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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

GABA ELISA

1. Introduction

1.1 Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of gamma-aminobutyric acid (GABA) in urine and various biological samples. After extraction and derivatization gamma-aminobutyric acid (GABA) is quantitatively determined by ELISA.

The 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 and the solid phase bound analyte compete for a fixed number of antiserum binding sites. When 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. The reaction is monitored at 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a standard curve prepared with known standards.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, guidelines and warnings

(1) This kit is intended for research 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 has to 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) have to 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. Avoid repeated freezing and thawing of reagents and specimens.
(5) For dilution or reconstitution purposes, use deionized, distilled, or ultra-pure water.
(6) 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.
(7) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(8) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
(9) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(10) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(11) A standard curve must be established for each run.
(12) 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.
(13) 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.
(14) 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.
(15) 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. Wash contaminated objects before reusing them.
(16) For information on 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.
(17) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.
(18) 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

Avoid excess of acid: excess of acid might exceed the buffer capacity of the dilution buffer. A **pH of 3.0** during the extraction is mandatory.

2.2.2 Drug interferences

It is recommended to avoid food supplements which might influence GABA levels (lemon balm, valerian, vitamin B6, L-theanine and kava) 24 hours before sampling.
Furthermore, additional diet restrictions before 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 the unopened reagents at 2 – 8 °C until expiration date. Do not use 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 has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Contents of the kit

BA D-0090 FOILS **Adhesive Foil** – Ready to use
Contents: Adhesive Foils in a resealable pouch
Volume: 3 x 4 foils

BA D-0033 48 **Macrotiter Plate** – Ready to use
Contents: 2 x 48 well plate, empty in a resealable pouch

BA E-2442 EXTRACT-PLATE 48 **Extraction Plate** – Ready to use
Contents: 2 x 48 well plate, precoated with cation exchanger in a resealable pouch

BA E-0030 WASH-CONC 50x **Wash Buffer Concentrate** – Concentrated 50x
Contents: Buffer with a non-ionic detergent and physiological pH
Volume: 1 x 20 ml/vial, light purple cap

BA E-0040 CONJUGATE **Enzyme Conjugate** – Ready to use
Contents: Goat anti-rabbit immunoglobulins conjugated with peroxidase
Volume: 1 x 12 ml/vial, red cap

BA E-0055 SUBSTRATE **Substrate** – Ready to use
Contents: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 ml/black vial, black cap

BA E-0080 STOP-SOLN **Stop Solution** – Ready to use
Contents: 0.25 M sulfuric acid
Volume: 1 x 12 ml/vial, light grey cap
Hazards identification:

H290 May be corrosive to metals.

BA E-2531 GABA **GABA Microtiter Strips** – Ready to use
Contents: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable foil pouch with desiccant

BA E-2510 AS GABA **GABA Antiserum** – Ready to use
Contents: Rabbit anti-GABA antibody, blue coloured
Volume: 1 x 6 ml/vial, blue cap

Standards and **Controls** – Concentrated

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	light yellow	75	727	4 ml
BA E-2503	STANDARD C	orange	250	2,425	4 ml
BA E-2504	STANDARD D	dark blue	750	7,275	4 ml
BA E-2505	STANDARD E	light grey	2,500	24,250	4 ml
BA E-2506	STANDARD F	black	7,500	72,750	4 ml
BA E-2551	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 ml
BA E-2552	CONTROL 2	dark red			4 ml

Conversion: GABA (ng/ml) x 9.7 = GABA (nmol/l)

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

BA E-2413 ASSAY-BUFF **Assay Buffer** – Ready to use

Contents: Buffer with alkaline pH

Volume: 1 x 20 ml/vial, yellow cap

BA E-2428 EQUA-REAG **Equalizing Reagent** – Lyophilized

Contents: Lyophilized protein

Volume: 1 vial, brown cap

BA E-2446 D-REAGENT **D-Reagent** – Ready to use

Contents: Crosslinking agent in dimethylsulfoxide

Volume: 1 x 3 ml/vial, white cap

Hazards identification:

H317 May cause an allergic skin reaction.

