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

Catalog No：E-BC-K839-M

Specification：96T(40 samples)

Measuring instrument：Microplate reader(450 nm)

Detection range：10.65-500 μmol/L

Elabscience® Alanine (Ala) 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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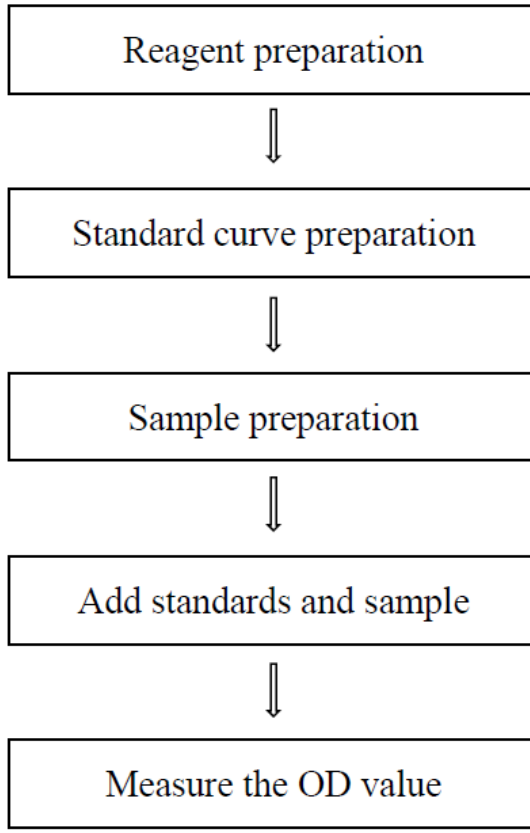
Assay summary



Intended use

This kit can be used to measure Alanine (Ala) content in serum (plasma), animal tissue and cell samples.

Detection principle

Alanine (Ala) is a non-essential amino acid, which is one of the 20 basic amino acids that make up proteins. Ala has a high content in human blood and plays an important role in human metabolic activities such as glucose-alanine cycle. It can also help the nervous system conduction and plays an important role in maintaining human health.

The detection principle of this kit is as follows: alanine is decomposed by enzymes to generate NADH, which is catalyzed by electron carrier to form a chromogenic substance with WST-8. The chromogenic substance has a maximum absorption at the wavelength of 450 nm, and the Ala content in the sample is calculated by measuring the OD value at 450 nm and the standard curve.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	11 mL ×1 vial	-20 ℃, 12 months, shading light
Reagent 2	Coenzyme A	0.22 mL ×1 vial	-20 ℃, 12 months, shading light
Reagent 3	Coenzyme B	Powder ×2 vials	-20 ℃, 12 months, shading light
Reagent 4	Enzyme Reagent A	Powder ×2 vials	-20 ℃, 12 months, shading light
Reagent 5	Enzyme Reagent B	1.32 mL ×1 vial	-20 ℃, 12 months, shading light
Reagent 6	Chromogenic Agent	11 mL ×1 vial	-20 ℃, 12 months, shading light
Reagent 7	10 mmol/L Standard	0.2 mL ×1 vial	-20 ℃, 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 (440-460 nm, optimum wavelength: 450 nm), Incubator

Consumer items:

10kDa MWCO Spin Filter

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of coenzyme B working solution:
Dissolve one vial of coenzyme B with 220 μL of double distilled water, mix well. Store at -20 ℃ for a week protected from light.

③ The preparation of enzyme reaction solution:
Dissolve one vial of enzyme reagent A with 275 μL of double distilled water, mix well, as enzyme reagent A working solution. For each well, prepare 17 μL of enzyme reaction solution (mix well 5 μL of enzyme reagent A working solution and 12 μL of enzyme reagent B). The enzyme reaction solution should be prepared on spot and the prepared solution should be used up on the same day. (Enzyme reagent A working solution will sink, mix before taking).

④ The preparation of enzyme reagent B working solution:
For each well, prepare 17 μL of enzyme reagent B working solution (mix well 12 μL of enzyme reagent B and 5 μL of double distilled water). The enzyme reagent B working solution should be prepared on spot and the prepared solution should be used up on the same day.

⑤ The preparation of reaction working solution:
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 515 μL of reaction working solution (mix well 250 μL of buffer solution, 5 μL of coenzyme A, 10 μL of coenzyme B working solution, and 250 μL of chromogenic agent). The reaction working solution should be prepared on spot and the prepared solution should be used up within 0.5 h.

⑥ The preparation of 500 μmol/L standard solution:
Before testing, please prepare sufficient 500 μmol/L standard solution according to the test wells. For example, prepare 800 μL of 500 μmol/L

standard solution (mix well 40 μL of 10 mmol/L standard and 760 μL of double distilled water).

⑦ The preparation of standard curve：

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

Dilute 500 μmol/L standard with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 25, 50, 100, 200, 300, 400, 500 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**25**	**50**	**100**	**200**	**300**	**400**	**500**
500 μmol/L standard (μL)	0	10	20	40	80	120	160	200
Double distilled water (μL)	200	190	180	160	120	80	40	0

Sample preparation

① Sample preparation:

Serum (plasma) samples:

Add serum (plasma) samples into 10 kDa MWCO Spin Filter and centrifuge at 12000 ×g for 25 min. Collect the filtrate, keep it on ice for detection.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 0.1 g).

② Wash tissue in normal saline (0.9% NaCl).

③ Homogenize 0.1 g tissue in 0.9 mL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000 × g for 10 min at 4℃ to remove insoluble material. Add supernatant into 10 kDa MWCO Spin Filter and centrifuge at 12000 ×g for 25 min. Collect filtrate and keep it on ice for detection.

