β-Hydroxybutyrate dehydrogenase (β-HBDH)

rp216193

Preparation and specification

Appearance	White amorphous powder,lyophilized
Protein purity	⩾90% (from SDS-PAGE)
Activity	⩾300 U/mg solid
Malate dehydrogenase	⩽0.002%
Lactate dehydrogenase	⩽0.002%
NADH oxidase	⩽0.002%

Properties

EC number	1.1.1.30(Recombinant from microorganism)	
Molecular weight	27 kDa (SDS-PAGE)	
Isoelectric point	6.1	
Michaelis constants	1.46×10^{-2} M (D-3-Hydroxybutyrate),9.5×10^{-5} M (NAD^+)	
Inhibitors	Hg^{2+}, Ag^+, SDS	
Optimum pH	8.5	Fig. 1
Optimum temperature	60℃	Fig. 2
pH stability	pH 5.0-10.0 (25 ℃, 16 h)	Fig. 3
Thermal stability	Below 50 ℃ (pH 8.5, 30 min)	Fig. 4
Storage stability	At least one year at -20℃	Fig. 5

Stability and Storage

Store at -20℃ long term (12 months). Upon delivery aliquot. Avoid freeze/thaw cycle

Applications

This enzyme is useful for enzymatic determination of ketone bodies (D-3-hydroxybutyrate and acetoacetate) in clinical analysis

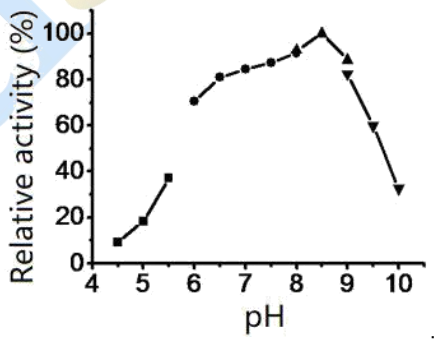

Fig. 1 Optimum pH

Buffer solution: pH 4.5-5.5, Acetate; pH 6.0-8.0, Na-phosphate; pH 8.0-9.0,

Tris-HCl. pH 9.0-10.0, Glycine-NaOH. Enzyme concentration: 1 mg/mL

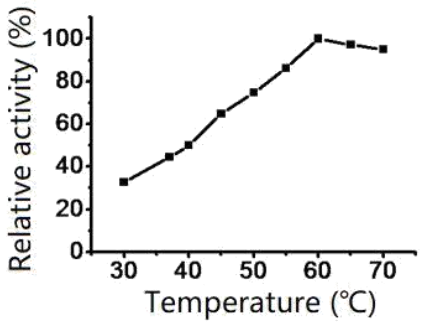

Fig. 2 Optimum temperature Reaction in 100 mM Tris-HCl buffer pH 8.5.
Enzyme concentration: 1 mg/mL

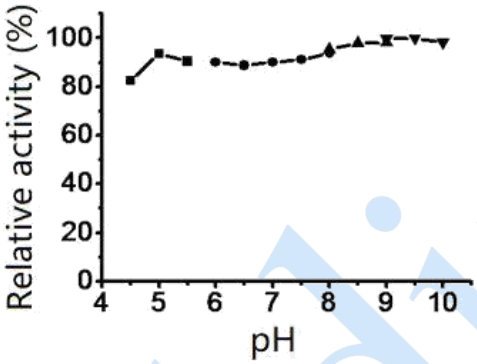

Fig. 3 pH Stability

25 ℃, 16 h-treatment with 50 mM buffer solution: pH 4.5-5.5, Acetate; pH 6.0-8.0, Na-phosphate; pH 8.0-9.0, Tris-HCl. pH 9.0-10.0, Glycine-NaOH. Enzyme concentration: 1 mg/mL

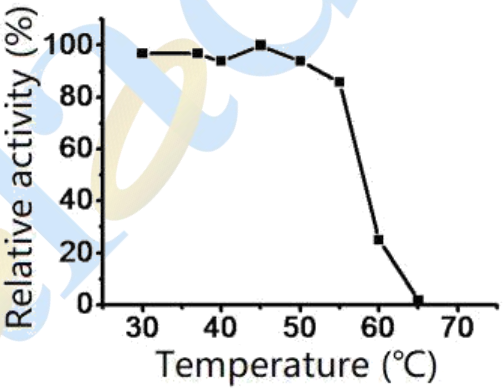

Fig. 4 Thermal stability

30 min-treatment with 100 mM Tris-HCl buffer, pH 8.5. Enzyme concentration: 1 mg/mL

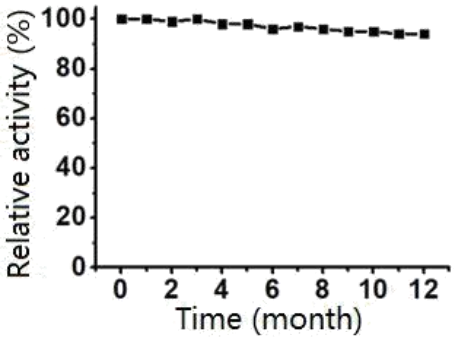

Fig.5 Storage stability (-20℃)

Assay principle

D-3-Hydroxybutyrate + NAD^+ $\xrightarrow{\text{D-3-hydroxybutyrate dehydrogenase}}$ Acetoacetate + NADH + H^+

The appearance of NADH is measured at 340 nm by spectrophotometry.

Unit definition

One unit (U) is defined as the amount of enzyme which produces 1 μmol of NADH per min under the conditions described below

Reagents preparation

Reagent I: 0.1 M pH 8.5 Tris-HCl buffer.

Reagent II:158mM D-3-Hydroxybutyrate solution, dissolved by Reagent I.

Reagent III: 27.9 mM NAD^+, dissolved by Reagent I.

Enzyme diluent: 100 mM Tris-HCl, pH 8.5, contains 0.1% BSA.

Sample: dilute the enzyme to 0.1-0.5 U/ml with enzyme diluent.

Procedure

1.Add 2.3ml Reagent I, 0.5ml Reagent II and 0.2ml Reagent III to the 3 mL cuvette (d=1.0 cm) in order, preincubated at 37℃ for 5min.

2.Add 0.1 mL the enzyme solution in the reaction mixture and mix to start the reaction, record the ΔA_s at 340 nm in 1 minute in a spectrophotometer thermostated at 37 ℃.

At the same time, measure the blank rate ΔA_b by using the same method as the test except that the enzyme diluent is added instead of the enzyme solution. $\Delta A = \Delta A_s - \Delta A_b$

Calculation

$$\text{Volume activity}(U/ml) = \frac{\Delta A \times vt \times df}{6.22 \times Vs \times 1.0} = \Delta A \times 4.98 \times df$$

$$\text{Weight activity}(U/mg) = \text{Volume activity} \times 1/C$$

Vt: Total volume (3.1mL)

Vs: Enzyme volume (0.1mL)
1.0: Light path length (cm)
df: dilution factor
C: Enzyme concentration (mg/mL)
6.22: Millimolar extinction coefficient of NADH under 340 nm ($cm^2/\mu mol$)

References

(1)F.P. Delafield, K.E. Cooksey and M. Doudoroff; J. Biol. Chem., 240, 4023 (1965).
(2)C.W. Shuster and M. Doudoroff; J. Biol. Chem., 237, 603 (1962).
(3)I. Sekuzu, P. Jurtshuk and D.E. Green; J. Biol. Chem., 238, 975 (1963).