CheKine™ Micro Glutaminase (GLS) Activity Assay Kit

Cat #: KTB3043 Size: 48 T/48 S 96 T/96 S

	Micro Glutaminase (GLS) Activity Assay Kit		
REF	**Cat #:** KTB3043	LOT	**Lot #:** Refer to product label
	Applicable sample: Animal and plant tissues, bacteria and cells, serum (plasma) or other biological fluids.		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Glutaminase (GLS), which is mainly found in higher animals, some bacteria and plant roots, catalyzes the hydrolysis of glutamine to glutamate and ammonia. It plays an important role in the regulation of nitrogen metabolism, especially the content of free ammonia and urea metabolism. CheKine™ Micro Glutaminase (GLS) Activity Assay Kit can detect biological samples such as animal and plant tissues, bacteria and cells, serum or plasma. In this kit, GLS catalyzes the hydrolysis of glutamine to L-glutamate and ammonia, and its enzymatic activity can be calculated by detecting the rate of ammonia increase using Nessler's reagent.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	70 mL	70 mL×2	4℃
Reagent Ⅱ	Powder×2 vials	Powder×2 vials	4℃, protected from light
Reagent Ⅲ	35 mL	70 mL	4℃, protected from light
Reagent Ⅳ	2.5 mL	5 mL	4℃
Reagent Ⅴ	1.5 mL	3 mL	4℃
Reagent Ⅵ	1.5 mL	3 mL	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	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 ultraviolet spectrophotometer capable of measuring absorbance at 420 nm
- 96-well microplate or microquartz cuvette, precision pipettes, disposable pipette tips,1.5 mL EP tube
- Water bath, freezing centrifuge
- Deionized water

• Mortar or homogenizer (for tissue samples)

Reagent Preparation

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃. Reagent Ⅰ has crystallization precipitation is normal. After returning to room temperature, mix well and use it.

Working Reagent II: Prepared before use. Add 12.5 mL deionized water to each Reagent Ⅱ for 48 T, and 25 mL deionized water to each Reagent Ⅱ for 96 T to fully dissolve. Working Reagent Ⅱ is freshly prepared.

Reagent III: Ready to use as supplied. Store at room temperature protected from light.

Reagent IV: Ready to use as supplied. Store at room temperature.

Reagent V: Ready to use as supplied. Store at room temperature.

Reagent VI: Ready to use as supplied. Store at room temperature protected from light.

Note: Reagent III has an irritating odor, Reagent IV is corrosive, and Reagent VI is toxic, so it is recommended to experiment in a fume hood.

Standard: Prepared before use. Add 1 mL deionized water to a bottle, dissolve thoroughly, that is 100 μmol/mL ammonia Standard. The remaining reagent can be stored at 4℃ for 1 month. Using 100 μmol/mL ammonia Standard solution, prepare standard curve dilution as described in the table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (nmol/mL)
Std.1	10 μL of 100 μmol/mL Standard	990	1,000
Std.2	500 μL of Std.1 (1,000 nmol/mL)	500	500
Std.3	500 μL of Std.2 (500 nmol/mL)	500	250
Std.4	500 μL of Std.3 (250 nmol/mL)	500	125
Std.5	500 μL of Std.4 (525 nmol/mL)	500	62.5
Std.6	500 μL of Std.5 (62.5 nmol/mL)	500	31.25
Std.7	500 μL of Std.6 (31.25 nmol/mL)	500	15.625
Blank	0	500	0

Note: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

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 Reagent Ⅰ 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 5×10^6 cells or bacteria into the EP tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent Ⅰ 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 ,or other Liquid samples: Test directly.

Note: If the protein concentration of the sample is need to determined, it is recommended to use Abbkine Cat #: KTD3001 Protein Quantification Kit (BCA Assay) to measure the protein concentration of the sample.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 420 nm, ultraviolet spectrophotometer was returned to zero with deionized water.

2. Operation table (The following operations are operated in the 1.5 mL EP tube):

Reagent	Blank Tube (μL)	Standard Tube (μL)	Control Tube (μL)	Test Tube (μL)
Sample	0	0	0	25
Deionized Water	0	0	25	0
Reagent Ⅰ	0	0	100	100
Working Reagent Ⅱ	0	0	400	400
Mix well and incubate at 37℃ for 1 h.				
Reagent Ⅲ	0	0	525	525
Mix well, centrifugation 8,000 g for 10 min at 25℃. The supernatant was removed and the following procedures were performed **in turn** in a 96-well plate or microglass cuvette:				
Supernatant	0	0	130	130
Std. Standard	0	130	0	0
Deionized Water	130	0	0	0
Reagent Ⅳ	30	30	30	30
Reagent Ⅴ	20	20	20	20
Reagent Ⅵ	20	20	20	20

4.Mix thoroughly, quiescence at room temperature for 15 min, and measure the absorbance value at 420 nm. The Blank Tube is recorded as A_{Blank}, the Standard Tube is marked as $A_{Standard}$, the Test Tube is marked as $A_{Test,}$ and the Control Tube is marked as $A_{Control.}$ Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank Tube, Standard Tube, Control Tube only need to be done once or twice. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.4, increase the sample quantity appropriately. If ΔA_{Test} is greater than $\Delta A_{Standard}$ of 1,000 noml/mL, the sample can be appropriately diluted with Reagent Ⅰ , the calculated result multiplied by the dilution factor, or decrease 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.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation. The determination of ΔA_{Test} is brought into the equation to get x (nmol/mL).

