（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K852-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（630-650 nm）

Detection range：0.06–10.0 μmol/mL

Elabscience® γ -Aminobutyric Acid (GABA) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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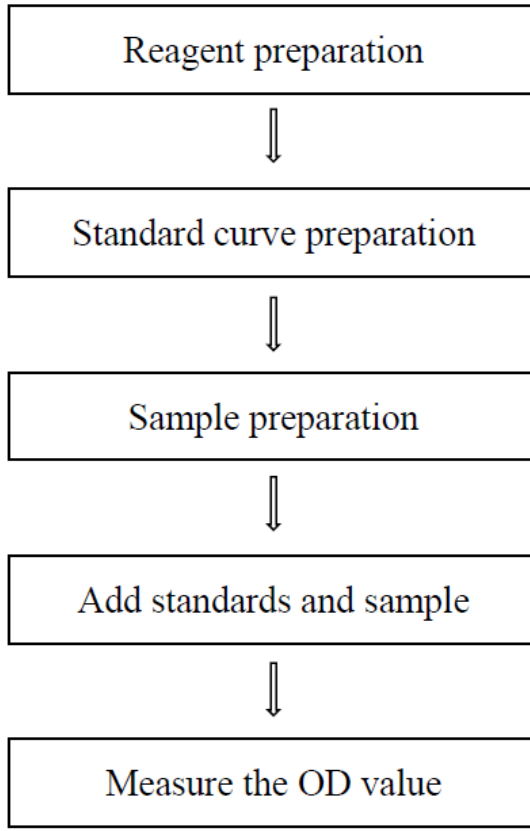
Assay summary



Intended use

This kit can be used to measure γ-Aminobutyric Acid (GABA) content in animal and plant tissue samples.

Detection principle

Phenol and sodium hypochlorite react with GABA to produce a blue-green product, which has maximum absorbance at 640 nm. GABA content can be calculated with the absorbance at 640 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	60 mL × 1 vial	60 mL × 2 vials	2-8℃, 12 months
Reagent 2	Buffer Solution	3 mL × 1 vial	6 mL × 1 vial	2-8℃, 12 months
Reagent 3	Chromogenic Agent A	2.4 mL × 1 vial	4.8 mL × 1 vial	2-8℃, 12 months, shading light,
Reagent 4	Chromogenic Agent B	3.6 mL × 1 vial	7.2 mL × 1 vial	2-8℃, 12 months, shading light
Reagent 5	Supplementary Solution	12 mL × 1 vial	24 mL × 1 vial	2-8℃, 12 months
Reagent 6	10 μmol/mL GABA Standard	1.6 mL × 1 vial	1.6 mL × 2 vials	2-8℃, 12 months
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Test tube, Micropipettor, Vortex mixer, Microplate reader (630-650 nm, optimum wavelength: 640 nm), Centrifuge, Water bath (95℃)

Reagent preparation

① Equilibrate all reagents to room temperature before use.

② The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 10 μmol/mL GABA standard solution with extracting solution to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 4, 5, 7, 9, 10 μmol/mL. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/mL)	**0**	**1**	**2**	**4**	**5**	**7**	**9**	**10**
10 μmol/mL GABA standard (μL)	0	20	40	80	100	140	180	200
Extracting solution (μL)	200	180	160	120	100	60	20	0

Sample preparation

① Sample preparation

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 50 mg tissue in 450 μL extracting solution with a dounce homogenizer at 4℃.

④ After homogenizing, transfer to EP tube and mark the liquid level scale. Heat in 95℃ water bath for 2 h (The EP tube cover is tight, and the tube cover is tied with a small hole for ventilation to prevent the cover from bursting under high temperature and spilling out of effective components).

⑤ The supernatant is supplemented with extracting solution to original volume and mix fully. Then centrifuge at 8000×g for 10 min and take the supernatant for detection.

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Epipremnum aureum tissue homogenization	1
10% Green pepper tissue homogenization	1
10% Chinese yam tissue homogenization	1
10% Rat heart tissue homogenization	1
10% Rat liver tissue homogenization	1
10% Rat kidney tissue homogenization	1

Note: The diluent is extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Please determine within 10 min after the reaction.

Operating steps

① Standard tube: Take 30 μL of standard with different concentrations to 1.5 mL EP tubes.

Sample tube: Take 30 μL of sample supernatant to 1.5 mL EP tubes.

② Add 50 μL of buffer solution and 40 μL of chromogenic agent A into each tube.

