Lysine Assay Kit (Fluorometric)

Lysine Assay Kit (Fluorometric) is a detection kit for the quantification of Lysine in serum, plasma, saliva, urine, cell lysate and tissue lysate.

Catalog number: ARG82654

Package: 100 assays

TABLE OF CONTENTS

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INTRODUCTION

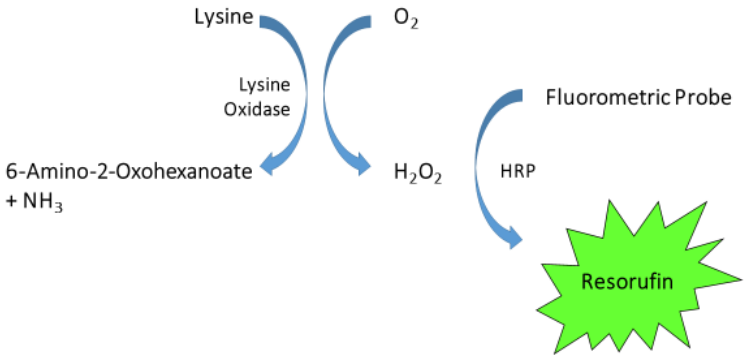
Lysine (symbol Lys or K) is an α-amino acid that is used in the biosynthesis of proteins. It contains an α-amino group (which is in the protonated –NH3+ form under biological conditions), an α-carboxylic acid group (which is in the deprotonated –COO– form under biological conditions), and a side chain lysyl ((CH2)4NH2), classifying it as a basic, charged (at physiological pH), aliphatic amino acid. The human body cannot synthesize lysine. It is essential in humans and must be obtained from the diet.

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.

Due to its importance in several biological processes, a lack of lysine can lead to several disease states including defective connective tissues, impaired fatty acid metabolism, anaemia, and systemic protein-energy deficiency. In contrast, an overabundance of lysine, caused by ineffective catabolism, can cause severe neurological disorders. [Provide by Wikipedia: Lysine]

PRINCIPLE OF THE ASSAY

This Lysine Assay Kit (Fluorometric) is a simple fluorometric assay that measures the amount of free lysine present in serum, plasma, saliva, urine, cell lysate and tissue lysate. The assay is based on the enzyme driven reaction. 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 Fluorometric plate reader. The concentration of free lysine in the samples is then determined by comparing the RFU of samples to the standard curve. (See the Figure below)



MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, 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.

Component	Quantity	Storage information
10X Assay Buffer	30 mL	-20°C
Lysine Standards (10 mM)	50 µL	-20°C
Fluorometric Probe (amber tube)	50 µL	-20°C
HRP (100 U/mL)	100 µL	-20°C
Lysine Oxidase	50 µL	-80°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader capable of reading excitation in the 530-570 nm range and emission in the 590-600 nm range.
- Standard 96-well black microplate
- Microcentrifuge tubes
- Deionized or Distilled water
- 1X PBS
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, 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.
- Prepare Reaction Mix only enough for immediate use by scaling the above example proportionally.
- 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.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue Lysates: Sonicate or homogenize tissue sample in cold 1X PBS or 1X Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.

Cell Lysates: Wash cells 3 times with cold 1X PBS prior to lysis. Lyse cells with sonication or homogenation in cold 1X PBS or 1X Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions in 1X Assay Buffer.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2,000 x g and 4°C for 10 minutes. The supernatant should be assayed directly or diluted as necessary in 1X Assay Buffer.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2,500 x g for 20 minutes. The supernatant should be assayed directly or diluted as necessary in 1X Assay Buffer.

Urine and Saliva: To remove insoluble particles, centrifuge at 10,000 x g for 10 minutes at 4°C. The supernatant should be assayed directly or diluted as necessary in 1X Assay Buffer.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum / plasma sample.
3. 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.

4. Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µM 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.
5. Avoid samples containing DTT or β-mercaptoethanol since the Fluorometric Probe is not stable in the presence of thiols (above 10 µM).

REAGENT PREPARATION

- **1X Assay Buffer:** Dilute the 10X Assay Buffer into deionized water to yield 1X Assay Buffer. (E.g., add 15 mL of 10X Assay Buffer into 135 mL of deionized water to a final volume of 150 mL) 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. (E.g., add 10 µL Fluorometric Probe stock solution, 2 µL HRP stock solution and 10 µL of Lysine Oxidase to 978 µL of 1X Assay Buffer for a final volume of 1 mL) This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4°C. Do not store the Reaction Reagent solution.
- **Standards:** Prepare fresh Lysine Standards before use by diluting in 1X Assay Buffer according to the Table below.

Standard tube	Final Lysine conc. (µM)	Volume of 1X Assay Buffer (µL)	Volume of 10 mM Lysine Standard (µL)
S1	100	495	5
S2	50	250	250 of S1
S3	25	250	250 of S2
S4	12.5	250	250 of S3
S5	6.25	250	250 of S4
S6	3.13	250	250 of S5
S7	1.56	250	250 of S6
S0	0	250	0

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add **50 µL** of **samples** or serial **diluted Lysine Standards** into 96-well microplate.
2. Add **50 µL** of prepared **Reaction Mix** to each well and mix well thoroughly.
3. Incubate for **10-30 minutes** at **room temperature** in the dark.

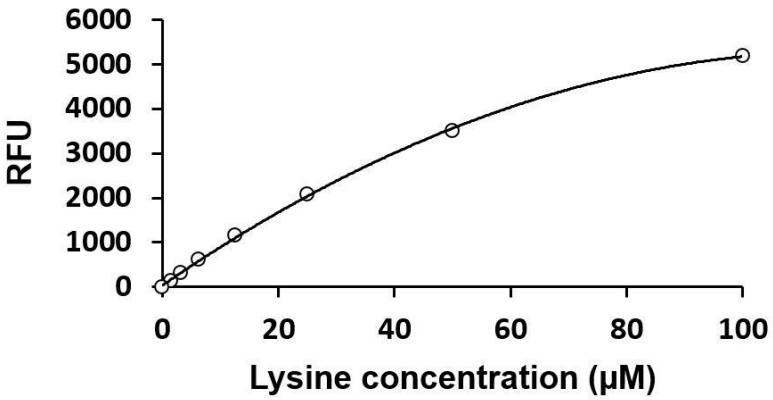
   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
4. Read the plate with a fluorescence microplate reader equipped for **excitation in the 530-570 nm range** and for **emission in the 590-600 nm range.**
5. Calculate the concentration of free lysine within samples by comparing the sample RFU to the standard curve.

CALCULATION OF RESULTS

1. Calculate the average fluorescence value for each set of standards and samples. Subtract the average Standard 0 value from itself and all standard and sample values. This is the corrected fluorescence.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Use the mean RF value for each sample determine the corresponding concentration from the standard curve.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Lysine Assay Kit (Fluorometric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

1.56 µM