# Human GABA ELISA Kit

Enzyme Immunoassay for the quantification of Gamma-aminobutyric acid (GABA) in urine

Catalog number: ARG82788

Package: 96 Wells

For research use only. Not for use in diagnostic procedures.

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## PRINCIPLE OF THE ASSAY

This assay employs the competitive quantitative sandwich enzyme immunoassay technique. An antigen has been pre-coated onto a microtiter plate. After extraction and derivatization, controls, standards or samples are pipetted into the wells together with specific GABA antiserum. The standards, controls and samples and the solid phase bound analyte antigen compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing.

Following a washing to remove unbound substances, anti-rabbit IgG conjugated to Peroxidase is added to each well and incubated. After washing away any unbound antibody conjugate, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of GABA present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of GABA in the sample is then determined by comparing the O.D of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| GABA-coated microtiter strips | 12 x 8 wells (Ready-to-use) | 4°C. |
| Reaction Plate | 2 x 48 wells (Ready-to-use) | 4°C |
| Extraction Plate | 2 x 48 wells (Ready-to-use) | 4°C |
| Standard A-F (0, 75, 250, 750, 2500, 7500 ng/ml) | 4 ml each (Ready-to-use) | 4°C |
| Control 1 (300 ng/mL ± 40%) | 4 ml (Ready-to-use) | 4°C |
| Control 2 (1000 ng/mL ± 40%) | 4 ml (Ready-to-use) | 4°C |
| GABA antiserum | 6 ml (Ready-to-use) | 4°C |
| Anti-rabbit IgG peroxidase conjugate | 12ml (Ready-to-use) | 4°C (Protect from light) |
| Diluent | 2 X 20 ml (Ready-to-use) | 4°C |
| 100X I-Buffer | 4 ml | 4°C |
| Elution Buffer | 20 ml (Ready-to-use) | 4°C |
| NaOH | 2 ml (Ready-to-use) | 4°C |
| Equalizing Reagent | 1 vial (Lyophilized) | 4°C |
| D-Reagent | 3 ml (Ready-to-use) | 4°C |
| Q-Buffer | 20 ml (Ready-to-use) | 4°C |
| Assay buffer | 20 ml (Ready-to-use) | 4°C |
| 50X Wash buffer | 20 ml | 4°C |
| TMB substrate | 12 ml (Ready-to-use) | 4°C (Protect from light) |
| STOP solution | 12 ml (Ready-to-use) | 4°C |
| Adhesive foil | 12 pieces | RT |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (Optional: 620-650 nm as reference)
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

## TECHNICAL HINTS AND PRECAUTIONS

- This kit is intended for professional 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.
- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All kit reagents and specimens should be brought to room temperature (20 – 25°C) and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
- If crystals are observed in the 50X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- 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.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- 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.
- Change pipette tips between the addition of different reagent or samples.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Urine –Spontaneous urine (second morning urine) stabilized with 10 µl 6 N HCl per 1 ml of urine sample can be used. Use samples immediately or within 6 hour when stored at 18-25°C. For a longer period storage, please store at2-8°C for up to 2 weeks and ≤ -20 °C for up to 6 months. Repeated freeze-thawing is not advisable. Avoid exposure to direct sunlight.

Note:

**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 urine sampling. Furthermore, additional diet restrictions before urine 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.

## REAGENT PREPARATION

- **1X Wash buffer**: Dilute **50X** Wash buffer into distilled water to yield 1X Wash buffer. E.g. dilute the 20 ml of 50X Wash Buffer into distilled water to a final volume of 1000 ml. The diluted wash buffer can be stored at 2 – 8 °C for 1 month.
- **Equalizing Reagent**: Reconstitute the lyophilized Equalizing Reagent with **12.5 ml** of **Assay Buffer**. Unused reconstituted equalizing reagent need to be stored in aliquots at-20 °C up to 1 month and may be thawed only once.
- **D-Reagent:** The D-Reagent has a freezing point of 18.5 °C. To ensure that the D-Reagent is liquid when being used, it must be ensured that the D-Reagent has reached room temperature and forms a homogeneous, crystal-free solution.
- **1X I-Buffer:** Dilute 100X I-Buffer into distilled water to yield 1X I-Buffer working solution. E.g. dilute the 4 ml of 100X I-Buffer into distilled water to a final volume of 400 ml. The diluted I-Buffer can be stored at 2 – 8°C for 1 month.
- **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.

