Lysine (Lys) Assay Kit

Catalog No.: abx298954

Size: 100 Assays

Storage: Store all components at 4°C.

Application: For quantitative detection of Lysine concentrations in serum, plasma, tissue homogenates, and cell and bacterial samples.

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

Introduction: Lysine (Lys) is an amphipathic alpha amino acid, and a constituent of many proteins. Humans are unable to synthesize Lysine, and so must be obtained from diet. This amino acid plays a crucial role in proteinogenesis, crosslinking of collagen polypeptides, uptake of minerals and epigenetic modifications. Lysine deficiency is implicated in connective tissue disorders, impaired fatty acid metabolism and anemia.

Abbexa's Lysine Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Lys concentrations. The dye reagents react with Lysine to create an absorption maximum at 478 nm. The intensity of the color is proportional to the concentration of Lysine, which can then be calculated.

Kit components

1. 96-well microplate
2. Assay Buffer 1: 2 × 30 ml
3. Assay Buffer 2: 2 × 30 ml
4. Dye Reagent: 1 vial
5. Reaction Buffer: 5 ml
6. Inhibitor Reagent: 2 ml
7. Standard: 1 vial
8. Plate Sealer: 3

Materials Required But Not Provided

1. Microplate reader (478 nm)
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. PBS/Double distilled water
6. Timer
7. Ice
8. Sonicator
9. Mortar
10. Convection oven
11. Water bath

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Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Dye Reagent Solution**
  Add 5 ml of distilled water into the Dye Reagent vial and mix thoroughly to prepare the Dye Reagent Solution. Any unused Dye Reagent Solution should be stored at 4°C.

- **Standard Solution**
  Add 1 ml of distilled water into the Standard vial and mix thoroughly. Ensure that the Standard has completely dissolved. Take 125 µl of this solution and add 875 µl of distilled water to prepare the Standard Solution (concentration 5 mmol/L).

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 1 ml of PBS or double distilled water for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 0.5 ml of Assay Buffer 1 on ice. Incubate in a water bath at 60°C for 1 hour. Add 0.5 ml of Assay Buffer 2, then centrifuge at 10,000 × g for 10 minutes. Transfer the supernatant to a new tube, then analyze immediately.

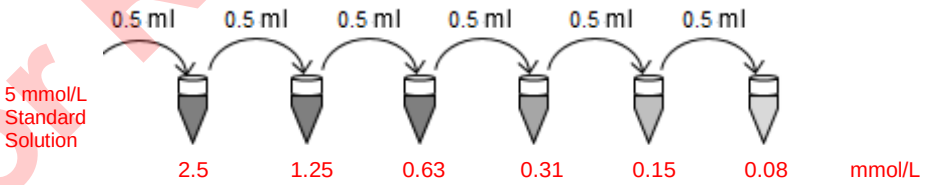
- **Serum and plasma samples**
  Centrifuge at 10,000 × g at 4°C for 5 minutes. Transfer the supernatant to a new pre-cooled tube, then analyze immediately. If dilutions are required, dilute with distilled water.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

If the expected concentration is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured concentration within the detection range of the kit.

1. Label 6 tubes with 2.5 mmol/L, 1.25 mmol/L, 0.63 mmol/L, 0.31 mmol/L, 0.15 mmol/L, and 0.08 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 5 µmol/L Standard Solution to the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on. The undiluted standard (5 mmol/L) serves as the top standard.



2. Set the sample, standard and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 80 µl of sample to the sample wells.
4. Add 80 µl of prepared standards to the standard wells.
5. Add 80 µl of distilled water to the blank wells.
6. Add 50 µl of Reaction Buffer Solution to all wells.
7. Add 20 µl of Inhibitor Reagent to all wells.
8. Add 50 µl of Dye Reagent Solution to all wells.
9. Tap the plate gently to mix. Incubate in a convection oven for 20 minutes at 90°C.
10. Read and record absorbance at 478 nm.

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C. Calculations

Lysine concentration per **µmol/ml** of protein:

$$\textbf{Lysine}\ (\textbf{µmol/ml}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}} \times \mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{5}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Lysine concentration per g of sample:

$$\textbf{Lysine}\ (\textbf{µmol/g}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{5}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Lysine concentration per 10^4 cells or bacteria:

$$\textbf{Lysine}\ (\textbf{µmol/10}^{\mathbf{4}}\ \textbf{cells}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{N}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{5}}{\mathbf{N}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Lysine concentration per ml of sample:

$$\textbf{Lysine}\ (\textbf{µmol/ml}) = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \mathbf{5} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in µmol/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (5 mmol/L= 5 µmol/ml)
W	Weight of the sample (in g)
N	Number of cells or bacteria (× 10^4)
$\mathbf{V_{Assay}}$	Volume of Assay Buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.08 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.08 ml)

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