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a ultrasonic cell disruptor at 4℃.

④ Centrifuge at 10000 × g for 10 min at 4℃ to remove insoluble material. Add supernatant into 10 kDa MWCO Spin Filter and centrifuge at 12000 × g for 25 min. Collect filtrate and keep it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
Human serum	3-10
Mouse serum	2-10
Guinea pig plasma	2-10
Pig serum	2-10
10% Mouse liver tissue homogenate	3-10
10% Mouse kindey tissue homogenate	2-10
1×10^6 Hela cells	1
1×10^6 RAW264.7 cells	1
1×10^6 Jurkat cells	1
1×10^6 293T cells	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: Add 20 μL of standard solution with different concentrations to the corresponding well.

Sample well: Add 20 μL of sample to the corresponding well.

Control well: Add 20 μL of sample to the corresponding well.

② Add 17 μL of enzyme reaction solution to the standard well and sample well, add 17 μL of enzyme reagent B working solution to the control well.

③ Add 200 μL of reaction working solution to each well.

④ Incubate at 37 ℃ for 30 min protected from light, measure the OD value of each well at 450 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. Serum (plasma) and liquid samples:

$$\text{Ala content (μmol/L)} = \frac{\Delta A - b}{a} \times f$$

2. Tissue samples :

$$\text{Ala content (μmol/kg wet weight)} = \frac{\Delta A - b}{a} \times V \div m \times f$$

3. Cell samples :

$$\text{Ala content (nmol/10^6)} = \frac{\Delta A - b}{a} \times V \div n \times f$$

[Note]

ΔA: $OD_{Sample} - OD_{Control}$.

V: The volume of the normal saline added to sample homogenization, mL

m: The weight of sample, g

n: The number of cell samples, 10^6

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 (μmol/L)	50.00	200.00	420.00
%CV	2.2	2.8	2.6

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 (μmol/L)	50.00	200.00	420.00
%CV	2.2	2.8	2.6

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 103.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (μmol/L)	50.00	200.00	420.00
Observed Conc. (μmol/L)	51.00	210.00	436.80
Recovery rate (%)	102	105	104

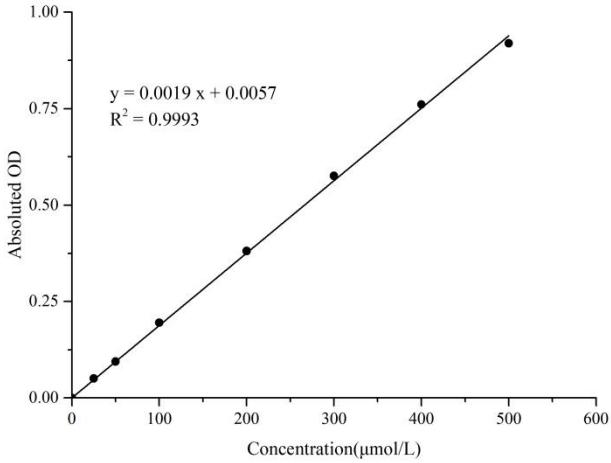
Sensitivity

The analytical sensitivity of the assay is 10.65 μmol/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（μmol/L）	**0**	**25**	**50**	**100**	**200**	**300**	**400**	**500**
OD	0.172	0.221	0.269	0.369	0.551	0.745	0.941	1.13
	0.172	0.224	0.264	0.365	0.555	0.751	0.925	1.053
Average OD	0.172	0.223	0.267	0.367	0.553	0.748	0.933	1.092
Absoluted OD	0.000	0.051	0.095	0.195	0.381	0.576	0.761	0.9195



Appendix Π Example Analysis

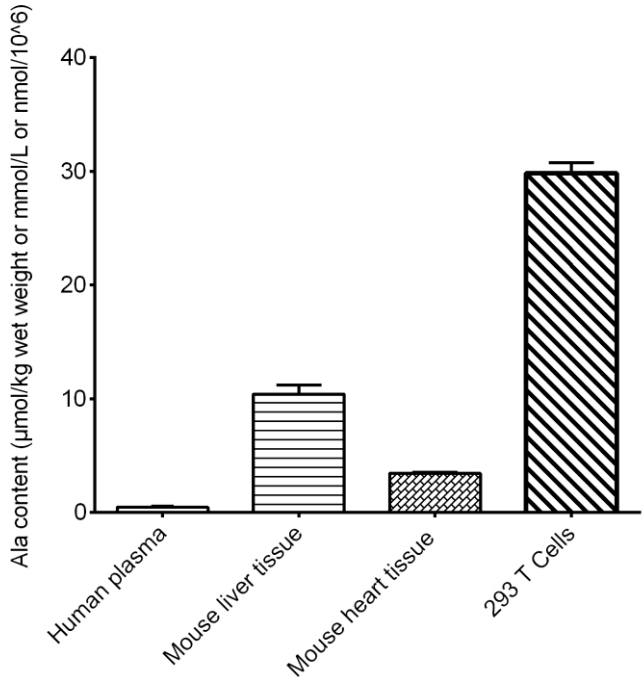
Example analysis:

Take 20 μL of human plasma (dilute for 5 times), carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0019 x + 0.0057, the average OD value of the sample well is 0.376, the average OD value of the control well is 0.202, and the calculation result is:

Ala content (μmol/L) = (0.376 - 0.202 - 0.0057) ÷ 0.0019 × 5 = 442.89 μmol/L

Detect human plasma (dilute for 5 times), 10% mouse liver homogenate (dilute for 10 times), 10% mouse heart homogenate (dilute for 10 times), 293 T cells (1.8×10^6), 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.