PRODUCT INFORMATION & MANUAL

GABA Assay Kit (Colorimetric)

NBP3-24553

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

GABA Assay Kit (Colorimetric)

Catalog No: NBP3-24553

Method: Colorimetric method

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.06 μmol/mL

Detection range: 0.06–10.0 μmol/mL

Average intra-assay CV (%): 4

Average inter-assay CV (%): 6.8

Verage recovery rate (%): 96

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure GABA content in animal and plant tissue samples.

▲Background

γ-Aminobutyric acid (GABA), a kind of 4-C non-protein amino acid, widely exists in animals and plants, and is effective inhibitory neurotransmitter in the animal central nervous system. It has the functions of lowering blood pressure, enhancing brain vitality, maintaining nerve stability, promoting growth hormone secretion and protecting liver and kidney, at present has been widely used in medicine and health food.

▲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	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 2 vials	2-8℃ , 12 months
Reagent 2	Buffer Solution	6 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Chromogenic Agent A	4.8 mL × 1 vial	2-8℃ , 12 months, shading light,
Reagent 4	Chromogenic Agent B	7.2 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 5	Supplementary Solution	24 mL × 1 vial	2-8℃ , 12 months
Reagent 6	10 μmol/mL GABA Standard	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. The amount of reagent 1 is based on the ratio of 0.05g:450uL for homogenation with an addition for volume maintenance during the extraction process if necessary.			

▲Materials prepared by users

Instruments

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

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

Please determine within 10 min after the reaction..

Pre-assay preparation

▲Reagent preparation

Bring all reagents to room temperature before use.

▲Sample preparation

Tissue: Weigh 0.05g tissue accurately, Add 450 uL of reagent 1 to homogenize mechanically. 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, If solvent volatilizes during the extraction process, supplement reagent 1 in time). The supernatant is supplemented with reagent 1 to original volume and mix fully. Then, centrifuge at 8000 g for 10 min and take the supernatant for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.06–10.0 μmol/mL).

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 reagent 1.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

[Note]：A–H, standard wells; S1–S80, sample wells.

▲Detailed operating steps

The preparation of standard curve

Dilute 10 μmol/mL GABA standard solution with reagent 1 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:

Number	Standard concentrations (μmol/mL)	10 μmol/mL Standard (μL)	Reagent 1 (μL)
A	0.0	0	200
B	1.0	20	180
C	2.0	40	160
D	4.0	80	120
E	5.0	100	100
F	7.0	140	60
G	9.0	180	20
H	10.0	200	0

The measurement of samples

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 reagent 2 and 40 μL of reagent 3 into each tube.

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

4) Add 60 μL of reagent 4 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 reagent 5 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.

▲Summary operation table

	Standard tube	Sample tube
Standard with different concentrations (μL)	30	
Sample supernatant (μL)		30
Reagent 2 (μL)	50	50
Reagent 3 (μL)	40	40
Mix fully and stand at room temperature for 5 min.		
Reagent 4 (μL)	60	60
Mix fully and heat in 95℃ water bath for 10 min, cool in ice bath.		
Reagent 5 (μL)	200	200
Mix fully, take 200 μL from each tube to the microplate and measure the OD value at 640 nm.		

▲Calculation

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

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

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0).

x: The concentration of Standard.

a: The slope of standard curve.

b: The intercept of standard curve.

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 Data

▲Example analysis

For green pepper tissue, take 30 μL of prepared 10% green pepper tissue supernatant and carry the assay according to the operation table. 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:

$$\text{GABA content (μmol/g wet weight)} = \frac{0.135 - 0.043 - 0.0032}{0.034} \times 0.9 \div 0.1 \times 1$$

$$= 23.49 \text{ μmol/g wet weight}$$

Appendix II References

1. Watanabe M, Maemura K, Kanbara K, et al. GABA and GABA Receptors in the Central Nervous System and Other Organs[J]. International Review of Cytology, 2002, 213(4):1-47.

2. Autoradiographic distribution of radioactivity from 14C-GABA in the mouse[J]. Microscopy Research & Technique, 2002.