

Instructions for use

REF

BA-E-2500



RUO

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 °C to 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 H₂SO₄. 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, LDN has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official 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 1 month when stored at 2 - 8 °C. 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** **48 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 4 ml/vial, brown 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

Urine stabilized with 10 µl 6N HCl per 1 ml of urine sample can be used.

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.

⚠ *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: 1 month 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 1 month at -20 °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: 1 month 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 – 2500 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 Microtiter 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).
8. 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 1ml 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 1ml 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).
8.	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

Measuring range	GABA	
	Test procedure 75 – 7500 ng/ml	59.1 – 7500 ng/ml
	Test procedure 25 – 2500 ng/ml	25 – 2500 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. spline, 4- parameter, akima).

⚠ *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

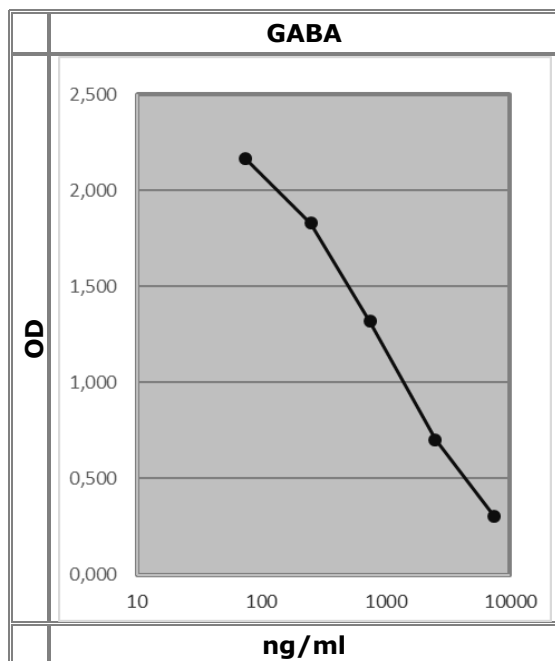
GABA (ng/ml) x 9.7 = GABA (nmol/l)

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

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
		GABA
	3-Aminobutanoic acid	< 0.1
	L-(+)-2-Aminobutyric acid	< 0.1
	β-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	1021 ± 56	5.5	4	2763 ± 195	7.1
5	2871 ± 200	7.2			
6	6327 ± 322	5.1			

Recovery		Range (ng/ml)	Range (%)	Mean (%)
	Urine		135 - 6327	103 - 117







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

9. References/Literature

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- (13) Strienz, J., *Nebennierenunterfunktion - Stress stört die Hormonbalance*. 3 ed. 2019: W. Zuckschwerdtverlag München.

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

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number	RUO	For research use only!