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Lysine assay kit

Product Number: LAK01

Shipping and Storage

Store at 4℃ in dark for 12 months

Description

Lysine is one of the essential amino acids in the human body, which can promote human development, enhance immune function, and improve the function of central nervous system tissues. Lysine is an essential alkaline amino acid. Due to the low content of lysine in cereal foods and their susceptibility to damage and deficiency during processing, it is called the first limiting amino acid. This reagent kit provides a simple detection method that can detect the content of lysine in various biological samples. The principle is that lysine in proteins has a free εNH2 reacts with ninhydrin reagent to generate a blue purple substance with a characteristic absorption peak at 570nm; Calculate the lysine content by measuring the absorbance at 570nm.

Components

Component	48T	96T	Storage
Extraction solution	50mL	100mL	4℃
Buffer 1	6.25mL	12.5mL	4℃
Buffer 2	Powder	Powder	4℃, avoid light
Lysine standard	Powder	Powder	4℃, avoid light

Self provided consumables

1. Enzyme reader or visible spectrophotometer (capable of measuring absorbance at 570nm) and water bath
2. 96 well plate or trace glass colorimetric dish, adjustable pipette and nozzle
3. Low temperature centrifuge
4. Deionized water, ethanol
5. Homogenizer (if it is a tissue sample)

Reagent preparation

Note: Before opening the cap of each component (small tube reagent), please centrifuge at low speed first.

1. Extraction solution: Ready to use; Before use, balance to room temperature; Store at 4 ℃.
2. Buffer 1: Ready to use type; Before use, balance to room temperature; Store at 4 ℃.
3. Buffer 2: Prepare before use. Dissolve 96T in 12.5mL of 95% ethanol and 48T in 6.25mL of 95% ethanol. Store unused and dissolved Buffer 2 at 4℃ in the dark for one week. If long-term storage is required, please store at 20℃ after packaging to avoid repeated freeze-thaw cycles.
4. Preparation of working solution: Prepare according to the required sample size in a ratio of Buffer 1: Dissolved Buffer 2=1:1.
5. Lysine standard: Prepare before use, add 1.71mL of deionized water, fully dissolve to obtain 40μmol/mL standard. Unused dissolved standard can be stored at 4 ℃ in the dark for one week. If long-term storage is required, please store at 20℃ after packaging to avoid repeated freeze-thaw cycles.
6. Standard curve setting: Dilute the 40μmol/mL standard with deionized water as shown in the table below.

	Standard volume	Extraction solution volume(μL)	Standard concentration(μmol/mL)
Std.1	20μL of 40μmol/mL	380	2
Std.2	200μL of Std.1(2μmol/mL)	200	1
Std.3	200μL of Std.2 (1μmol/mL)	200	0.5
Std.4	200μL of Std.3 (0.5μmol/mL)	200	0.25
Std.5	200μL of Std.4 (0.25μmol/mL)	200	0.125
Std.6	200μL of Std.5 (0.125μmol/mL)	200	0.0625

Std.7	200μL of Std.6 (0.0625μmol/mL)	200	0.0313

Note: Please use newly prepared standard samples for each experiment.

Sample preparation

1. **Animal tissue:**Weigh approximately 0.1g of the sample, add 1mL of Extraction solution, homogenize thoroughly at room temperature, transfer to a 1.5mL EP tube, cover tightly (to prevent water loss), and extract in an 80 ℃ water bath for 20 minutes; After cooling, centrifuge 10000g at room temperature for 10 minutes, and take the supernatant for testing.
2. **Plant tissue:**Weigh approximately 0.1g of the sample, add 1mL of Extraction solution and crush. Crush at room temperature with ultrasound for 5 minutes (power of 20% or 200W, ultrasound for 3 seconds, interval of 7 seconds, repeat 30 times), transfer to a 1.5mL EP tube, cover tightly (to prevent water loss), and extract in an 80 ℃ water bath for 20 minutes; After cooling, centrifuge 10000g at room temperature for 10 minutes, and take the supernatant for testing.
3. **Cells or bacteria:**Collect 5 million cells or bacteria into a centrifuge tube, wash the cells with cold PBS, discard the supernatant after centrifugation, add 1mL Extraction solution, crush at room temperature with ultrasound for 5 minutes (power of 20% or 200W, ultrasound for 3 seconds, interval of 7 seconds, repeat 30 times), transfer to a 1.5mL EP tube, cover tightly (to prevent water loss), and extract in an 80 ℃ water bath for 20 minutes; After cooling, centrifuge 10000g at room temperature for 10 minutes, and take the supernatant for testing.
4. **Cell supernatant or serum (plasma):** In a 1.5mL EP tube, take 0.5mL of liquid and add 0.5mL of extraction solution. Cover tightly (to prevent water loss) and place in an 80 ℃ water bath for extraction for 20 minutes; After cooling, centrifuge 10000g at room temperature for 10 minutes, and take the supernatant for testing.

   Note: It is recommended to use fresh samples. If the experiment is not conducted immediately, the samples can be stored at 80℃ for 6 months. It is recommended to use the BCA protein quantification kit for sample protein concentration determination.

