CheKine™ Micro Glutamine Synthetase (GS) Activity Assay Kit

Cat #: KTB3042 Size: 48 T/24 S 96 T/48 S

	Micro Glutamine Synthetase (GS) Activity Assay Kit		
REF	**Cat #:** KTB3042	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and Plant Tissues, Cells or Bacteria, Plasma, Serum or other Liquid samples		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Glutamine synthase (GS, EC 6.3.1.2) is mainly present in plants and is one of the key enzymes for ammonia assimilation in organisms. It can catalyze the synthesis of glutamine from ammonium ions and glutamic acid, not only preventing excessive ammonium ions from being toxic to organisms, but also being the main storage and transportation form of ammonia. GS catalyzes the synthesis of glutamine from ammonium ions and glutamic acid in the presence of ATP and Mg^{2+}, glutamine is further converted into γ-Glutamoyl hydroxamic acid, a complex formed with iron under acidic conditions, has a maximum absorption peak at 540 nm and can be determined by a spectrophotometer.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	60×2 mL	4℃
Reagent I	6 mL	12 mL	-20℃
Reagent II	6 mL	12 mL	-20℃
Reagent III	Powder×1 vial	Powder×2 vials	-20℃, protected from light
Reagent IV	7.5 mL	15 mL	4℃, protected from light

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 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Thermostatic water bath, ice maker, centrifuge, incubator
- Deionized water
- Mortar or homogenizer

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. Preheat at 37℃ for 20 min before use, mix thoroughly. If there is any sediment, let it stand for 10 min. Take the supernatant and set aside for use. Store at -20℃.

Reagent II: Ready to use as supplied. Preheat at 37℃ for 20 min before use, mix thoroughly. If there is any sediment, let it stand for 10 min. Take the supernatant and set aside for use. Store at -20℃.

Working Reagent III: Prepared before use. Take one tube Reagent III and add 5 mL of deionized water, fully dissolve and set aside for use. Unused reagents can be stored for 4 weeks when packaged at -20℃, protected from light to avoid repeated freeze-thaw cycles.

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

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.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells or Bacteria: Collect 2×10^7 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum or other Liquid samples: Test directly.

Note: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Cat #: KTD3002, if it is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm, visible spectrophotometer was returned to zero with deionized water.
2. Enzymatic reaction (The following operations are operated in the 1.5 mL EP tube):

Reagent	Test Tube (μL)	Control Tube (μL)
Reagent I	160	0
Reagent II	0	160
Working Reagent III	70	70
Sample supernatant	70	70
Mix thoroughly and place in an accurate water bath at 37℃ (for mammals) or 25℃ (for other species) for 30 min		
Reagent IV	100	100

Mix well, let stand at 25℃ for 10 min, then 8,000 g, centrifuge at 25℃ for 10 min, take 200 μL of supernatant and transfer it to a microglass cuvette or a 96 well plate, and measure the absorbance value at 540 nm. The absorbance of test tube, control tube were recorded as A_{Test}, $A_{Control}$. Calculate $\Delta A=A_{Test}-A_{Control}$.

Note: Each measuring tube needs to be equipped with a control tube. Before the experiment, it is suggested that 2-3 samples with large expected differences should be selected for pre-experiment. If ΔA is less than 0.1, the sample volume can be appropriately increased. If ΔA is greater than 1.0, the sample can be further diluted with Extraction Buffer before proceeding with the experiment, and the final dilution factor should be taken into account in the calculations.

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.

1. Calculated by protein concentration

Active unit definition: The change in absorbance value at 540 nm by 0.005 per min per mg of tissue protein in the reaction system is defined as a unit of enzyme activity.

GS (U/mg prot)=$\Delta A \times V_{Total} \div (Cpr \times V_{Sample}) \div 0.005 \div T$**=38×ΔA÷Cpr**

2. Calculated by sample fresh weight

Active unit definition: The change in absorbance value at 540 nm by 0.005 per min per g of tissue in the reaction system is defined as a unit of enzyme activity.

GS (U/g fresh weight)=$\Delta A \times V_{Total} \div (W \times V_{Sample} \div V_{Total\ Sample}) \div 0.005 \div T$**=38×ΔA÷W**

3. Calculated by number of cells or bacteria

Active unit definition: The change in absorbance value at 540 nm by 0.005 per min per 10^4 bacteria or cells in the reaction system is defined as a unit of enzyme activity.

GS (U/g 10^4)=$\Delta A \times V_{Total} \div (2{,}000 \times V_{Sample} \div V_{Total\ Sample}) \div 0.005 \div T$**=0.019×ΔA**

4. Calculated by sample volume

Active unit definition: The change in absorbance value at 540 nm by 0.005 per min per mL serum (lasma) in the reaction system is defined as a unit of enzyme activity.

GS (U/mL)=$\Delta A \times V_{Total} \div V_{Sample} \div 0.005 \div T$**=38×ΔA**

V_{Total}: total volume of enzymatic reaction, 0.4 mL; V_{Sample}: sample volume added, 0.07 mL; $V_{Total\ Sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; T: reaction time, 30 min; W: sample weight, g; 2,000: Total number of bacteria or cells, 2×10^7.

Typical Data

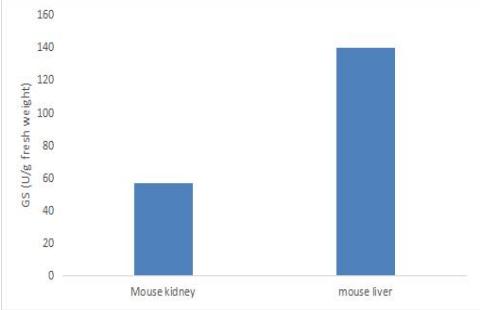

Figure 1. Determination GS activity in mouse kidney and mouse liver by this assay kit

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.