Lysine Microplate Assay Kit

Catalog # AS0159

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

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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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 react 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
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 1mlAssay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s,repeat 30 times); 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 1mlAssay buffer 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.

3. For serum or plasma samples

Add 1 ml Assay buffer for 0.1 ml serum or plasma; mix; centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

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.			

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

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

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

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, 1 ml;

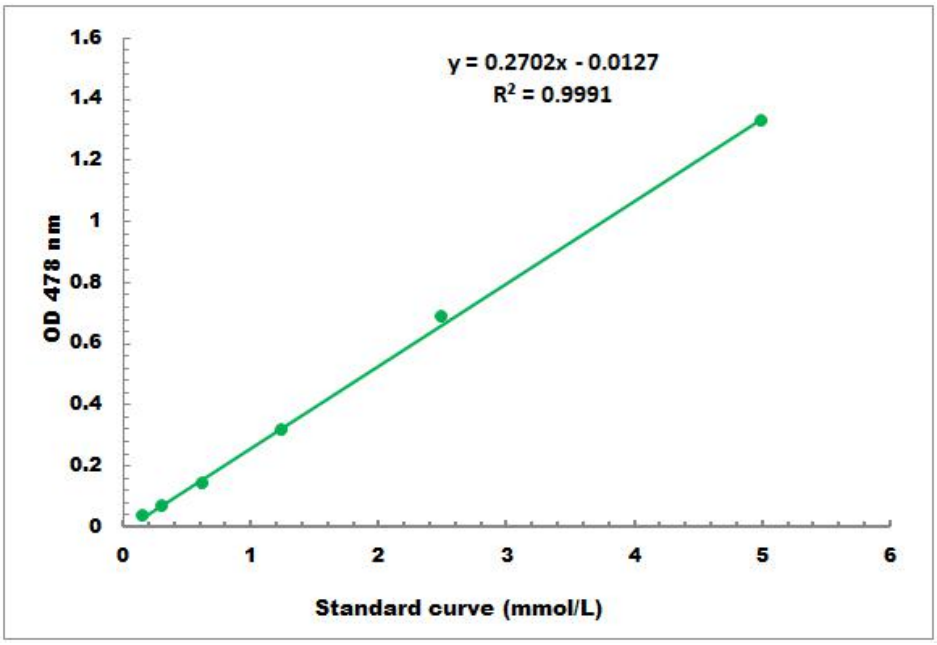
$C_{Protein}$: the protein concentration, μmol/ml;

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 to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES