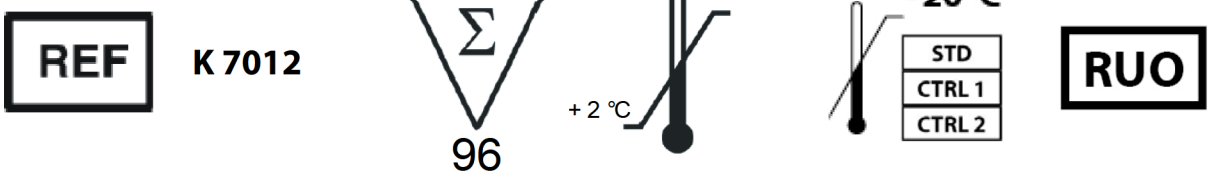
Manual

GABA ELISA Kit

For the determination of GABA in human EDTA plasma, serum and urine

For research use only

Valid from 07.04.2011



1. INTENDED USE

The GABA ELISA Kit is intended for the quantitative determination of GABA in human EDTA plasma, serum and urine. It is for research use only.

2. PRINCIPLE OF THE TEST

This assay is based on the method of competitive enzyme linked immunoassays. The sample preparation includes the addition of a derivatization reagent for GABA derivatization. Afterwards, the treated samples and the polyclonal GABA antiserum are incubated in wells of a microtiter plate coated with a GABA-derivative (tracer). During the incubation period, the target GABA in the sample competes with the tracer immobilized on the wall of the microtiter wells for the binding of the polyclonal antibodies. The GABA in the sample displaces the antibodies out of the binding to the tracer. Therefore, the concentration of the tracer-bound antibody is inverse proportional to the GABA concentration in the sample. During the second incubation step, a peroxidase-conjugated antibody is added to each microtiter well to detect the anti-GABA 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 color changes from blue to yellow, and the absorbance is measured in a photometer at 450 nm. The intensity of the yellow color is inverse proportional to the GABA concentration in the sample; this means, high GABA concentration in the sample reduces the concentration of tracer-bound antibodies and lowers the photometric signal.

A dose response curve of 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. The ELISA results are normalized to the creatinine concentration of the urine sample. For this reason, a parallel determination of the creatinine concentration is required.

3. MATERIAL SUPPLIED

Catalog No	Content	Kit Components	Quantity
K7012MTP	PLATE	One holder with precoated strips	12 x 8 wells
K7012ST	STD	Standards diluted in reaction buffer (ready to use)	6 x 1 vial
K7012KO1 K7012KO2	CTRL 1 CTRL 2	Controls diluted in reaction buffer (ready to use)	2 x 1 vial
K7012WP	WASHBUF	Wash buffer concentrate (10-fold)	2 x 100 ml
K7012AK	AB	Anti-GABA antibody (lyophilized)	3 x 1 vial
K7012K	2.AB	POD antibody (concentrate)	120 µl
K7012CSP	2.ABDIL	Conjugate stabilizing buffer	24 ml
K7012RP	DERBUF	Reaction buffer	2 x 25 ml
K7012DR	DER	Derivatization reagent	3 x 1 vial
K7012LM	DMSO	Dimethylsulfoxide (DMSO)	3 ml
K7012SL	CODIL	Dilution buffer after derivatization	28 ml
K7012TMB	SUB	TMB substrate	25 ml
K7012AC	STOP	Stop solution	15 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Double distilled water (aqua bidest.)
- Precision pipettors and disposable tips to deliver 10-1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 10000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm
  (reference wave length 620 or 690 nm)

5. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay**. The kit can be used up to 3 times within the expiry date stated on the label.

- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.

- Dilute the **wash buffer concentrate (WASHBUF)** with aqua bidest. **1:10** before use (**100 ml WASHBUF + 900 ml aqua bidest**.), mix well. Crystals may occur due to high salt concentration in the stock solution. The crystals must be redissolved at room temperature or at 37°C using a water bath before dilution. The WASHBUF is stable at **2-8°C** until the expiry date stated on the label. Diluted buffer solution can be stored in a closed flask at **2-8°C for one month**.

- **Standards (STD) and controls (CTRL1, CTRL2)** are already diluted in reaction buffer (REABUF). Store standards and controls frozen at -20°C, thaw before use in the test, and re-freeze immediately after use. Standards and controls can be re-frozen up to 3 times.

- **DMSO** could crystallize at 4°C. Dissolve the crystals at 20-25°C in a water bath.

- Dissolve the content of one vial of **derivatization reagent (DER)** in **750 µl DMSO.** Put the vial on a horizontal shaker for 5 min. Discard any rest of the reagent after use. DER must be **prepared immediately before use**. The ELISA kit can be separated into three performances by providing three DER vials. Please note: **DMSO attacks all plastics but not polypropylene products and laboratory glass.**

- Dissolve the **anti-GABA antibody (AB)** in **6 ml of diluted wash buffer**. The ELISA kit can be separated into three performances by providing three AB vials. Diluted anti-GABA antibody is stable over a longer period. It can be stored at **2-8°C for 4 weeks.**

- Dilute the **POD antibody (2.AB) 1:200** with conjugate stabilizing buffer (2.ABDIL) (**e.g. 110 µl 2.AB + 22 ml 2.ABDIL, prepare only the required amount**). The undiluted POD antibody (2.AB) is stable at **2-8°C** until the expiry date stated on the label. Diluted POD antibody (2.AB) is not stable over a longer period. It can be stored at **2-8°C for only 5 days.**

- All other test reagents are ready for use. Test reagents are stable until the expiry date (see label of test package) when stored at 2-8°C.

6. PRECAUTIONS

- For research use only.
- Human materials used in kit components were tested and found to be negative for HIV and Hepatitis B. However, for safety reasons all kit components should be treated as if potentially infectious.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on kit label.

7. SPECIMEN COLLECTION AND PREPARATION

EDTA plasma, serum and urine

- Venous fasting blood and urine are suited for this test system. Blood samples are stable for one week at 2-8°C. In urine samples GABA is stable for 72 h at room temperature. Therefore urine samples can be sent without cooling. For longer storage, blood and urine samples should be frozen at -20°C. We recommend acidifying the urine samples.
- Lipemic or hemolytic samples may give erroneous results and should not be used for analysis.
- The EDTA plasma, serum and urine samples are diluted for derivatization.

  Samples with visible amounts of **precipitates** should be **centrifuged** at least for 5 min at 10000 x g. The resulting supernatant is used in the assay.
- For sample preparation, a derivatization reagent (DER) for derivatization of GABA is added (details are given in the sample preparation procedure).

8. ASSAY PROCEDURE

Procedural notes

- Quality control guidelines should be observed.

- Incubation time, incubation temperature, and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure that are not coordinated with the producer may influence the test results. Immundiagnostik AG can therefore not be held reliable for any damage resulting from this.
- The assay should always be performed according to the enclosed manual.

Sample preparation procedure

Derivatization of standards (STD), controls (CTRL) and diluted samples (SAMPLE) is carried out in single analysis.

Dilute **EDTA plasma and serum samples** with reaction buffer by **factor 1:4**, i.e. **100 µl** sample + **300 µl** reaction buffer (DERBUF). These vials, containing 400 µl diluted sample, are used for derivatization (see step 2.)

Dilute **urine samples** with reaction buffer by **factor 1:50**, i.e. **20 µl** urine sample + **980 µl** reaction buffer (DERBUF). Take out 400 µl for derivatization (see step 2.)

1.	Bring all reagents and samples to room temperature (18-26°C).
2.	Add **400 µl of ready to use standards (STD), 400 µl of ready to use controls (CTRL)** and **400 µl of diluted samples (SAMPLE)** in the corresponding vial.
3.	Add **25 µl** of freshly prepared **derivatization reagent (DER)** into each vial (standards, controls and samples), mix well and incubate **for 60 min** on a shaker (180-240 rpm) **at room temperature (18-26°C)**.
4.	Afterwards add **500 µl of dilution buffer (CODIL)** into each vial, mix well and incubate for **30 min** on a shaker (180-240 rpm) **at room temperature (18-26°C)**.

2 x 100 µl of each treated sample (STD, CTRL, SAMPLE) are used in the ELISA as duplicates.

