Product Manual

Lysine Assay Kit (Colorimetric)

Catalog Number

MET-5130 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Lysine is an amino acid containing amine (-NH_2) and carboxyl (-COOH) functional groups, as well as a lysyl group side chain ((CH_2)$_4$$NH_2$). Lysine is an essential amino acid since it is not synthesized in the body and therefore must be obtained from food sources.

Lysine is one of 22 natural amino acids that serve as fundamental building blocks for the production of proteins. Lysine also plays a part in protein structure: its side chain contains a positively charged group on one end connected to a long hydrophobic carbon tail. Because of these properties, lysine is considered relatively amphipathic and can be found either buried within the core of a protein or in solvent channels as well as on the exterior of proteins, where it can interact with the aqueous environment. Lysine can also aid in protein stabilization since its ε-amino group forms hydrogen bonds, salt bridges, and covalent interactions to form a Schiff base. In addition, lysine is involved in epigenetic regulation by means of direct histone modification. There are several covalent histone modifications, often involving lysine residues located in the extending tail of histones. These modifications effect gene regulation, in which genes can be either activated or repressed. Lysine has also been shown to be involved in structural proteins of connective tissues, calcium homeostasis, and fatty acid metabolism. Lysine has been shown to play a role in the crosslinking between the three helical polypeptides of collagen, resulting in collagen stabilization and increased tensile strength. Lysine has also been suggested to play a role in calcium intestinal absorption and renal retention, and therefore might be involved in calcium homeostasis. Finally, lysine is a known precursor of carnitine, a molecule that transports fatty acids to the mitochondria to be oxidized resulting in energy release.

Cell Biolabs' Lysine Assay Kit is a simple colorimetric assay that measures the amount of free lysine present in foods or biological samples in a 96-well microtiter plate format. Lysine found in polypeptide chains (peptides and proteins) are not detected. Each kit provides sufficient reagents to perform up to 100 assays*, including blanks, lysine standards and unknown samples. Sample lysine concentrations are determined by comparison with a known lysine standard. The kit has a detection sensitivity limit of 1.56 µM lysine.

Assay Principle

Cell Biolabs' Lysine Assay Kit measures lysine within food or biological samples. Lysine is converted by lysine oxidase into 6-amino-2-oxohexanoate plus ammonia and hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific colorimetric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples and standards are read with a standard 96-well colorimetric plate reader. Samples are compared to a known concentration of lysine standard within the 96-well microtiter plate format (Figure 1).

Note: Each sample replicate requires 2 assays, one treated with lysine oxidase (+LO) and one without (-LO). Lysine is calculated from the difference in OD readings from the 2 wells.

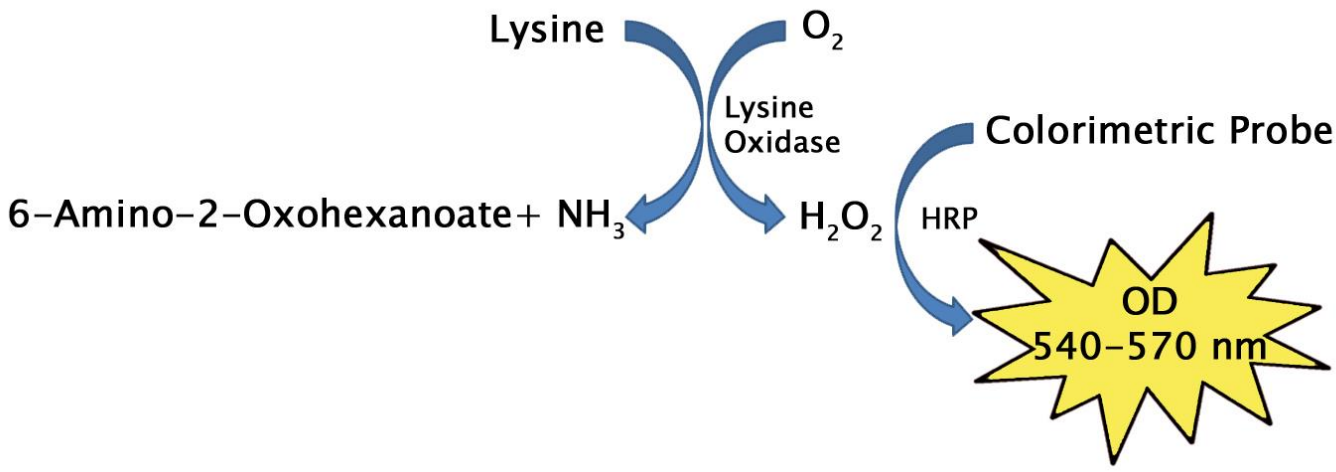

Figure 1. Lysine Assay Principle.

Related Products

1. MET-5053: Total Thiol Assay Kit
2. MET-5054: L-Amino Acid Assay Kit (Colorimetric)
3. MET-5070: Glycine Assay Kit
4. MET-5071: Taurine Assay Kit
5. MET-5080: Glutamate Assay Kit (Colorimetric)
6. MET-5151: S-Adenosylhomocysteine (SAH) ELISA Kit
7. MET-5152: S-Adenosylmethionine (SAM) ELISA Kit
8. STA-675: Hydroxyproline Assay Kit

Kit Components

1. Lysine Standard (Part No. 51291C): One 50 µL tube at 10 mM.
2. 10X Assay Buffer (Part No. 51192A): One 30 mL bottle.
3. Colorimetric Probe (Part No. 50222C): One 50 µL amber tube.
4. HRP (Part No. 234402): One 100 µL tube at 100 U/mL in glycerol.
5. Lysine Oxidase (Part No. 51292D): One 50 µL tube containing recombinant Lysine Oxidase from *Trichoderma viride*.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. Microcentrifuge tubes

4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Standard 96-well clear microtiter plate and/or cell culture microplate
7. Multichannel micropipette reservoir
8. Spectrophotometric microplate reader capable of reading in the 540-570 nm range.

Storage

Upon receipt, store the 10X Assay Buffer at room temperature. Store the Lysine Oxidase at -80ºC. Store all other components at -20ºC. The Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 10X Assay Buffer to 1X with deionized water. Mix to homogeneity. Store the 1X Assay Buffer at 4ºC.
- Reaction Mix: Prepare a Reaction Mix by diluting the Colorimetric Probe 1:100, HRP 1:500, and Lysine Oxidase 1:100 in 1X Assay Buffer. For example, add 10 µL Colorimetric Probe stock solution, 2 µL HRP stock solution, and 10 µL of Lysine Oxidase to 978 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

- Control Mix: Prepare a Control mix by diluting the Colorimetric Probe 1:100 and HRP 1:500 in 1X Assay Buffer. For example, add 10 µL of Colorimetric Probe and 2 µL of HRP to 988 µL of 1X Assay Buffer for a total of 1 mL. This Control Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4ºC.

  Note: Scale the described example up or down appropriately and prepare only enough for immediate use.

Preparation of Samples

- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.
- Cell lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer.
- Saliva or Urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. Dilute the supernatant 1:10 to 1:20 in 1X Assay Buffer just prior to performing the assay.
- Serum or Plasma: Deproteinate the sample by running it through a centrifugal filter unit (e.g. Amicon Ultra 0.5 mL 10K Cat. No. UFC501024) and collecting the flow through. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. Dilute the supernatant as necessary into 1X Assay Buffer just prior to performing the assay.

Notes:

- *All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.*
- *Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM will oxidize the Colorimetric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2).*
- *Avoid samples containing DTT or β-mercaptoethanol since the Colorimetric Probe is not stable in the presence of thiols (above 10 µM).*

Preparation of Standard Curve

Prepare fresh Lysine Standards before use by diluting in 1X Assay Buffer according to Table 2 below.

Standard Tubes	10 mM Lysine Solution (µL)	1X Assay Buffer (µL)	Lysine (µM)
1	5	495	100
2	250 of Tube #1	250	50
3	250 of Tube #2	250	25
4	250 of Tube #3	250	12.5
5	250 of Tube #4	250	6.25
6	250 of Tube #5	250	3.13
7	250 of Tube #6	250	1.56
8	0	250	0

Table 2. Preparation of Lysine Standards.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each sample replicate requires two paired wells, one to be treated with Lysine Oxidase (Reaction Mix) and one without the enzyme (Control Mix) to measure endogenous sample background.
2. Add 50 µL of each Lysine Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50 µL of Reaction Mix to the standards and to one half of the paired sample wells, and mix the well contents thoroughly.
4. Add 50 µL of Control Mix to the other half of the paired sample wells and mix thoroughly.
5. Incubate for 10 to 30 minutes at room temperature protected from light.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
6. Read the plate with a spectrophotometric microplate reader in the 540-570 nm range.

Example of Results

The following figures demonstrate typical Lysine Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

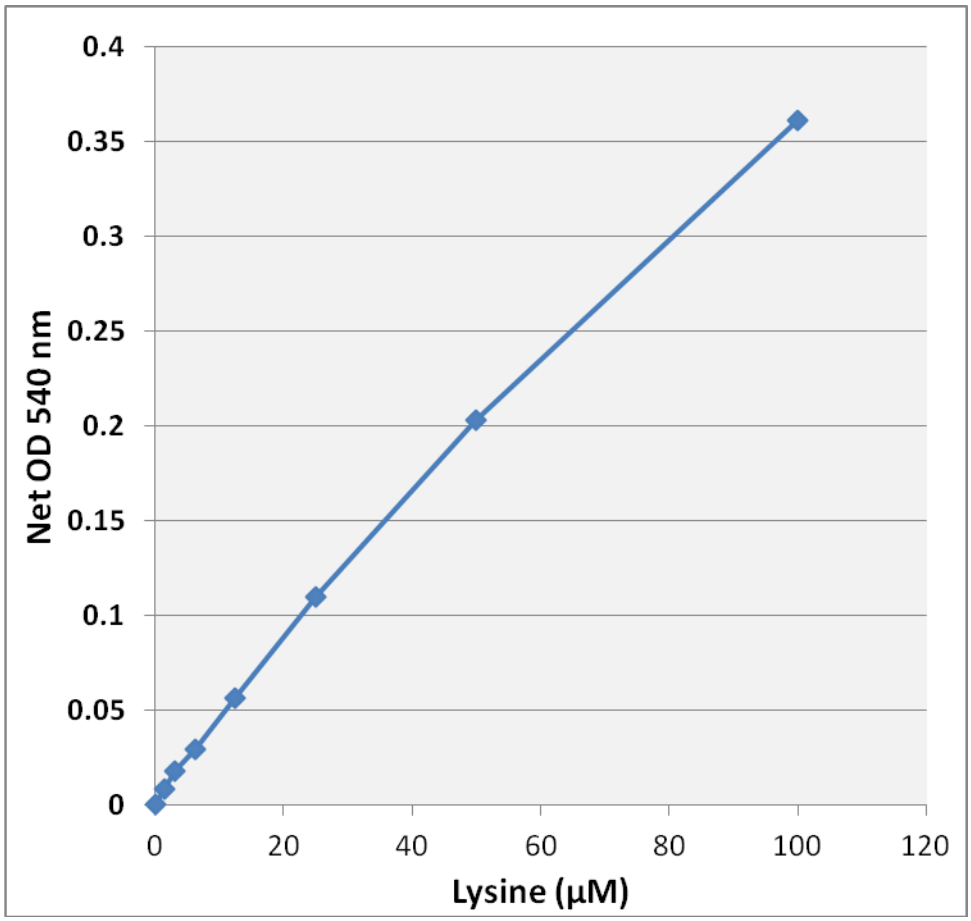

Figure 2: Lysine Standard Curve.

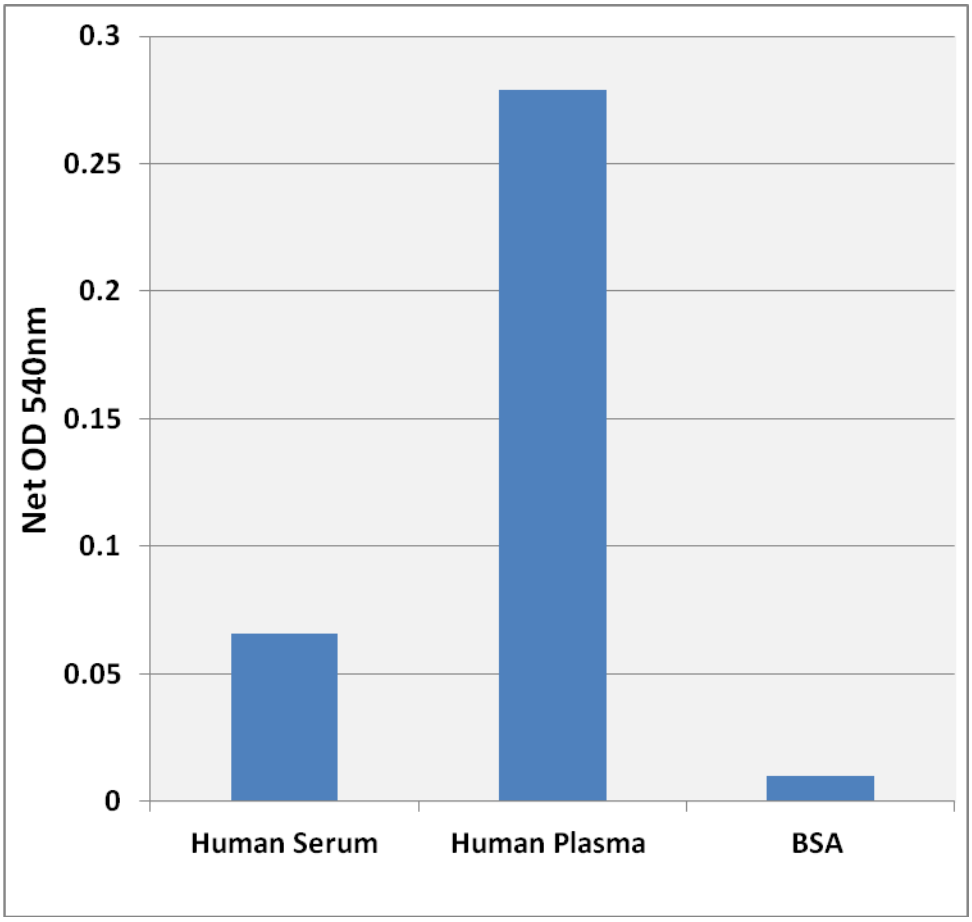

Figure 3: Lysine Detection in human serum, human plasma, or BSA using the Lysine Assay Kit. Fifty microliters of human serum, human plasma, or 10 mg/mL BSA were tested according to the Assay Protocol.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without Lysine Oxidase (-LO) from the sample well values containing enzyme (+LO) to obtain the difference. The absorbance difference is due to the Lysine Oxidase activity:

$$\Delta OD = (OD_{+LO}) - (OD_{-LO})$$

5. Compare the ΔOD of each sample to the standard curve to determine and extrapolate the quantity of lysine present in the sample. Only use values within the range of the standard curve.

References

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Recent Product Citation

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Warranty

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com