Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Lysine Assay Kit (Fluorometric)

(Catalog # K2005-100; 100 Assays; Store at -20°C)

I. Introduction:

Lysine is one of the 20 naturally-occuring proteogenic amino acids and an essential dietary amino acid. In addition to its indispensable nutritional role, lysine is a critical component of the fibrous connective tissue proteins such as collagen and elastin. Lysine residues in fibril proteins are subjected to post-translational oxidation, forming reactive aldehydes that crosslinks adjacent polypeptide strands. Post-translational methylation and acetylation of the ε-amino moiety in histone lysine residues is a primary mechanism of epigenetic transcriptional regulation. Lysine is also required for the synthesis of carnitine, which assists in mitochondrial fatty acid oxidation. Dietary lysine deficiency, frequent in impoverished populations with limited access to high-quality protein sources, results in anemia and the suppression of growth and immune function. Additionally, lysine deficiency severely impairs the ability to cope with stress-induced anxiety or recovery from injuries. BioVision's Lysine Assay Kit allows the highly sensitive quantification of L-lysine levels in various biological samples. The assay is based on the selective enzymatic metabolism of lysine, yielding an oxidized intermediate which reacts with a fluorogenic probe to form a stable fluorophore (Ex/Em = 538/587 nm). The assay is not affected by the physiological concentration of other amino acids and is high-throughput adaptable. The kit can detect less than 5 µM Lysine in samples.

II. Applications:

- Estimation of L-Lysine concentration in various biological samples

III. Sample Type:

- Human or animal biological fluids (plasma, serum, etc.)
- Soft tissue homogenates (*i.e*. liver, brain, etc.)
- Cultured cell lysates (adherent or suspension cells) or cell culture growth/fermentation medium

IV. Kit Contents:

Components	K2005-100	Cap Code	Part Number
Lysine Assay Buffer	25 ml	WM	K2005-100-1
Lysine Probe	200 µl	Red	K2005-100-2
Lysine Enzyme Mix	1 vial	Green	K2005-100-3
Developer Mix	1 vial	Violet	K2005-100-4
L-Lysine Standard	1 vial	Yellow	K2005-100-5

V. User Supplied Reagents and Equipment:

- Multiwell fluorescence microplate reader
- Black 96-well plates with flat bottom
- 10 kDa Spin Columns (Cat# 1997 or equivalent)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the Lysine Assay Buffer to warm to room temperature (RT) prior to use. Read the entire protocol before performing the assay procedure.

- **Lysine Probe:** Provided as a solution in DMSO. Divide into aliquots and store at -20°C, protected from light. Prior to use, warm solution to RT. After use, promptly retighten the cap to minimize adsorption of airborne moisture.
- **Lysine Enzyme Mix:** Reconstitute with 220 µl of Lysine Assay Buffer. Divide into aliquots and store at -20°C, protected from light. Avoid repeated freeze-thaw cycles.
- **Developer Mix:** Reconstitute with 220 µl of ddH_2O. Divide into aliquots and store at -20°C, protected from light. Avoid repeated freeze-thaw cycles.
- **L-Lysine Standard:** Reconstitute with 110 µl of ddH_2O to prepare a 10 mM L-Lysine Standard stock solution. At -20°C, reconstituted standard is stable for 5 freeze-thaw cycles.

VII. Lysine Assay Protocol:

1. Sample Preparation:

a. Biological fluid Samples (such as plasma and serum) should be clarified by centrifugation at 10,000 x *g* and 4°C for 5 min to remove any insoluble materials. Soft tissues (~10 mg) or cultured cells (~1 x 10^6) should be rapidly homogenized on ice with 100 µl ice cold Lysine Assay Buffer. Centrifuge at 15,000 x *g* and 4°C for 10 min and transfer the supernatant to a new micro-centrifuge tube.

b. Various enzymes found in biological Samples may interfere with the assay. To eliminate potential enzymatic interference, Samples should be deproteinized using 10 kDa MWCO Spin Columns (Cat. # 1997 or equivalent). Transfer clarified samples to Spin Columns, centrifuge at 10,000 x *g* for 10 min at 4°C and collect the filtrate. *Once deproteinized, Samples may be stored at -20°C for future experiments for at least 2 months.*

c. For each Test Sample, add the same volume (2-20 µl) of Sample into *three* parallel wells in a black, flat bottom 96-well plate. One of the Sample wells will be used as a Sample Background Control. The other two wells will be used for Unspiked Sample and Spiked Sample (containing Sample spiked with 400 pmoles of internal L-Lysine Standard) measurements, respectively. Use of a single-point Standard addition method (in which unspiked and spiked Test Sample wells are assayed in parallel) is required to ensure accurate quantification in different types of Samples that may impart matrix effects.

2. **Internal Spike Preparation:** Prepare a 100 µM L-Lysine Standard solution by adding 10 µl of the 10 mM L-Lysine Standard stock solution to 990 µl of Lysine Assay Buffer. Add 4 µl of the 100 µM L-Lysine Standard solution (400 pmoles L-Lysine Standard) to all of the Spiked Sample wells. Adjust the volume of all wells to 60 µl/well with Lysine Assay Buffer.
3. **Reaction Mix Preparation:** Prepare Sample Reaction Mix (used for both Unspiked Sample and Spiked Sample wells) and Sample Background Mix (used for Sample Background Control wells) according to the table below. Make a sufficient amount of each type of mix to add 40 µl to all assay wells of that type.

