Technical Manual

γ-Aminobutyric Acid (GABA) Colorimetric Assay Kit

- Catalogue Code: MAES0202
- Size: 96T
- Research Use Only

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

96T

Range:

0.06–10.0 μmol/mL

Sensitivity:

0.06 μmol/mL

Storage:

2-8°C for 3 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 3 months.

Do not use components from different batches of kit.

2. 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.

3. Intended Use:

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

4. 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.

5. Kit Components & Storage:

Item	Specification	Storage
Extracting Solution	40 mL × 2 vials	2-8°C, 3 months
Buffer Solution	6 mL × 1 vial	2-8°C, 3 months
Chromogenic Agent A	1.6 mL × 3 vials	2-8°C, 3 months, avoid direct sunlight
Chromogenic Agent B	7.2 mL × 1 vial	2-8°C, 3 months, avoid direct sunlight
Supplementary Solution	24 mL × 1 vials	2-8°C, 3 months
GABA Standard (10 μmol/mL)	1.6 mL × 2 vials	2-8°C, 3 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Water bath (95℃)
- Centrifuge
- Microplate Reader (630-650 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)

6. Assay Notes:

Determine within 10 min after the reaction.

7. Reagent Preparation:

Bring all reagents to room temperature before use.

8. Sample Preparation:

1. Tissue sample:

Weigh about 0.1 g tissue. Add 0.9 mL of extracting solution, homogenize mechanically, after homogenizing, transfer to EP tube. Heat in 95°C 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 extracting solution in time). The supernatant is supplemented with extracting solution to 0.9 mL and mix fully. Then, centrifuge at 8000 g for 10 min and take the supernatant for detection.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. 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 extracting solution.

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 640 nm

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.

10. Operation Steps:

The preparation of standard curve

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

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 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°C 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.

Operation Table

	Standard tube	Sample tube
Standard of different concentrations (μL)	30	
Sample supernatant (μL)		30
Buffer solution (μL)	50	50
Chromogenic agent A (μL)	40	40
Mix fully with vortex mixer and stand at room temperature for 5 min.		
Chromogenic agent B (μL)	60	60
Mix fully with vortex mixer and heat in 95°C water bath for 10 min, cool in ice bath		
Supplementary solution (μL)	200	200
Mix fully, take 200 μL from each tube to the microplate and measure the OD value of each well at 640 nm with microplate reader.		

11. Calculations:

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.

Tissue sample:

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

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.

12. Performance Characteristics:

Detection Range	0.06–10.0 μmol/mL
Sensitivity	0.06 μmol/mL
Average recovery rate (%)	96
Average inter-assay CV (%)	6.8
Average intra-assay CV (%)	4

Analysis

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:

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

$$= 23.49 \text{ μmol/g wet weight}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching 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.

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

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