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ab241028
L-Arginine Assay Kit

For the measurement of L-Arginine in biological samples and beverages.

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

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1. Overview

The L-Arginine Assay Kit (ab241028) provides a quick, specific, and easy method for the measurement of total L-arginine concentrations in a wide variety of samples. In this enzyme-based assay, L-arginine is converted into a series of intermediates, which will further react with a probe producing a stable colorimetric signal (OD: 450 nm).

The kit is simple to use, sensitive and high-throughput adaptable and can detect as low as 1 nmol/well of L-arginine in biological samples.

2. Protocol Summary

Prepare all samples, controls and standards as instructed.

Prepare the Arginine standard curve.

Add 2-40 µL of sample to desired wells, adjust volume to 40 µL with Assay Buffer 5.

Prepare the Enzyme mix and add 10 µL to each well containing Standard, Sample, Sample Background and Spiked wells.

Prepare the Reaction Mix. Add 200 µL to Standard, Sample, Sample Background and Spiked wells.

Measure the absorbance (450 nm) in a microplate in end point mode.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- All components in this kit are shipped on blue ice and are suitable for storage at -20°C, unless reconstituted. Upon receipt, immediately store kit at -20°C in the dark. Individual components may be stored at alternative temperatures as show in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
Assay Buffer 5	25 mL	-20°C
Arginine Enzyme Mix	1 vial	-20°C
Probe V	12 mL	-20°C or +4°C
Arginine Probe Mix B	12 mL	-20°C
Converter Mix D	1 vial	-20°C
Arginine	1 vial	-20°C

PLEASE NOTE: Assay Buffer 5 was previously labelled as Assay Buffer V and Arginine Assay Buffer, and Converter Mix D as Converter Enzyme V and Sample Cleanup Mix. The composition has not changed.

5. Materials Required, Not Supplied

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

- 96-well clear plate with flat bottom.
- Multi-well spectrophotometer.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Assay Buffer 5:

Ready to use as supplied. Warm to room temperature before use. Store at 4 °C or -20 °C.

6.2 Arginine Enzyme Mix:

Reconstitute each vial with 220 µL Assay Buffer 5. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw. Use within two months.

6.3 Probe V:

Ready to use as supplied. Warm to room temperature before use. Store at 4°C or -20°C. Keep away from light.

6.4 Arginine Probe Mix B:

Ready to use as supplied. Warm to room temperature before use. Store at -20°C. Keep away from light.

6.5 Converter Mix D:

6.6 Reconstitute each vial with 220 µL Assay Buffer 5. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze and thaw. Use within two months

6.7 Arginine:

Reconstitute with 500 µL of dH_2O to make a 100 mM stock solution. Store at -20°C.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Prepare a 4 mM solution of Arginine by adding 40 µL of the 100 mM Arginine stock to 960 µl of dH_2O.

7.2 Add 0, 2, 4, 6, 8 and 10 µL of the 4 mM standard into a series of standard wells on a 96-well plate.

7.3 Adjust the volume to 40 µL/well with Assay Buffer 5 to generate 0, 8, 16, 24, 32, and 40 nmol/well of the Arginine.

Standard #	4 mM Arginine Standard (µL)	Assay Buffer 5 (µL)	Arginine nmol/well
1	10	30	40
2	8	32	32
3	6	34	24
4	4	36	16
5	2	38	8
6	0	40	0

8. Sample Preparation

- For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
- For samples having high background, prepare a parallel sample well as the background control to correct the interference from the samples.

8.1 Biological Fluids:

- Add 2 µL of Converter Mix D per 100 µl of sample. Incubate at 37 °C for 1 hour.
- Centrifuge the treated sample in a 10kDa MWCO Spin Column at 13,000 x g for 10 min at 4 °C and collect the filtrate.

8.2 Beverages:

- Centrifuge the sample at 13,000 x g to discard the precipitate. Collect the supernatant and centrifuge in a 10kDa MWCO Spin Column at 13,000 x g for 10 min at 4 °C and collect the filtrate.

8.3 For all samples:

- Prepare duplicates by adding 2-40 µL of the pretreated, filtered samples in parallel wells. Bring the volume of all wells to 40 µL with Assay Buffer 5. Label them as "sample" and "sample background".

∆**Note:** Arginine varies over a wide range for different samples. For unknown samples, we recommend performing a pilot experiment with a few dilutions to ensure readings are within the standard curve range. For normal human serum, average arginine concentration 10-150 µM and can range to 250-1500 µM for patients with argininemia.

∆**Note:** For samples with arginine concentration close to the detection limit (25 µM), we recommend running two samples in parallel and spiking one with a known amount of Arginine (e.g. 4 nmol) to ensure accurate determination of L-arginine.

9. Assay Procedure

9.1 **Enzyme Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 10 µL Mix containing:

	Reaction Mix	Background control Mix
Arginine Enzyme Mix	2 µL	/
Assay Buffer 5	8 µL	10 µL

9.2 Mix well and add 10 µL of the Enzyme Mix to each well containing the Standard, Sample and Spiked wells.

9.3 Add 10 µL of Background mix to the Sample background wells. Mix well and incubate the plate for 30 min at 37°C

9.4 **Reaction Mix:** Prepare enough Reaction Mix for the number of assays to be performed (including Arginine standard curve and Positive Control wells).

9.5 For each well, prepare 200 µL Reaction Mix containing:

	Reaction Mix
Probe V	100 µL
Arginine Probe Mix B	100 µL

9.6 Mix and add 200 µL of the Reaction Mix to each well containing the Standard, Sample, Sample Background and Spiked wells. Mix well and incubate the plate for 60 min at 37 °C. Protect from light.

9.7 **Measurement:** Measure the absorbance (OD_{450}) in a microplate reader in end point mode.

10. Data Analysis

10.1 Subtract the zero Standard curve reading from all Standard readings. Plot the Standard Curve.

10.2 The sample background control reading is significant, subtract the background control reading from the sample.

10.3 For unspiked samples apply the corrected sample reading to the Arginine Standard Curve to get to get B nmol of L-Arginine in the well.

$$\textbf{L-Arginine concentration} = \frac{B}{V} \times D = \textbf{nmol/µL =mM}$$

Where:

B is the L-Arginine amount from Standard Curve (nmol)

V is sample volume added into the reaction well (µL)

D is sample dilution factor

For spiked samples:

For spiked L-arginine samples, subtract Sample background from both of the Sample reading ($Fs = OD_{sample} - OD_{sbc}$) and the Spiked sample reading and Spike reading ($F_{spike} = OD_{spike} - OD_{sbc}$). Calculate amount of L-arginine (B) as follows.

$$\boldsymbol{L - Arginine\ Amount\ (B) = \frac{F_s}{F_{Spike} - F_s} \times Arginine\ Spike\ (nmol)}$$

11. Typical Data

Typical data provided **for demonstration purposes only**.

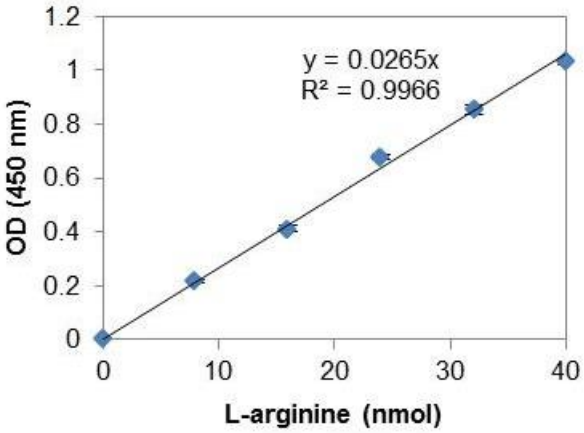

Figure 1. L-Arginine Standard Curve.

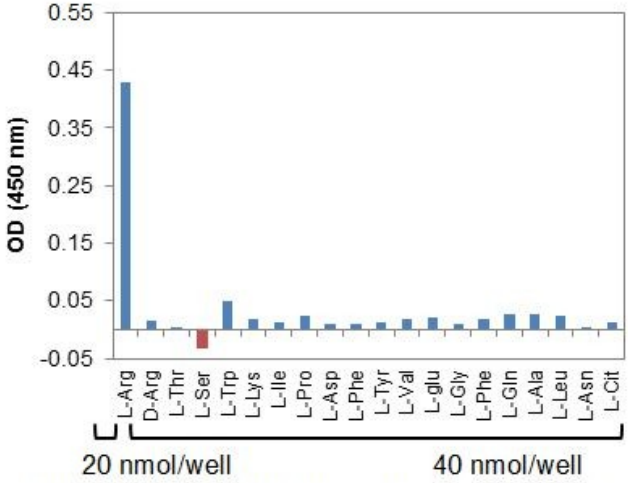

Figure 2. Specificity of the detection of L-arginine over other amino acids: D-arginine and other L-amino acids were tested at a 2-fold molar excess (each AA: 40 nmol) vs L-arginine (20 nmol).

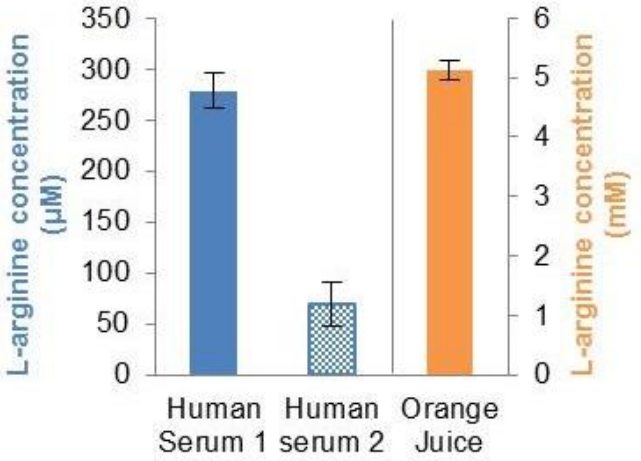

Figure 3. Estimations of L-arginine in two human serum sample (30 µL) and orange juice (1.6 µL). L-arginine concentrations were 0.278 mM and 0.069 mM in the two human serum samples, and 5.113 mM in orange juice. Assays were performed following the kit protocol.

12. Notes

13. Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)