CheKine™ Micro Lysine (LYS) Assay Kit

Cat #: KTB1470 **Size:** 96 T/96 S

	Micro Lysine (LYS) Assay Kit		
REF	**Cat #:** KTB1470	LOT	**Lot #:** Refer to product label
	Detection range: 0.125-8 μmol/mL		**Sensitivity:** 0.03125 μmol/mL
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Lysine is one of the essential amino acids of human body, which can promote human development, enhance immune function and improve the function of central nervous system. Lysine is a basic essential amino acid. Because the lysine content in cereal food is very low, and it is easy to be destroyed and lacking during processing, it is called the first restricted amino acid. CheKine™ Micro Lysine (LYS) Assay Kit provides a convenient tool for detection of lysine. The principle is that the lysine in protein has a free ε-NH_2, which can react with Ninhydrin hydrate to produce blue-purple substances, which has a maximum absorption peak at 570 nm. The lysine content of the sample can be calculated by measuring the absorbance at 570 nm.

Materials Supplied and Storage Conditions

Kit components	Size	Storage conditions
	96 T	
Extraction Buffer	120 mL	4℃
Assay Buffer	2 mL	4℃
Substrate	Powder×1 vial	4℃, protected from light
Substrate Cofactor	Powder×1 vial	4℃, protected from light
Standard	Powder×1 vial	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 570 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Centrifuge, water bath
- Deionized water, 95% ethanol, 60% ethanol

• Homogenizer (for tissue samples)

Reagent Preparation

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

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

Working Substrate: Add 2 ml 95% ethanol for 96 T to dissolve before use. It is recommended to bathe in 37℃ water for 5 min to promote dissolution. The remaining reagent can be stored at 4℃ for one week.

Working Substrate Cofactor: Add all Assay Buffer to dissolve before use. The remaining reagent can be stored at 4℃ for one week.

Note: Substrate or Substrate Cofactor has certain irritation, so personal protection is recommended during use.

Working Reagent: Prepare according to the ratio as Working Substrate: Working Substrate Cofactor=1:1.

Standard: Add 1.71 mL deionized water to dissolve before use. The concentration is 40 μmol/mL. This solution can be stored at 4℃, protected from light for one week.

Standard curve setting: Dilute 40 μmoL/mL Standard with deionized water to 8, 4, 2, 1, 0, 5, 0.25, 0.125 μmol/mL standard solution as shown in the table below.

Num.	Volume of Standard	Volume of deionized water (μL)	The concentration of Standard
Std.1	40 μL of 40 μmol/mL	160	8 μmol/mL
Std.2	100 μL of Std.1 (8 μmol/mL)	100	4 μmol/mL
Std.3	100 μL of Std.2 (4 μmol/mL)	100	2 μmol/mL
Std.4	100 μL of Std.3 (2 μmol/mL)	100	1 μmol/mL
Std.5	100 μL of Std.4 (1 μmol/mL)	100	0.5 μmol/mL
Std.6	100 μL of Std.5 (0.5 μmol/mL)	100	0.25 μmol/mL
Std.7	100 μL of Std.6 (0.25 μmol/mL)	100	0.125 μmol/mL

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

Sample Preparation

Note: Fresh samples are recommended. If not assayed immediately, samples can be stored at -80℃ for 1 month.

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize at room temperature. Transfer to 1.5 mL EP tube, cover tightly and place in 80℃ incubator for 20 min. After cooling, centrifuge at 10,000 g for 10 min at room temperature. Use supernatant for assay.

2. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break at room temperature 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Transfer to 1.5 mL EP tube, cover tightly and place in 80℃ incubator for 20 min. After cooling, centrifuge at 10,000 g for 10 min at room temperature. Use supernatant for assay.

3. Cells or bacteria: Collect 5×10^6 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 at room temperature 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Transfer to 1.5 mL EP tube, cover tightly and place in 80℃ incubator for 20 min. After cooling, centrifuge at 10,000 g for 10 min at room temperature. Use supernatant for assay.

4. Cell supernatant, serum, or plasma: Add 0.5 mL samples and 0.5 mL Extraction Buffer to 1.5 mL EP tube, cover tightly and place in 80℃ incubator for 20 min. After cooling, centrifuge at 10,000 g for 10 min at room temperature. Use supernatant for assay.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content 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 570 nm, visible spectrophotometer was returned to zero with deionized water.

