Labor Diagnostika Nord GmbH & Co. KG
Am Eichenhain 1, 48531 Nordhorn
Telefon: +49-5921-8197 0
Telefax: +49-5921-8197 222
e-mail: info@ldn.de
Internet: http://www.ldn.de

Instructions for use

GABA Research ELISA

REF **BA E-2600**

96

+2 +8 °C

RUO For Research use only- Not for use in diagnostic procedures

GABA Research ELISA

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Gamma-aminobutyric acid (GABA) in biological samples.

After extraction and derivatization Gamma-aminobutyric acid (GABA) is quantitatively determined by ELISA.
The competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples and the solid phase bound analyte 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. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

2. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test; the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.
All reagents, however, should be treated as potential biohazards in use and for disposal.

3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

4.1 Contents of the kit

BA D-0033	TU 48	**Macrotiter Plate**	2 x 48 wells	ready for use
BA D-0090	FOILS	**Adhesive Foil**	2 x 4	ready for use
BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 mL	concentrate, dilute content with dist. water to a final volume of 1000 mL
BA E-0040	CONJUGATE	**Enzyme Conjugate**	1 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4
BA E-2428	EQUA-REAG	**Equalizing Reagent**	1 x	lyophilized
BA E-2442	EXTRACT-PLATE 48	**Extraction Plate**	2 x 48 wells	ready for use
BA E-2446	D-REAGENT	**D-Reagent**	1 x 4 mL	ready for use
BA E-2458	Q-BUFFER	**Q-Buffer**	1 x 20 mL	ready for use
BA E-2561	I-BUFFER	**I-Buffer**	1 X 4 mL	concentrate, dilute content with dist. water to a final volume of 400 mL
BA E-2501	STANDARD A	**Standard A**	1 x 4 mL	ready for use*
BA E-2502	STANDARD B	**Standard B**	1 x 4 mL	ready for use*
BA E-2503	STANDARD C	**Standard C**	1 x 4 mL	ready for use*
BA E-2504	STANDARD D	**Standard D**	1 x 4 mL	ready for use*
BA E-2505	STANDARD E	**Standard E**	1 x 4 mL	ready for use*
BA E-2506	STANDARD F	**Standard F**	1 x 4 mL	ready for use*
BA E-2510	AS GABA	**GABA Antiserum**	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-2513	ASSAY-BUFF	**Assay Buffer**	1 x 20 mL	ready for use
BA E-2531	TU GABA	**GABA Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, precoated
BA E-2541	ELUTION-BUFF	**Elution Buffer**	1 x 50 mL	ready for use
BA E-2551	CONTROL 1	**Control 1**	1 x 4 mL	ready for use*
BA E-2552	CONTROL 2	**Control 2**	1 x 4 mL	ready for use*
BA E-2560	DILUENT	**Diluent**	2 x 20 mL	ready for use
BA E-2787	NAOH	**NaOH**	1 x 2 ml	ready for use

***** For the determination of serum and plasma, standards and controls should always be diluted 1:3 (e.g. 100 µL standard + 200 µL destilled water). Any predilution of standards and controls has to be considered in the calculation of results.

4.2 Additional materials and equipment required but not provided with the kit

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1000 µL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Vortex mixer
- Absorbent material (paper towel)
- Distilled water

5. Sample collection and storage

In general this assay is dedicated for any biological sample such as plasma, serum, tissue homogenates and other biological samples.

Storage: up to 24 hours at 2 – 8 °C, for longer periods (up to 6 months) at - 20°C.

6. Test procedure

Allow all reagents and samples to reach room temperature. Duplicate determinations are recommended.

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.
Storage: up to 6 months 2–8°C.

Equalizing Reagent
Reconstitute the Equalizing Reagent with **10 mL** of **Assay Buffer**.
Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquotes at -20°C and may be thawed only once.

I-Buffer
Dilute the 4 mL I-Buffer Concentrate with distilled water to a final volume of 400 mL.
Storage: up to 2 months 4-8°C.

