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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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D-3-Hydroxybutyrate Dehydrogenase (3-HBDH)

Cat no. LDG0022RG

Product Overview

Specification

Appearance	White amorphous powder, lyophilized
Activity	300 U/mg or more (containing approx. 10% of stabilizers)

Properties

Stability	Stable at −20℃ for at least one year
Molecular weight	27.9 kDa
Isoelectric point	6.55

Applications

1. Enzymatic determination of ketone bodies (D-3-Hydroxybutyrate)
2. Detection of the presence of ketone bodies [1]

Assay

1. Assay principle

D-3-Hydroxybutyrate + NAD^+ —(D-3-Hydroxybutyrate dehydrogenase)→ Acetoacetate + NADH + H^+

2. Unit definition

One unit causes the formation of one micromole of NADH per minute under the conditions described below.

3. Reagents

A. Tris-HCl buffer, pH 8.5 (25℃)	0.1 M
B. 3-Hydroxybutyrate solution	158 mM [200 mg D,L-3-Hydroxybutyrate Na salt (MW = 126.09)/10 mL of Tris-HCl buffer (A)](Stable at least 5 days if stored at 4℃)
C. NAD^+ solution	27.9 mM [80 mg NAD^+ (MW = 663.43)/ 4.0 mL of Tris-HCl buffer (A)](Stable for at least 5 days if stored at 4℃)
D. Enzyme diluent	0.1 M Tris-HCl buffer, pH 8.5 containing 0.1% BSA

4. Procedure

(1) Prepare the following **working solution** immediately before use and equilibrate at 37℃ for approximately 5 minutes (for 4 reactions).

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Working Solution

Tris-HCl buffer, pH 8.5 (Reagent A)	4.6 mL
3-Hydroxybutyrate solution (Reagent B)	1 mL
NAD^+ solution (Reagent C)	0.4 mL
Total	6 mL

(2) Pipette 1.5 mL of **working solution** into a tube.

(3) Add 0.05 mL of the enzyme solution* and mix by gentle inversion.

Concentration in a reaction	
Tris-HCl buffer	0.1 M
3-Hydroxybutyrate solution	25 mM
NAD^+	1.8 mM

(4) Pipette the mixture into a cuvette (d=1.0 cm). Record the increase in optical density at 340 nm against water for 1 to 5 minutes in a spectrophotometer at room temperature and calculate the ΔOD per minute from the initial linear portion of the curve (ΔOD test). At the same time, measure the blank rate (ΔOD blank) by using the same method as the test except that the enzyme diluent is added instead of enzyme solution.

* Dissolve the enzyme preparation in ice-cold enzyme diluent **(Reagent D)** dilute to **0.1–0.5 U/mL** with the same buffer and store on ice.

(5) Activity can be calculated by using the following formula:

Volume activity (U/mL) =

$$\frac{\Delta OD/\text{ min } (\Delta OD \text{ test} - \Delta OD \text{ blank}) \times Vt \times df}{6.22 \times 1.0 \times Vs}$$

$$= \Delta OD/\text{ min} \times 4.98 \times df$$

Weight activity (U/mg) = (U/mL)×1/C

Vt: Total volume (1.55 mL)

Vs: Sample volume (0.05 mL)

6.22 : Millimolar extinction coefficient of NADH at 340 nm (cm^2/micromole)

1.0: Light path length (cm)

df: Dilution factor

C: Enzyme concentration in dissolution (mg/ mL)

Reference

1. *Md Mominul Hoque. et al.* Structure of D-3-hydroxybutyrate dehydrogenase prepared in the presence of the substrate D-3-hydroxybutyrate and NAD+. *Structure communication* (2009).

The effect of different conditions on D-3-Hydroxybutyrate Dehydrogenase

A.

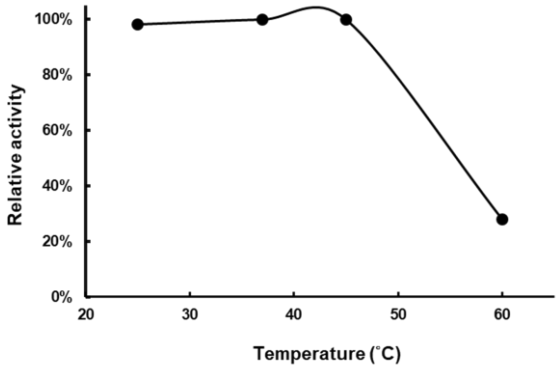

Figure A. Temperature activity of 3-HBDH. The enzyme reactions in 0.1 M Tris-HCl buffer, pH 8.5, were carried out under different temperatures.

B.

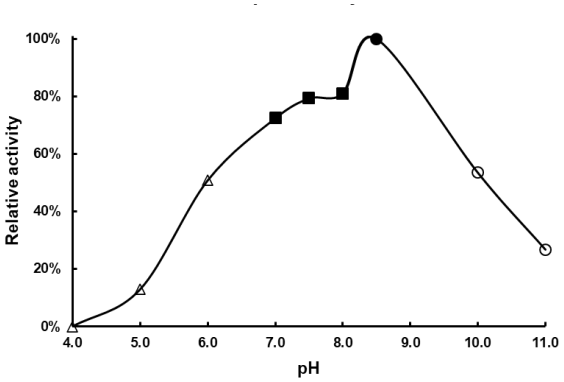

Figure B. pH activity of 3-HBDH. The buffer conditions with various pH values were used in the reaction at 37°C. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

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C.

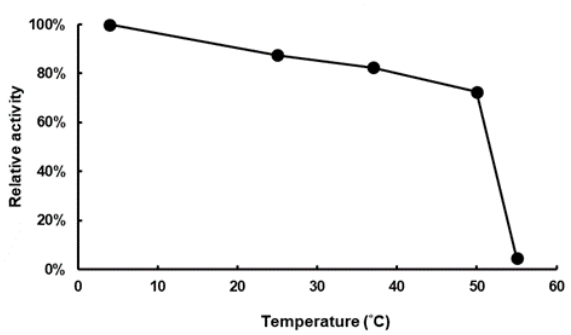

Figure C. Thermal stability of 3-HBDH. The enzyme powder was reconstituted by double-distilled water and treated with different temperatures for 15 minutes. Final concentration: 15.9 U/ mL

D.

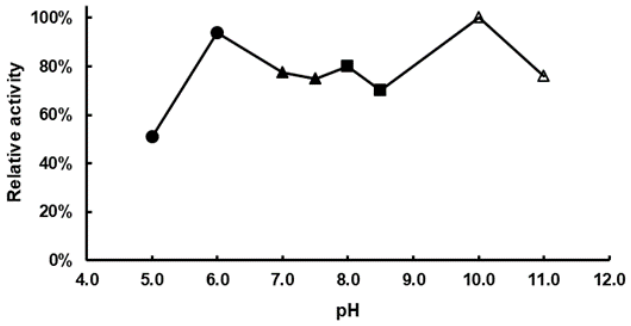

Figure D. pH stability of 3-HBDH. The enzyme powder was reconstituted by double-distilled water and treated with different pH buffer condition for 20 hours. pH 4.0-6.0, 0.1 M Sodium citrate buffer; pH 7.0-8.0, 0.1 M Potassium phosphate buffer; pH 8.5, 0.1 M Tris-HCl buffer; pH 10.0-11.0, 0.1 M Carbonate-bicarbonate buffer.

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