MA-0157

Branched Chain Amino Acid Assay

(100 wells, Colorimetric, OD 450 nm, Store at -20°C)

Introduction:

The branched-chain amino acids, comprising leucine, isoleucine, and valine, are deemed essential, and must be obtained through diet as the body cannot produce them independently. BCAAs are particularly important for muscle health and growth. Leucine, in particular, acts as a powerful stimulator of protein synthesis and insulin secretion. BCAAs also act as an energy source during periods of intense exercise, preserving muscle mass and preventing their breakdown. BCAAs are unique in their ability to bypass the liver and be directly utilized by muscles for energy production. An imbalance in BCAA metabolism is linked to various health conditions like insulin resistance, obesity, and muscle wasting. Some of this appears to occur through mTORC impairment. On the other hand, maintaining an appropriate BCAA ratio is associated with improved insulin sensitivity and a reduced risk of chronic diseases. AkrivisBio's BCAA Assay is a simple direct method of measuring the BCAA's in a variety of sample types with a sensitivity down into the low nanomole range vs normal serum concentrations in the 50-300 nmole/ml range.

Assay Principle:

1 - Leucine dehydrogenase which recognizes all of the BCAA's as substrates oxidizes Leu, Ile and Val with the formation of NADH.
2 - NADH transfers its reducing equivalents to the redox dye, WST-8 converting it from its nearly colorless tetrazolium form to its intensely colored formazan.

Assay Components:

Assay Buffer	25 ml	WM	MA-0157A
Leucine Dehydrogenase	lyoph	Green	MA-0157B
WST-8 Reagent	lyoph	Red	MA-0157C
Leu Standard	100 µl	Yellow	MA-0157D

Storage and Handling Considerations:

Store the unopened kit at -20°C. Allow all components to come to room temperature and centrifuge all vials briefly prior to opening.
Assay Buffer: Ready to use as supplied. Store at 4°C.
Leucine Dehydrogenase: Reconstitute with 220 µl of Assay Buffer. Store at 4°C.
WST-8 Mix: Add 220 µl of DI H_2O and let stand for several minutes, vortex briefly and its ready to use. Store at 4°C
Leucine Standard: 0.4 mM solution, ready to use as supplied. Store at 4°C.

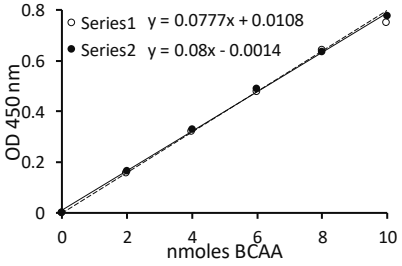
Assay Protocol:

1. Standard Curve: Transfer 0 – 5 – 10 – 15 – 20 – 25 of the Leucine Standard to a series of wells in a 96-well plate, giving 0 – 2 – 4 – 6 – 8 – 10 nmol of the Standard. Adjust well volumes to 50 µl with Assay Buffer.
2. Sample Preparation:
Homogenize tissue (10 mg) or cells (1 x 10^6) using 100 µl of Assay buffer. Centrifuge at 16,000 x g for 10 minutes to pellet insoluble materials. Transfer the clear supernatant to a fresh tube. Add up to 50 µl of sample to wells in a 96-well plate and adjust all well volumes to 50 µl with Assay Buffer. Serum concentrations are generally 60 – 300 µM so sample volume should be around 2-10 µl.
3. Initiate Reaction:
Each well requires 50 µl of Reaction Mix. Prepare sufficient material for the total number of wells to be analyzed, including all standards and test samples, containing:

	Reaction Mix
Assay Buffer	46 µl
Leucine Dehydrogenase	2 µl
WST-8 Reagent	2 µl

Add 50 µl of Reaction Mix to all wells.
4. Measurement: Start monitoring all wells at 450 nm at room temperature. Acquire data for at least 30 minutes.
5. Typical Results:

nmol Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0546	0.0508	0	0
2	0.2115	0.2170	0.1569	0.1662
4	0.3742	0.3810	0.3196	0.3302
6	0.5299	0.5417	0.4753	0.4909
8	0.6949	0.6847	0.6403	0.6339
10	0.8026	0.8263	0.7480	0.7755



6. Calculation: Subtract the zero standard value from all other standards and samples. Plot the Standard Curve. Determine the slope of the Standard Curve. This slope defines the sensitivity of the detection system. Divide the absorbance values for the test samples by the slope of the standard curve to obtain nanomoles of BCAA in the well. To convert back to nanomoles in the original sample:

A. Divide the values per well by the volume of sample added to each well = nanomoles BCAA per µl of sample
B. Multiply the nanomoles BCAA per µl of sample by the total volume of the supernatant in step 2 above = total nanomoles of BCAA per sample
C. Divide the total nanomoles of BCAA per sample by the mg tissue or # of cells used initially = nanomoles BCAA per mg tissue (or per # of cells, etc.)

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