2. Add the following reagents respectively into each EP tube:

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Deionized Water	10	0	0
Std. with Different Concentration	0	10	0
Sample	0	0	10
Working Reagent	20	20	20

3. Mix well, then cover tightly and place in boiling water bath for 5 min. After cooling with running water for 10 s, add 170 μL 60% ethanol. Then reverse the EP tube several times, centrifuge at 5,000 g for 10 min at room temperature, and transfer 150 μL of each reaction to separate wells in a 96-well plate or microglass cuvette. Then reading the values at 570 nm. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$; $\Delta A_{Standard}=A_{Standard}-A_{Blank}$ (blank well needs to be detected one time). Be sure to finish the measurement within 30 min after color development.

Note: The Blank Well and the Standard Well 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 ΔA_{Test} is less than 0.002, increase the sample quantity appropriately. If ΔA_{Test} is greater than 1.8, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor.

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 y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{Test} into the equation to obtain the y value (μmoL/mL).

2. Calculate the content of Lysine in sample

(1) By sample fresh weight

Lysine (μmol/g)=$y\div(W\div V_{Extraction})\times n$**=$y\div W\times n$**

(2) Calculated by protein concentration

Lysine (μmol/mg prot)**=$y\div Cpr\times n$**

(3) Calculated by cells or bacteria number

Lysine (μmol/10^4)=$y\div$(Cells or Bacteria number$\div V_{Extraction}$)$\times n=y\div 500$**=$0.002\times y\times n$**

(4) Calculated by liquid volume

Lysine (μmol/mL)**=$y\times 2\times n$**

Where: W: sample weight, g; $V_{Extraction}$: Extraction Buffer volume added, 1 mL; n: dilution multiple of sample further dilution; Cpr: sample protein concentration, mg/mL; 500: Total number of bacteria or cells, 5×10^6; 2: the dilution multiple of Extraction the liquid (0.5 mL+0.5 mL)/0.5 mL=2.

Typical Data

Typical standard curve

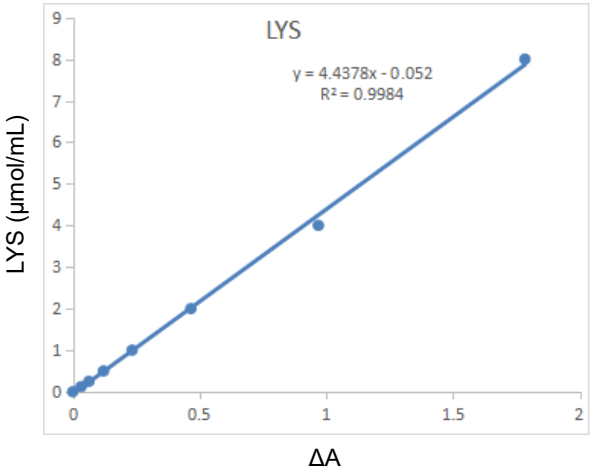

Figure 1. Standard curve for Lysine.

Examples:

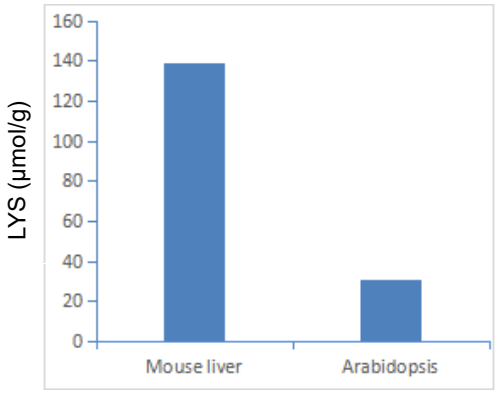

Figure 2. Lysine content in mouse liver and arabidopsis respectively. Assays were performed following kit protocol.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Assay Kit
KTB1440	CheKine™ Micro Glutamate (Glu) Assay Kit
KTB1450	CheKine™ Micro Cysteine (Cys) Assay Kit
KTB1460	CheKine™ Micro Amino Acid (AA) 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.