6.2 Preparation of samples

The GABA ELISA is a flexible test system for various biological sample types and samples. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to fit the protocol to his specific needs:

- ⚠ For the determination of samples 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 <u>not</u> dilute samples!**

  ⚠ For the determination of samples in a **range between 75 – 7 500 ng/mL**, ***do <u>not</u> dilute standards, controls or samples.***

- 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 in 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.

If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer directly!

6.3 Test procedure (75 – 7 500 ng/mL)

6.3.1 Extraction

1.	Pipette **100 µL** of the **standards, controls** and **samples** into the appropriate wells of the **Extraction Plate.**
2.	Add **100 µL** of the **Diluent** to all wells. Cover plate with **Adhesive Foil** and **shake** for **15 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
3.	**Discard** and blot dry by tapping the inverted plate on absorbent material. **Wash** each well with **500 µL** of distilled water and shake for 5 min at RT (20-25°C) on a shaker (approx. 600 rpm).
4.	**Discard** the wash and blot dry by tapping the inverted plate on absorbent material.
5.	Pipette **400 µL** of **Elution Buffer** into the appropriate wells of the **Extraction Plate.** Cover plate with **Adhesive Foil** and **shake** for **10 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6.	Use **100 µl** for the subsequent derivatization!

6.3.2 Derivatization

1.	Pipette **100 µL** of the **extracted standards, controls and samples** into the appropriate wells of the **Macrotiter Plate.**
2.	Pipette **10 µL** of the **NaOH** into all wells.
3.	Add **50 µL** of the **Equalizing Reagent** (fresh prepared before assay) to all wells and **shake** for **1 min** on a shaker (600rpm).
4.	Pipette **10 µL** of the **D-Reagent** into all wells.
5.	Cover plate with **Adhesive Foil** and shake for **2 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6.	Pipette **200 µL Q-Buffer** into all wells.
7.	Shake for **10 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
8.	Use **50 µl** for the subsequent ELISA!

6.3.3 GABA ELISA

1.	Pipette **50 µL** of the **derivatized standards, controls** and **samples** into the appropriate wells of the **GABA Microtiter Strips.**
2.	Pipette **50 µL** of the **GABA Antiserum** into all wells and mix shortly.
3.	Cover plate with **Adhesive Foil** and incubate for **15 - 20 hours** (overnight) at **2 – 8 °C**. ***Alternatively*** *incubate* ***2 hours*** *at* ***RT*** *(20-25°C) on a* ***shaker*** *(approx. 600rpm).*
4.	Remove the foil and discard. Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
5.	Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
6.	Incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
7.	Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
8. ⚠	Pipette **100 µL** of the **Substrate** into all wells and incubate for **20-30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). ***Avoid exposure to direct sun light!***
9.	Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** and a reference wavelength between 620 nm and 650 nm.

6.4 Test procedure (25 – 2 500 ng/mL)

6.4.1 Extraction

1.	Pipette **300 µL** of the **diluted standards, controls** and **<u>undiluted</u> samples** into the appropriate wells of the **Extraction Plate.**
2.	Add **300 µL** of the **Diluent** to all wells. Cover plate with **Adhesive Foil** and **shake** for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
3.	**Washing step (2 cycles):** **Discard** and blot dry by tapping the inverted plate on absorbent material. **Add 1mL** of **I-Buffer** to each well and incubate the plate for **5 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). **Discard** and blot dry by tapping the inverted plate on absorbent material. **Add 1mL** of **I-Buffer** to each well and incubate the plate for **5 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
4.	**Discard** and blot dry by tapping the inverted plate on absorbent material.
5.	Pipette **250 µL** of **Elution Buffer** into the appropriate wells of the **Extraction Plate.** Cover plate with **Adhesive Foil** and **shake** for **10 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6.	Use **100 µl** for the subsequent derivatization!

6.4.2 Derivatization

1.	Pipette **100 µL** of the **extracted standards, controls and samples** into the appropriate wells of the **Macrotiter Plate.**
2.	Pipette **10 µL** of the **NaOH** into all wells.
3.	Add **50 µL** of the **Equalizing Reagent** (fresh prepared before assay) to all wells and **shake** for **1 min** on a shaker (600rpm).
4.	Pipette **10 µL** of the **D-Reagent** into all wells.
5.	Cover plate with **Adhesive Foil** and incubate for **2 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6.	Pipette **150 µL Q-Buffer** into all wells.
7.	Incubate for **10 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
8.	Use **25 µl** for the subsequent ELISA!

