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Product Information

Branched Chain Amino Acid Assay Kit

Catalog Number **MAK003**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The branched-chain amino acids (BCAA), leucine, isoleucine, and valine, are the most common essential amino acids in proteins. The BCAA may function in both energy production and as nutrient signals. Dietary supplementation with BCAA may aid in the treatment of various disorders such as Amyotrophic Lateral Sclerosis (ALS), latent and chronic hepatic encephalopathies, and to prevent muscle atrophy in cancer patients. BCAA may increase the rate of protein synthesis and sensitize cells to insulin and insulin-like growth factor.

In this assay, BCAA concentration is determined using a coupled enzyme reaction, which results in a colorimetric (450 nm) product, proportional to the BCAA present. The BCAA Assay Kit provides a simple convenient means of measuring the BCAAs in a variety of biological samples.

Components

The kit is sufficient for 100 assays in 96 well plates.

BCAA Assay Buffer Catalog Number MAK003A	25 mL
BCAA Enzyme Mix Catalog Number MAK003B	1 vl
WST Substrate Mix Catalog Number MAK003C	1 vl
Leucine Standard, 1 µmole Catalog Number MAK003D	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate. It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

BCAA Assay Buffer- Allow buffer to come to room temperature before use.

BCAA Enzyme Mix - Reconstitute with 220 µL of BCAA Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at 2–8 °C. Use within 2 months of reconstitution and keep cold while in use.

WST Substrate Mix - Reconstitute with 220 µL of water. Mix well by pipetting, then aliquot and store, protected from light, at 2–8 °C. Use within 2 months of reconstitution and keep cold while in use.

Leucine Standard- Ready-to-use as supplied. Store at 2–8 °C.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, unless otherwise indicated, protected from light, is recommended.

Procedure

Leucine Standards for Colorimetric Detection

Dilute 10 µl of the 1 µmole (10 mM) Leucine Standard with 90 µl of water to generate a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µl of the 1 mM standard solution into a 96 well plate to generate 0, 2, 4, 6, 8, and 10 nmole/well standards. Add BCAA Buffer to each well to bring the volume to 50 µL.

Sample Preparation

Tissue (10 mg) or cells (2×10^6) should be rapidly homogenized in 100 µL of cold BCAA Assay buffer. Centrifuge at 13,000 × g for 10 minutes at 4 °C to remove insoluble material.

Serum and other liquid samples can be directly added to the wells.

Bring samples to a final volume of 50 µL with BCAA Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Note: NADH or NADPH from cell or tissue extracts generates background for this assay. To remove the effect of NADH or NADPH background, a blank may be set up for each sample by omitting the BCAA Enzyme Mix. The blank readings can then be subtracted from the sample and standard readings.

Assay Reaction

1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Blank Sample
BCAA Assay Buffer	46 µL	48 µL
BCAA Enzyme Mix	2 µL	–
WST Substrate Mix	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

3. Measure the absorbance at 450 nm (A_{450}).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Leucine Standard. Correct for the background by subtracting the 0 (blank) value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Leucine standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample readings to obtain the corrected measurement. Using the corrected measurements, determine the amount of BCAA present in the sample from the standard curve.

Concentration of BCAA

$$S_a/S_v = C$$

S_a = Amount of BCAA in unknown sample (nmole) from standard curve

S_v = Sample volume (μL) added into the wells

C = Concentration of BCAA in sample

Sample Calculation

Amount of BCAA (S_a) = 5.84 nmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of BCAA in sample

$$5.84 \text{ nmole}/50 \ \mu\text{L} = 0.1168 \text{ nmole}/\mu\text{L}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay Not Working	Assay Buffer Ice Cold	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96-well plate used	For fluorescence assays, use black plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the BCAA Assay Buffer
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/Higher Readings in Samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Always check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Always prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear Standard Curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the reaction mix	Prepare a master reaction mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to the dilutions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so that it is in the correct linear range

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