Test procedure

5.	Mark the positions of standards (STD)/ controls (CTRL)/ samples (SAMPLE) in duplicate on a protocol sheet.
6.	Take as many microtiter strips (PLATE) as needed from kit. Store unused strips covered at 2-8°C. Strips are stable until the expiry date stated on the label.
7.	Wash each well **5 times** by dispensing **250 µl of diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate (PLATE) should be firmly tapped on absorbent paper to remove excess solution.
8.	For the analysis in duplicate, take **2 x 100 µl of standards (STD) / controls (CTRL) / samples (SAMPLE)** out of the vial and add into the respective wells of the microtiter plate (PLATE).
9.	Add **100 µl** diluted **anti-GABA antibody (AB)** into each well. Cover the plate tightly.
10.	Incubate overnight **(15-20 hours) at 2-8°C.**
11.	Aspirate the contents of each well. Wash each well **5 times** by dispensing **250 µl of diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate (PLATE) should be firmly tapped on absorbent paper to remove excess solution.
12.	Add **200 µl** diluted **POD antibody (2. AB)** into each well.
13.	Cover plate tightly and incubate for **1 hour at room temperature (18-26°C)** on a horizontal shaker (180-240 rpm).
14.	Aspirate the contents of each well. Wash each well **5 times** by dispensing **250 µl of diluted wash buffer** into each well. After the final washing step, the inverted microtiter plate (PLATE) should be firmly tapped on absorbent paper to remove excess solution.
15.	Add **200 µl** of **TMB substrate (SUB)** into each well.

16. Incubate for **6-12 min at room temperature (18-26°C)** in the dark*.
17. Add **100 µl of stop solution (STOP)** into each well, mix thoroughly.
18. Determine **absorption** immediately with an ELISA reader at **450 nm**. If the highest extinction of the standards **(STD)** is above the range of the photometer, absorption must be measured immediately at **405 nm** and the obtained results used for evaluation. If possible, the extinctions from each measurement should be compared with extinctions obtained at a reference wavelength, e. g. 595 nm, 620 nm, 630 nm, 650 nm and 690 nm can be used.

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

9. EVALUATION OF RESULTS

If the test is performed in strict compliance with the manufacturer's instructions (i.e. with the exact volumes for standards, controls, samples, and with correct sample treatment), standards, controls, and blood samples are equally diluted. Therefore, **no dilution factor is required for the calculation of results from plasma and serum samples.**

For **urine samples** with 1:50 dilution, the values calculated from the calibration curve **have to be multiplicated by a factor of 12.5** to obtain the true results. The results must be related to the creatinine content of the urine samples.

$$GABA\left[\frac{\mu g}{g_{creatinine}}\right] = dilutionfactor \times \frac{c_{GABA}\left[\frac{\mu mol}{l}\right]}{c_{creatinine}\left[\frac{mmol}{l}\right]} \times \frac{MW_{GABA}\left[\frac{g}{mol}\right]}{MW_{creatinine}\left[\frac{g}{mol}\right]}$$

The resulting factor of 11395 is multiplicated with the concentration of GABA [µmol/l] divided by the concentration of creatinine [mmol/l].

$$GABA\left[\frac{\mu g}{g_{creatinine}}\right] = 11395 \times \frac{c_{GABA}\left[\frac{\mu mol}{l}\right]}{c_{creatinine}\left[\frac{mmol}{l}\right]}$$

Calculation of 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 calibrator must be specified with a value less than 1 (e.g. 0.01).

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 logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e.g. 0.01).

