Recombinant Human BCAT1

Catalog Number: 9536-BA

DESCRIPTION

Source	*E. coli*-derived Lys2-Ser386 Accession # P54687-1 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	44 kDa

SPECIFICATIONS

SDS-PAGE	40-45 kDa, reducing conditions
Activity	Measured by its ability to convert leucine and alpha-ketoglutarate to alpha-ketoisocaproate and glutamate. The specific activity is >9,000 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 0.05% Tween-20, pH 8.0
- Recombinant Human BCAT1 (rhBCAT1) (Catalog # 9536-BA)
- Recombinant Human NQO-1 (rhNQO-1) (Catalog # 7567-DH)
- Glutamate dehydrogenase (GIDH) (Sigma, Catalog # G7882), 200 U/mL sock in 50 mM Tris, 0.05% Tween-20, pH 8.0
- Nicotinamide adenine dinucleotide (β-NAD) (Sigma, Catalog # N6522), 100 mM stock in deionized water
- Resazurin (Catalog # AR002)
- α-Ketoglutaric Acid (Sigma, Catalog # K2010), 1 M stock in deionized water
- L-Leucine (EMD Biosciences, Inc., Catalog # 4330), 100 mM stock in deionized water
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhBCAT1 to 0.04 µg/mL in Assay Buffer.
2. Dilute α-Ketoglutaric Acid to 100 mM in Assay Buffer.
3. Prepare Substrate Mixture containing 100 U/mL GIDH, 2 mM β-NAD, 40 µM Resazurin, 1 mM α-Ketoglutaric Acid, 4 mM L-Leucine, and 4 µg/mL rhNQO-1 in Assay Buffer.
4. Load 50 µL of 0.04 µg/mL rhBCAT-1 in a plate, and start the reaction by adding 50 µL of Substrate Mixture. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of Substrate Mixture.
5. Read at excitation and emission wavelengths of 540 nm and 585 nm (top read), respectively, in kinetic mode for 8 minutes with a 3 minute lag time in kinetic mode.
6. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank.

**Derived using calibration standard Resorufin (Sigma, Catalog # R3257).

Final Assay Conditions

Per Well:

- rhBCAT1: 0.002 µg
- GIDH: 5 U
- β-NAD: 1 mM
- rhNQO-1: 0.2 µg
- Resazurin: 0.02 mM
- α-Ketoglutaric Acid: 0.5 mM
- L-Leucine: 2 mM

PREPARATION AND STORAGE

Shipping

The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage

Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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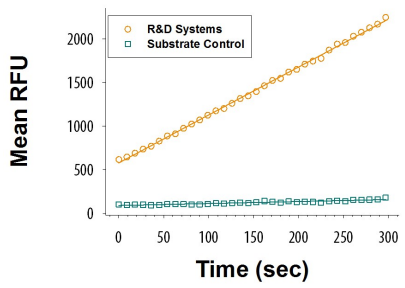
Enzyme Activity



Recombinant Human BCAT1 (Catalog # 9536-BA) is measured by its ability to convert leucine and alpha-ketoglutarate to alpha-ketoisocaproate and glutamate.

BACKGROUND

Branched-chain-amino-acid aminotransferases (BCATs) are enzymes that catalyze the first reaction in the catabolism of the essential branched-chain amino acids leucine, isoleucine, and valine to their respective keto-acids while concurrently producing glutamate. BCATs belong to the class-IV pyridoxal-phosphate-dependent (PLP-dependent) aminotransferase family of enzymes (1). There are two BCAT isozymes in humans and mammals, a mitochondrial form known as BCATm or BCAT2 and a cytosolic form known as BCATc or BCAT1 that share 55% sequence identity. In humans and rodents, BCATm is found in most tissues whereas BCATc is almost exclusively present in the nervous system (2,3) where it is thought to play a role in glutamate neurotransmitter metabolism (1, 4). The 43 kDa human BCATc exists as an active homodimer and has a unique CXXC active site near the dimerization domain (4). BCATc can be regulated by redox and is implicated as a marker for oxidative stress (5,6) and linked to Alzheimers Disease and Dementia (7). BCATc has been shown to cause cell proliferation (8) and significant evidence has been found implicating BCATc in several types of cancer (8-10). In many reports, BCATc can be further used as a marker or for diagnostic purposes in cancers (11-13).

References:

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