUFF**.
Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquots for max. 2 months at < -15 °C and may be thawed only once.

I-Buffer

Dilute the 4 ml **I-BUFFER** with water (deionized, distilled, or ultra-pure) to a final volume of 400 ml.
Storage: 2 months at 2 – 8 °C

D-Reagent

The **D-REAGENT** has a freezing point of 18.5 °C. Make sure that the **D-REAGENT** has reached room temperature and forms a homogeneous, crystal-free solution.

GABA Microtiter Strips

In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

Extraction Plate

In rare cases residues of the cation exchanger can be seen in the wells as small, black dots or lines. These residues do not influence the quality of the product.

6.2 Preparation of samples – Extraction

1.	Pipette **100 µl** of the **standards, controls** and **samples** into the appropriate wells of the **EXTRACT-PLATE** **48**.
2.	Add **100 µl** of the **DILUENT** to all wells. Cover plate with **FOILS** and incubate for **15 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
3.	**Discard** and blot dry by tapping the inverted plate on absorbent material. **Add 500 µl** of **I-Buffer** to each well and incubate for **5 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
4.	**Discard** the wash and blot dry by tapping the inverted plate on absorbent material.
5.	Pipette **150 µl** of **ELUTION-BUFF** into the appropriate wells of the **EXTRACT-PLATE** **48**. Cover plate with **FOILS** and incubate for **10 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
6.	Use **100 µl** for the subsequent **derivatization**!

6.3 Derivatization

1.	Pipette **100 µl** of the **extracted standards, controls** and **samples** into the appropriate wells of the **Macrotiter Plate** **48**.
2.	Pipette **10 µl** of the **NAOH** into all wells.
3.	Add **50 µl** of the **Equalizing Reagent** (fresh prepared before assay) to all wells.
4.	Incubate for **1 min** on a shaker (approx. 600 rpm).
5.	Pipette **10 µl** of the **D-REAGENT** into all wells.
6.	Cover plate with **FOILS** and incubate for **2 h** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
7.	Pipette **100 µl** **Q-BUFFER** into all wells.
8.	Shake for **10 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
9.	**Use 50 µl for the subsequent ELISA!**

6.4 GABA ELISA

1.	Pipette **50 µl** of the **derivatized standards, controls** and **samples** into the appropriate wells of the **GABA Microtiter Strips** **GABA**.
2.	Pipette **50 µl** of the **AS** **GABA** into all wells and mix shortly.
3.	Cover plate with **FOILS** and incubate for **15 – 20 h** (overnight) at **2 – 8 °C**.
4.	Remove the foil. Discard or aspirate the content of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µl** of the **CONJUGATE** into all wells.
6.	Incubate for **30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
7.	Discard or aspirate the content of the wells. Wash the plate **3 x** by adding **300 µl** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8.	Pipette **100 µl** of the **SUBSTRATE** into all wells and incubate for **20 – 30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). ⚠ ***Avoid exposure to direct sunlight!***
9.	Add **100 µl** of the **STOP-SOLN** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	GABA
	59.1 – 7,500 ng/ml

The standard curve, which can be used to determine the concentration of the unknown samples, is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis) using a concentration of 0.001 ng/ml for Standard A (this alignment is mandatory because of the logarithmic presentation of the data). Use non-linear regression for curve fitting (e.g. 4-parameter, marquardt).

