

Version 1 Last updated 4 June 2020

# ab273311

## Lysine Assay Kit (Fluorometric)

View Kit datasheet: <https://www.abcam.com/ab273311>  
(use <https://www.abcam.cn/ab273311> for china, or  
<https://www.abcam.co.jp/ab273311> for Japan)

For the estimation of lysine concentration in plasma, serum, tissue homogenates and cell lysates.

This product is for research use only and is not intended for diagnostic use.

## Table of Contents

1. Overview	1
2. Protocol Summary	2
3. Precautions	3
4. Storage and Stability	3
5. Limitations	4
6. Materials Supplied	4
7. Materials Required, Not Supplied	4
8. Technical Hints	5
9. Reagent Preparation	6
10. Sample Preparation	7
11. Assay Procedure	8
12. Calculations	9
13. Typical Data	10
14. FAQ / Troubleshooting	12
15. Notes	13

## 1. Overview

Lysine Assay Kit (Fluorometric) (ab273311) 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  $\mu\text{M}$  Lysine in samples.

## 2. Protocol Summary

Prepare all reagents as directed.



Prepare samples and internal spikes as directed.



Add Samples, Spiked Samples and Background Control to appropriate wells and adjust volume to 60  $\mu$ L.



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



Incubate plate at 25°C for 45 mins protected from light.



Measure fluorescence in end point mode.

### 3. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

### 4. Storage and Stability

**Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 6 months from receipt.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

Item	Quantity	Storage temperature (before and after prep)
Lysine Assay Buffer	25 mL	-20°C
Lysine Probe	200 µL	-20°C
Lysine Enzyme Mix (Lyophilized)	1 vial	-20°C
Developer Mix (Lyophilized)	1 vial	-20°C
Lysine Standard (Lyophilized)	1 vial	-20°C

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well spectrophotometer capable of measuring fluorescence at Ex/Em= 538/587 nm
- Black 96-well plate with flat bottom
- Dounce Homogenizer
- 10 kDa Spin Columns

## 8. Technical Hints

- **This kit is sold based on number of tests. A “test” simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

## 9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

### 9.1 **Lysine Assay Buffer:**

Ready to use as supplied. Store at  $-20^{\circ}\text{C}$ . Thaw and bring to room temperature before use.

### 9.2 **Lysine Probe:**

Provided as a solution in DMSO. Divide into aliquots and store at  $-20^{\circ}\text{C}$ , protected from light. Prior to use, warm solution to room temperature. After use, promptly retighten the cap to minimize adsorption of airborne moisture.

### 9.3 **Lysine Enzyme Mix (Lyophilized):**

Reconstitute with 220  $\mu\text{L}$  Lysine Assay Buffer. Divide into aliquots and store at  $-20^{\circ}\text{C}$ , protected from light. Avoid repeated freeze-thaw cycles.

### 9.4 **Lysine Developer (Lyophilized):**

Reconstitute with 220  $\mu\text{L}$  of ddH<sub>2</sub>O. Divide into aliquots and store at  $-20^{\circ}\text{C}$ , protected from light. Avoid repeated freeze-thaw cycles.

### 9.5 **Lysine Standard (Lyophilized):**

Reconstitute with 110  $\mu\text{L}$  of ddH<sub>2</sub>O to prepare a 10 mM L-Lysine Standard stock solution. At  $-20^{\circ}\text{C}$ , reconstituted standard is stable for 5 freeze-thaw cycles.

## 10. Sample Preparation

### Plasma and Serum:

- 10.1 Biological fluid Samples (such as plasma and serum) should be clarified by centrifugation at 10,000 x *g* and 4°C for 5 minutes to remove any insoluble materials.

### Soft tissues and cultured cells:

- 10.2 Soft tissues (~10 mg) or cultured cells (~1 x 10<sup>6</sup>) should be rapidly homogenized on ice with 100 µL ice cold Lysine Assay Buffer.
- 10.3 Centrifuge at 15,000 x *g* and 4°C for 10 minutes and transfer the supernatant to a new micro-centrifuge tube.

### Deproteinization:

- 10.4 Various enzymes found in biological Samples may interfere with the assay.
- 10.5 To eliminate potential enzymatic interference, Samples should be deproteinized using 10 kDa MWCO Spin Columns.
- 10.6 Transfer clarified samples to Spin Columns, centrifuge at 10,000 x *g* for 10 minutes at 4°C and collect the filtrate.
- 10.7 Once deproteinized, Samples may be stored at -20°C for future experiments for at least 2 months.

## 11. Assay Procedure

Thaw all reagents thoroughly and mix gently.

- 11.1 For Sample (S), prepare three wells for each sample labeled Sample Background Control (SBC), Unspiked Sample and Spiked Sample). Add 2-20  $\mu\text{L}$  Sample into each of these wells.
- 11.2 **Internal Spike Preparation:** Prepare a 100  $\mu\text{M}$  L-Lysine Standard solution by adding 10  $\mu\text{L}$  of the 10 mM L-Lysine Standard stock solution to 990  $\mu\text{L}$  of Lysine Assay Buffer. Add 4  $\mu\text{L}$  of the 100  $\mu\text{M}$  L-Lysine Standard solution (400 pmoles L-Lysine Standard) to all of the Spiked Sample wells.
- 11.3 Adjust the volume of all wells to 60  $\mu\text{L}$ /well with Lysine Assay Buffer.
- 11.4 **Reaction Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 40  $\mu\text{L}$  of Reaction Mix. Mix well.

