Technical Bulletin

Lysine Assay Kit (Fluorometric)

Catalog Number MAK421

Product Description

Lysine is one of the 20 naturally occurring 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 crosslink 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.

The 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 (λ_{Ex} = 538 nm/ λ_{Em} = 587 nm). The assay is not affected by the physiological concentration of other amino acids and is suitable for high-throughput applications. The method can detect less than 5 μM lysine in samples.

The kit is suitable for the estimation of L-lysine concentration in human or animal biological fluids (plasma, serum, etc.), soft tissue homogenates (e.g., liver, brain) and cultured cell lysates (adherent or suspension cells) or cell culture growth/fermentation medium.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Lysine Assay Buffer 25 mL
  Catalog Number MAK421A
- Lysine Probe 200 μL
  Catalog Number MAK421B
- Lysine Enzyme Mix 1 vial
  Catalog Number MAK421C
- Developer Mix 1 vial
  Catalog Number MAK421D
- L-Lysine Standard 1 vial
  Catalog Number MAK421E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- Black flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

Lysine Assay Buffer: Ready to use. Allow the Lysine Assay Buffer to warm to room temperature prior to use. Chill an appropriate amount of Lysine Assay Buffer for use in Sample Preparation.

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 room temperature. 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 purified water. Divide into aliquots and store at -20 °C, protected from light. Avoid repeated freeze-thaw cycles.

L-Lysine Standard: Reconstitute with 110 µL of purified water to prepare a 10 mM L-Lysine Standard stock solution. At -20 °C, reconstituted standard is stable for five freeze-thaw cycles.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Biological fluid samples such as plasma and serum should be clarified by centrifugation at 10,000 × *g*, 4 °C for 5 minutes to remove any insoluble materials.
2. Soft tissues (~10 mg) or cultured cells (~1 × 10^6 cells) should be rapidly homogenized on ice with 100 µL of ice-cold Lysine Assay Buffer. Centrifuge at 15,000 × *g*, 4 °C for 10 minutes and transfer the supernatant to a new micro-centrifuge tube.
3. Various enzymes found in biological Samples may interfere with the assay. To eliminate potential enzymatic interference, Samples should be deproteinized using a 10 kDa Spin Column such as Corning Spin-X UF concentrator.
   a. Transfer clarified samples to Spin Columns.
   b. Centrifuge at 10,000 × *g* for 10 minutes at 4 °C and collect the filtrate.
   c. Once deproteinized, Samples may be stored at -20 °C for future experiments for at least two months.

4. 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. Designate as "Sample Background Control" (SBC), "Sample" (S), and "Spiked Sample" (Sample + L-Lysine Spike; SS). The Spiked Sample contains Sample spiked with 400 pmoles of internal L-Lysine Standard. Use of a single-point Standard addition method (in which unspiked and spiked Sample wells are assayed in parallel) is required to ensure accurate quantification in different types of samples that may impart matrix effects.
5. 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.
6. Add 4 µL of the 100 µM L-Lysine Standard solution (400 pmoles L-Lysine Standard) to all of the Spiked Sample (SS) wells. Adjust the total volume of all wells to 60 µL/well with Lysine Assay Buffer.

Reaction Mixes

1. Mix enough reagents for the number of assays to be performed.
   a. For each well containing unspiked Sample (S) or Spiked Sample (SS), prepare 40 µL of Sample Reaction Mix according to Table 1, mix well.
   b. For each Sample Background Control well (SBC), prepare 40 µL of SBC Reaction Mix according to Table 1, mix well.

Table 1.
Preparation of Reaction Mixes

Reagent	Sample Reaction Mix	SBC Reaction 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

2. Add 40 µL of the Sample Reaction Mix to all unspiked Sample (S) and Spiked Sample (SS) wells and 40 µL of the SBC Reaction Mix to Sample Background Control (SBC) well(s).

Measurement

Incubate the plate at 25 °C for 45 minutes, protected from light. Measure the fluorescence (RFU) of all wells at λ_{Ex} = 538 nm/ λ_{Em} = 587 nm in endpoint mode.

Results

1. For both unspiked Sample (S) and Spiked Sample (SS) wells, calculate the net fluorescence signal (F) by subtracting the Sample Background Control (SBC) RFU reading from each of the corresponding Sample RFU readings:

   $F_{SS} = RFU_{SS} - RFU_{SBC}$

   $F_{Sample} = RFU_{Sample} - RFU_{SBC}$.

2. Determine the amount of Lysine (B pmol) in the unspiked Sample wells using the following formula:

   Lysine amount (B) in unspiked Sample =

$$\left[\frac{F_{Sample}}{F_{ss} - F_{Sample}}\right] \times 400 \text{ pmol}$$

3. 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 (S) well exceeds 1000 pmoles should be diluted in Lysine Assay Buffer and retested.

   Sample L-Lysine Concentration (pmol/µL or µM) =

   (B/V) × D

where

B = Amount of Lysine, calculated from the standard addition formula above (in pmol)

V = Volume of sample added to the well (in µL)

D = Dilution factor (if applicable; D = 1 for undiluted samples)

Figure 1.
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.

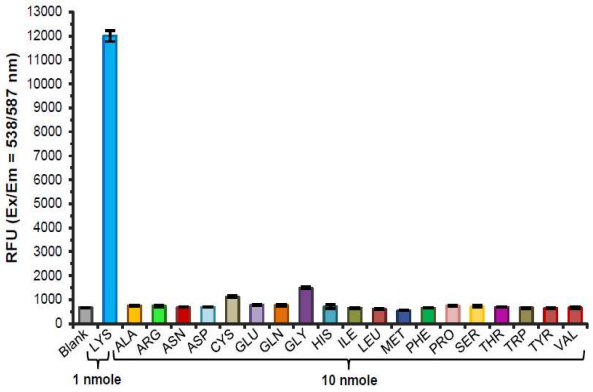
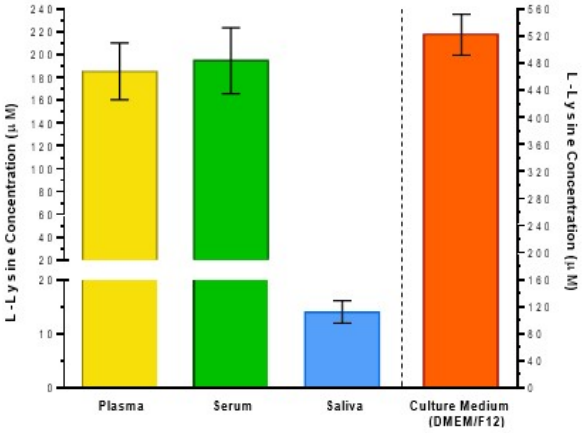


Figure 2.
Estimation of total L-Lysine in pooled normal human plasma (5 µL/well), single donor off-the-clot human serum (5 µL/well), single donor human saliva (5 µL/well) and cell culture growth medium (DMEM/F12 with 10% FBS, 2 µL/well). L-Lysine concentrations for plasma, serum and saliva samples were 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 and assayed according to the kit protocol.



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