Cat. No.: HBDH-003

D-3-Hydroxybutyrate Dehydrogenase

(ETERBIO-EZ-HBDH-003)

Lot No.:_____

Expiry Date:

Store at -20℃

Origin

Microorganism

Specification

Appearance	Lyophilized powder
Activity	≧30 U/mg solid at 37℃

Properties

Molecular wight	approx. 27 kDa	
Isoelectric point	6.6	
Optimum pH	6.5	(Fig.1)
Optimum temperature	37℃	(Fig.2)
pH stability	7.4-8.0 (25℃, 21 hr)	(Fig.3)
Thermal stability	Below 40℃	(Fig.4)

Assay

D-3-Hydroxybutyrate + NAD^+ $\xrightarrow{\text{D-3-hydroxybutyrate dehydrogenase}}$ Cholest-4-en-3-one + NADH + H^+

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

Unit definition

One unit enzyme will cause the formation of one micromole of NADH per minute at 37℃ under the activity assay.

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Method

Reagent：

(A) 100 mM Tris-HCl buffer, pH 8.5

(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〔77.49 mg β-Nicotinamide adenine dinucleotide sodium salt (MW＝685.41) / 4.0ml of Tris-HCl buffer (A)〕(Stable for at least 5 days if stored at 4℃)

(D) Enzyme solution：Dissolve in 100 mM Tris-HCl buffer, pH 8.5

Procedure：

1. Prepare 230 µl (A), 50 µl (B), 20 µl (C), mix well and equilibrate 37℃ for about 5 minutes.
2. Add 10 µl of the enzyme solution (D) and mix in a 96 well plate.
3. Record the increase in optical density at 340 nm for 2 to 3 minutes in a spectrophotometer at 37℃, and calculate the ΔOD per minute.

Calculation：

$$\text{Volume activity (U/ml)} = \frac{(\Delta A_{340nm}/\text{min}) \times Vt \times df}{6.22 \times 1.0 \times Vs}$$

$$\text{Weight activity (U/mg)} = \frac{\text{U/ml}}{\text{mg/ml protein}}$$

Vt ：Total volume (310 µl)

Vs ：Sample volume (10 µl)

6.22 ：Millimolar extinction coefficient of NADH at 340 nm

1.0 ：Light path length (cm)

df ：Dilution factor

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Characteristics

Fig. 1 (37℃, 5 min treatment in 0.1 M buffer)

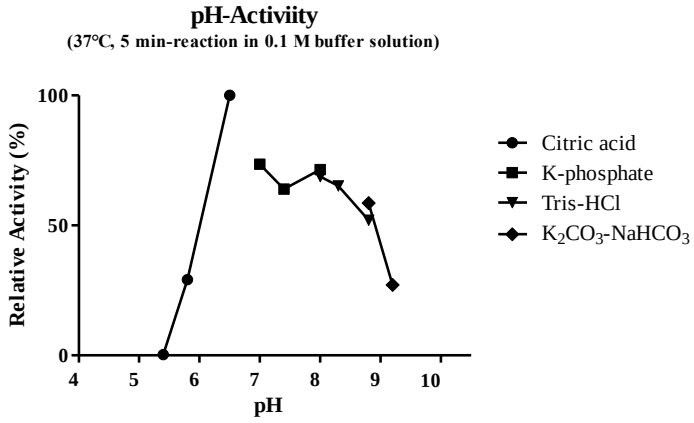


Fig. 2 (5 min in 0.1 M Citric acid buffer, pH 6.5)

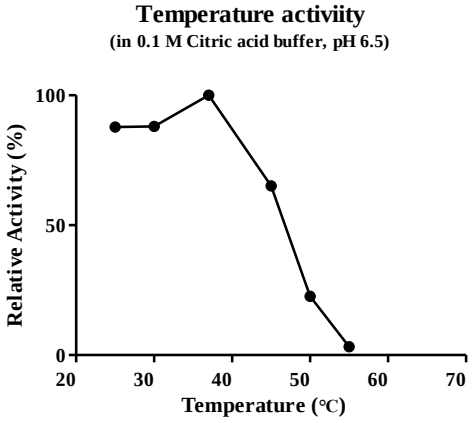


Fig. 3 (25℃, 21-hr treatment in 50 mM buffer)

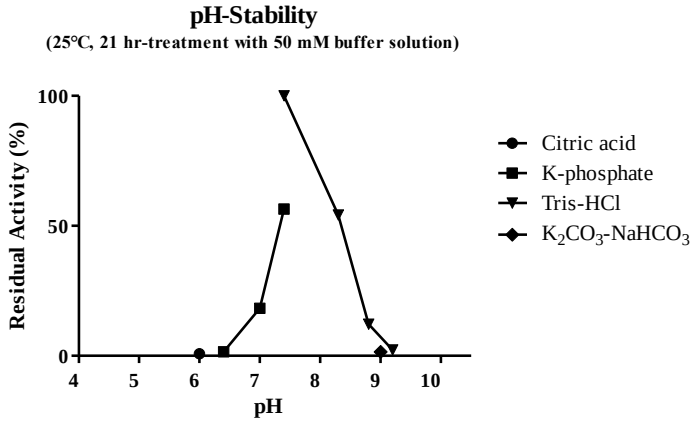
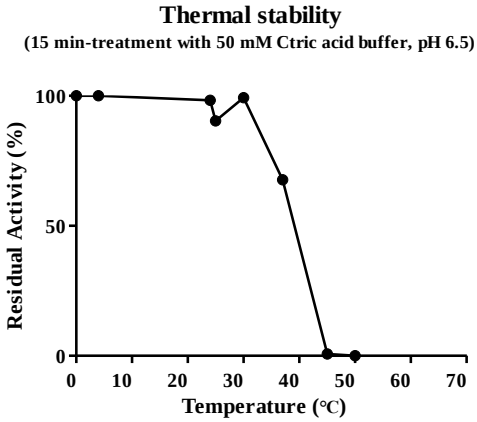


Fig. 4 (15 min in 50 mM Citric acid buffer, pH 6.5)



Substrate specificity

Table 1. The Substrate Specificity of D-3-Hydroxybutyrate dehydrogenase

Substrate	Relative activity(%)
3-Hydroxybutyrate	100
3-Hydroxypropionate	0
D,L-Lactate	0
2-Hydroxybutyrate	0
D,L-Malate	0
Gluconate	0
Glycolate	0