Technical Bulletin

Branched Chain Amino Acid Kit

Catalogue number MAK562

Product Description

Leucine, isoleucine and valine, known collectively as branched-chain amino acids (BCAA) are unique amongst the essential amino acids in that they undergo oxidation to a greater extent in the peripheral tissues than in liver[1]. Concentrations of these amino acids in plasma are altered by moderate and exhausting exercise, by diet and the nutritional status of the individual[2].

In this assay, BCAA concentration is determined using a coupled enzyme reaction to catalyze the conversion of a BCAA molecule to its ketone form, which results in the conversion of NAD+ to NADH.3 NADH is detected by a colorimetric indicator, with an absorption maximum of 450 nm, proportional to the amount of BCAA present. BCAA Kit has been used to quantify the 3 BCAAs in various types of samples, such as food, dietary supplements, blood, serum, etc.

Components

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

- Assay Buffer 25 mL
  Catalogue Number MAK562A
- Enzyme Mix 0.22 mL
  Catalogue Number MAK562B
- WST Probe 0.44 mL
  Catalogue Number MAK562C
- Leucine Standard 0.11 mL
  Catalogue Number MAK562D

Reagents and Equipment Required but Not Provided

- 96-well plates, clear, flat bottom. It is recommended to use clear plates for colorimetric assays.
- Plate reader that is capable to read wavelength of 490 nm.
- Pipettors and Pipettes
- Vortex Mixer

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product is shipped on wet ice. Store at -20°C upon receipt.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. Avoid repeated freeze/thaw cycles.

Assay Buffer: Allow buffer to come to room temperature.

Enzyme Mix: Reconstitute with 220 µL Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at 2–8° C. Suitable for use 2 months after reconstitution. Keep cold while in use.

WST Probe: Allow to thaw, then vortex and aliquot while cold. Store, at 2–8° C, shielded from light. Use within 2 months and keep cold while in use.

Leucine Standard: Allow to thaw. Store at 2–8 °C.

Procedure

All Samples and Standards should be run in triplicates.

Preparation of Leucine Standards

1. Dilute 10 µL of the 10 mM Leucine Standard with 90 µL ultrapure water to generate a 1 mM standard solution.
2. Add 0, 2, 4, 6, 8, and 10 µL standard solution into a 96-well plate to generate 0, 2, 4, 6, 8, and 10 nmole/well calibration points. Add Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation

Tissues (10 mg) or cells (~2 X 10^6) should be rapidly homogenized in 100 µL cold assay buffer. Centrifuge at 13,000 x g for 10 minutes at 4° C to remove solids.

Serum and other liquid samples may be assayed directly.

If necessary, add Assay Buffer to each well to bring the volume to 50 µL. It is recommended to test several sample dilutions to ensure the readings are within the linear range of the calibration curve.

Note: NADH or NADPH from cells and tissue extracts generate background reading for this assay. Always run a blank sample without the enzyme mix (as shown in right column of Table 1) and subtract its reading from that of the sample, in order to counter this effect.

Assay Reaction

1. Prepare the reaction mixes according to Table 1. 50 µL of the appropriate reaction mix is required for each well.

Table 1.

Preparation of Reaction Mixes

Component	Samples and Standards	Blank
Assay Buffer	44 µL	46 µL
Enzyme Mix	2 µL	
WST Probe	4 µL	4 µL

2. Add 50 µL of the appropriate reaction mix to each well. Mix by using a horizontal shaker or pipetting.
3. Incubate the reaction for 30 minutes at room temperature (18-35ºC) while protected from light.
4. Measure the absorbance at 450 nm (A_{450}) using a plate reader.

Results

Calculations

The background for the assay is the value obtained for the 0 (blank) leucine standard. Subtract the blank value from all readings to eliminate background.

Use the values from the 6 Leucine concentration points (0-10 nmole/well) to plot a calibration curve and determine its slope.

Note: A fresh standard curve must be set up and read every time the assay is performed.

Subtract the blank sample (no enzyme mix) 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 (in nmol/µL or mM)

Sa/Sv = C

Sa = Concentration of BCAA in unknown sample, as calculated from the calibration curve

Sv = Sample volume (µL) added into the wells

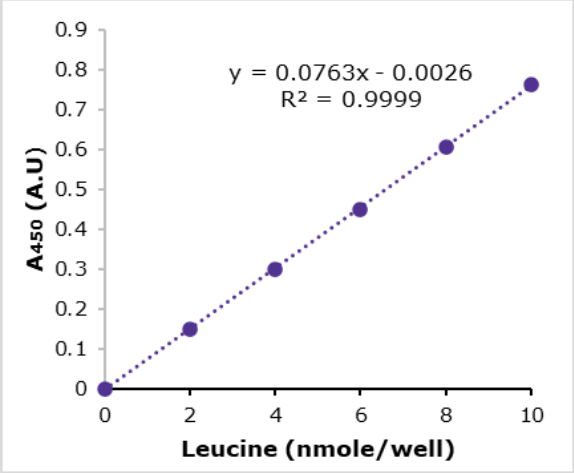
C = BCAA concentration in sample

For example, if the calculated concentration of the sample from the calibration curve is 6.2 nmole, and the amount of sample added to the well is 40 µL, then:

C = 6.24 nmole / 40 µL

BCAA concentration in sample = 0.16 nmole/µL.

Figure 1:
Example of a Leucine calibration curve.



References

1. Gleeson, M. & Maughan R. J. *Clinica Chimica Acta*, 166: 163 (1987)
2. Lemon, P. W. R & Nagle, F. J. Med. Sci. Sports Exercise, 13: 141Yue F, Zhang J, Xu J, Niu T, Lü X, Liu M. (1981)
3. Livesey G & Lund P. *Biochem J*, 188: 705 (1980)

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	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 colorimetric assays, use flat bottom, clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	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
	Presence of interfering substance in the sample	If possible, dilute sample further
	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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each 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 plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions 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 readings are in the linear range

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Technical Service

Visit the tech service page on our web site at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.