2. Calculation of GLS activity:

(1) Calculated by protein concentration

Active unit definition: Catalysis of glutamine to 1 nmol ammonia per minute per milligram of protein was defined as one unit of enzyme activity.

$GLS(U/mg\ prot)=x\times V_{Total}\div(V_{Sample}\times Cpr)\div T$**$=0.7x\div Cpr$**

(2) Calculated by sample fresh weight

Active unit definition: Catalysis of glutamine to 1 nmol ammonia per minute per gram of sample was defined as one unit of enzyme activity.

GLS(U/g fresh weight)=x×V_{Total}÷(W×V_{Sample}÷$V_{Total\ sample}$)÷T=**0.7x÷W**

(3) Calculated by number of cells or bacteria

Active unit definition: Catalysis of glutamine to 1 nmol ammonia per minute per 10^4 cells or bacteria was defined as one unit of enzyme activity.

GLS(U/10^4)=x×V_{Total}÷(n×V_{Sample}÷$V_{Total\ sample}$)÷T=**0.7x÷n**

(4) Calculated by volume of liquid sample

Active unit definition: Catalysis of glutamine to 1 nmol ammonia per minute per milliliter of liquid was defined as one unit of enzyme activity.

GLS(U/mL)=x×V_{Total}÷V_{Sample}÷T=**0.7x**

V_{Total}: total reaction volume, 1.05 mL; $V_{Sample:}$ sample volume added, 0.025 mL; $V_{Total\ sample}$: Extraction Buffer volume added, 1 mL; Cpr: sample protein concentration, mg/mL; T: reaction time, 60 min. N: The number of bacteria or cells, in tens of thousands; W: weight of sample, g.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

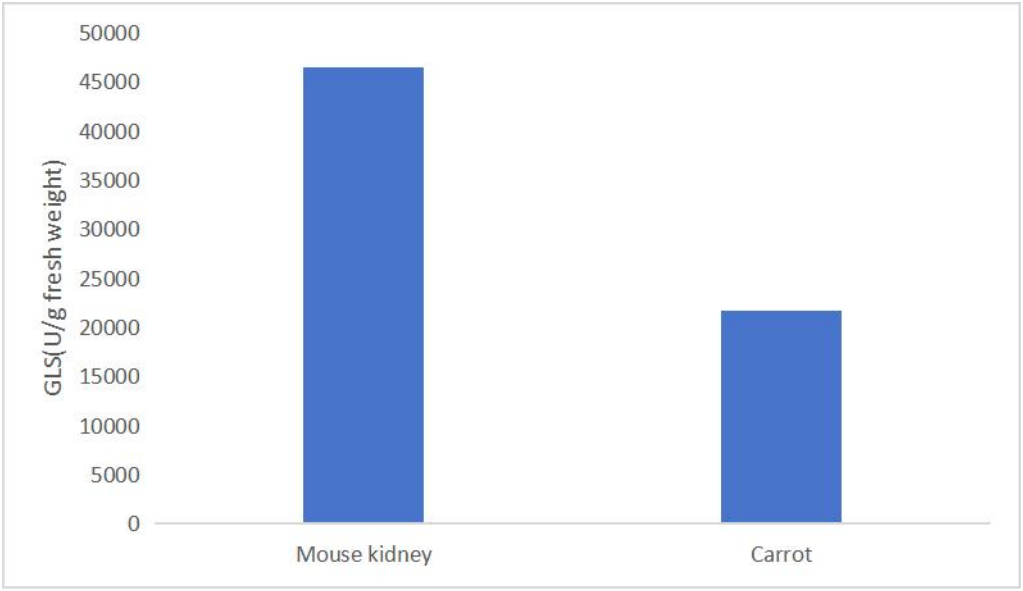

Figure 1. Determination GLS activity in mouse kidney and carrot by this assay kit.

Recommended Products

Catalog No.	Product Name
KTB4010	CheKine™ Micro Soil Nitrate Reductase (S-NR) Activity Assay Kit
KTB3041	CheKine™ Micro Glutamic Acid Dehydrogenase（GDH）Assay Kit
KTB3050	CheKine™ Water and Soil Nitrite Content Assay Kit
KTB3051	CheKine™ Micro Food Nitrite 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.