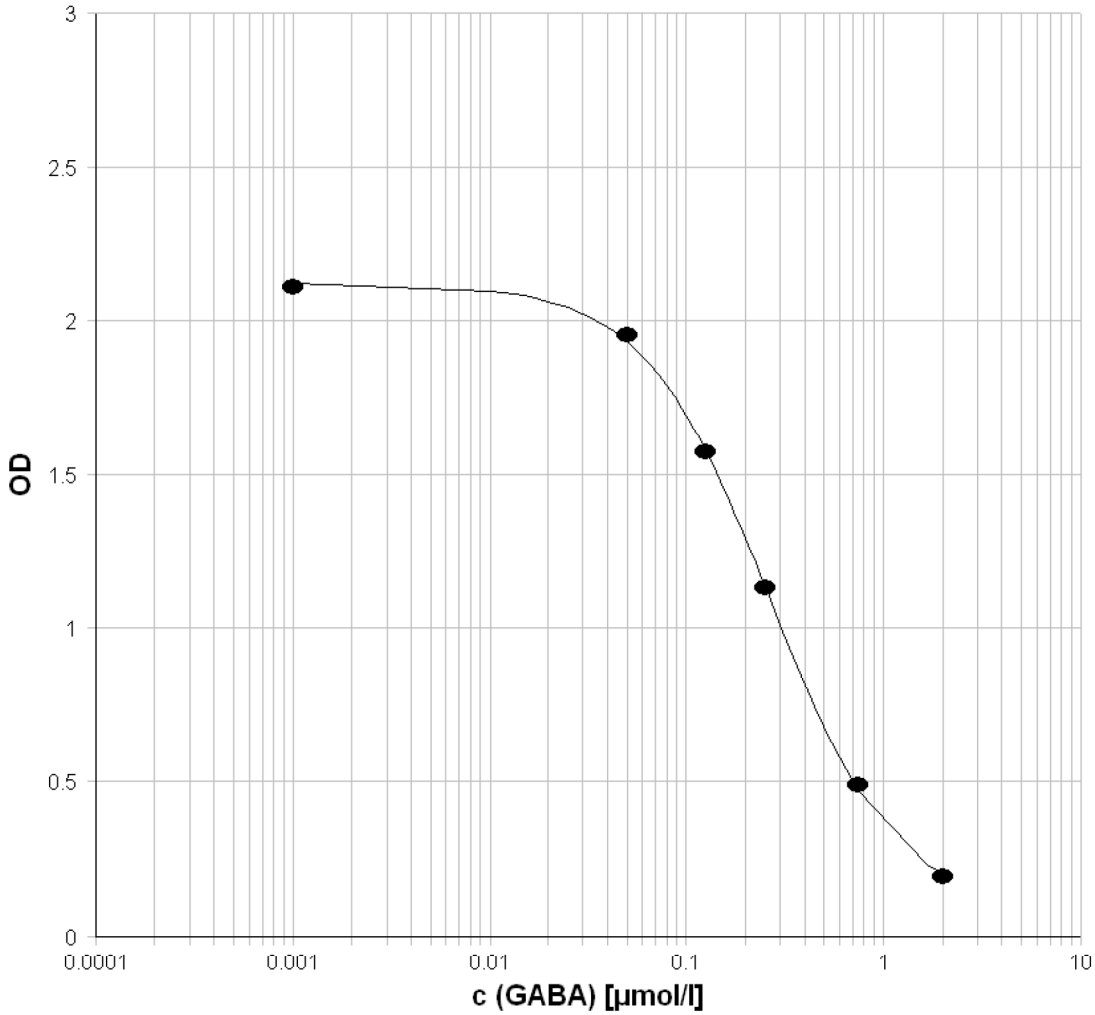
Plausibility of the measured pairs of values should be examined before automatically evaluating the results. If this option is not available within the used program, the pairs of values should be controlled manually.

Controls

Control samples or plasma pools should be analyzed 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 the acceptable limits.

The concentration of controls and patient samples can be determined directly from the calibration curve. In the following, an example of a calibration curve is given, do not use it for the calculation of your results.

Example of calibration curve



Expected results

Based on internal studies with plasma samples of evidently healthy persons **(n=20)** a mean value of 0.182 µmol/l was calculated. The standard deviation was 0.053 µmol/l.

Plasma mean value ± 2 x standard deviation: **0.182 ± 0.106 µmol/l**

Normal range: **0.076 – 0.288 µmol/l**

We recommend each laboratory to develop its own normal range. The values mentioned above are only for orientation and can deviate from other published data.

10. PERFORMANCE CHARACTERISTICS

Cross reactivity

β-alanine	< 0.4 %
α-aminobutyric acid	< 0.01 %

Precision and reproducibility

EDTA plasma:

Intra-assay (n=12)		
sample	GABA [µmol/l]	coefficient of variation (CV) [%]
1	0.122	7.1
2	0.197	9.2

Inter-assay (n=6)		
sample	GABA [µmol/l]	coefficient of variation (CV) [%]
1	0.089	13.5
2	0.420	8.0

Sensitivity

The sensitivity was set as B_0 + 2SD. The zero-standard was measured 48 times.

Sample	GABA mean value [OD]	2 x Standard deviation (SD)	Detection limit [µmol/l]
zero-standard	2.28	0.18	0.024

Recovery

One sample was spiked with different GABA concentrations and measured using this assay. The analytical recovery rate was determined by the expected and measured GABA levels. The expected levels were calculated as the sum of the measured GABA concentration in the original sample and the spiked GABA amount. The mean recovery rate for all concentrations was 99.5 % (n=10).

