

Manual

# GABA ELISA Kit

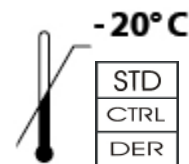
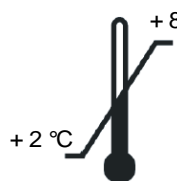
*For the determination of GABA in human EDTA plasma, serum and urine*

*For research use only*

Valid from 28.02.2014



IMM-K 7012



## 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 are incubated in wells of a microtiter plate coated with a polyclonal antibody against GABA-derivative, together with assay reagent containing GABA-derivative (tracer). During the incubation period the target GABA in the sample competes with the tracer for the binding of the polyclonal antibodies on the wall of the microtiter wells. GABA in the sample displaces the tracer out of the binding to the antibodies. Therefore, the concentration of antibody-bound tracer is inverse proportional to the GABA concentration in the sample.

During the second incubation step, a peroxidase conjugate is added to each microtiter well to detect the tracer. 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 antibody-bound tracer 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 (serum or EDTA plasma) is determined directly from this curve. For urine samples the ELISA results are normalized to the creatinine concentration in the samples. Therefore, a parallel determination of the creatinine concentration is required.

### 3. MATERIAL SUPPLIED

Catalog No	Label	Kit Components	Quantity
K7012MTP	PLATE	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 (see specification for range)	2 x 1 vial
K7012WP	WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 ml
K7012AR	ASYREAG	Assay reagent, lyophilized	3 x 1 vial
K7012K	CONJ	POD conjugate (concentrate 200x)	120 $\mu$ l
K7012KV	CONJBUF	Conjugate stabilizing buffer, ready to use	24 ml
K7012RP	DERBUF	Reaction buffer, ready to use	2 x 25 ml
K7012DR	DER	Derivatization reagent	3 x 1 vial
K7012LM	DMSO	Dimethylsulfoxide (DMSO)	2 ml
K7012SL	CODIL	Dilution buffer after derivatization, ready to use	28 ml
K7012TMB	SUB	TMB substrate (Tetramethylbenzidine), ready to use	25 ml
K7012AC	STOP	ELISA stop solution, ready to use	15 ml

### 4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water\*
- Precision pipettors and disposable tips to deliver 10-1000  $\mu$ l
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm or 405nm

\* Immundiagnostik AG recommends the use of Ultra Pure 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. 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 ultra pure water **1:10** before use (**100 ml WASHBUF + 900 ml ultra pure water**), 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 buffer concentrate 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 room temperature or in a water bath.
- Store the **derivatization reagent (DER)** at **-20°C**. Bring to room temperature before opening. Dissolve the content of one vial in **550 µl DMSO**. Allow the content of the vial to dissolve for 10 min and mix thoroughly with a vortex-mixer. DER must be **prepared immediately before use.** When more than one vial is to be used, combine the contents and mix prior to use. Discard any rest of the reagent after 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 content of one vial of **assay reagent (ASYREAG)** in **4 ml of diluted wash buffer.** When more than one vial is to be used, combine the contents and mix prior to use. The ELISA kit can be separated into three performances by providing three ASYREAG vials. Dissolved assay reagent can be stored at **-20°C for 4 weeks.**

- Dilute the **POD conjugate (CONJ) 1:200** with conjugate stabilizing buffer (CONJBUF) (**e.g. 110 µl CONJ + 22 ml CONJBUF, prepare only the required amount**). Undiluted POD conjugate is stable at 2-8°C until the expiry date stated on the label. Diluted POD conjugate can be stored at **2-8°C for 1 week**.
- 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.
- Kit reagents contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- 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 (see sample preparation procedure).

**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

- The assay should always be performed according to the enclosed manual.
- Do not interchange different lot numbers of any kit component within the same assay.
- 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.