## ASSAY PROCEDURE

- Extraction

1. Add **100 µl** of **undiluted standards**, **controls and samples** into the Extraction Plate.
2. Add **100 µl** of the **Diluent** into each well. Cover the plate with adhesive foil and incubate for **15 minutes at RT** on a microplate shaker. (600 rpm).
3. Discard and blot dry by tapping the inverted plate on absorbent material.
4. Add **500 µl** of **I-Buffer** to each well and incubate for **5 min at RT (20 – 25 °C)** on a microplate shaker. (600 rpm).
5. Discard the wash and blot dry by tapping the inverted plate on absorbent material.
6. Add **150 µl** of **Elution Buffer** to the appropriate well of the Extraction Plate. Cover plate with Adhesive Foil and incubate for **10 minutes at RT** on a microplate shaker. (600 rpm).
7. Use **100 µl** for the subsequent derivatization

- Derivatization

1. Add **100 µl** of **extracted standards, controls and samples** in duplicate into the **Reaction Plate**.
2. Add **10 µl** of **NaOH** into each well.
3. Add **50 µl** of the **Equalizing Reagent (fresh prepared before assay)** into all wells and shake on a microplate at 600rpm **for 1 min** on a microplate shaker. (600 rpm).
4. Add **10 µl** of the **D-Reagent** into all wells.

5. Cover the plate with adhesive foil and incubate for **2 hours at RT** on a microplate shaker. (600 rpm).
6. Add **100 µl** of the **Q-Buffer** into all wells. Shake for **10 min at RT** on a microplate shaker. (600 rpm).
7. Use **50 µl** for the subsequent ELISA process.

- GABA ELISA

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame (GABA-coated microplate), return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl** of the **derivatized standards, controls and samples** into the GABA-coated microtiter strips.
3. Add **50 µl** of the **GABA antiserum** into each well and mix shortly.
4. Cover the plate with adhesive foil and incubate for **15-20 hours** at **2-8°C**.
5. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µl** of the **Enzyme-Conjugate Antibody** (Anti-rabbit IgG peroxidase conjugate) into each well. Incubate **for 30 min at RT** on a microplate shaker. (600 rpm).
7. Aspirate each well and **wash as step 5**.

8. Add **100 µl** of **TMB Substrate** to each well. Incubate for **20-30 minutes at RT** on a microplate shaker. (600 rpm) in dark.
9. Add **100 µl** of **Stop Solution** to each well and shake lightly to ensure homogeneous mixing.
10. Read the OD with a microplate reader at **450 nm** immediately (with a reference wavelength between 620nm and 650nm) within 10 minutes.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for the detail. (https://www.arigobio.com/elisa-analysis)
6. Urine samples and controls: The concentrations of the samples and controls can be read directly from the standard curve.
7. If the initial assay found samples contain GABA higher than the highest standard, the samples can be diluted with distilled water and then re-assay the

samples. For the calculation of the concentrations this dilution factor has to be taken into account.

8. Expected reference value: It is strongly recommended that each laboratory should determine its own reference value.

206- 1548 µg/g creatinine

2 – 15 µmol/g creatinine

0.23–1.7mmol/mol creatinine

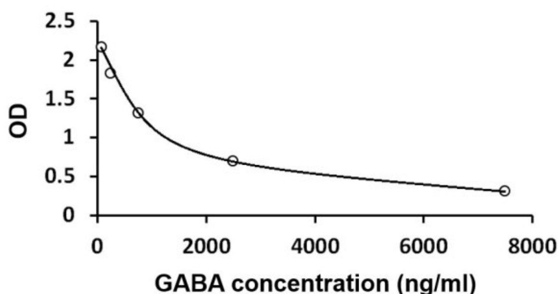
9. Conversion: GABA (ng/ml) x 9.7 = GABA (nmol/l)

10. Refer to the table below for molar conversion:

| | Concentration of standards | | | | | |
|---|---|---|---|---|---|---|
| Standard | A | B | C | D | E | F |
| GABA (ng/ml) | 0 | 75 | 250 | 750 | 2500 | 7500 |
| GABA (nmol/L) | 0 | 727 | 2425 | 7275 | 24250 | 72750 |
| Conversion | GABA (ng/ml) x 9.7 = GABA (nmol/L) | | | | | |

## EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



## QUALITY ASSURANCE

### Sensitivity

The standard range of this GABA ELISA kit from 0-7500 ng/ml.

Limit of Blank (LOB): 19.6 ng/ml

Limit of Detection (LOD): 30.4 ng/ml

Limit of Quantification (LOQ): 59.1 ng/ml

Measuring range: 59.1-7500 ng/ml.

### Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 9.3% and inter-assay precision was 9.05%.

### Recovery

103-117% (Mean: 112%)

### Linearity

98-123% (Mean: 110%)

### Cross Reactivity

| Substance | Cross Reactivity (%) |
|---|---|
| | GABA |
| 3-Aminobutanoic acid | < 0.1 |
| L-(+)-2-Aminobutyric acid | < 0.1 |
| beta-Alanine | 0.8 |
| L-Aspartic acid | < 0.1 |
| (S)-(+)-Glutamine | < 0.1 |
| Glycine | < 0.1 |
| L-Glutamine | < 0.1 |