	Sample Reaction Mix	Sample Background Mix
Lysine Assay Buffer	35 µl	37 µl
Lysine Enzyme Mix	2 µl	—
Developer Mix	2 µl	2 µl
Lysine Probe	1 µl	1 µl

Mix well. Add 40 µl of the Sample Reaction Mix to all Unspiked and Spiked Sample wells and 40 µl of the Sample Background Mix to Sample Background Control well(s).

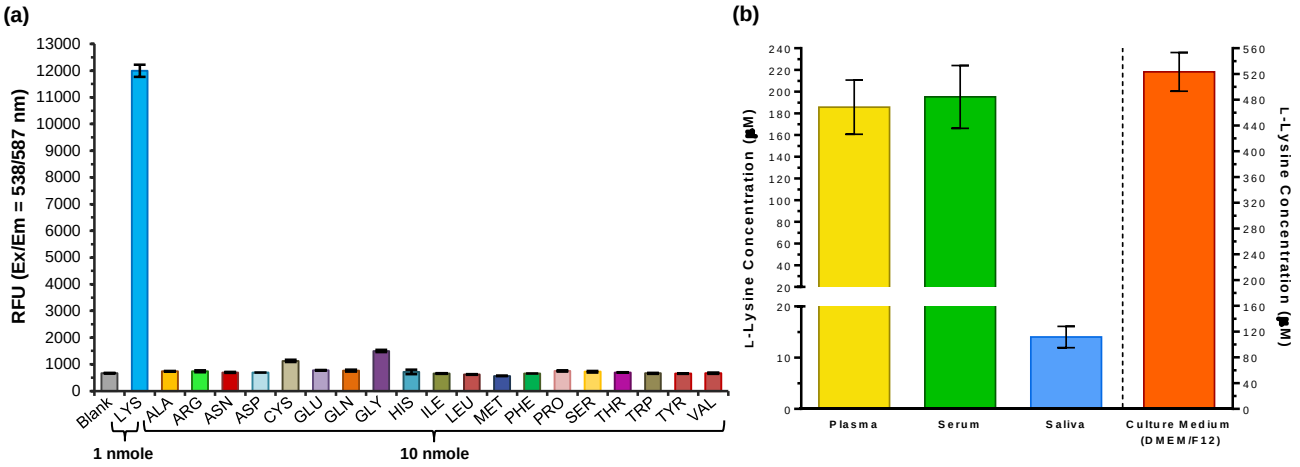
4. **Measurement:** Incubate the plate at 25°C for 45 min, **protected from light**. Measure the fluorescence of all wells at Ex/Em = 538/587 nm in endpoint mode.
5. **Calculation:** For both Unspiked and Spiked Sample wells, calculate the net fluorescence signal (*F*) by subtracting the Sample Background (BC) RFU reading from each of the corresponding Sample RFU readings: $F_{Spiked} = RFU_{Spiked} - RFU_{BC}$ and $F_{Unspiked} = RFU_{Unspiked} - RFU_{BC}$. Determine the amount of Lysine (*B* pmol) in the Unspiked Sample wells using the following formula:

$$\text{Amount of L-Lysine in Unspiked Sample wells } (\boldsymbol{B}) = \left(\frac{F_{Unspiked}}{(F_{Spiked} - F_{Unspiked})} \times \mathbf{400\ pmoles}\right)$$

Note: In order to ensure that readings are within the linear range of the assay, samples for which the calculated amount of L-Lysine in the Unspiked Sample well exceeds 1000 pmoles should be diluted in Lysine Assay Buffer and retested.

$$\textbf{Sample L-Lysine Concentration} = \frac{\boldsymbol{B}}{\boldsymbol{V}} \times \boldsymbol{D} = \textbf{pmol/µl = µM}$$

Where: ***B*** is the amount of Lysine, calculated from the standard addition formula above (in pmol)
V is the volume of sample added to the well (in µl)
D is the sample dilution factor (if applicable, *D*=1 for undiluted samples)



Figures: (a) Specificity for detection of L-Lysine (LYS) over other common amino acids. At a 10-fold molar excess (10 nmole/well) versus L-Lysine (1 nmole/well), all other amino acids tested contributed ≤10% interference. **(b)** Estimation of total L-Lysine in pooled normal human plasma (5 µl/well), single donor off-the-clot human serum (5 µl/well), single donor human saliva (5 µl/well) and cell culture growth medium (DMEM/F12 with 10% FBS, 2 µl/well). L-Lysine concentrations for plasma, serum and saliva samples were 185.7 ± 24.9 µM, 195.1 ± 28.9 µM and 14.03 ± 1.31 µM, respectively, whereas the concentration for DMEM/F12 culture medium was 518.6 ± 32.4 µM. Data are mean ± SD of at least 3 replicates. Samples were deproteinized using 10 kDa MWCO spin columns (Cat # 1997) and assayed according to the kit protocol.

VIII. Related Products:

Glycine Assay Kit (K589)	Glutamine Assay Kit (K556)	Cysteine Assay Kit (K558)	Glutamate Assay Kit (K629)
Alanine Assay Kit (K652)	Tyrosine Assay Kit (K573)	Aspartate Assay Kit (K552)	Phenylalanine Assay Kit (K572)
DL-Serine Assay Kit (K743)	Homocysteine Assay Kit (K531)	Methionine Assay Kit (K442)	Tryptophan Assay Kit (K557)
BCAA Assay Kit (K564)	Taurine Assay Kit (K988)	Threonine Assay Kit (K463)	Arginine Assay Kit (K384)

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