BA E-2458 Q-BUFFER **Q-Buffer** – Ready to use

Contents: TRIS buffer

Volume: 1 x 20 ml/vial, white cap

BA E-2561 I-BUFFER **I-Buffer** – Concentrated

Contents: Buffer with non-ionic detergent and non-mercury preservative

Volume: 1 x 4 ml/vial, light red cap

BA E-2541 ELUTION-BUFF **Elution-Buffer** – Ready to use

Contents: Buffer with citric acid

Volume: 1 x 20 ml/vial, dark green cap

BA E-2560 DILUENT **Diluent** – Ready to use

Contents: Buffer with acidic pH

Volume: 2 x 20 ml/vial, blue cap

BA E-2787 NAOH **NaOH** – Ready to use

Contents: Sodium hydroxide solution

Volume: 1 x 2 ml/vial, purple cap

Hazards identification:

H290 May be corrosive to metals.
H315 Causes skin irritation.
H319 Causes serious eye irritation.

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 – 500 µl; 1 ml; 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
- Absorbent material (paper towel)
- Water (deionized, distilled, or ultra-pure)

5. Sample collection and storage

Various biological samples can be used with this assay. The validation procedure was done for urine samples.

Urine
Spontaneous urine (second morning urine) stabilized with 10 µl 6 N 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. Duplicate determinations are recommended. It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.
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.

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.*

⚠ *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.*

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 ml Wash Buffer Concentrate with water (deionized, distilled, or ultra-pure) to a final volume of 1000 ml.
Storage: 2 months at 2 – 8 °C

Equalizing Reagent
Reconstitute the Equalizing Reagent with **12.5 ml** of **Assay Buffer**.
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 Concentrate 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

The GABA ELISA is a flexible test system for various biological sample types and volumes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to adapt the protocol to his specific needs:

⚠ *For the determination of samples in a range **between 25 – 2,500 ng/ml**, standards and controls should always be **diluted 1:3** with water [e.g. 100 µl standard + 200 µl water (deionized, distilled, or ultra-pure)]. This predilution of the standards has to be taken into account in the calculation of results. The standards are diluted to make sure that the samples fall into the linear part of the standard curve. **Do not dilute samples**!*

⚠ *For the determination of samples in a **range between 75 – 7,500 ng/ml, do not dilute standards, controls or samples.***

- Avoid excess of acid: excess of acid might exceed the buffer capacity of the dilution buffer. A **pH of 3.0** during the extraction is mandatory.
- It is advisable to perform a **Proof of Principle** to determine the recovery of GABA from the samples. Prepare a stock solution of GABA. Add small amounts (to change the native sample matrix as less as possible) of the stock solutions to the sample matrix and check the recovery.
- Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with water (deionized, distilled, or ultra-pure) and have to be re-assayed.

If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer directly!

6.3 Test procedure (75 – 7,500 ng/ml)

Extraction

1.	Pipette **100 µl** of the **standards, controls** and **samples** into the appropriate wells of the **Extraction Plate.**
2.	Add **100 µl** of the **Diluent** to all wells. Cover plate with **Adhesive Foil** 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 Buffer** into the appropriate wells of the **Extraction Plate.** Cover plate with **Adhesive Foil** and incubate for **10 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
6.	Use **100 µl** for the subsequent **derivatization**!

Derivatization

1.	Pipette **100 µl** of the **extracted standards, controls** and **samples** into the appropriate wells of the **Macrotiter Plate.**
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 **Adhesive Foil** 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!**

GABA ELISA

1.	Pipette **50 µl** of the **derivatized standards, controls** and **samples** into the appropriate wells of the **GABA Microtiter Strips.**
2.	Pipette **50 µl** of the **GABA Antiserum** into all wells and mix shortly.
3.	Cover plate with **Adhesive Foil** 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 **Enzyme 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 Solution** 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).

