Recombinant Human Biotinidase/BTD

Catalog Number: 7839-BT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Biotinidase/BTD protein Ala42-Asp543, with a C-terminal 6-His tag Accession # P43251
N-terminal Sequence Analysis	Ala42
Predicted Molecular Mass	58 kDa

SPECIFICATIONS

SDS-PAGE	65-80 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze the substrate biotin 4-Nitrophenyl ester (BNP). The specific activity is >190 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in NaH_2PO_4, NaCl, EDTA and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 0.2 M NaCl, 0.1% Triton, pH 7.5
- Recombinant Human Biotinidase/BTD (rhBTD) (Catalog # 7839-BT)
- (+)-Biotin 4-Nitrophenyl ester (BNP) (Sigma, Catalog # 861650), 50 mM stock in DMSO
- Dimethyl Sulfoxide (DMSO) (Sigma, Catalog # 34869)
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhBTD to 20 µg/mL in Assay Buffer.
2. Dilute room temperature BNP to 1 mM in DMSO. Mix well.
3. Load 50 µL of 20 µg/mL rhBTD in a clear strip well plate, and start the reaction by adding 50 µL of 1 mM BNP. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 1 mM BNP.
4. Incubate sealed plate at room temperature for 10 minutes in the dark.
5. Read at 405 nm (absorbance) in endpoint mode.
6. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Abs* (OD) x Conversion Factor** (pmol/OD)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard p-Nitrophenol (Sigma, Catalog # 241326).

Final Assay Conditions

Per Well:

- rhBTD: 1 µg
- BNP: 0.5 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, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

Biotinidase (BTD) is a member of the nitrilase superfamily, which consists of 12 families of nitrilases, amidases, carbamylases, and N-acyltrasferases (1). It is a thiol hydrolase releasing biotin from biotinamide, biotin-lysine, biotin-peptide conjugates and biotin methylester. It is expressed in most mammalian tissues, with high activity being present in liver, kidney, serum, intestine, and adrenal glands (2). BTD with two other proteins, sodium-dependent multivitamin transporter, and holocarboxylase synthetase, play major roles in the homeostasis of biotin (3). BTD contributes to the homeostasis through the intestinal release of free biotin from digested biotin-containing proteins and plasma transport and the recycling of biotin from breakdown products of biotinylated carboxylases. BTD deficiency can lead to a decrease in biotin bioavailability due to failure in releasing biotin from dietary proteins. It can be caused by gene mutations or from decreased secretion of BTD into the intestinal lumen (4). A recent study indicates that the BTD level in human plasma is a potential biomarker for the detection of breast cancer (5).

References:

1. Pace, H. C. and C. Brenner (2001) Genome. Biol. **2**:reviews/0001.1.
2. Chauhan, J. and K. Dakshinamurt (1986) J. Biol. Chem. **261**:4286.
3. Wolf, B. (2005) J Nur Biochem. **16**:441.
4. Zempleni, J. *et al.* (2008) Expert Rev Endocrinol Metab. **3**:715.
5. Kang, U. B. *et al.* (2010) BMC Cancer **10**:114.

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