CheKine™ Micro γ-Aminobutyric Acid (GABA) Content Assay Kit

Cat #: KTB3045 Size: 48 T/48 S 96 T/96 S

	Micro γ-Aminobutyric Acid (GABA) Content Assay Kit		
REF	**Cat #:** KTB3045	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

γ-Aminobutyric Acid (GABA) is a non-proteinogenic amino acid consisting of four carbon atoms, which is widely present in the organisms of plants, animals, and microorganisms. Within plants, GABA is primarily generated through decarboxylation of L-glutamate catalyzed by glutamate decarboxylase. In the mammalian brain, GABA serves as an efficacious inhibitory neurotransmitter, exhibiting functions such as lowering blood pressure, enhancing brain vitality, nourishing nerve cells, maintaining neural stability, promoting growth hormone secretion, and benefiting liver and kidney health. It has already found extensive applications in both pharmaceuticals and health food products.Principle of Determination: GABA reacts with phenol and sodium hypochlorite, giving rise to a blue-green product that exhibits a maximum absorbance at 640 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Reagent Ⅰ	3 mL	6 mL	4℃
Reagent Ⅱ	2.5 mL	5 mL	4℃, protected from light
Reagent Ⅲ	4 mL	8 mL	4℃, protected from light
Reagent Ⅳ	12.5 mL	25 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 640 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, ice maker, centrifuge, seal film
- Deionized water

• Homogenizer or mortar (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent II: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Note: Reagent II is toxic, Reagent III is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.

Reagent IV: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month. When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

Weigh 0.1 g tissue sample, add 1 mL Extraction Buffer, homogenize thoroughly, then transfer the mixture to an EP tube. Incubate at a water bath set at 95°C for 2 h (ensuring that the tube is securely capped and sealed with a seal film around the opening to prevent water evaporation). After cooling, centrifuge at 8,000 g at 25°C for 10 min. Collect the supernatant for further analysis.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 640 nm, visible spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are carried out in a 1.5 mL EP tube.):

Reagent	Test Tube (μL)	Blank Tube (μL)
Sample supernatant	30	0
Extraction Buffer	0	30
Reagent I	50	50
Reagent II	40	40
Mix well and allow to stand at room temperature for 5 min		
Reagent III	60	60
Mix thoroughly, incubate at a water bath set to 95°C for 10 min, and then cool on ice.		
Reagent IV	200	200
Mix thoroughly, aspirate 200 μL into a 96-well plate, measure the absorbance values A_{Test} and A_{Blank} at 640 nm, and calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$.		

Note: The Blank Tube only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If A_{Test} is less than 0.05, increase the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

Regression equation determined under standard conditions: y=1.557x-0.004, R^2=0.992, x represents the standard (mg/mL), y denotes ΔA.

(1) Calculated by protein concentration

GABA (mg/mg prot)=(ΔA+0.004)÷1.557÷Cpr**=0.642×(ΔA+0.004)÷Cpr**

(2) Calculated by sample fresh weight

GABA (mg/g fresh weight)=(ΔA+0.004)÷1.557÷W**=0.642×(ΔA+0.004)÷W**

Cpr: protein concentration, mg/mL; W: sample weight, g.

Precautions

1. Sample processing times can be lengthy, so ensure that tube caps are tightly secured or wrap the tube openings with seal film.

Typical Data

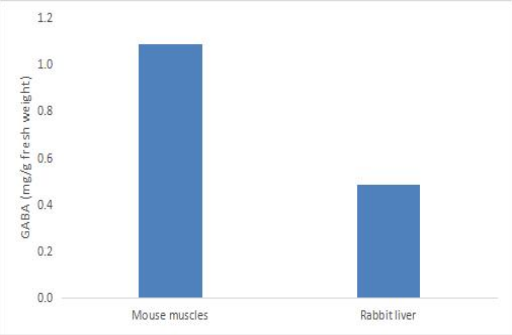

Figure 1. Determination GABA content in Mouse muscles and Rabbit liver by this assay kit

Recommended Products

Catalog No.	Product Name
KTB1015	CheKine™ Micro α-Glucosidase Activity Assay Kit
KTB1121	CheKine™ Pyruvate Acid (PA) Colorimetric Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.