6.4 Test procedure (25 – 2,500 ng/ml)

Extraction

1.	Pipette **300 µl** of the **diluted standards, controls** and **undiluted samples** into the appropriate wells of the **Extraction Plate.**
2.	Add **300 µl** of the **Diluent** to all wells. Cover plate with **Adhesive Foil** and incubate for **30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
3.	**Washing step (2 cycles):** **Discard** and blot dry by tapping the inverted plate on absorbent material. **Add 1 ml** of **I-Buffer** to each well and incubate the plate for **5 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm). **Discard** and blot dry by tapping the inverted plate on absorbent material. **Add 1 ml** of **I-Buffer** to each well and incubate the plate 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 Buffer** into the appropriate wells of the **Extraction Plate.** Cover plate with **Adhesive Foil** and incubate for **10 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 600 rpm).
6.	Use **100 µl** for the subsequent **derivatization**!

Derivatization

1.	Pipette **100 µl** of the **extracted standards, controls** and **samples** into the appropriate wells of the **Macrotiter Plate.**
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 **Adhesive Foil** 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!**

GABA ELISA

1.	Pipette **50 µl** of the **derivatized standards, controls** and **samples** into the appropriate wells of the **GABA Microtiter Strips.**
2.	Pipette **50 µl** of the **GABA Antiserum** into all wells and mix shortly.
3.	Cover plate with **Adhesive Foil** 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 **Enzyme 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 Solution** 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

	GABA	
Measuring range	Test procedure 75 – 7,500 ng/ml	59.1 – 7,500 ng/ml
	Test procedure 25 – 2,500 ng/ml	25 – 2,500 ng/ml

The standard curve 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).
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.*

Samples and Controls
The concentrations of the controls can be read directly from the standard curve.

The concentrations of the samples (Test procedure 75 – 7,500 ng/ml) can be read directly from the standard curve.

The concentrations of the samples (Test procedure 25 – 2,500 ng/ml) have to be **divided by 3.**

Samples found with concentrations higher than the highest standard (Standard F) should be diluted accordingly with water (deionized, distilled, or ultra-pure) and have to be re-assayed.

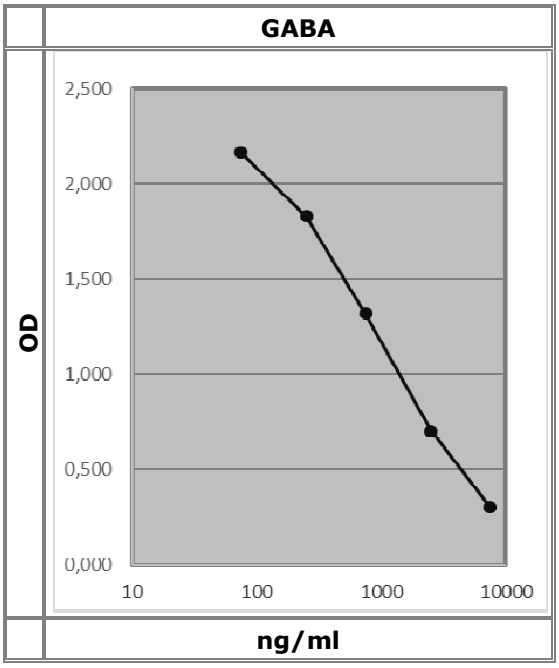
Conversion
GABA (ng/ml) x 9.7 = GABA (nmol/l)

7.1 Quality control

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

7.2 Typical standard curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

Various biological samples can be used for GABA determination. The following data had been taken from the validation report and refer to urine samples.

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

	Substance	**Cross Reactivity (%)**
		GABA
Analytical Specificity (Cross Reactivity)	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**		
Sample	Mean ± SD (ng/ml)	CV (%)	Sample	Mean ± SD (ng/ml)	CV (%)
1	135 ± 32	23.7	1	205 ± 25	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			

Recovery		Range (ng/ml)	Range (%)	Mean (%)
	Urine	135 – 6,327	103 – 117	112

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

9. References/Literature

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

Symbols:

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