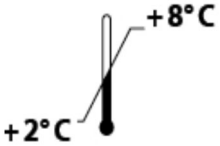
Manual

GABA ELISA

For the in vitro determination of GABA in urine

Valid from 2023-03-01

REF **K 7012**



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1. INTENDED USE

The GABA (urine) ELISA is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of the concentration of free gamma-aminobutyric acid (GABA) in urine.

The assay is an *in vitro* diagnostic medical device and intended to be used by professional users in a laboratory environment. It can be performed manually or using an automated platform.

The test serves as an aid to diagnosis of a reduced GABA concentration in urine.

2. INTRODUCTION

Gamma-aminobutyric acid (GABA) is the most prevalent and most important inhibitory neurotransmitter in the central nervous system. It counteracts the excitatory catecholamines and likewise attenuates the endocrine stress response. GABA stabilises blood pressure, regulates appetite, has anxiolytic and sleep-inducing effects [1]. It is synthesised from glutamic acid, a non-essential amino acid, which in turn functions as an excitatory neurotransmitter in the central nervous system and can also be considered as an antagonist of GABA.

Of clinical interest are very low GABA concentrations found in depression [2,3], autism [4], or hypertension [5].

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit Components	Quantity
K 7012	PLATE	Microtiter plate, pre-coated	12 x 8 wells
K 7012	STD	Standards, ready-to-use (0, 0.3, 1, 3, 10, 30 µmol/l)	6 x 500 µl
K 7012	CTRL 1	Control, ready-to-use (see specification for range)	1 x 500 µl
K 7012	CTRL 2	Control, ready-to-use (see specification for range)	1 x 500 µl
K 0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
K 7012	AB	GABA antibody, peroxidase-labelled, ready-to-use	1 x 6 ml
K 7012	REABUF	Reaction buffer, ready-to-use	1 x 70 ml
K 7012	DER	Derivatisation reagent, lyophilised	1 vial

K 0008.10	DMSO	Dimethylsulfoxide (DMSO)	1 x 10 ml
K 0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
K 0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipets and 10-1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label.
- **Preparation of the wash buffer**: The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** can be used until the expiry date stated on the label when stored at **2-8 °C**. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2-8 °C for 1 month**.
- **DMSO** crystallises at 2-8 °C. Before use, bring to room temperature to dissolve the crystals.
- The **lyophilised derivatisation reagent (DER)** can be used until the expiry date stated on the label when stored at **2-8 °C**. Bring to room temperature before opening and dissolve the content of the vial in **DMSO** as stated on the label. Allow to dissolve for **15 min** and mix thoroughly with a vortex-mixer. The **derivatisation reagent** (reconstituted DER) can be stored at **2-8 °C for**

2 months. Bring to room temperature before reuse. Please note: DMSO attacks all plastics but not polypropylene products and laboratory glass.

- All other test reagents are ready-to-use. Test reagents can be used until the expiry date stated on the label when stored at **2-8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

In urine, GABA is stable for 4 days at 2-8°C or for 72 h at room temperature. For longer storage keep frozen at -20°C. We recommend acidifying the urine samples.

Urine samples are **diluted** for derivatisation (see derivatisation procedure).

For sample preparation, a derivatisation reagent for derivatisation of GABA is added (see derivatisation procedure).

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of GABA in urine. This assay is based on the method of competitive enzyme linked immunoassays.

The sample preparation includes the addition of a derivatisation reagent for GABA derivatisation. Afterwards, the treated samples and a peroxidase-conjugated polyclonal GABA antibody are incubated in wells of a microtiter plate coated with GABA derivative (tracer). During the incubation period, the target GABA in the sample competes with the tracer, immobilised on the wall of the microtiter wells, for the binding of the polyclonal antibodies.

After washing away the unbound components, tetramethylbenzidine (TMB) is added as a peroxidase substrate. Finally, the enzymatic reaction is terminated by an acidic stop solution. The colour changes from blue to yellow, and the absorbance is measured in a photometer at 450 nm. The intensity of the yellow colour is inverse proportional to the GABA concentration in the sample; this means, high GABA concentration in the sample reduces the concentration of tracer-bound antibody and lowers the photometric signal. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standards. GABA, present in the patient samples, is determined directly from this curve.

