



## CheKine™ Micro Glutaminase (GLS) Activity Assay Kit

Cat #: KTB3043

Size: 48 T/96 T

	<b>Micro Glutaminase (GLS) Activity Assay Kit</b>		
<b>REF</b>	<b>Cat #:</b> KTB3043	<b>LOT</b>	<b>Lot #:</b> Refer to product label
	<b>Applicable sample:</b> Animal and plant tissues, bacteria and cells, serum (plasma) or other biological fluids.		
	<b>Storage:</b> Stored at 4°C 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	60 mL	60 mL×2	4°C
Reagent II	1	1×2	4°C, protected from light
Reagent III	30 mL	60 mL	RT, protected from light
Reagent IV	2.5 mL	5 mL	RT
Reagent V	1.5 mL	3 mL	RT
Reagent VI	1.5 mL	3 mL	RT, protected from light

### 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°C.

**Reagent II:** Prepared before use. Add 25 mL deionized water to a bottle, dissolve thoroughly. Store at 4°C. This reagent 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.

## Sample Preparation

**Note:** We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80°C 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°C. Use supernatant for assay, and place it on ice to be tested.

2. Cells or Bacteria: Collect  $5 \times 10^6$  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°C. 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 centrifugal tube):

Reagent	Blank Well (μL)	Test Well (μL)
Sample	0	25
Standard	0	0
Deionized Water	25	0
Reagent I	100	100
Reagent II	400	400

Mix well and incubate at 37°C for 1 h.

Reagent III	525	525
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Mix well, centrifugation 8,000 g for 10 min at 25°C. The supernatant was removed and the following procedures were performed in a 96-well plate or microglass cuvette:

Supernatant	130	130
Reagent IV	30	30
Reagent V	20	20
Reagent VI	20	20

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

**Note:** Each test well needs to be equipped with a control well, standard curve and blank well 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  $\Delta A_{\text{Test}}$  is less than 0.4, increase the sample quantity appropriately. If  $\Delta A_{\text{Test}}$  is greater than 2, 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

The regression equation determined under standard conditions is  $y = 1.9244x + 0.0057$ ,  $R^2 = 0.9983$ ; X is the standard concentration ( $\mu\text{mol/mL}$ ), and y is the absorbance value A.

### 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.

$$\text{GLS(U/mg prot)} = (\Delta A - 0.0057) \div 1.9244 \times V_{\text{Total}} \div (V_{\text{Sample}} \times \text{Cpr}) \div T \times 1,000 = \mathbf{363.7 \times (\Delta A - 0.0057) \div \text{Cpr}}$$

#### (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.

$$\text{GLS(U/g fresh weight)} = (\Delta A - 0.0057) \div 1.9244 \times V_{\text{Total}} \div (W \times V_{\text{Sample}} \div V_{\text{Total sample}}) \div T \times 1,000 = \mathbf{363.7 \times (\Delta A - 0.0057) \div W}$$

#### (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.

$$\text{GLS(U/10}^4\text{)} = (\Delta A - 0.0057) \div 1.9244 \times V_{\text{Total}} \div (n \times V_{\text{Sample}} \div V_{\text{Total sample}}) \div T \times 1,000 = \mathbf{363.7 \times (\Delta A - 0.0057) \div n}$$

#### (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.

$$\text{GLS(U/mL)} = (\Delta A - 0.0057) \div 1.9244 \times V_{\text{Total}} \div V_{\text{Sample}} \div T \times 1,000 = \mathbf{363.7 \times (\Delta A - 0.0057)}$$

$V_{\text{Total}}$ : total reaction volume, 1.05 mL;  $V_{\text{Sample}}$ : sample volume added, 0.025 mL;  $V_{\text{Total sample}}$ : 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; 1000: conversion factor, 1  $\mu\text{mol} = 1,000 \text{ nmol}$ .

## Typical Data

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

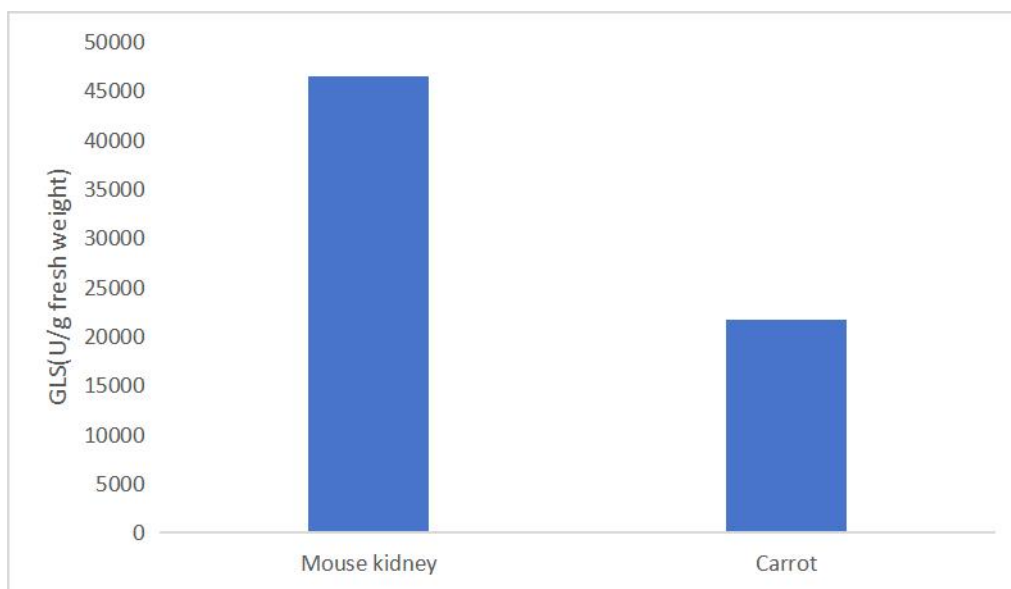


Figure 1. Determination GLS activity in mouse kidney and carrot by this assay kit.

## 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.