Human GABA ELISA Kit

Enzyme Immunoassay for the quantification of Gamma-aminobutyric acid (GABA) in serum, plasma and urine

Catalog number: ARG80451

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MANUFACTURED BY:

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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
Reaction Plate	2 x 48 wells (Ready-to-use)	4°C
Extraction Plate	2 x 48 wells (Ready-to-use)	4°C
GABA-coated microtiter strips	12 x 8 wells (Ready-to-use)	4°C.
Adhesive foil	3 X 4 pieces	RT

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
Diluent	2 X 20 ml (Ready-to-use)	4°C
100X I-Buffer	4 ml	4°C
Elution Buffer	50 ml (Ready-to-use)	4°C
NaOH	2 ml (Ready-to-use)	4°C
Equalizing Reagent	1 vial (Lyophilized)	4°C
D-Reagent	4 ml (Ready-to-use)	4°C
Q-Buffer	20 ml (Ready-to-use)	4°C
Assay buffer	20 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)
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

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (Optional: 620-650 nm as reference and 405nm if needed)
- 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 4°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 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.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C up to 6 month. Avoid repeated freeze-thaw cycles.

Note:

Fasting specimens or pre-feed specimens for children (2- 3 hours after last meal) are advised.

Haemolytic and especially lipemic samples should not be used for the assay

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C up to 6 month. Avoid repeated freeze-thaw cycles.

Note:

Fasting specimens or pre-feed specimens for children (2- 3 hours after last meal) are advised.

Haemolytic and especially lipemic samples should not be used for the assay

Urine – Collect urine in a bottle containing 10-15 ml of 6M HCl, and use it immediately or within 24 hour. For a longer period storage, please store at ≤ -20 °C up to 6 months. Repeated freeze-thawing is not advisable.

Note:

If the percentage of the final concentration of acid is too high, the buffer

capacity of the Diluent is insufficient. As a consequence interfering factors are not extracted quantitatively.

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 at 2 – 8 °C for 1 month.
- Equalizing Reagent: Reconstitute the lyophilized Equalizing Reagent with 10 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 4°C for 1 month.

SAMPLE PREPARATION

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 (Ex. Serum/plasma) in a range between 25 - 2500 ng/ml:

Standards and controls should always be diluted 1:3 with distilled water (e.g. 100 µl standard + 200 µl distilled water). 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 (ex. Urine) in a range between 75 - 7500 ng/ml:

Do not dilute standards, controls or samples.

• Other suggestions:

-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.

- The sample volume determines the sensitivity of this test. Determine the sample volume needed to determine GABA in your sample by testing different amounts of sample volumes.

ASSAY PROCEDURE

A. For Urine or samples might contain 75- 7500 ng/ml of GABA

- Extraction
  1. Add **100 µl** of **undiluted standards** (standard range between 75- 7500 ng/ml), **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 inversed plate on absorbent material. Wash each well with **500 µl** of **distilled water** and incubate for **5min at RT** on a microplate shaker. (600 rpm). After washing, discard and blot dry by tapping the inversed plate on absorbent material.
  4. Add **400 µl** of **Elution Buffer** to each well. Incubate for **10 minutes at RT** on a microplate shaker. (600 rpm).
  5. 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.
  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 **200 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.
4. Cover the plate with adhesive foil and incubate for **15-20 hours** at **2-8°C** (or incubate for **2 hours at RT**) on a microplate shaker. (600 rpm).
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. Note: In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.

B. For Serum/Plasma or samples might contain 25- 2500 ng/ml of GABA):

- **Extraction**

1. Dilute **standards and controls 1:3** with distilled water. (e.g. 100 µl standard + 200 µl distilled water, in turn to get standard range between 25 - 2500 ng/ml.) **DO NOT dilute samples.**
2. Add **300 µl** of **diluted standards, controls** and **undiluted samples** into the Extraction Plate.
3. Add **300 µl** of the **Diluent** into each well. Cover the plate with adhesive foil and incubate for **30 minutes at RT** on a microplate shaker. (600 rpm).
4. Discard and blot dry by tapping the inversed plate on absorbent material. Wash each well with **1 ml** of **1X I-Buffer** for **5min at RT** on a microplate shaker. (600 rpm). After washing, discard and blot dry by tapping the inversed plate on absorbent material. Repeat this step for total **2 times**.
5. Add **250 µl** of **Elution Buffer** into the appropriate wells of the Extraction Plate. Cover the plate with adhesive foil and incubate for **10 minutes at RT** on a microplate shaker (600 rpm).
6. 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 into all wells and shake on a microplate shaker at 600rpm for 1 min.
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 150 µl of the Q-Buffer into all wells. Incubate for 10 min at RT on a microplate shaker (600 rpm).
7. Use 25 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 25 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.
4. Cover the plate with adhesive foil and incubate for 15-20 hours at 2-8°C (or incubate for 2 hours at RT) on a microplate shaker. (600 rpm).
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.**

Note: In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.

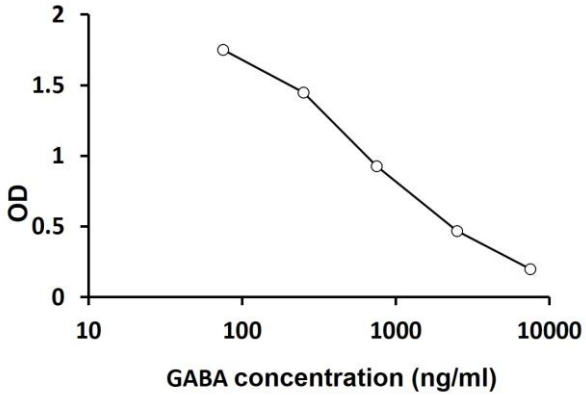
CALCULATION OF RESULTS

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. Serum/plasma: The read concentrations of plasma samples have to be divided by 3.
6. Urine samples and controls: The concentrations of the samples and controls can be read directly from the standard curve.
7. The total amount of GABA excreted in urine during 24 h is calculated as following: µg/24h = µg/L x L/24h
8. Conversion: GABA (ng/ml) x 9.7 = GABA (nmol/l)
9. 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 minimum detectable dose (MDD) of GABA from Urine ranged from 0-7500 ng/ml. The mean MDD was 49 ng/ml

The minimum detectable dose (MDD) of GABA from Serum/Plasma ranged from 0-2500 ng/ml. The mean MDD was 25 ng/ml

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 9.3% and inter-assay precision was 10.3%.

Recovery

96-116%

Linearity

74-119% (Mean: 93%)

Cross Reactivity

Substance	Cross Reactivity (%)
	GABA
GABA	100
β-Alanine	1.6
α-Aminobutyric acid	< 0.09
Glycine	< 0.09
L-Glutamine	< 0.09
B-Aminobutyric acid	< 0.09