ab83374

Branched Chain Amino Acid Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Branched Chain Amino Acids in various samples

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 6a, Last updated 5 June 2023

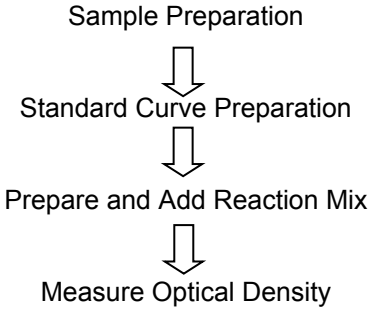
Table of Contents

1. Overview

The branched-chain amino acids or BCAAs, refer to the amino acids with non-linear aliphatic side-chains, namely leucine, isoleucine and valine. These three essential amino acids make up approximately 1/3 of skeletal muscle in the human body. BCAAs are currently used clinically to aid in the recovery of burn victims, as well as for strength supplementation for athletes. BCAAs, primarily Leu, can stimulate insulin secretion. The BCAAs have also been implicated in a wide range of other physiological effects.

Abcam's Branched Chain Amino Acid Assay Kit provides a simple convenient means of measuring the BCAA's in a variety of biological samples. The kit utilizes an enzyme assay in which BCAA is oxidatively deaminated, producing NADH which reduces the probe, generating a colored product (λ_{max} = 450 nm). Abcam's Branched Chain Amino Acid Assay Kit measures BCAAs in the range of 0 to 10 nmol per sample with a detection limit of ~0.2 nmol (~10 µM BCAA in sample). BCAAs are present in serum ~0.1-0.4 mM each (~0.125-1.5 mM combined).

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer IV/BCAA Assay Buffer	25 mL
BCAA Enzyme Mix/BCAA Enzyme Mix (Lyophilized)	1 vial
Developer Solution III/WST Substrate Mix (Lyophilized)	1 vial
Leucine Standard/Leu Standard (1 µmol)	100 µL

* Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

BCAA ENZYME MIX: Dissolve with 220 µL Assay Buffer IV/BCAA Assay Buffer. Pipette up and down to dissolve. Stable at 4°C for two months.

DEVELOPER SOLUTION III/WST SUBSTRATE MIX: Dissolve with 220 µL of dH_2O before use. Mix well, store at 4°C, protect from light. Stable for 2 months.

LEUCINE STANDARD: Ready to use as supplied. Store at 4°C.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

Note: This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

4. Assay Protocol

1. Sample Preparation:

Tissue (40 mg) or cells (4 x 10^6) can be homogenized with 200 µL assay buffer. Centrifuge at 15,000 x g for 10 min to remove cell debris and other insoluble materials.

Typical volume for serum samples should be in the range of 1-20µL.

We recommend to test cell lysate samples in the range of 0.1-1x10^6 cells/well.

Add samples to sample wells in a 96-well plate and bring the volume to 50 µL/well with assay buffer.

Typical volume for serum samples should be in the range of 1-20 µL.

We suggest testing several doses of your sample to make sure the readings are within the standard curve range. We recommend to run background controls (pg 7) for all samples.

2. Standard Curve Preparation:

Dilute 10 µL of the 10 mM Leucine Standard with 90 µL dH_2O to generate 1 mM Leucine standard. Add 0, 2, 4, 6, 8, 10 µL of the diluted Standard into a 96-well plate to generate 0, 2, 4, 6, 8, 10 nmol/well standard. Bring the volume to 50 µL with Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µL Reaction Mix containing:

	Amino Acid Measurement	Bkgd Control
Assay Buffer	46 µL	48 µL
Enzyme Mix	2 µL	---
Developer Solution III/WST Substrate Mix	2 µL	2 µL

4. Add 50 µL of the Reaction Mix to each well containing the leucine standard and test samples. Mix well. Incubate the reaction for 30 min at room temperature, protect from light.

Note:

NADH and NADPH can generate significant background. If these compounds are suspected of being in your sample at significant concentration, perform a simple background control by replacing the Enzyme Mix with 2 µL Assay Buffer. The background reading should be subtracted from the BCAA test sample readings.

5. Measure OD at 450 nm in a microplate reader.

5. Data Analysis

Correct background by subtracting the value derived from the zero BCAA standards from all readings. The background reading can be significant and must be subtracted from sample readings.

Plot the standard curve. Apply sample readings to the standard curve.

BCAA concentrations of the test samples can then be calculated:

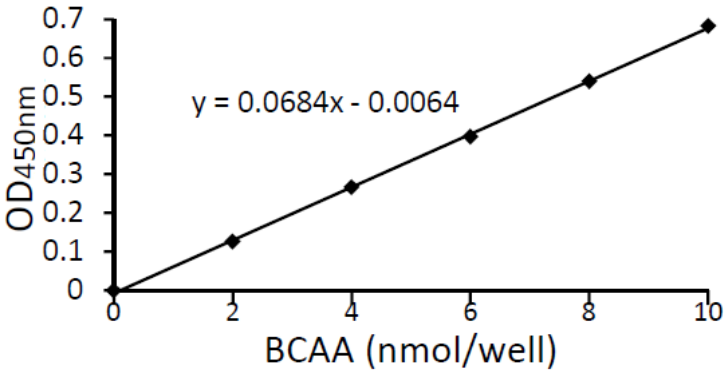
Concentration = Sa / Sv (nmol/µL, or mM)

Where:

Sa is BCAA content of unknown samples (nmol) from standard curve

Sv is sample volume (µL) added into the assay wells

BCAA molecular weights are: Leu 131.18, Ile 131.18, Val 117.15 g/mol.



Leucine Assay performed according to this protocol

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)