Recombinant Human BCAT2

Catalog Number: 9537-BA

DESCRIPTION

Source	*E. coli*-derived Ala28-Val392 Accession # O15382-1 with an N-terminal Met and 6-His tag
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	42 kDa

SPECIFICATIONS

SDS-PAGE	37-42 kDa, reducing conditions
Activity	Measured by its ability to convert leucine and alpha-ketoglutarate to alpha-ketoisocaproate and glutamate. The specific activity is >3,500 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 BCAT2 (rhBCAT) (Catalog # 9537-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 rhBCAT2 to 0.1 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 0.1 µg/mL rhBCAT2 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 three 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:

- rhBCAT2: 0.005 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 polar packs. 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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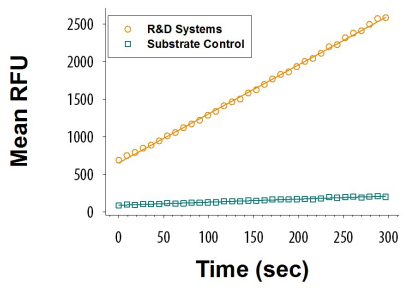
Enzyme Activity



Recombinant Human BCAT2 (Catalog # 9537-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, BCATc is almost exclusively present in the nervous system (2,3) while BCATm is constitutively expressed in most tissues and is generally thought to be important in body nitrogen metabolism (1,2). The 41 kDa human BCATm exists as an active homodimer and has a unique CXXC active site near the dimerization domain (4). Knockouts display decreased adiposity and obesity through alterations of leucine-dependent mTOR signaling making it a potential therapeutic target for obesity (5). BCATm can be regulated by oxidative stress, interfering with its ability to interact with protein disulfide isomerase (6). BCATm overexpression in the brain is detectable in Alzheimers disease and dementia (7,8) suggesting it plays an important role in glutamate toxicity, a key pathogenic feature of these diseases.

References:

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