To normalise the ELISA results to the creatinine concentration in the samples, a parallel determination of the creatinine concentration is required.

Derivatisation procedure

Bring **all reagents and samples to room temperature** (15-30 °C) and mix well.

Dilute the urine samples **1:3** with reaction buffer as follows:

50 µl sample + **100 µl** reaction buffer (REABUF), mix well.

Derivatisation of standards, controls, and diluted samples is carried out in reaction vials (e.g. 1.5 ml polypropylene vials).

We recommend preparing one derivatisation per standard, control and sample and transferring it in duplicate determinations into the wells of the microtiter plate.

1.	Add **25 µl standard** (STD)/ **control** (CTRL)/ **diluted sample** into the respective vials.
2.	Add **500 µl reaction buffer** (REABUF) into each vial (STD, CTRL, sample).
3.	Add **50 µl derivatisation reagent** into each vial (STD, CTRL, sample) and **mix thoroughly** by repeated inversion or several seconds on a vortex mixer.
4.	Incubate for **30 min** at room temperature (15-30 °C) on a **horizontal shaker**.

2 x 50 µl of the derivatised standards, controls and samples are used in the ELISA as duplicates.

Test procedure

Mark the positions of standards/controls/samples in duplicate on a protocol sheet.

Take as many microtiter strips (PLATE) as needed from the kit. Store unused strips covered with foil at 2-8 °C. Strips are stable until expiry date stated on the label.

5.	For the analysis in duplicate take **2 x 50 µl** of the **derivatised standards/ controls/ samples** out of the vials and add into the respective wells of the microtiter plate.
6.	Add **50 µl GABA antibody** (AB) into each well of the microtiter plate.
7.	Cover the strips tightly with foil and incubate for **1 hour** at room temperature (15-30 °C) on a **horizontal shaker**.
8.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.

9.	Add **100 µl substrate** (SUB) into each well.
10.	Incubate for **8-12 min*** at room temperature (15-30 °C) in the **dark**.
11.	Add **100 µl stop solution** (STOP) into each well and mix well.
12.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm (690 nm) as a reference.

* The intensity of the colour change is temperature sensitive. We recommend to observe the colour change and to stop the reaction upon good differentiation.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. Attention should be paid to the following points:

Derivatisation of the samples is possible in 30 minutes on a horizontal shaker or, after thorough mixing, in 90 minutes without shaking in an automated processor. In automated processing, the volumes of samples and diluents may be scaled while maintaining the respective dilutions. Device-specific minimum and maximum volumes must be taken into account. The homogeneity of the resulting dilution must be ensured.

For further details please contact your supplier or Immundiagnostik AG.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. **4 parameter algorithm**

   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e.g. 0.001).

2. **Point-to-point calculation**

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

3. **Spline algorithm**

   We recommend a linear ordinate for optical density and a linear abscissa for concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the program used, the duplicate values should be evaluated manually.

Urine samples

The obtained reasults have to be multiplied by the **dilution factor of 3** to get the actual concentrations.

In case another dilution factor has been used, multiply the obtained result by the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range can be further diluted with reaction buffer and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

analytical sensitivity × sample dilution factor to be used

Analytical sensitivity see chapter "Performance Characteristics".

Biotin interference

Samples containing a biotin concentration of ≤ 743 ng/ml show a change of the results of < 25 %. Higher concentrations of biotin can lead to false results. Patients taking > 5 mg biotin per day should wait at least 24 hours after taking biotin to have their samples collected. Results of patients taking biotin supplements or receiving a high-dose biotin therapy should generally be interpreted along with the total clinical picture.

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within

the same assay one or more values of the quality control samples are outside of the acceptable limits.