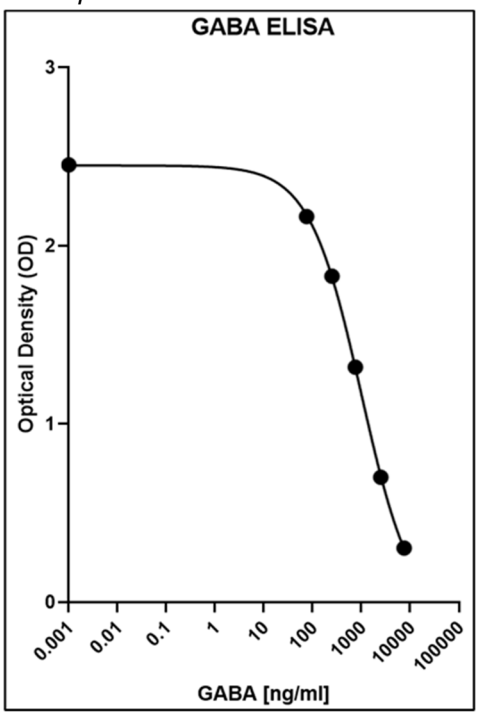
⚠*This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample and have to be reported as being positive.*

Urine samples and controls
The concentrations of the samples and controls can be read directly from the standard curve.
Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with water (deionized, distilled, or ultra-pure) and must be re-assayed. For the calculation of the concentrations this dilution factor has to be taken into account.

Conversion:
GABA [ng/ml] x 9.7 = GABA [nmol/l]

7.1 Typical standard curve

⚠*Example: Do not use for calculation!*



8. Control samples

The confidence limits of the kit controls are indicated on the QC-Report.

9. Assay characteristics

9.1 Performance data

Analytical Sensitivity	
	GABA
Limit of Blank (LOB)	19.6 ng/ml
Limit of Detection (LOD)	30.4 ng/ml
Limit of Quantification (LOQ)	59.1 ng/ml

Analytical Specificity (Cross Reactivity)	
Substance	**Cross Reactivity [%]**
	GABA
3-Aminobutanoic acid	< 0.1
L-(+)-2-Aminobutyric acid	< 0.1
ß-Alanine	0.8
L-Aspartic acid	< 0.1
(S)-(+)-Glutamine	< 0.1
Glycine	< 0.1
L-Glutamic acid	< 0.1

Precision					
Intra-Assay			**Inter-Assay**		
Urine, n = 12			**Urine**, n = 13		
Sample	Mean ± SD [ng/ml]	CV [%]	Sample	Mean ± SD [ng/ml]	CV [%]
1	135 ± 32	23.7	1	205 ± 25.4	12.4
2	241 ± 30	12.6	2	426 ± 40	9.4
3	451 ± 41	9.2	3	944 ± 69	7.3
4	1,021 ± 56	5.5	4	2,763 ± 195	7.1
5	2,871 ± 200	7.2			
6	6,327 ± 322	5.1			

Lot-to-Lot			
	Sample	Mean ± SD [ng/ml]	CV [%]
GABA in urine (n = 3)	1	826 ± 82.5	10.0
	2	2,526 ± 129	5.1
GABA in artificial matrix (n = 3)	3	1,229 ± 21.0	1.7

Recovery was determined according to the CLSI standard EP 34 1st ed.

Recovery			
	Range [ng/ml]	Mean [%]	Range [%]
Urine	157 – 4,832	101	86 – 111

Linearity			
	Serial dilution up to	Mean [%]	Range [%]
Urine	1:64	110	98 – 123

9.2 Metrological Traceability

The values assigned to the standards and controls of the GABA ELISA are traceable to SI Units by weighing with quality-controlled analyte.

Standards and Controls	
	Uncertainty [%]
GABA	1.6

GABA ELISA	
Concentration [ng/ml]	Expanded Uncertainty [%] k = 2*
205	25.0
426	19.1
944	15.0
2,763	14.6

* This defines an interval about the measured result that will include the true value with a probability of 95%.

10. References/Literature

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For updated literature or any other information please contact your local supplier.

11. Changes

Version	Release Date	Chapter	Change
17.0-r	2024-05-22	4.1 7.2 9.1 9.2	- Hazard labelling updated according to SDS - Typical standard curve updated - Lot-to-Lot added, Recovery updated - Chapter Metrological Traceability added

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Use-by date		Batch code		
	Consult instructions for use		Content		
	Caution		Catalogue number		Distributor
	Date of manufacture				For research use only!