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Product Manual

# Lysine Assay Kit (Fluorometric)

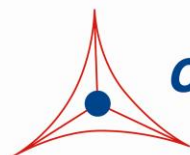
Catalog Number

MET-5129

100 assays

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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## **Introduction**

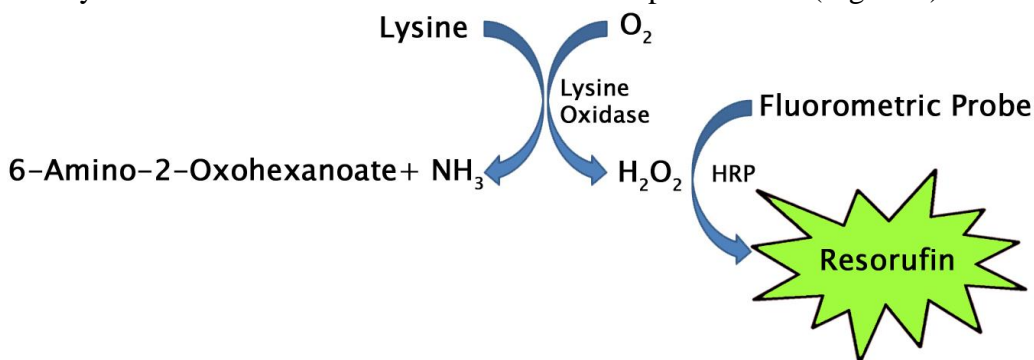
Lysine is an amino acid containing amine ( $\text{-NH}_2$ ) and carboxyl ( $\text{-COOH}$ ) functional groups, as well as a lysyl group side chain ( $(\text{CH}_2)_4\text{NH}_2$ ). Lysine is an essential amino acid since it is not synthesized in the body and therefore must be obtained from food sources.

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  $\epsilon$ -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 fluorometric 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  $1.56\text{ }\mu\text{M}$  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 fluorometric 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 fluorometric plate reader. Samples are compared to a known concentration of lysine standard within the 96-well microtiter plate format (Figure 1).



**Figure 1. Lysine Assay Principle.**

## **Related Products**

1. MET-5053: Total Thiol Assay Kit
2. MET-5055: L-Amino Acid Assay Kit (Fluorometric)
3. MET-5071: Taurine Assay Kit
4. MET-5070: Glycine Assay Kit
5. STA-674: Glutamate Assay Kit (Fluorometric)

## **Kit Components (shipped on blue ice)**

1. Lysine Standard (Part No. 51291C): One 50  $\mu$ L tube at 10 mM.
2. 10X Assay Buffer (Part No. 51192A): One 30 mL bottle.
3. Fluorometric Probe (Part No. 50231C): One 50  $\mu$ L amber tube.
4. HRP (Part No. 234402): One 100  $\mu$ L tube at 100 U/mL in glycerol.
5. Lysine Oxidase (Part No. 51292D): One 50  $\mu$ L tube containing recombinant Lysine Oxidase from *Trichoderma viride*.

## **Materials Not Supplied**

1. Distilled or deionized water
2. 1X PBS
3. Standard 96-well black microtiter plate and/or cell culture microplate

## **Storage**

Upon receipt, store the 10X Assay Buffer at room temperature. Store the Lysine Oxidase at -80°C. Store all other components at -20°C. The Fluorometric 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 4°C.
- Reaction Mix: Prepare a Reaction Mix by diluting the Fluorometric Probe 1:100, HRP 1:500, and Lysine Oxidase 1:100 in 1X Assay Buffer. For example, add 10  $\mu$ L Fluorometric Probe stock solution, 2  $\mu$ L HRP stock solution, and 10  $\mu$ L of Lysine Oxidase to 978  $\mu$ L 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 4°C.

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

## **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<sup>6</sup> 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: Deproteinize 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  $\mu\text{M}$  and glutathione concentrations above 50  $\mu\text{M}$  will oxidize the Fluorometric 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  $\beta$ -mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10  $\mu\text{M}$ ).

## **Preparation of Standard Curve**

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

| Standard Tubes | 10 mM Lysine Solution ( $\mu\text{L}$ ) | 1X Assay Buffer ( $\mu\text{L}$ ) | Lysine ( $\mu\text{M}$ ) |
|----------------|---|-----------------------------------|--------------------------|
| 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**

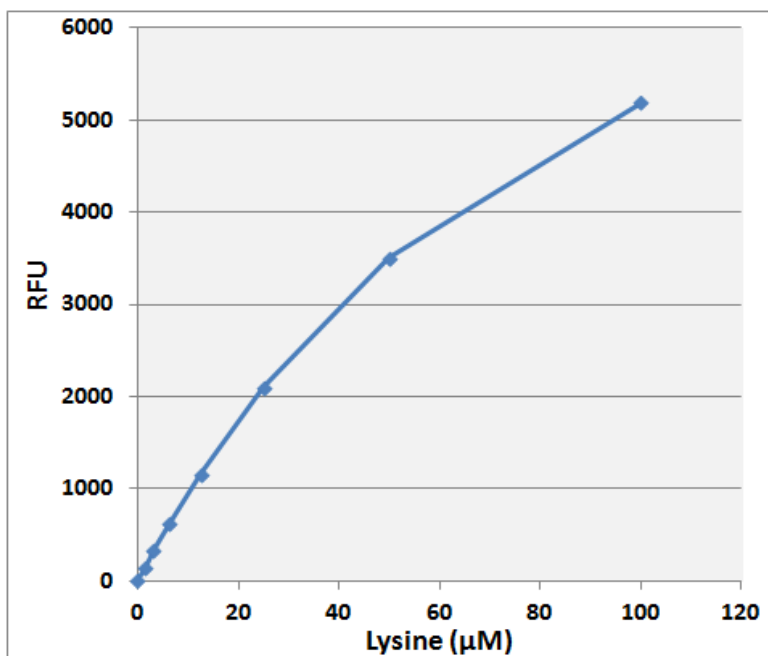
1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50  $\mu\text{L}$  of each Lysine Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 50  $\mu\text{L}$  of Reaction Mix to each well. Mix the well contents thoroughly and 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.*

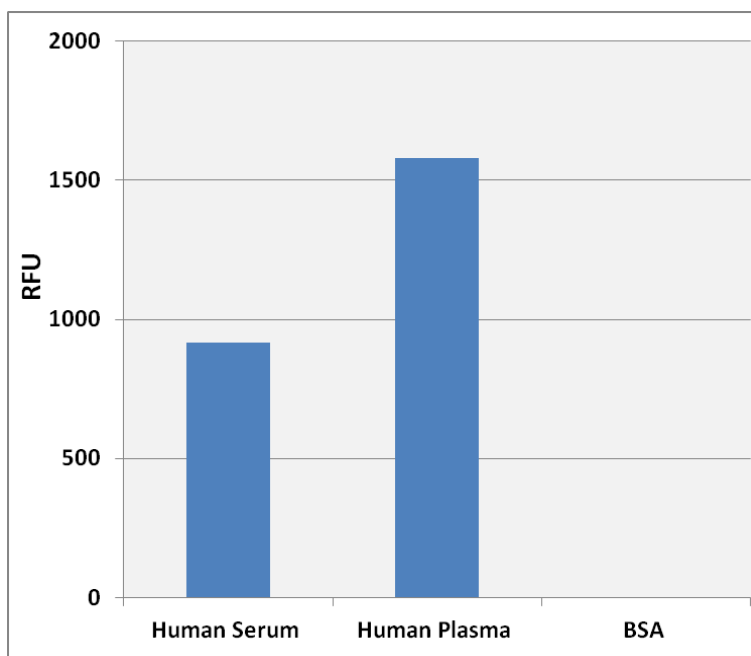
4. Read the plate with a fluorescence microplate reader equipped for excitation in the 530-570 nm range and for emission in the 590-600 nm range.
5. Calculate the concentration of lysine within samples by comparing the sample fluorescence to the standard curve.

## 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.** 50 μL of neat human serum, human plasma, or 10 mg/mL BSA was tested according to the Assay Protocol.

## References

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## **Warranty**

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