LysineMicroplate Assay Kit

User Manual

Catalog # CAK1205

(Version 1.4A)

Detection and Quantification of Lysine Content in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Lysine (symbol Lys or K) is an α-amino acid that is used in the biosynthesis of proteins. It contains an α-amino group (which is in the protonated −NH3+ form under biological conditions), an α-carboxylic acid group (which is in the deprotonated −COO− form under biological conditions), and a side chain lysyl ((CH2)4NH2), classifying it as a basic, charged (at physiological pH), aliphatic amino acid.

The human body cannot synthesize lysine, so it is essential in humans and must be obtained from the diet. In organisms that synthesise lysine, it has two main biosynthetic pathways, the diaminopimelate and α-aminoadipate pathways, which employ different enzymes and substrates and are found in different organisms.

Lysine catabolism occurs through one of several pathways, the most common of which is the saccharopine pathway.

Lysine plays several roles in humans, most importantly proteinogenesis, but also in the crosslinking of collagen polypeptides, uptake of essential mineral nutrients, and in the production of carnitine, which is key in fatty acid metabolism. Lysine is also often involved in histone modifications, and thus, impacts the epigenome. The ε-amino group often participates in hydrogen bonding and as a general base in catalysis. The ε-ammonium group (NH3+) is attached to the fourth carbon from the α-carbon, which is attached to the carboxyl (C=OOH) group.

Lysine reacts with ninhydrin, the reaction products can be measured at a colorimetric readout at 478 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 2	4 °C
Assay Buffer II	30 ml x 2	4 °C
Reaction Buffer	5 ml x 1	4 °C
Inhibitor	2 ml x 1	4 °C
Dye Reagent	Powderx 1	4 °C
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Dye Reagent:add 5 ml distilled water to dissolve before use, store at 4 °C.

Standard:add 1 mldistilled water to dissolve before use, then then add 125 µl into 875 µldistilled water, mix; the concentration will be 5mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 478 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Convection oven
7. Centrifuge
8. Timer

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlPBS/ddH2O for 5 $\times 10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times) on ice; centrifuged at 8000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weigh0.1 g tissue, homogenize with 0.5mlAssay Buffer I in thecentrifuge tube, incubate at 60°Cwater bath for 1 hour; then add 0.5mlAssay Buffer II, centrifuged at 10,000g for 10minutes, take the supernatant into a new centrifuge tube for detection.

3. For liquid samples

Centrifuged at 10,000 g and 4°C for 5 minutes to removeany insoluble materials.Detect directly, or dilute with distilled water.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	80 µl	--	--
Standard	--	80 µl	--
Distilled water	--	--	80 µl
Reaction Buffer	50 µl	50 µl	50 µl
Inhibitor	20 µl	20 µl	20 µl
Dye Reagent	50 µl	50 µl	50 µl
Mix, put it into the convection oven,90 °Cfor 20 minutes, record absorbance measured at 478 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the protein concentration of sample

$$Lysine(\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the quantity of cells or bacteria

$$Lysine(\mu mol/10^4\ cell) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay})$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

3. According to the weight of sample

$$Lysine(\mu mol/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

4. According to the volume of sample

$$Lysine\ (\mu mol/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$$

$$= 5 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the standard concentration, 5mmol/L = 5μmol/ml;

V_{Sample}: the volume of the sample, 0.08 ml;

$V_{Standard}$: the volume of the standard, 0.08 ml;

V_{Assay}: the volume of Assay Buffer I and Assay Buffer II, 1 ml;

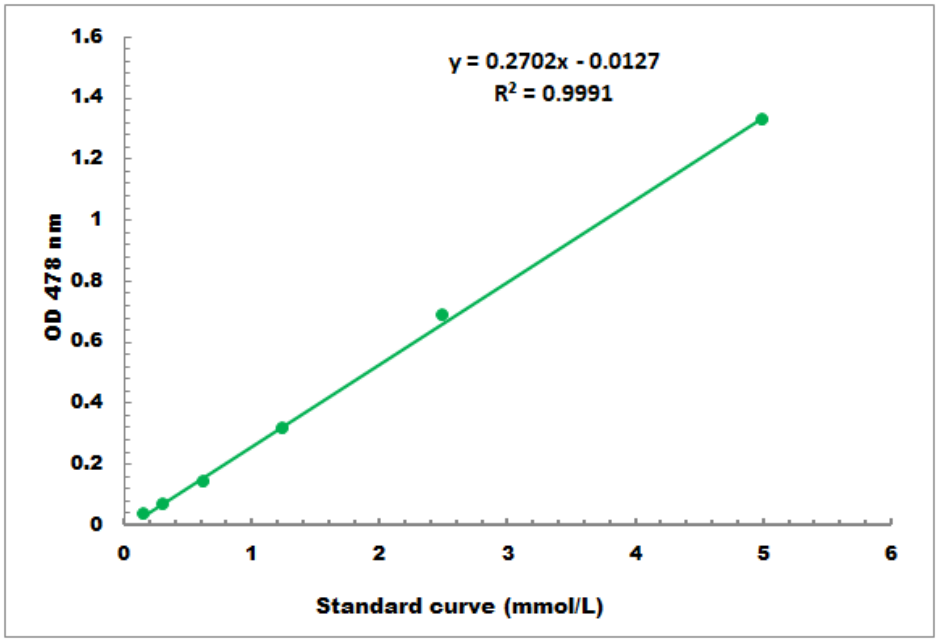
$C_{Protein}$: the protein concentration, μmol/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1mmol/L - 5mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES