Product Information

Branched Chain Amino Acid (Leu/Ile/Val) Colorimetric Assay Kit

I. Kit Contents:

Components	K2085-100 100 assays	Cap Color	Part Number
Hydrolysis Buffer	25 ml	NM	K2085-C-1
Development Buffer Hydrolysis Enzyme	25 ml	WM	K2085-C-2
Mix (Lyophilized) Development Enzyme	1 vial	Blue	K2085-C-3
Mix (Lyophilized)	1 vial	Green	K2085-C-4
Developer (Lyophilized)	1 vial	Red	K2085-C-5
Gln Standard (Lyophilized)	1 vial	Yellow	K2085-C-6

II. Introduction:

The branched-chain amino acids (BCAAs) are amino acids having aliphatic side-chains with a branch, namely leucine (Leu), isoleucine (Ile) and valine (Val). They are essential amino acids and make up approximately 1/3 of skeletal muscle in the human body. BCAAs are used for strength supplementation for athletes or to aid in the recovery of burn victims. BCAAs are also involved in a wide range of other physiological effects. In addition, Leu can stimulate insulin secretion.

The Branched Chain Amino Acid (Leu/Ile/Val) Colorimetric Assay Kit provides a sensitive, simple and convenient way for detection of BCAAs in various biological fluids based on colorimetric method. The assay is based on an enzyme assay in which BCAA is oxidatively deaminated to produce NADH, which reduces the probe to generate a colored product (λmax = 450 nm). The kit can measure 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 in serum are about 0.1 - 0.4 mM each (0.125 - 1.5 mM combined).

III. Storage and Handling:

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.

IV. Reagent Reconstitution and General Consideration:

BCAA Enzyme Mix: Dissolve with 220 µl BCAA Assay Buffer. Pipette up and down to dissolve. Stable at 4°C for two months.

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

V. BCAA Assay Protocol:

1. Standard Curve: 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.

2. Sample Preparation: Tissue (20 mg) or cells (2 x 10^6) can be homogenized with 100 µl Assay buffer. Centrifuge at 15,000g for 10 minutes to remove cell debris and other insoluble materials. Add samples to sample wells in a 96-well plate and bring the volume to 50 µl/well with Assay Buffer. We suggest testing several doses of your sample to make sure the readings are within the standard curve range. Typical volume for serum samples should be in the range of 1 – 20 µl.

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	---
WST Substrate Mix	2 µl	2 µl

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. 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.

4. Measure O.D. at 450 nm in a microplate reader

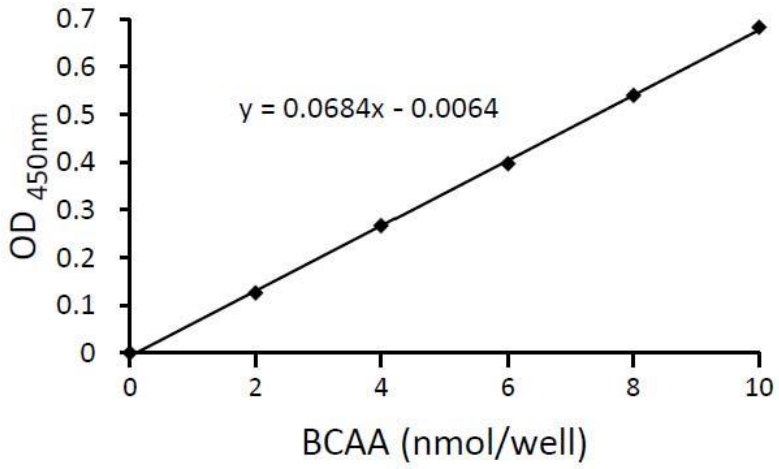
5. Calculation: Correct background by subtracting the value derived from the 0 BCAA standards from all readings (The background reading can be significant and must be subtracted from sample readings). Plot standard curve. Apply sample readings to the standard curve. BCAA concentrations of the test samples can then be calculated:

C = Sa/Sv (nmol/µl, or mM)

Where: Sa = BCAA content of unknown samples (nmol) from standard curve,

Sv = 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

General Troubleshooting Guide:

Problems	Cause	Solution
Assay not working	• Use of a different buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates ; Luminescence: White plates; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in d atasheet)	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as

	• Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer data sheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

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Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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