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（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K850-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader（510-530 nm nm）

Detection range：0.008-1 mmol/L

Elabscience®L-Arginine (L-Arg) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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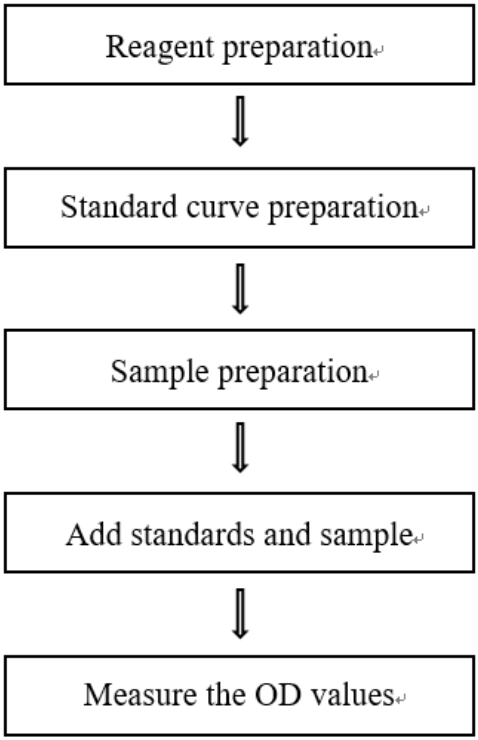
Assay summary



Intended use

This kit can measure L-Arginine (L-Arg) content in serum, plasma, urine, plant and animal tissue samples.

Detection principle

Arginine (Arg) is an essential amino acid in the human body and can also be absorbed in food. Natural arginine is L-type, which is alkaline and easily reacts with acids to form salts. Arginine is an intermediate metabolite in the ornithine cycle, which can promote the conversion of ammonia into urea, thereby reducing blood ammonia content.

Arginine generates urea under the action of enzymes, and urea can complex with oxime reagents under strong acidic conditions by boiling. The complex has a maximum absorption peak at 520 nm, and the arginine content can be calculated by measuring the OD value at 450 nm.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution A	50 mL ×1 vial	-20°C, 12 months
Reagent 2	Buffer Solution B	1.8 mL ×1 vial	-20°C, 12 months, shading light
Reagent 3	Enzyme Reagent A	Powder ×2 vials	-20°C, 12 months, shading light
Reagent 4	Enzyme Reagent B	Powder ×2 vials	-20°C, 12 months, shading light
Reagent 5	Chromogenic Agent A	24 mL ×1 vial	-20°C, 12 months, shading light
Reagent 6	Chromogenic Agent B	24 mL ×1 vial	-20°C, 12 months, shading light
Reagent 7	1 mmol/L Standard	1 mL ×2 vials	-20°C, 12 months, shading light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (510-530 nm, optimum wavelength: 520 nm), Incubator, 10 KD ultrafiltration tube

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Keep enzyme reagent A on ice during use Equilibrate other reagents to room temperature before use.

② Preparation of enzyme reagent A working solution:
Dissolve one vial of enzyme reagent A with 0.7 mL of buffer solution B, mix well to dissolve. Keep enzyme reagent A working solution on ice during use. Store at -20℃ for 7 days protected from light.

③ Preparation of enzyme reagent A reaction working solution:
For each well, prepare 60 μL of reagent A reaction working solution (mix well 40 μL of buffer solution A and 20 μL of enzyme reagent A working solution). Keep enzyme reagent A reaction working solution on ice during use. The prepared solution should be used up within the same day.

④ Preparation of enzyme reagent B working solution:
Dissolve one vial of enzyme reagent B with 2 mL of double distilled water, mix well to dissolve. Keep enzyme reagent B working solution on ice during

use. Store at -20℃ for 3 days protected from light.

⑤ Preparation of chromogenic working solution：

For each well, prepare 400 μL of chromogenic working solution (mix well 200 μL of chromogenic agent A and 200 μL of chromogenic agent B). The prepared solution should be used up within the same day.

⑥ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mmol/L standard solution with buffer solution A to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.5, 0.6, 0.8, 1.0 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.5**	**0.6**	**0.8**	**1**
1 mmol/L standard (μL)	0	20	40	60	100	120	160	200
Buffer solution A (μL)	200	180	160	140	100	80	40	0

Sample preparation

① Sample preparation

Plasma, serum or urine samples:

① For each well，add 80 μL of supernatant and 20 μL of enzyme reagent B working solution, mix well. Incubate at 37℃ for 20 min. After incubation, centrifuge the incubated sample at 12000×g for 10 min at 4℃

② Centrifuge the supernatant with a 10 KD ultrafiltration tube at 12000×g for 15 min at 4 ℃, take the filtrate from the outer tube for use.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃. Centrifuge at 12000×g for 10 minutes. Collect supernatant for use.

④ For each well，add 80 μL of supernatant and 20 μL of enzyme reagent B working solution, mix well. Incubate at 37℃ for 20 min. After incubation, centrifuge the incubated sample at 12000×g for 10 min at 4℃

⑤ Centrifuge the supernatant with a 10 KD ultrafiltration tube at 12000×g for 15 min at 4℃， take the filtrate from the outer tube for use.

Note: Before ultrafiltration, the volume of each sample supernatant or liquid sample should not exceed 300 μL.

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Rat liver tissue homogenate	1-2
10% Rat kidney tissue homogenate	1-2
10% Rat heart tissue homogenate	1-2
10% Rat lung tissue homogenate	1-2
Human serum	1-2
Human plasma	1-2
Bovine serum	1-2
10% Nartjie tissue homogenate	1-2

Note: The diluent is buffer solution A. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① When boiling, pay attention to avoid the introduction of water into the reaction system.

② The product has a pungent odor, and the addition of reagents should be carried out in a fume cupboard.

Operating steps

① Standard tube: Add 40 μL of standard solution with different concentrations into the corresponding tube.

Control tube: Add 40 μL of sample into control tube.

Sample tube: Add 40 μL of sample into sample tube.

② Add 60 μL of enzyme reagent A reaction working solution into standard well and sample tube. Add 60 μL of buffer solution A into control tube.

③ Mix fully and incubate at 60℃ for 2h.

④ Add 400 μL of chromogenic working solution into each tube and incubate in 98 ℃ water bath for 30 min.(Note: When boiling, exhaust gas can be carried out 1-2 times or tie a small hole in the EP tube mouth to avoid external water entering the reaction system).

⑤ Cool the tubes to room temperature and take 200μL of supernatant to the microplate. Measure the OD values of each well at 520 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Tissue sample:

$$\text{L-Arg content (mmol/kg wet weight)} = (\Delta A_{520} - b) \div a \div (m \div V) \div 0.8^* \times f$$

2. Serum (plasma) and urine samples:

$$\text{L-Arg content (mmol/L)} = (\Delta A_{520} - b) \div a \div 0.8^* \times f$$

[Note]

ΔA_{520}: $OD_{Sample} - OD_{Control}$.

m: The weight of sample, g.

V: The volume of homogenate, mL.

0.8*: Dilution ratio of enzyme reagent B working solution to the sample.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.05	0.32	0.74
%CV	3.8	1.9	1.8

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	0.05	0.32	0.74
%CV	8.0	6.0	7.6

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 96%.

	Standard 1	Standard 2	Standard 3
Expected Conc. （mmol/L）	0.15	0.42	0.7
Observed Conc. （mmol/L）	0.1	0.4	0.7
recovery rate(%)	98	94	96

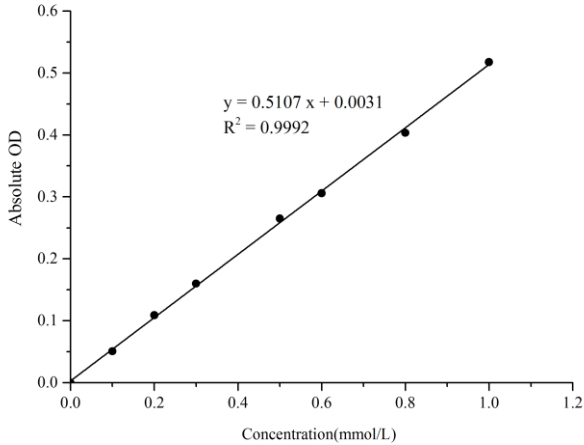
Sensitivity

The analytical sensitivity of the assay is 0.008 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	**0**	**0.1**	**0.2**	**0.3**	**0.5**	**0.6**	**0.8**	**1.0**
OD Value	0.064	0.115	0.173	0.224	0.330	0.370	0.464	0.579
	0.064	0.115	0.173	0.224	0.328	0.370	0.471	0.584
Average OD	0.064	0.115	0.173	0.224	0.329	0.370	0.468	0.582
Absoluted OD	0	0.051	0.109	0.160	0.265	0.306	0.404	0.518



Appendix II Example Analysis

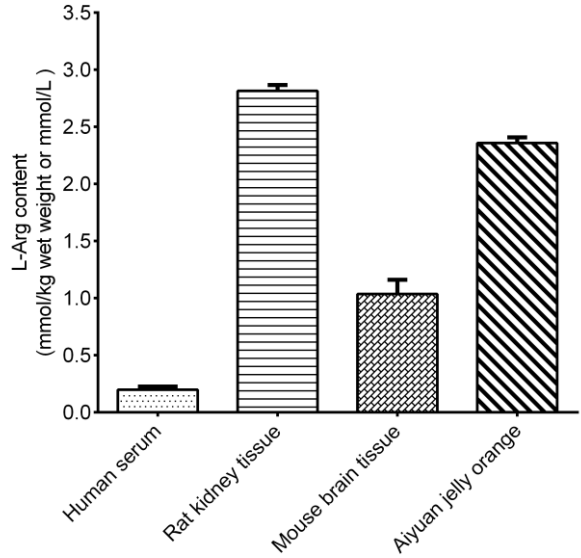
Example analysis:

Take 40 μL of human serum which dilute for 2 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.5107 x +0.0031, the average OD value of the control is 0.081, the average OD value of the sample is 0.135, ΔA_{520}= OD_{Sample} – $OD_{control}$ = 0.135 – 0.081 = 0.054, and the calculation result is:

$$\text{L-Arg content (mmol/L)} = (0.054 - 0.0031) \div 0.5107 \div 0.8 \times 2 = 0.250 \text{ mmol/L}$$

Detect human serum (dilute for 2 times), 10% rat kidney tissue homogenate (dilute for 2 times), 10% mouse brain tissue homogenate (dilute for 2 times), aiyuan jelly orange (dilute for 2 times) according to the protocol, the result is as follows:



Statement

1. This assay kit is for research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.