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

Catalog No：E-BC-K847-M

Specification：48T (32 samples)/96T (80 samples)

Measuring instrument：Microplate reader (440-460 nm)

Detection range：0.009-2 mmol/L

Elabscience® Branched Chain Amino Acids (BCAA) 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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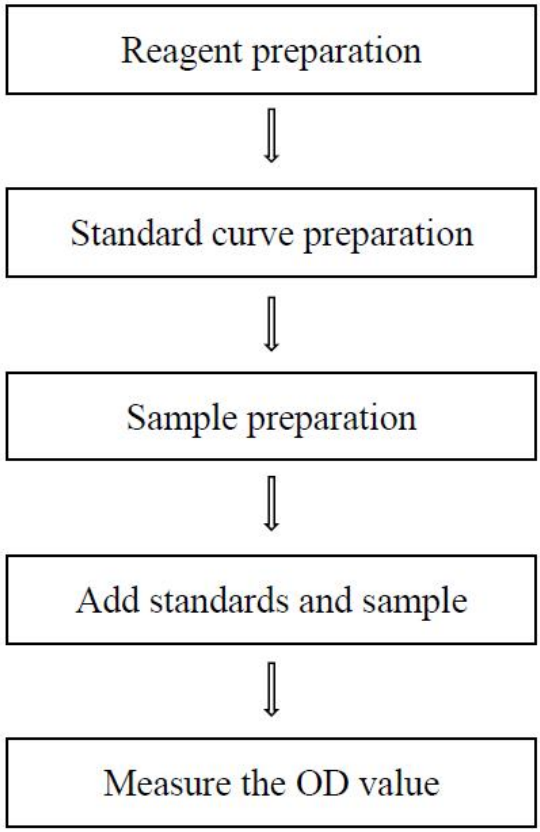
Assay summary



Intended use

This kit can be used to measure branched chain amino acids (BCAA) content in serum, plasma, cell, animal and plant tissue samples.

Detection principle

Branched Chain Amino Acids (BCAA), including leucine, isoleucine and valine, are essential amino acids for the human body, which have the functions of muscle synthesis, anti-protein breakdown and stimulating insulin production.

The detection principle of this kit is: the branched chain amino acid under the catalysis of enzymes to generate the product of reaction with chromogenic agent generated color material, which has maximum absorption at 450 nm. The content of BCAA in samples was calculated by measuring OD value at 450 nm and standard curve.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	50 mL × 1 vial	-20°C, 12 months, shading light
Reagent 2	Enzyme Reagent	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months, shading light
Reagent 3	Coenzyme	Powder × 2 vials	Powder × 4 vials	-20°C, 12 months, shading light
Reagent 4	Chromogenic Agent	3 mL × 1 vial	6 mL × 1 vial	-20°C, 12 months, shading light
Reagent 5	50 mmol/L Standard Solution	0.5 mL × 1 vial	1 mL × 1 vial	-20°C, 12 months, shading light
	Microplate	48 wells	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(37℃)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to 25℃ before use.

② The preparation of enzyme working solution:
Dissolve one vial of enzyme reagent with 275 μL of double distilled water, mix well to dissolve. Store at -20°C for 7 days protected from light.

③ The preparation of coenzyme working solution:
Dissolve one vial of coenzyme with 1375 μL of buffer solution, mix well to dissolve (If the coenzyme is difficult to dissolve, it can be dissolved by ultrasound at 40°C for 5 min). Store at -20°C for 7 days protected from light.

④ The preparation of reaction working solution:
Before testing, please prepare sufficient reaction working solution according to the test wells. For example, prepare 135 μL of reaction working solution (mix well 10 μL of enzyme working solution and 125 μL of coenzyme working solution). Keep it on ice during use protected from light and used up within same day.

⑤ The preparation of 2 mmol/L standard solution:
Before testing, please prepare sufficient 2 mmol/L standard solution. For example, prepare 500 μL of 2 mmol/L standard solution (mix well 20 μL of 50 mmol/L standard solution and 480 μL of double distilled water). Store at -20°C for 3 months protected from light.

⑥ The preparation of standard curve:
Always prepare a fresh set of standards. Discard working standard dilutions

after use.

Dilute 2 mmol/L standard solution with double distilled water diluent to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**0.2**	**0.4**	**0.6**	**0.8**	**1.2**	**1.6**	**2**
2 mmol/L standard (μL)	0	10	20	30	40	60	80	100
Double distilled water (μL)	100	90	80	70	60	40	20	0

Sample preparation

① Sample preparation

Serum or plasma samples: detect directly.

Tissue sample:

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

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

③ Homogenize 20 mg tissue in 180 μL 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. Collect supernatant and keep it on ice for detection. The supernatant should be used up within same day.

⑤ Meanwhile, determine the protein concentration of supernatant. (animal tissue:E-BC-K318-M; plant tissue:E-BC-K168-M).

Cell (adherent or suspension) 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. Collect supernatant and keep it on ice for detection. The supernatant should be used up within same day.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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

Sample type	Dilution factor
Human serum	1
10% Mouse liver tissue homogenate	5-10
10% Mouse kidney tissue homogenate	5-10
10% Mouse heart tissue homogenate	5-10
10% Mouse brain tissue homogenate	1
10% Mouse spleen tissue homogenate	1
1×10^6 Jurkat cells	1
1×10^6 HL-60 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 wells: Add 10 μL of standard solution with different concentrations to the corresponding wells.
Sample well: Add 10 μL of samples to each well.

② Add 50 μL of reaction working solution to each well.

③ Add 50 μL of chromogenic agent to each well.

④ Mix fully with microplate reader for 5 s and incubate at 37°C for 30 min. Measure the OD value of each well at 450 nm.

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 and plasma samples:

$$\text{BCAA content (mmol/L)} = (\Delta A_{450} - b) \div a \times f$$

2. Tissue and cell samples:

$$\text{BCAA content (mmol/gprot)} = (\Delta A_{450} - b) \div a \div C_{pr} \times f$$

[Note]

ΔA_{450}: $OD_{sample} - OD_{blank}$.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

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)	0.65	0.78	1.02
%CV	0.6	1.0	0.8

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)	0.65	0.78	1.02
%CV	3.4	8.5	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 100.3%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (mmol/L)	0.5	1	1.5
Observed Conc. (mmol/L)	0.47	1.04	1.55
Recovery rate (%)	94	104	103

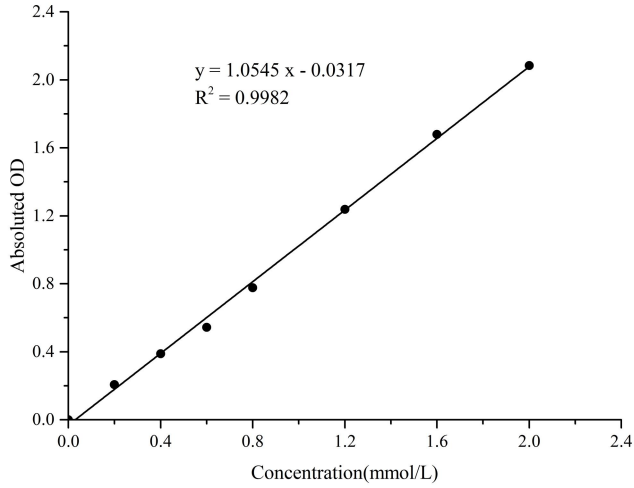
Sensitivity

The analytical sensitivity of the assay is 0.009 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	0.2	0.4	0.6	0.8	1.2	1.6	2
OD value	0.122	0.327	0.513	0.675	0.894	1.354	1.804	2.203
	0.124	0.331	0.511	0.657	0.905	1.368	1.801	2.211
Average OD value	0.123	0.329	0.512	0.666	0.890	1.361	1.803	2.207
Absoluted OD value	0	0.206	0.389	0.543	0.777	1.238	1.680	2.084



Appendix II Example Analysis

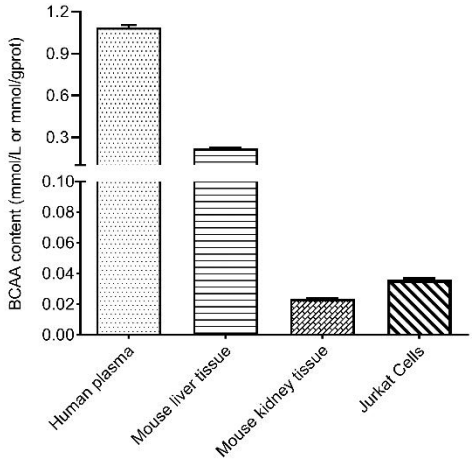
Example analysis：

Take 10 μL of 10% mouse liver tissue homogenate which dilute for 5 times and carry the assay according to the operation steps. The results are as follows:

Standard curve: y =1.0545 x - 0.0317, the OD of the blank well is 0.121, the OD of the sample well is 0.564, ΔA_{450} = 0.564 - 0.121 = 0.443, the concentration of protein is 10.12 gprot/L, and the calculation result is:

BCAA content (mmol/gprot) = (0.443 + 0.0317) ÷ 1.0545 ÷10.12 × 5 = 0.222 mmol/gprot

Detect human serum (dilute for 10 times), 10% mouse liver tissue homogenate (the concentration of protein is 10.12 gprot/L, dilute for 5 times), 10% mouse kindey tissue homogenate (the concentration of protein is 8.02 gprot/L, dilute for 5 times) and 2×10^6 Jurkat cells (the concentration of protein is 1.02 gprot/L) 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.