Datasheet for ABIN5067587

Branched Chain Amino Acid Assay Kit

3 Images

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Overview

Quantity:	192 tests
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	Branched Chain Amino Acid Assay Kit measures BCCAs within food or biological samples.
Sensitivity:	15.6 µM
Characteristics:	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 µ M BCAAs. *Each unknown sample replicate requires two paired wells, one positive well and one endogenous control well.
Components:	1. L-Leucine Standard : One 30 µL tube at 100 mM. 2. 5X Assay Buffer : One 12 mL bottle. 3. NAD+ : One 400 µL tube. 4. WST-1 Reagent : One 2 mL amber tube. 5. Leucine Dehydrogenase : 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. 3

Target Details

Background:	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

Target Details

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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Protocol:	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 .
Reagent Preparation:	• 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. • Reaction Mix: Prepare the Reaction Mix by diluting the WST-1 Reagent 1:10, NAD+ 1:100, and Leucine Dehydrogenase 1:100 in 1X Assay Buffer. For example, add 200 µL WST-1 reagent, 20 µL of NAD+, and 20 µL of Leucine Dehydrogenase to 1760 µL of 1X Assay Buffer for a total of 2 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4 °C. Note: Prepare only enough for immediate use by scaling the above example proportionally. • Endogenous Control Mix: Prepare the Endogenous Control Mix by diluting the WST-1 Reagent 1:10 and NAD+ 1:100, in 1X Assay Buffer. For example, add 200 µL WST-1 reagent

Application Details

	and 20 µL of NAD+ to 1780 µL of 1X Assay Buffer for a total of 2 mL. This Endogenous Control Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4 °C. Note: Prepare only enough for immediate use by scaling the above example proportionally.
Sample Preparation:	• 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 106 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. 4 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.
Assay Procedure:	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 100 µL of Reaction Mix to all standard wells and one half of the paired unknown sample wells. 5. Add 100 µL of Endogenous Control Mix to the remaining paired unknown sample wells. 6. Mix all well contents thoroughly and incubate for 5 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. 7. Read the plate with a spectrophotometric microplate reader at 450 nm.
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 (Control) from the sample well values containing Leucine Dehydrogenase (LD) to obtain the difference. The absorbance difference is due to the enzyme Leucine Dehydrogenase activity (Figure 3): ΔA = ALD - AControl 5. Compare the change in absorbance ?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.
Restrictions:	For Research Use only

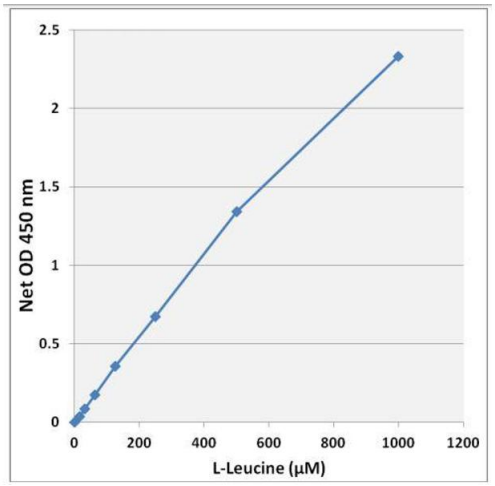
Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.

Handling

Storage:	-20 °C/-80 °C
Storage Comment:	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 4°C.

Images



Biochemical Assay

Image 1. L-Leucine Standard Curve.

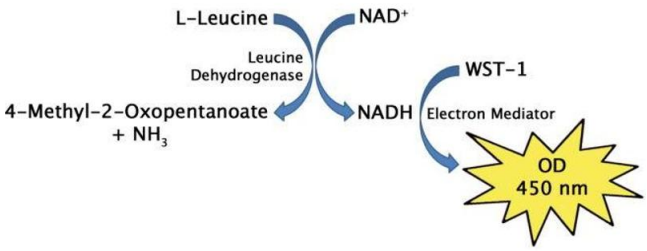
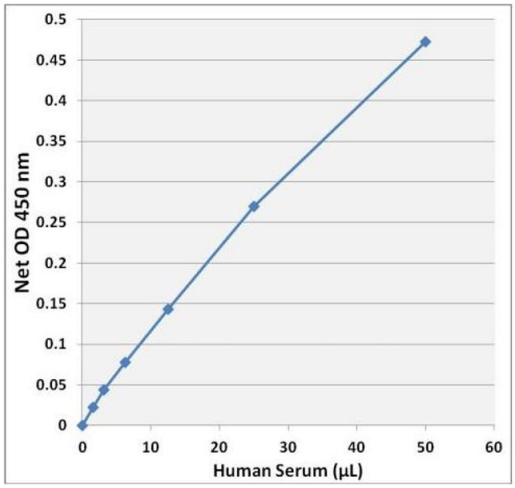


Image 2. Branched Chain Amino Acid Assay Principle.



Biochemical Assay

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