Component	Reaction Mix ( $\mu\text{L}$ )	Sample Background Mix ( $\mu\text{L}$ )
Lysine Assay Buffer	35	37
Lysine Enzyme Mix	2	---
Developer Mix	2	2
Lysine Probe	1	1

- 11.5 Add 40  $\mu\text{L}$  of the Sample Reaction Mix to all Unspiked and Spiked Sample wells and 40  $\mu\text{L}$  of the Sample Background Mix to Sample Background Control well(s).
- 11.6 Incubate the plate at 25°C for 45 minutes, protected from light.
- 11.7 Measure fluorescence (Ex/Em = 538/587 nm) in end point mode.

## 12. Calculations

- 12.1 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:

$$F_{\text{Spiked}} = \text{RFU}_{\text{Spiked}} - \text{RFU}_{\text{BC}} \text{ and } F_{\text{Unspiked}} = \text{RFU}_{\text{Unspiked}} - \text{RFU}_{\text{BC}}.$$

- 12.2 Determine the amount of Lysine (B pmol) in the Unspiked Sample wells using the following formula:

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

**Δ 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.

$$\text{Sample L - Lysine Concentration (C)} = \frac{B}{V} \times D = \text{pmol} / \mu\text{L} = \mu\text{M}$$

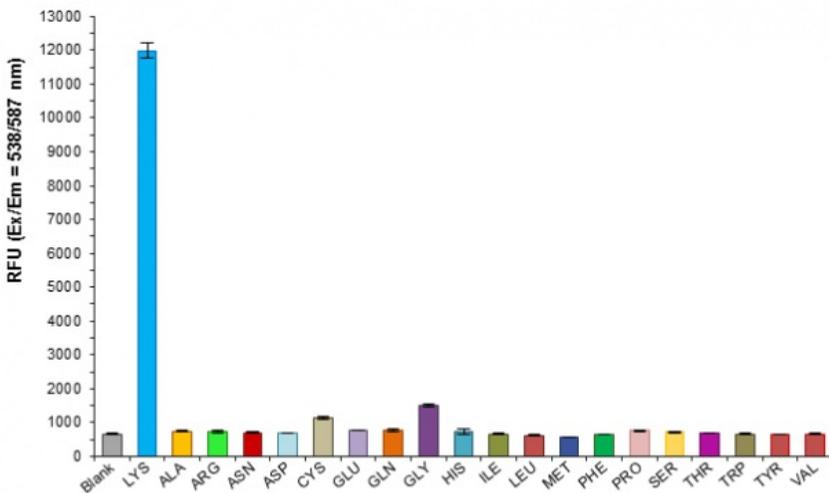
B = amount of Lysine, calculated from the standard addition formula above (in pmol)

V = sample volume added to the well (in  $\mu\text{L}$ ).

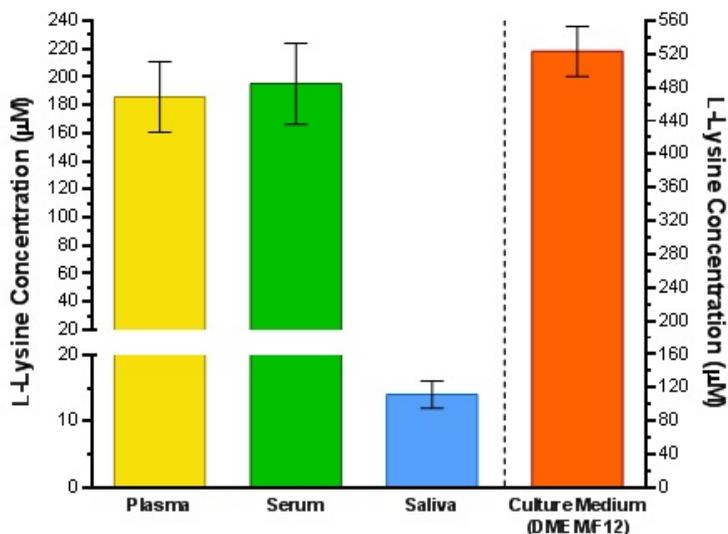
D = sample dilution factor (if applicable, D=1 for undiluted samples)

### 13. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



**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  $\leq 10\%$  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.

## 14. FAQ / Troubleshooting

General troubleshooting points are found at [www.abcam.com/assaykitguidelines](http://www.abcam.com/assaykitguidelines).

## 15. Notes

## Technical Support

Copyright © 2020 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

**For all technical or commercial enquiries please go to:**

[www.abcam.com/contactus](http://www.abcam.com/contactus)

[www.abcam.cn/contactus](http://www.abcam.cn/contactus) (China)

[www.abcam.co.jp/contactus](http://www.abcam.co.jp/contactus) (Japan)