Protocol

1. Preheat the enzyme-linked immunosorbent assay (ELISA) reader or visible spectrophotometer for more than 30 minutes, adjust the wavelength to 570nm, and zero the visible spectrophotometer with deionized water.
2. Sample determination (add the following reagents in sequence to the EP tube):

Reagent	Blank tube(μL)	Standard tube(μL)	Measuring tube(μL)
Reagent	100	0	0
Extraction solution	0	100	0
Different concentration standards	0	0	100
Sample	200	200	200

3. Mix well, take an 80 ℃ water bath for 30 minutes (cover tightly to prevent moisture loss), and cool to room temperature. Add 300uL of 60% ethanol and mix well. Take 200uL and transfer it to a 96 well plate or a trace quartz colorimetric dish. Measure the absorbance value at 570nm and calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$ (only one blank tube needs to be made). After color development, it is necessary to measure it within 30 minutes.

   Note: Before the experiment, it is recommended to choose 2-3 samples with significant expected differences for the pre experiment. If the A-test is less than 0.001, the sample size can be increased appropriately. If the A-test is greater than 1.2, the sample can be further diluted with deionized water, and the calculated result should be multiplied by the dilution factor.

Data Analysis

1. Drawing of standard curve

   With the concentration of the standard Solution as the y-axis and the ΔAStandard 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

   2.1. By sample fresh weight

Lysine (μmol/g)=y×V_{Sample}÷(V_{Sample}÷$V_{Extraction}$×W)×n=y÷W×n

2.2. Calculated by protein concentration

Lysine (μmol/mg prot)==y×V_{Sample}÷(Cpr×V_{Sample})×n=y÷Cpr×n

2.3. Calculated by Cells or Bacteria number

Lysine (μmol/104 cells)=y×V_{Sample}÷(Cells or Bacteria number×V_{Sample}÷$V_{Extraction}$)×n=y÷500×n=y÷500×n=0.002y×n

2.4. Calculated by liquid volume

Lysine (μmol/mL)=y×V_{Sample}÷V_{Sample}×2×n=y×2×n

Tips

1. V_{Sample}: sample volume added, 0.1mL;
2. $V_{Extraction}$: Extraction system volume, 1mL;
3. W: sample weight, g;
4. n: dilution multiple of sample further dilution;
5. Cpr: sample protein concentration, mg/mL;
6. 500: Total number of bacteria or cells, 5×106;
7. 2: the dilution multiple of Extraction the liquid (0.5 mL+0.5 mL)/0.5 mL=2.

Typical Data

Typical standard curve

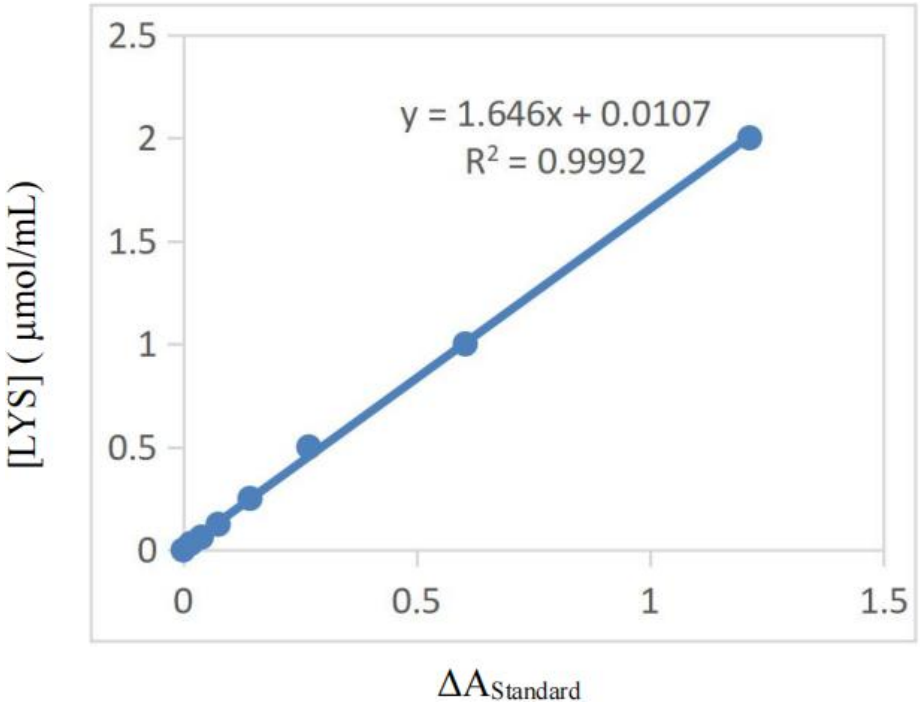

Figure1. Standard Curve of Lysine in 96-well plate assay-data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Note

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. The Buffer 1s only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.
3. The kit should not be used beyond the expiration date on the kit label.
4. Do not mix or use components from other lots.
5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.