GABA ELISA Kit

(For Human)

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

Catalog number: ARG80451

distributed in the US/Canada by:
EAGLE BIOSCIENCES, INC.
20A NW Blvd, Suite 112 Nashua, NH 03063
Phone: 617-419-2019 FAX: 617-419-1110
www.EagleBio.com • info@eaglebio.com

EAGLE BIOSCIENCES

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

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

PRINCIPLE OF THE ASSAY

This assay employs the competitive enzyme immunoassay technique. An antigen GABA has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any GABA present compete for the fixed number of antibody binding site. After washing away any unbound substances, the antibody bound to the solid phase is detected by using TMB as a substrate. The reaction is monitored at 450 nm±2 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

MATERIALS PROVIDED & STORAGE INFORMATION

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

Component	Quantity	Storage information
Macrotiter Plate	2 x 48 wells	Ready for use
Extraction Plate	2 x 48 wells	Ready for use
GABA-coated microtiter strips	12 x 8 wells	4°C.
Standard A-F	6 vials/4 ml	4°C, ready for use
Control 1	1 vial	4°C, ready for use
Control 2	1 vial	4°C, ready for use
Equalizing Reagent	1 vial	Lyophilized
D-Reagent	4 ml	Ready for use
Q-Buffer	20 ml	Ready for use
100X I-Buffer	4 ml	4°C
Enzyme-conjugated Antibody	1 vial	4°C

GABA antiserum	6 ml	4°C, ready for use
Assay buffer	20 ml	4°C, ready for use
Elution Buffer	50 ml	Ready for use
50X Wash buffer	20 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
Diluent	2 x 20 ml	Ready for use
NaOH	2 ml	Ready for use
STOP solution	12 ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- 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.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be

assayed in duplicates.

- 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 or 24-hour urine, collected in a bottle containing 10-15 ml of 6 M HCL, should be used. Store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin 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. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer.
- **Equalizing Reagent**: Reconstitute the Equalizing Reagent with 10 ml of Assay Buffer.
- **1X I-Buffer:** Dilute 100X Wash buffer into distilled water to yield 1X Wash buffer.

ASSAY PROCEDURE

- Extraction (Urine)

1. Add 100 µl of 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. Incubate for 15 minutes at RT.
3. Discard and blot dry by tapping the inversed plate on absorbent material. Wash each well with 500 µl of distilled water for 5min. at RT. 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 room temperature.

- Derivatization (Urine)

1. Add 100 µl of extracted standards, controls and samples in duplicate into the Macrotiter Plate.
2. Add 10 µl of NaOH into each well.
3. Add 50 µl of the Equalizing Reagent into all wells and shake 600rpm for 1 min.
4. Add 10 µl of the D-Reagent into all wells.
5. Cover the wells and incubate for 2 hours at RT.
6. Add 200 µl of the Q-Buffer into all wells. Shake 600rpm for 10 min. at RT.

- GABA ELISA (Urine)

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

1. Add 50 µl of the derivatized standards, controls and samples into the GABA-coated microtiter strips.

2. Add 50 µl of the GABA antiserum into each well.
3. Cover the wells and incubate for 15-20 hours at 2-8°C (or incubate for 2 hours at RT).
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (350 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.
5. Add 100 µl of the Enzyme-Conjugate Antibody into each well. Incubate for 30 min. at RT.
6. Aspirate each well and wash as step 4.
7. Add 100 µl of TMB Reagent to each well. Incubate for 20 minutes at room temperature in dark.
8. Add 100 µl of Stop Solution to each well.
9. Read the OD with a microplate reader at 450 nm immediately.

- Extraction (Serum/Plasma)

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.)
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. Incubate for 30 minutes at RT.
4. Discard and blot dry by tapping the inversed plate on absorbent material. Wash each well with 1 ml of I-Buffer for 5min. at RT. After washing,

discard and blot dry by tapping the inversed plate on absorbent material. Repeat this step 2 times.

5. Add 250 µl of Elution Buffer to each well. Incubate for 10 minutes at room temperature.

- **Derivatization (Serum/Plasma)**

1. Add 100 µl of extracted standards, controls and samples in duplicate into the Macrotiter Plate.
2. Add 10 µl of NaOH into each well.
3. Add 50 µl of the Equalizing Reagent into all wells and shake 600rpm for 1 min.
4. Add 10 µl of the D-Reagent into all wells.
5. Cover the wells and incubate for 2 hours at RT.
6. Add 150 µl of the Q-Buffer into all wells. Shake 600rpm for 10 min. at RT.

- **GABA ELISA (Serum/Plasma)**

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

1. Add 25 µl of the derivatized standards, controls and samples into the GABA-coated microtiter strips.
2. Add 50 µl of the GABA antiserum into each well.
3. Cover the wells and incubate for 15-20 hours at 2-8°C (or incubate for 2 hours at RT).
4. Aspirate each well and wash, repeating the process 2 times for a total 3 washes. Wash by filling each well with 1× Wash Buffer (350 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.

5. Add 100 µl of the Enzyme-Conjugate Antibody into each well. Incubate for 30 min. at RT.
6. Aspirate each well and wash as step 4.
7. Add 100 µl of TMB Reagent to each well. Incubate for 20 minutes at room temperature in dark.
8. Add 100 µl of Stop Solution to each well.
9. Read the OD with a microplate reader at 450 nm immediately.

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.

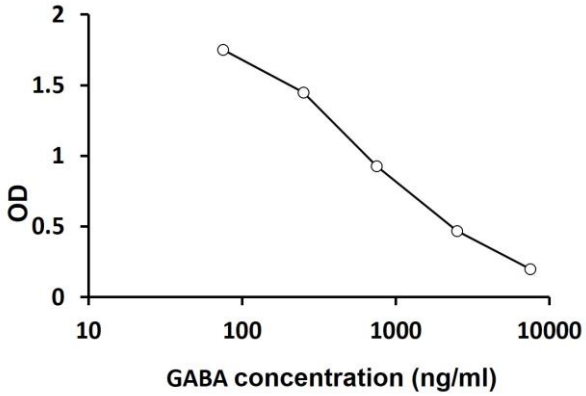
5. The total amount of GABA excreted in urine during 24 h is calculated as following:

µg/24h = µg/l x l/24h

6. 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 ranged from 0-7500 ng/ml. The mean MDD was 49 ng/ml

The minimum detectable dose (MDD) of GABA 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	Range (ng/mL)	Range (%)	Mean (%)
	35-4048	74-119	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

distributed in the US/Canada by:

EAGLE BIOSCIENCES, INC.

20A NW Blvd, Suite 112 Nashua, NH 03063

Phone: 617-419-2019 ● FAX: 617-419-1110

www.EagleBio.com ● info@eaglebio.com

For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.