6.4.3 GABA ELISA

1.	Pipette **25 µL** of the **derivatized standards, controls** and **samples** into the appropriate wells of the **GABA Microtiter Strips.**
2.	Pipette **50 µL** of the **GABA Antiserum** into all wells and mix shortly.
3.	Cover plate with **Adhesive Foil** and incubate for **15 - 20 hours** (overnight) at **2 – 8 °C**. ***Alternatively*** *incubate* ***2 hours*** *at* ***RT*** *(20-25°C) on a* ***shaker*** *(approx. 600rpm).*
4.	Remove the foil and discard. Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
5.	Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
6.	Cover plate with **Adhesive Foil** and incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600
7.	Remove the foil and discard. Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer**. Blot dry by tapping the inverted plate on absorbent material.
8. ⚠	Pipette **100 µL** of the **Substrate** into all wells and incubate for **20-30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). ***Avoid exposure to direct sun light!***
9.	Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
GABA (ng/mL)	0	75	250	750	2 500	7 500
GABA (µmol/L)	0	727	2 425	7 275	24 250	72 750
Conversion:	GABA (ng/mL) x 9.7 = GABA (nmol/L)					

The calibration curve from which the concentrations in the samples can be taken is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the standards (linear, y-axis) against the corresponding concentrations (logarithmic, x-axis).
Use non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

Test procedure (25 – 2 500 ng/mL):
The read concentrations of **plasma samples** have to be **divided by 3.**
The concentrations of the controls can be read directly from the standard curve.

Test procedure (75 – 7 500 ng/mL):
The concentrations of the samples and controls can be read directly from the standard curve.

7.1 Quality control

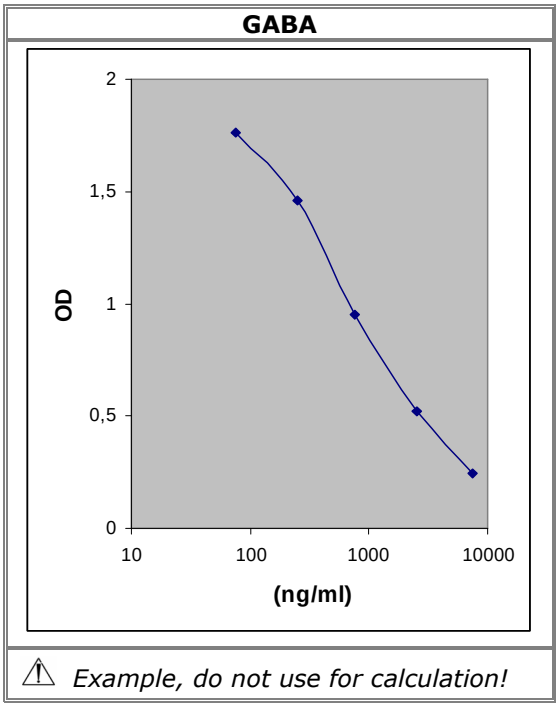
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC Report.

7.2 Calibration

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.

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

7.3 Typical calibration curve



⚠ *Example, do not use for calculation!*

8. Assay characteristics

Analytical Specificity (Cross Reactivity)	Substance	Cross Reactivity (%)
		GABA
	GABA	100
	ß-Alanine	0.50
	α-Aminobutyric acid	< 0.001

Precision					
Intra-Assay			**Inter-Assay**		
Sample	Range (ng/mL)	CV (%)	Sample	Range (ng/mL)	CV (%)
1 (n = 32)	296 ± 34	11	1 (n = 41)	509 ± 82	16
2 (n = 32)	1 237 ± 127	10	2 (n = 40)	1 233 ± 172	14

		Mean (%)	Range (%)	% Recovery after spiking
Recovery	GABA male	109	104 - 111	
	GABA female	113	105 - 118	

⚠ ***For current literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	I V D	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!