TECHNICAL MANUAL

GABA Colorimetric Assay Kit

- **SKU CODE:** MAES0202
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Color development
- Measure absorbance at 640 nm

2. Intended use

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

3. 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 using the absorbance at 640 nm.

4. 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°C, 12 months
Reagent 2	Buffer Solution	3 mL × 1 vial	6 mL × 1 vial	2-8°C, 12 months
Reagent 3	Chromogenic Agent A	2.4 mL × 1 vial	4.8 mL × 1 vial	2-8°C, 12 months, protect from light
Reagent 4	Chromogenic Agent B	3.6 mL × 1 vial	7.2 mL × 1 vial	2-8°C, 12 months, protect from light
Reagent 5	Supplementary Solution	12 mL × 1 vial	24 mL × 1 vial	2-8°C, 12 months
Reagent 6	10 μmol/mL GABA Standard	1.6 mL × 1 vial	1.6 mL × 2 vials	2-8°C, 12 months
	Microplate	48 wells	96 wells	No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

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

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **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 create a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 4, 5, 7, 9, 10 µmol/mL. Standard Preparation Reference: Item ①-⑧: Concentrations (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

7. Sample preparation

1. **Tissue sample preparation:** 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°C. 4. After homogenizing, transfer to EP tube and mark the liquid level. Heat in 95°C water bath for 2 h (ensure the EP tube cover is tight, and pierce a small hole for ventilation to prevent the cover from bursting under high temperature). 5. Supplement the supernatant with extracting solution to original volume and mix thoroughly. Then centrifuge at 8000×g for 10 min and collect the supernatant for detection.
2. **Sample dilution:** The recommended dilution factor for different samples is as follows (for reference only): 10% Epipremnum aureum tissue homogenate: 1 10% Green pepper tissue homogenate: 1 10% Chinese yam tissue homogenate: 1 10% Rat heart tissue homogenate: 1 10% Rat liver tissue homogenate: 1 10% Rat

kidney tissue homogenate: 1 Note: The diluent is extracting solution. For other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

Please determine the results within 10 minutes after the reaction is complete.

9. Operating steps

1. Standard tube: Add 30 μL of standard with different concentrations to 1.5 mL EP tubes. Sample tube: Add 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 thoroughly with vortex mixer and incubate at room temperature for 5 min.
4. Add 60 μL of chromogenic agent B into each tube.
5. Mix thoroughly with vortex mixer and heat in 95°C water bath for 10 min, then cool in ice bath.
6. Add 200 μL of supplementary solution into each tube and mix thoroughly.
7. Transfer 200 μL from each tube to the microplate and measure the OD value of each well at 640 nm with a microplate reader.

10. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using absolute OD value of standards and corresponding concentrations as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

Tissue sample:

GABA content (μmol/g wet weight) = (ΔA_640 - b) ÷ a × V ÷ m × 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 testing.

11. Appendix I Performance Characteristics

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).

Intra-assay Precision

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.

Inter-assay Precision

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

Recovery

Three samples of high, medium, and low concentrations were tested 6 times in parallel to obtain an average recovery rate of 96%.

Recovery

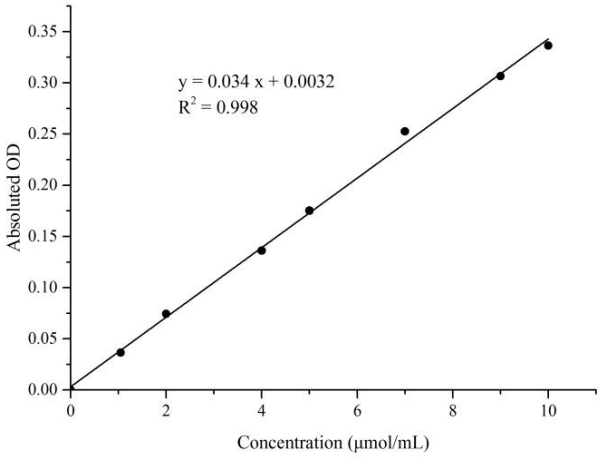
	Standard 1	Standard 2	Standard 3
Expected Conc. (µmol/mL)	1.5	4.6	8.5
Observed Conc. (µmol/mL)	1.5	4.4	8.1
Recovery rate (%)	98	95	95

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.

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), the standard curve and data are provided below for reference only:



Standard curve

Concentration (µmol/mL)	Average OD	Absolute OD
0	0.043	0.000
1.0	0.079	0.037

Concentration (μmol/mL)	Average OD	Absolute OD
2.0	0.117	0.074
4.0	0.179	0.136
5.0	0.218	0.175
7.0	0.295	0.253
9.0	0.349	0.307
10.0	0.379	0.336

12. Appendix II Example Analysis

Example analysis:

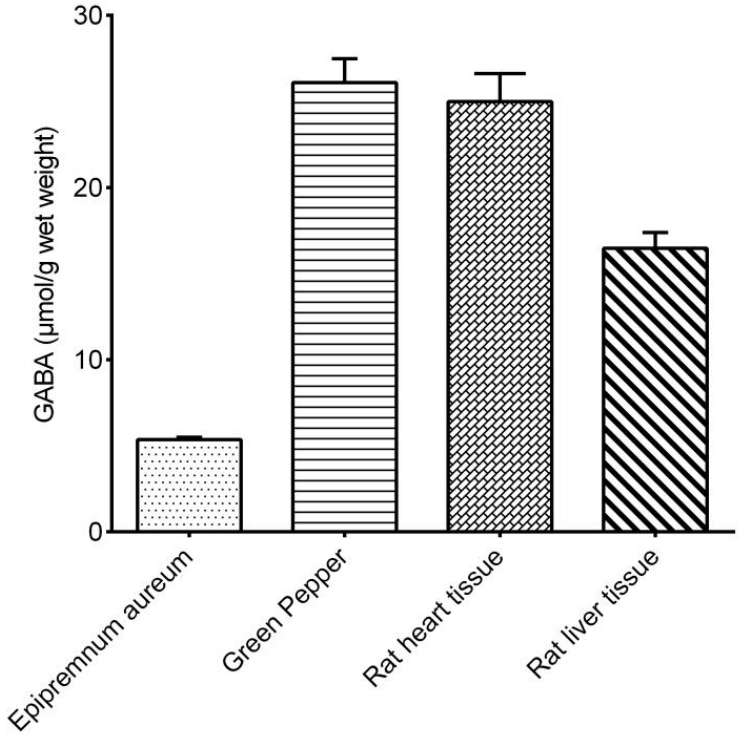
For green pepper tissue, take 30 μL of prepared 10% green pepper tissue supernatant and perform the assay according to the operating steps. The results are as follows:

Standard curve: y = 0.034x + 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

Testing results for 10% epipremnum aureum tissue homogenate, 10% green pepper tissue homogenate, 10% rat heart tissue homogenate and 10% rat liver tissue homogenate according to the protocol are shown below:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.