TECHNICAL MANUAL

Branched Chain Amino Acids (BCAA) Colorimetric Assay Kit

- **SKU CODE:** MAES0526
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate and measure OD value

2. Intended use

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

3. 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: branched chain amino acids under the catalysis of enzymes generate products that react with a chromogenic agent to produce a colored material, which has maximum absorption at 450 nm. The content of BCAA in samples is calculated by measuring the OD value at 450 nm using a standard curve.

4. 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

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
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	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (440-460 nm, optimum wavelength: 450 nm), Incubator (37°C)

Reagents:

Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to 25°C before use.
2. **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. **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. **Preparation of reaction working solution:** Before testing, 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 use within the same day.
5. **Preparation of 2 mmol/L standard solution:** Before testing, 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. **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 to serial concentrations. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.6, 0.8, 1.2, 1.6, 2 mmol/L.

7. Sample preparation

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°C.

4. Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used within the same day.

5. Meanwhile, determine the protein concentration of supernatant (animal tissue: MAES0177; plant tissue: MAES0126).

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 an ultrasonic cell disruptor at 4°C.

4. Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be used within the same day.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

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

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 perform a pretest to confirm the dilution factor.

8. Operating steps

1. Standard wells: Add 10 µL of standard solution with different concentrations to the corresponding wells. Sample wells: 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.

9. Calculation

The standard curve:

1. Average the duplicate readings for each standard.

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

3. Plot the standard curve using absolute OD value of standards and corresponding concentrations 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:

BCAA content (mmol/L) = (ΔA_450 - b) ÷ a × f

2. Tissue and cell samples:

BCAA content (mmol/gprot) = (ΔA_450 - b) ÷ a ÷ C_{pr} × f

[Note]

ΔA_450: $OD_{sample} - OD_{blank}$

f: Dilution factor of sample before test

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

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Inter-assay Precision

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

Recovery

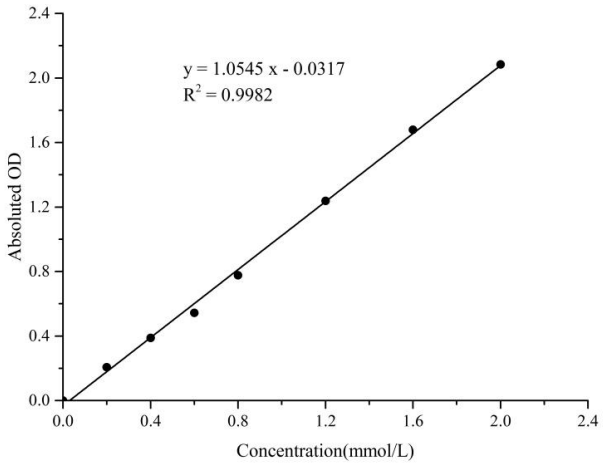
Three samples of high concentration, middle concentration and low concentration were tested with each concentration assayed 6 times in parallel to obtain an average recovery rate of 100.3%.

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.

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), the standard curve and data are provided below for reference only.



11. Appendix II Example Analysis

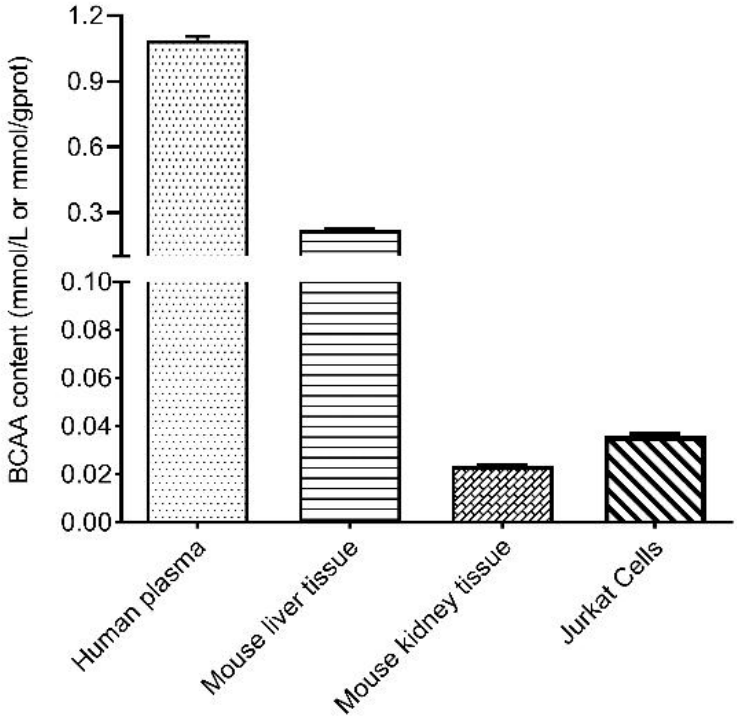
Example analysis:

Take 10 µL of 10% mouse liver tissue homogenate diluted 5 times and carry out the assay according to the operating steps. The results are as follows:

Standard curve: y = 1.0545x - 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 (diluted 10 times), 10% mouse liver tissue homogenate (protein concentration is 10.12 gprot/L, diluted 5 times), 10% mouse kidney tissue homogenate (protein concentration is 8.02 gprot/L, diluted 5 times) and 2×10^6 Jurkat cells (protein concentration is 1.02 gprot/L) according to the protocol, the results are as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance is not within the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.