### *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** (15-30°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** (15-30°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** (15-30°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** with **250 µl** of diluted **wash buffer**. After the final washing step, the inverted microtiter plate should be firmly tapped on absorbent paper.
8. For the analysis in duplicate take **2 x 100 µl** of the **derivatized standards/ controls/ samples (STD/ CTRL/ SAMPLE)** out of the vials and add into the respective wells.
9. Add **100 µl** of dissolved **assay reagent (ASYREAG)** 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** with **250 µl** of diluted **wash buffer**. After the final washing step the inverted microtiter plate should be firmly tapped on absorbent paper.
12. Add **200 µl** diluted **POD conjugate (CONJ)** into each well.
13. Cover plate tightly and incubate for **1 hour at room temperature** (15-30°C) on a horizontal shaker (180-240 rpm).

14. Aspirate the contents of each well. Wash each well **5 times** with **250 µl** of diluted **wash buffer**. After the final washing step the inverted microtiter plate should be firmly tapped on absorbent paper.
15. Add **200 µl** of **TMB substrate (SUB)** into each well.
16. Incubate for **6-12 min at room temperature (15-30°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** 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 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 **multiplied by 12.5**. The results must be related to the creatinine content of the urine samples.

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



Or, simplified: the resulting factor of 11395 is multiplied with the concentration of GABA [ $\mu\text{mol/l}$ ] and divided by the concentration of creatinine [ $\text{mmol/l}$ ]:

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

### 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. 0.001).

#### 2. Point-to-point-calculation

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

#### 3. Spline-algorithm

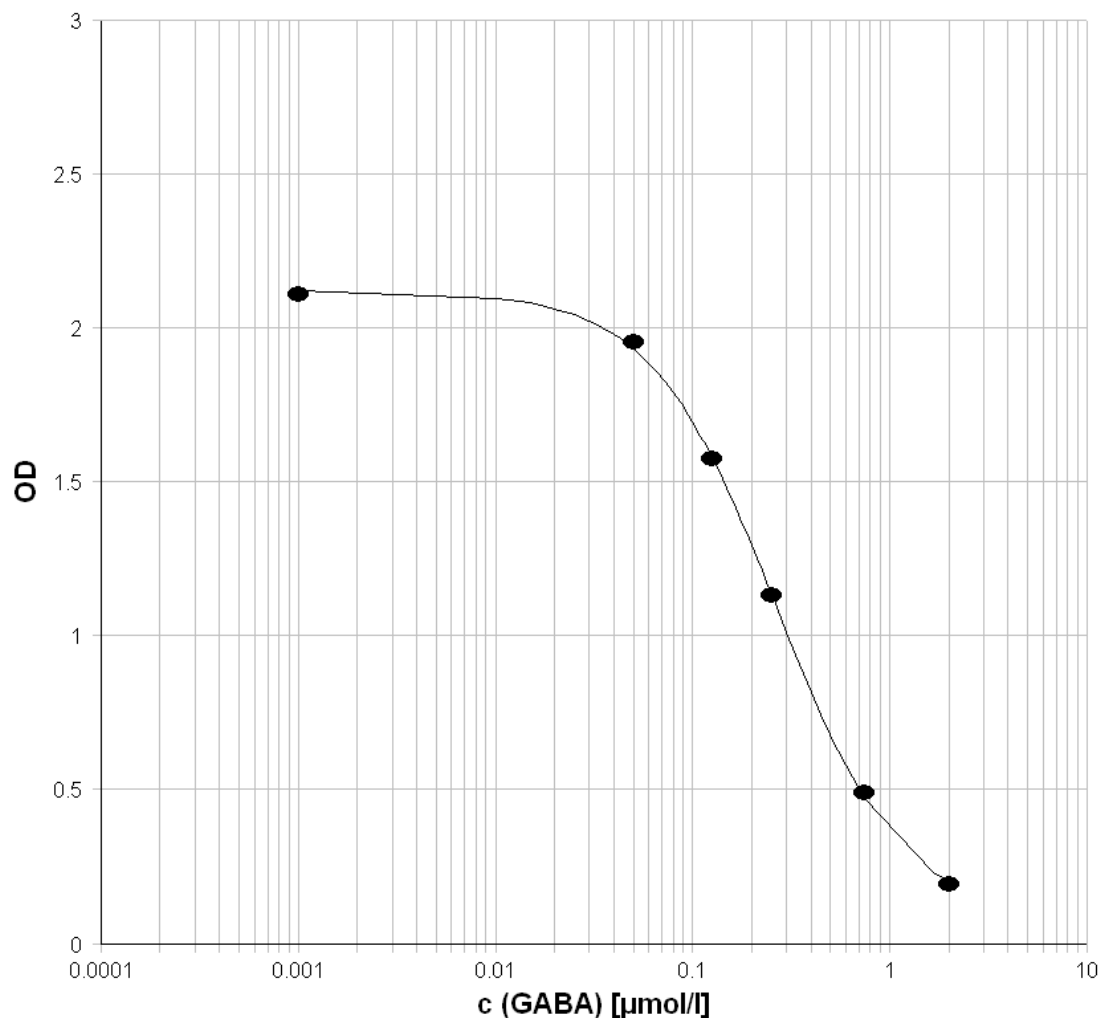
We recommend a linear ordinate for optical density and a linear abscissa for concentration.

