Branched Chain Amino Acid Assay Kit

Branched Chain Amino Acid Assay Kit is a detection kit for the quantification of free Branched Chain Amino Acid in serum, plasma, urine, cell lysate and tissue lysate.

Catalog number: ARG82227

Package: 192 assays

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

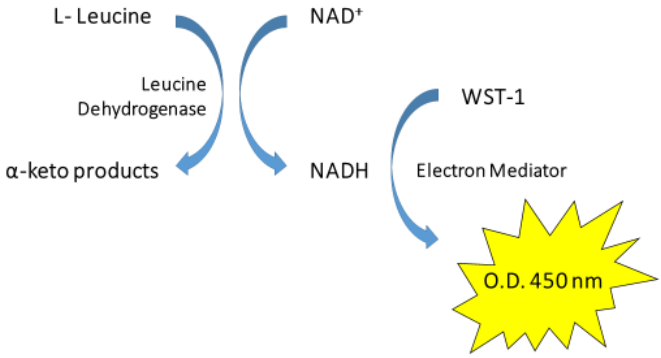
A branched-chain amino acid (BCAA) is an amino acid having an aliphatic side-chain with a branch (a central carbon atom bound to three or more carbon atoms). Among the proteinogenic amino acids, there are three BCAAs: leucine, isoleucine, and valine. Non-proteinogenic BCAAs include 2-aminoisobutyric acid.

The three proteinogenic BCAAs are among the nine essential amino acids for humans, accounting for 35% of the essential amino acids in muscle proteins and 40% of the preformed amino acids required by mammals. Synthesis for BCAAs occurs in all locations of plants, within the plastids of the cell, as determined by presence of mRNAs which encode for enzymes in the metabolic pathway.

BCAAs fill several metabolic and physiologic roles. Metabolically, BCAAs promote protein synthesis and turnover, signaling pathways, and metabolism of glucose. Oxidation of BCAAs may increase fatty acid oxidation and play a role in obesity. Physiologically, BCAAs take on roles in the immune system and in brain function. BCAAs are broken down effectively by dehydrogenase and decarboxylase enzymes expressed by immune cells, and are required for lymphocyte growth and proliferation and cytotoxic T lymphocyte activity. Lastly, BCAAs share the same transport protein into the brain with aromatic amino acids (Trp, Tyr, and Phe). Once in the brain BCAAs may have a role in protein synthesis, synthesis of neurotransmitters, and production of energy. [Provide by Wikipedia: Branched-chain amino acid]

PRINCIPLE OF THE ASSAY

This Branched Chain Amino Acid Assay Kit employs a simple colorimetric assay to measure the total amount of free BCAAs (Leucine, Isoleucine and Valine) in food or biological sample. The assay is based on an enzyme driven reaction: L-Leucine, L-valine, and L-isoleucine are converted by Leucine Dehydrogenase (in the presence of excess NAD+) into their corresponding α-keto products (α-ketoisocaproate, α-ketovalerate, or α-ketoisovalerate) plus ammonia and NADH. The converted NADH is then detected colorimetrically with WST-1 which is converted to the formazan form in the presence of an electron mediator. Samples are compared to a known concentration of L-Leucine standard within the 96-well microtiter plate format. The concentration of BCAAs in the samples is then determined by comparing the 450 nm absorbance of samples to the standard curve.



MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store the L-Leucine Standard, WST-1 Reagent, and Leucine Dehydrogenase at -20°C. The WST-1 reagent is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles. Store the NAD+ at -80°C. Store the 5X Assay Buffer at room temperature.

Component	Quantity	Storage information
L-Leucine Standards (100 mM)	30 µL	-20°C
5X Assay Buffer	12 mL	Room temperature
NAD+	400 µL	-80°C
WST-1 Reagent (amber tube)	2 mL	-20°C
100X Leucine Dehydrogenase (30 U/mL)	100 µL	-20°C

Note: One unit is defined as the amount of enzyme that will form 1 micromole of NADH per minute.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate Reader capable of reading at O.D. 450 nm
- Deionized or Distilled water
- Standard 96-well clear microtiter plate or clear cell culture microplate
- 1X PBS
- Pipettes and pipette tips
- Multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, store the L-Leucine Standard, WST-1 Reagent, and Leucine Dehydrogenase at -20°C. The WST-1 reagent is light sensitive and must be stored accordingly. Avoid multiple freeze / thaw cycles. Store the NAD+ at -80°C. Store the 5X Assay Buffer at room temperature.
- This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue Lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Perform dilutions in PBS.

Cell Lysates: Resuspend cells at 1-2 x 10^6 cells/mL in PBS. Homogenize or sonicate the cells on ice. Centrifuge at 10,000 x g for 10 minutes at 4°C to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in PBS.

Plasma: Collect blood with EDTA, heparin or citrate and centrifuge at 2000 x g and 4°C for 10 minutes.

Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum / plasma sample.
3. All samples should be assayed immediately or stored at -80°C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.

REAGENT PREPARATION

- **1X Assay buffer:** Dilute the 5X Assay Buffer into deionized water to yield 1X Assay Buffer. (E.g., add 12 mL of 5X Assay Buffer into 48 mL of deionized water to a final volume of 60 mL)
- **1X Leucine Dehydrogenase Solution:** Dilute the 100X Leucine Dehydrogenase into 1X Assay Buffer to yield 1X Leucine Dehydrogenase. (E.g., add 10 µL of 100X Leucine Dehydrogenase into 0.99 mL of 1X Assay Buffer to a final volume of 1 mL)

  Note: Prepare only enough for immediate use.
- **Reaction Mix:** Dilute the WST-1 Reagent 1:10 and NAD+ 1:100 in 1X PBS. (E.g., add 200 µL WST-1 reagent and 20 µL of NAD+ to 1780 µL of 1X PBS to a final volume of 2 mL) This Reaction Mix volume is enough for 20 assays.

  Note: Prepare only enough for immediate use.
- **Standards:** Prepare fresh L-Leucine standards by diluting in 1X PBS according to the Table below.

Standard tubes	Final L-Leucine conc. (µM)	Volume of 1X PBS (µL)	Volume of L-Leucine Standard (µL)
S1	1000	495	5 of 100 mM L-Leucine Standard
S2	500	250	250 of S1
S3	250	250	250 of S2
S4	125	250	250 of S3
S5	62.5	250	250 of S4
S6	31.3	250	250 of S5
S7	15.6	250	250 of S6
S0	0	250	0

ASSAY PROCEDURE

Prepare and mix all reagents thoroughly before use. Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Note: Each sample replicate requires two paired wells, one positive well and one endogenous control well.

1. Add **50 µL** of **samples** or serial **diluted Bile Acid Standards** into 96-well microplate. (Each sample add into two separate wells)
2. Add **50 µL** of **Reaction Mix** to each well.
3. Add **50 µL** of **1X Leucine Dehydrogenase Solution** to all standards and half of the paired sample wells. (Positive wells)
4. Add **50 µL** of **1X Assay Buffer** to the remaining half of paired sample wells. (endogenous control wells)
5. Mix well and incubate for **5-30 minutes** at **room temperature** on a microplate shaker.

   Note: This assay is **continuous** (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
6. Read the plate at **450 nm** using a microplate reader.

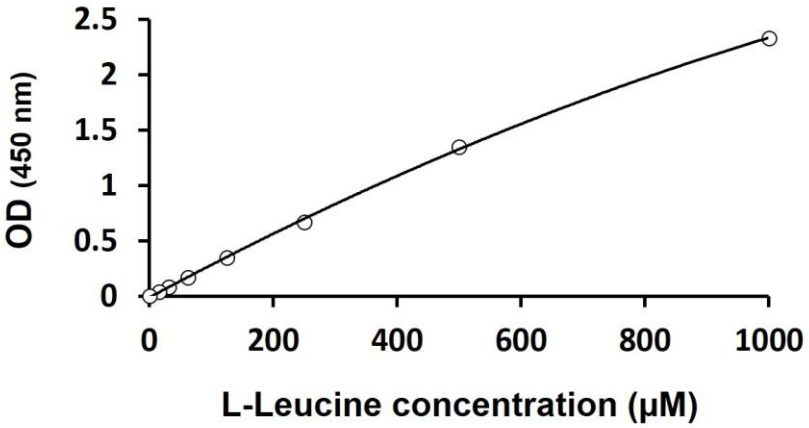
CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of standards and samples.
2. Subtract the average value of Standard 0 from all standard values.
3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
4. Subtract the sample well values without Leucine Dehydrogenase (endogenous control wells) from the sample well values containing Leucine Dehydrogenase (Positive wells) to obtain the difference. The absorbance difference is due to the enzyme Leucine Dehydrogenase activity:

   $\Delta A = A_{Positive} - A_{Control}$
5. Compare the change in absorbance Δ A of each sample to the standard curve to determine and extrapolate the quantity of BCAA present in the sample. Only use values within the range of the standard curve.

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Branched Chain Amino Acid Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

15.6 µM

Intra-assay and Inter-assay precision

The CV value of intra-assay and inter-assay was ≤ 10 %