EDTA plasma:

spike [µmol/l]	GABA measured [µmol/l]	GABA expected [µmol/l]	recovery [%]
0	x = 0.104	x	100.0
0.15	0.252	0.104+0.15 = 0.254	99.2
0.3	0.401	0.104+0.3 = 0.404	99.3

Linearity

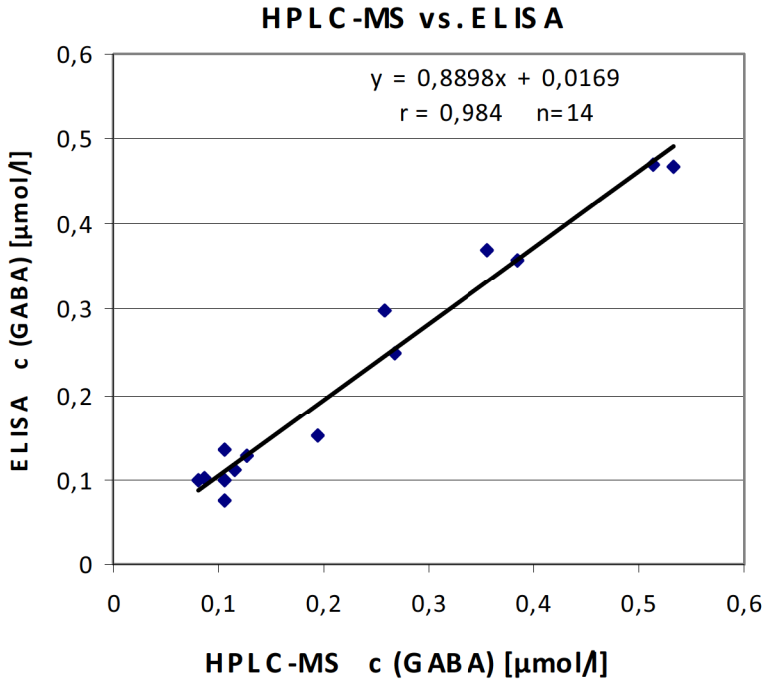
The linearity of the ELISA was determined by the dilution of a spiked patient sample. The mean linearity was 107 % (n=10).

EDTA-plasma

dilution	measured [µmol/l]	expected [µmol/l]	recovery [%]
original	0.478	0.478	100.0
1+1	0.252	0.239	105.4
1+3	0.138	0.120	115.5

Correlation with HPLC-MS

14 samples were measured with this ELISA and HPLC-MS. The correlation was r = 0.984.



11. LIMITATIONS

Hemolytic and lipemic samples may give erroneous results. Do not measure hemolytic and lipemic samples.

12. REFERENCES

- Arrúe A, Dávila R, Zumárraga M, Basterreche N, González-Torres MA, Goienetxea B, Zamalloa MI, Anguiano JB, Guimón J: GABA and homovanillic acid in the plasma of Schizophrenic and bipolar I patients. Neurochem Res. 2010 Feb;35(2):247-53.
- Cai HL, Zhu RH, Li HD, Zhang XH, Hu L, Yang W, Ye HS: Elevated plasma γ-aminobutyrate/glutamate ratio and responses to risperidone antipsychotic treatment in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2010 Oct 1;34(7):1273-8. Epub 2010 Jul 15.
- Küçükibrahimoğlu E, Saygin MZ, Calişkan M, Kaplan OK, Unsal C, Gören MZ: The change in plasma GABA, glutamine and glutamate levels in fluoxetine- or S-citalopram-treated female patients with major depression. Eur J Clin Pharmacol. 2009 Jun;65(6):571-7.

- Vaiva G, Boss V, Ducrocq F, Fontaine M, Devos P, Brunet A, Laffargue P, Goudemand M, Thomas P: Relationship between posttrauma GABA plasma levels and PTSD at 1-year follow-up. Am J Psychiatry. 2006 Aug;163(8):1446-8.

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- Test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for research use only.
- Reagents should not be used after the date of expiry stated on the label.
- Single components with different lot numbers should not be mixed or exchanged.
- Guidelines for medical laboratories should be observed.
- 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 wrong use.

Used symbols:


Temperature limitation

Catalogue Number

For research use only

Contains sufficient for <n> tests


Manufacturer


Use by

Lot number