③ Mix fully with vortex mixer and stand at room temperature for 5 min.

④ Add 60 μL of chromogenic agent B into each tube.

⑤ Mix fully with vortex mixer and heat in 95℃ water bath for 10 min, cool in ice bath.

⑥ Add 200 μL of supplementary solution into each tube and mix fully.

⑦ Take 200 μL from each tube to the microplate and measure the OD value of each well at 640 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

Tissue sample:

$$\text{GABA content (μmol/g wet weight)} = (\Delta A_{640} - b) \div a \times V \div m \times f$$

[Note]

ΔA_{640}: $OD_{Sample} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

m: The weight of tissue, g.

V: The volume of extraction solution, mL.

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three rat heart tissue samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/mL)	0.64	2.60	7.20
%CV	4.3	3.9	3.8

Inter-assay Precision

Three rat heart tissue samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μmol/mL)	0.64	2.60	7.20
%CV	6.6	7.2	6.6

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 96%.

	Standard 1	Standard 2	Standard 3
Expected Conc. （μmol/mL）	1.5	4.6	8.5
Observed Conc. （μmol/mL）	1.5	4.4	8.1
recovery rate(%)	98	95	95

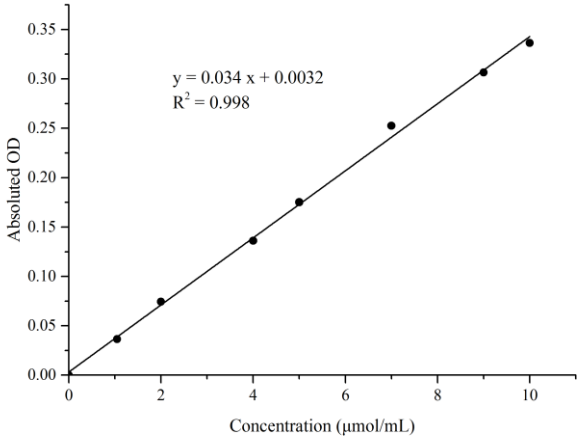
Sensitivity

The analytical sensitivity of the assay is 0.06 μmol/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/mL)	**0**	**1.0**	**2.0**	**4.0**	**5.0**	**7.0**	**9.0**	**10.0**
Average OD	0.043	0.079	0.117	0.179	0.218	0.295	0.349	0.379
Absoluted OD	0.000	0.037	0.074	0.136	0.175	0.253	0.307	0.336



Appendix II Example Analysis

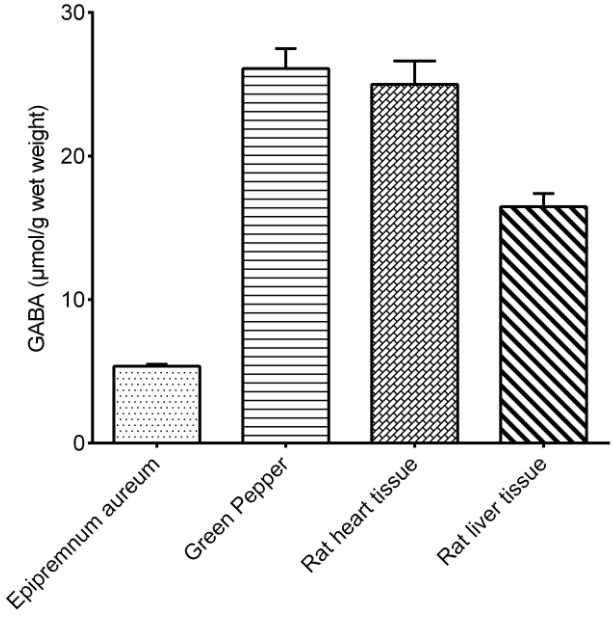
Example analysis：

For green pepper tissue, take 30 μL of prepared 10% green pepper tissue supernatant and carry the assay according to the operation steps. The results are as follows: Standard curve: y = 0.034 x + 0.0032, the OD value of the sample is 0.135, the OD value of the blank is 0.043, and the calculation result is:

GABA content (μmol/g wet weight) =（0.135 - 0.043 - 0.0032）÷0.034 ×0.9 ÷0.1 ×1

= 23.49 μmol/g wet weight

Detect 10% epipremnum aureum tissue homogenization, 10% green pepper tissue homogenization, 10% rat heart tissue homogenization and 10% rat liver tissue homogenization according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.