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  $0.182 \mu\text{mol/l}$  was calculated. The standard deviation was  $0.053 \mu\text{mol/l}$ .

**Plasma mean value  $\pm$  2  $\times$  standard deviation:**  **$0.182 \pm 0.106 \mu\text{mol/l}$**

**Normal range:**  **$0.076 - 0.288 \mu\text{mol/l}$**

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*

$\beta$ -alanine	< 0.4 %
$\alpha$ -aminobutyric acid	< 0.01 %

### *Precision and reproducibility*

EDTA plasma:

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

Inter-assay (n=6)		
sample	GABA [ $\mu$ mol/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 [ $\mu$ mol/l]
zero-standard	2.28	0.18	0.024

### Recovery

One plasma sample was spiked with different GABA concentrations and measured in 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 99.3 % (n=10).

spike [ $\mu\text{mol/l}$ ]	GABA measured [ $\mu\text{mol/l}$ ]	GABA expected [ $\mu\text{mol/l}$ ]	recovery [%]
0	0.104		
0.15	0.252	0.254	99.2
0.3	0.401	0.404	99.3

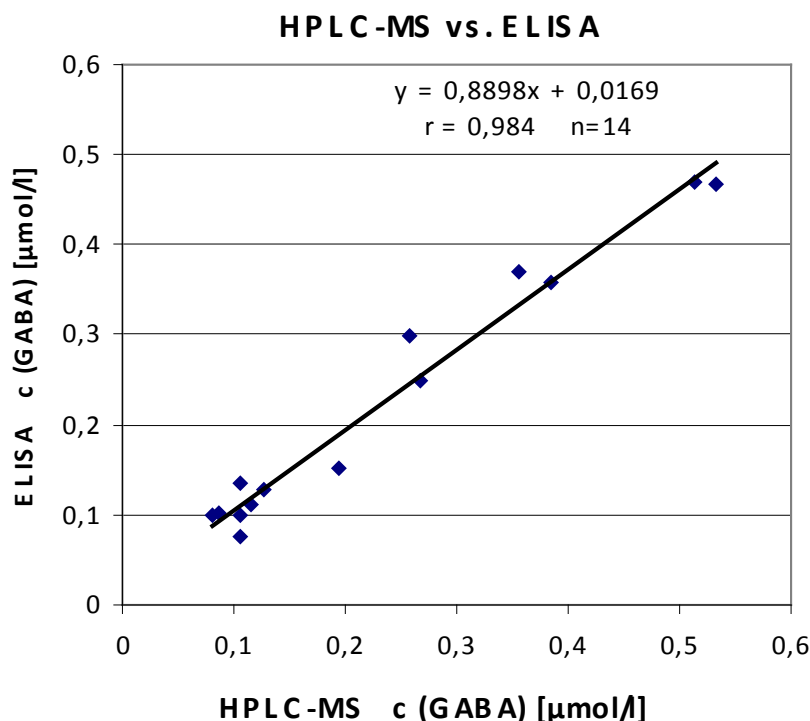
### Linearity

The linearity of the ELISA was determined by the dilution of a spiked patient sample. The mean linearity was 110 % (n=10).

dilution	measured [ $\mu\text{mol/l}$ ]	expected [ $\mu\text{mol/l}$ ]	recovery [%]
original	0.478		
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  $\gamma$ -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

- 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		

Li StarFish distribuisce: