NOTE: Revisions to "Preparation of Reagents" and Assay Protocol

Product Manual

Branched Chain Amino Acid Assay Kit

Catalog Number

MET-5056	192 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Amino acids are organic compounds that contain amine (-NH2) and carboxyl (-COOH) functional groups, as well as a side-chain (R group) which confers uniqueness to each amino acid. The main elements of an amino acid are carbon, hydrogen, oxygen, and nitrogen, although other elements can be found in some amino acids. About 500 amino acids are known, but only 20 are coded in the human genome. A Branched Chain Amino Acid (BCAA) contains a branch structure side chain with one central carbon connected to at least three other carbon atoms. The only three BCAAs coded by the human genome are leucine, valine, and isoleucine.

BCAAs play key roles in animal metabolism and physiology. BCAAs enhance protein synthesis, signaling pathways, and glucose metabolism. On a physiological level, BCAAs function within the immune system. BCAAs are degraded by dehydrogenase and decarboxylase enzymes, are found in immune cells, and are required for lymphocyte growth, proliferation, and activity. BCAAs are transported into the brain by the same enzyme used to transport aromatic amino acids (tryptophan, tyrosine, and phenylalanine). Once in the brain BCAAs contribute to protein synthesis, neurotransmitter synthesis, and energy production. In humans, rats and mice models, BCAA levels in the blood are elevated in obese, insulin resistant models of diet-induced diabetes. This result suggests the possibility that BCAAs promote obesity and diabetes pathogenesis. In mice fed limited amounts of BCAAs, glucose tolerance is improved and weight loss is observed.

Cell Biolabs' Branched Chain Amino Acid Assay Kit is a simple colorimetric assay that measures the total amount of free BCAAs (Leucine, Isoleucine, and Valine) present in foods or biological samples in a 96-well microtiter plate format. BCAAs in polypeptide chains (peptides and proteins) are not detected. Each kit provides sufficient reagents to perform up to 192 assays*, including blanks, L-Leucine standards and unknown samples. Sample BCAA concentrations are determined by comparison with a known L-Leucine standard. The kit has a detection sensitivity limit of 15.6 µM BCAAs.

**Each unknown sample replicate requires two paired wells, one positive well and one endogenous control well.*

Assay Principle

Cell Biolabs' Branched Chain Amino Acid Assay Kit measures BCCAs within food or biological samples. 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. Samples and standards are then read with a standard 96-well colorimetric plate reader (Figure 1).

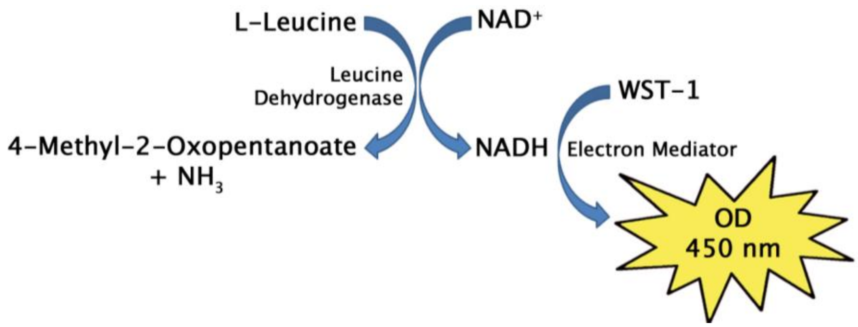

Figure 1. Branched Chain Amino Acid Assay Principle.

Related Products

1. STA-674: Glutamate Assay Kit
2. STA-675: Hydroxyproline Assay Kit
3. STA-680: Glucose Assay Kit (Colorimetric)
4. STA-682: Total Carbohydrate Assay Kit
5. MET-5054: L-Amino Acid Assay Kit (Colorimetric)

Kit Components (shipped on blue ice)

1. L-Leucine Standard (Part No. 50561C): One 30 µL tube at 100 mM.
2. 5X Assay Buffer (Part No. 50562A): One 12 mL bottle.
3. NAD+ (Part No. 50563D): One 400 µL tube.
4. WST-1 Reagent (Part No. 50564C): Two 1 mL amber tubes.
5. Leucine Dehydrogenase (Part No. 50565C): One 100 µL tube at 30 U/mL

   Note: One unit is defined as the amount of enzyme that will form 1.0 micromole of NADH per minute.

Materials Not Supplied

1. Distilled or deionized water
2. 1X PBS
3. Standard 96-well clear microtiter plate and/or clear cell culture microplate

Storage

Upon receipt, store the L-Leucine Standard, WST-1 Reagent, and Leucine Dehydrogenase at -20ºC. 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.

Preparation of Reagents

- 1X Assay Buffer: Dilute the 5X Assay Buffer 1:5 with deionized water (48 mL) to make 60 mL of a 1X solution. Stir or vortex to homogeneity. Store at room temperature.

- 1X Leucine Dehydrogenase Solution: Dilute Leucine Dehydrogenase stock 1:100 in 1X Assay Buffer.
  Note: Prepare only enough for immediate use.
- Reaction Mix: Dilute the WST-1 Reagent 1:10 and NAD+ 1:100 in 1X PBS. For example, add 200 µL WST-1 reagent and 20 µL of NAD+ to 1780 µL of 1X PBS for a total of 2 mL. This Reaction Mix volume is enough for 20 assays.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Tissue lysates: Sonicate or homogenize tissue sample in cold PBS and centrifuge at 10000 xg 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 to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in PBS.
- Serum, plasma or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary in PBS.

  Notes: 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.

Preparation of Standard Curve

Prepare fresh L-Leucine standards before use by diluting in 1X PBS according to Table 2 below.

Standard Tubes	100 mM L-Leucine Solution (µL)	1X PBS (µL)	L-Leucine (µM)
1	5	495	1000
2	250 of Tube #1	250	500
3	250 of Tube #2	250	250
4	250 of Tube #3	250	125
5	250 of Tube #4	250	62.5
6	250 of Tube #5	250	31.3
7	250 of Tube #6	250	15.6
8	0	250	0

Table 2. Preparation of L-Leucine Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each unknown sample replicate requires two paired wells, one positive well and one endogenous control well.
2. Add 50 µL of each standard into wells of a 96-well microtiter plate.
3. Add 50 µL of each unknown sample to each of two separate wells.
4. Add 50 µL of Reaction Mix to all standards and unknown samples.

5. Add 50 µL of 1X Leucine Dehydrogenase Solution to all standards and half of the paired unknown sample wells (positive wells).
6. Add 50 µL of 1X Assay Buffer to the remaining half of the paired unknown sample wells (endogenous control wells).
7. Mix all well contents thoroughly and incubate for 5-30 minutes at room temperature on an orbital shaker.

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

Example of Results

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

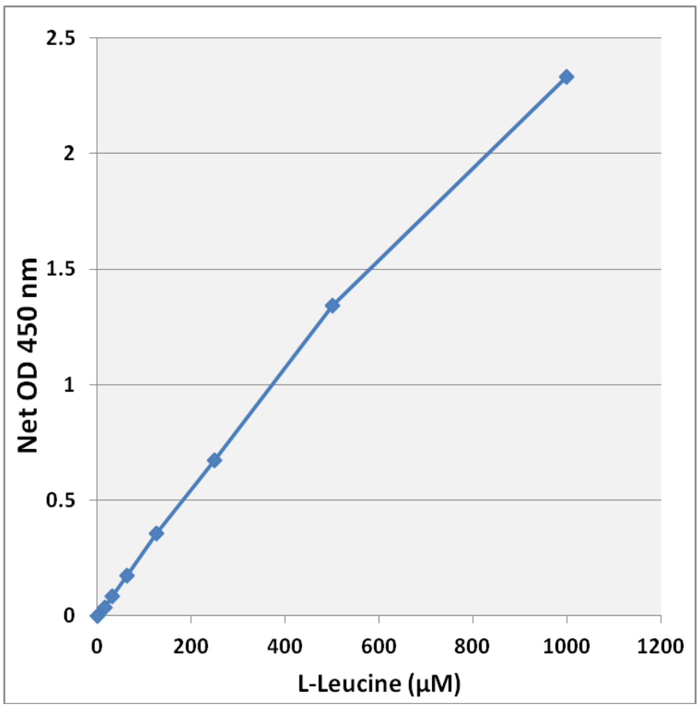

Figure 2: L-Leucine Standard Curve.

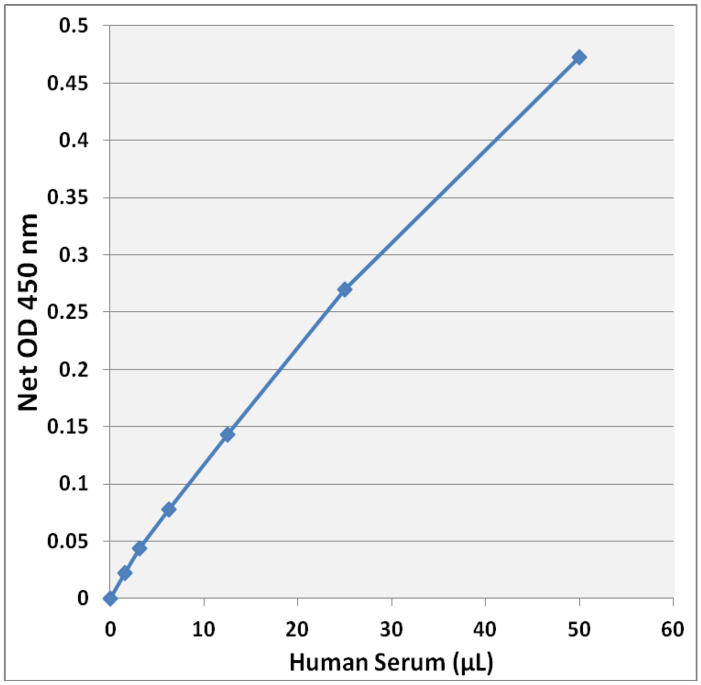

Figure 3: BCCA Detection in Human Serum using the Branched Amino Acid Assay Kit.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
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 ΔA 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.

References

1. Wagner I, Musso H (1983). *Angew. Chem. Int.* **22**: 816–828.
2. Sakami W, Harrington H (1963). *Annual Review of Biochemistry*. **32** (1): 355–98.
3. Monirujjaman, Md. (2014). *Advances in Molecular Biology*.1–6
4. Babchia, N (2010). *Investigative Ophthalmology & Visual Science*. **51**: 421–429.
5. Lynch CJ Adams SH (2014). *Nat Rev. Endocrin*. **10**: 723–736.
6. Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M (2009). *Cell Metabolism*. **9**: 311–326
7. Fontana L, Cummings NE, Arriola Apelo SI, Neuman JC, Kasz I, Schmidt BA, Cava E, Spelta F, Tosti V (2016). *Cell Reports*. **16**: 520–30.
8. White PJ, Lapworth AL, An Ji, Wang L, McGarrah RW, Stevens RD, Ilkayeva O, George T, Muehlbauer MJ. *Molecular Metabolism*. **5**: 538–551.

Recent Product Citations

1. Park, S. et al. (2025). Epigenetic regulation of intracellular branched-chain amino acid homeostasis maintains a normal lifespan. *iScience*. **28**(7):112846. doi: 10.1016/j.isci.2025.112846.
2. Nemoto, S. et al. (2025). Tetraspanin7 in Adipose Tissue Remodeling and Its Impact on Metabolic Health. *Mol Metab*. doi: 10.1016/j.molmet.2025.102168.
3. Jo, C. et al. (2023). Construction and Modeling of a Coculture Microplate for Real-Time Measurement of Microbial Interactions. *mSystems*. **8**(2):e0001721. doi: 10.1128/msystems.00017-21.
4. Sing, C.N. et al. (2022). Identification of a modulator of the actin cytoskeleton, mitochondria, nutrient metabolism and lifespan in yeast. *Nat Commun*. **13**(1):2706. doi: 10.1038/s41467-022-30045-9.
5. Akasu, R. et al. (2022). Calpain-mediated proteolytic production of free amino acids in vascular endothelial cells augments obesity-induced hepatic steatosis. *J Biol Chem*. doi: 10.1016/j.jbc.2022.101953.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com