Reference range

Based on in-house studies with samples of apparently healthy persons, a median of 3.2 µmol/g_{crea} was determined (n = 40). The 10th percentile was 2,0 µmol/g_{crea}, resulting in the following normal range:

GABA concentration in urine: > 2,0 µmol/g_{crea}

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n = 16

The repeatability was assessed with 4 samples under constant parameters (same operator, measurement system, day and kit lot).

sample	mean value [µmol/l]	CV [%]
1	13.2	5.2
2	23.6	4.5
3	37.9	5.2
4	51.4	7.1

Reproducibility (Inter-Assay); n = 8

The reproducibility was assessed with 4 samples under varying parameters (different operators, measurement systems, days and kit lots).

sample	mean value [µmol/l]	CV [%]
1	12.2	11.4
2	20.8	8.5
3	31.2	7.4
4	41.3	9.1

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, 3 different samples (spikes) were added to 3 samples. The expected values result from the mean of sample and spike.

sample [µmol/l]	spike [µmol/l]	expected [µmol/l]	obtained [µmol/l]	recovery [%]
23.3	4.9	14.1	15.9	112.7
	9.0	16.2	16.9	104.4
	13.6	18.5	19.2	104.0
36.3	4.9	20.6	22.0	106.7
	9.0	22.7	23.5	103.7
	13.6	24.9	23.3	93.4
48.7	4.9	26.8	27.6	102.8
	9.0	28.9	29.2	101.1
	13.6	31.2	31.9	102.2

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed with a serial dilution of 4 urine samples.

The following values have been estimated based on the consentrations of the standard curve without considering possibly used sample dilution factors.

For GABA in urine, the method has been demonstrated to be linear from 1.5 to 18.2 µg/ml, showing a recovery rate of 97.4 – 118.3 % in this interval.

sample [µmol/l]	dilution	expected [µmol/l]	obtained [µmol/l]	recovery [%]
A	1:3		5.0	
	1:4	3.7	4.0	108.0
	1:6	2.5	2.8	112.2
	1:8	1.9	2.0	107.4
	1:10	1.5	1.6	109.9
B	1:3		8.9	
	1:4	6.7	7.2	107.4
	1:6	4.5	4.9	109.1
	1:8	3.4	3.7	111.5
	1:10	2.7	3.0	111.5
C	1:3		13.6	
	1:4	10.2	11.2	110.5
	1:6	6.8	7.3	107.5
	1:8	5.1	5.7	111.1
	1:10	4.1	4.8	118.3

D	1:3		18.2	
	1:4	13.7	13.3	97.4
	1:6	9.1	9.1	100.2
	1:8	6.8	7.0	102.0
	1:10	5.5	6.1	111.1

Analytical sensitivity

The zero standard was measured 48 times. The detection limit was set as B_0 - 2 SD and estimated to be 0.27 µmol/l.

Analytical specificity

The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to GABA. The specificity is calculated in percent in relation to the GABA-binding activity:

β-alanine	< 0.14 %
β-aminobutyric acid	no cross reactivity was observed
α-aminobutyric acid	no cross reactivity was observed
glycine	no cross reactivity was observed
glutamine	no cross reactivity was observed

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are harmful to health and the environment. Substrates for enzymatic colour reactions can also cause skin and/or respiratory irritation. Any contact with the substances should be avoided. Further safety information can be found in the safety data sheet, which is available from Immundiagnostik AG on request.
- The 10x Wash buffer concentrate (WASHBUF) contains surfactants which may cause severe eye irritation in case of eye contact.

  Warning: Causes serious eye irritation. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.
- The stop solution consists of diluted sulfuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be

wiped up immediately with copious quantities of water. Do not breathe vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore, we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be sent to Immundiagnostik AG along with a written complaint.

15. REFERENCES

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Used symbols:

	Temperature limitation		Catalogue number
	In Vitro Diagnostic Medical Device		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Contains plasma derivatives or human blood		Consult instructions for use
	Consult specification data sheet		Do not re-use
	Unique Device Identification		Contains material of animal origin
	Medicinal substance		Contains material of human origin