(Diagnostic Reagent Grade)

T-68

3-HYDROXYBUTYRATE DEHYDROGENASE [3-HBDH Ⅱ]

from *Alcaligenes faecalis*
(D-3-Hydroxybutyrate: NAD^+ oxidoreductase, EC 1.1.1.30)

D-3-Hydroxybutyrate + NAD^+ ⟶ Acetoacetate + NADH + H^+

Preparation and Specification

Appearance : White amorphous powder, lyophilized
Specific activity : More than 1,500 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 60±5 kDa (TSK G-3000SW) 30±5 kDa (SDS-PAGE)	
Isoelectric point	: pH 5.0±0.2	
Michaelis constant	: D-3-Hydroxybutyrate 1.6 × 10^{-3}M	
Optimum pH	: 8.5	Figure 1
pH stability	: 5.5-11.0 (37℃, 60 min)	Figure 2
Optimum temperature	: 45℃ (Tris-HCl buffer)	Figure 3
Thermal stability	: Stable at 37℃ and below (pH 8.5, 10 min)	Figure 4
Effect of metal ions	: See Table 2	
Effect of detergents	: See Table 3	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **ketone bodies** when coupled with acetoacetate decarboxylase (AADC), thio-NAD and NADH.

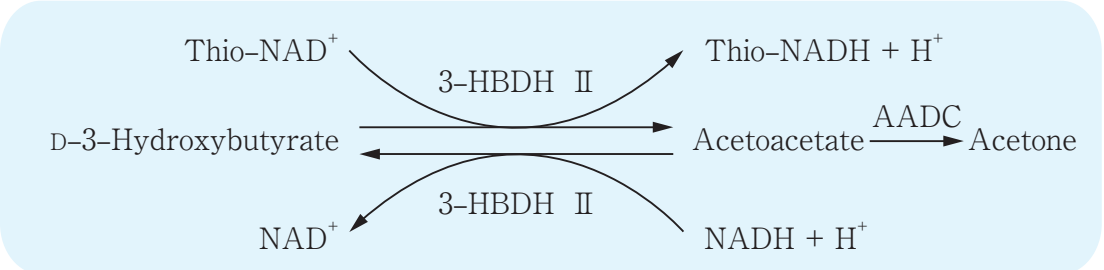

Table 1. Substrate specificity

Substrate	Relative activity (%)
3-Hydroxybutyric acid	100
2-Hydroxybutyric acid	0
D,L-Lactic acid	0
D,L-Malic acid	0
Gluconic acid	0
Glycolic acid	0

Table 2. Effect of metal ions on 3-HBDH Ⅱ activity

Metal ion (1mM)	Relative activity (%)
None	100
LiCl	104
NaCl	101
NH_4Cl	101
KCl	98
CsCl	100
$CuCl_2$	13
$BaCl_2$	107
$ZnCl_2$	88
$PbCl_2$	60
$NiCl_2$	49
$CoCl_2$	44
$MnCl_2$	40
$CaCl_2$	91
$MgCl_2$	94
$FeSO_4$	91
$FeCl_3$	103
EDTA	85
NaN_3	102

Table 3. Effect of detergents on 3-HBDH Ⅱ activity

Detergent (0.1%)		Relative activity (%)
None		100
Pluronic	L-71	57.3
	P-103	94.7
	F-68	68.7
Adekatol	SO-120	110
	LO-7	109
	NP-690	112
	PC-8	93.9
	NP-720	54.2
Nikkol	SL-10	62.9
	TL-10	74
	MGO	55.7
	TMGO5	54.2
	MYO-6	75.6
	MYL-10	32.8
	BL-20TX	101
	NP-18TX	99.2
	OP-10	104
	HCD-100	91.6
TX-100		100
Tween 80		65.6

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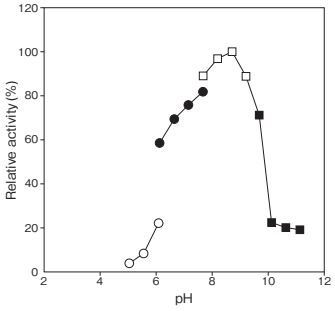
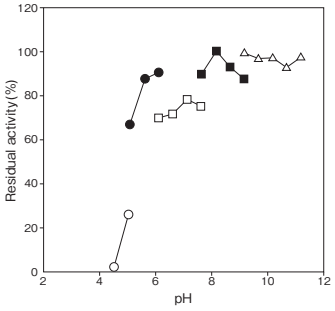
Fig.1 pH Optimum



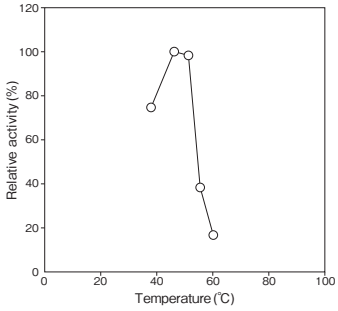
○ : Acetate buffer
● : Phosphate buffer
□ : Tris-HCl buffer
■ : Glycine-NaOH buffer

Fig.2 pH Stability



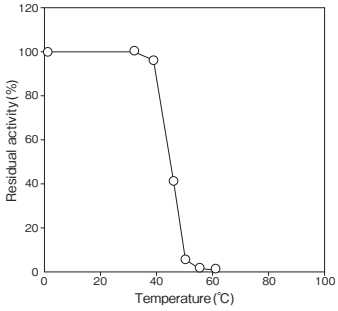
37℃, 60min
○ : Citrate buffer
● : Acetate buffer
□ : Phosphate buffer
■ : Tris-HCl buffer
△ : Glycine-NaOH buffer

Fig.3 Optimum Temperature



pH 8.5
50 mM Tris-HCl buffer

Fig.4 Thermal Stability



pH 8.5, 10min.
50 mM Tris-HCl buffer

Assay

■ Principle

The assay is based on the increase in absorbance at 340 nm as the formation of NADH proceeds in the following reaction:

$$\text{3–Hydroxybutyrate} + \text{NAD}^{+} \xrightarrow{\text{3–HBDH Ⅱ}} \text{Acetoacetate} + \text{NADH} + \text{H}^{+}$$

NAD: Nicotineamido adenine dinucleotide

■ Unit definition

One unit is defined as the amount of enzyme which converts 1 μ mole of 3–Hydroxybutylate to acetoacetate per minute at 37℃ under the conditions specified in the assay procedure.

■ Reagents

1. Reaction mixture
   Dissolve 126 mg of D–(–)–3–hydroxybutylic acid with 12.5 ml of 0.2 M Tris–HCl buffer pH 8.5 and add 25 ml of distilled water and 12.5 ml of 10 mM NAD solution.
2. Enzyme dilution buffer
   20 mM Tris–HCl buffer pH 8.5 containing 0.1% (W/V) BSA.
3. Reagents
   NAD: NACALAI TESQUE, INC. #24334–84
   D–(–)–3–Hydroxybutylic acid (Na salt):
   Sigma Chemical Co. #29836-0
   BSA: Millipore Fraction V pH5.2 #81–053

■ Enzyme solution

Accurately weigh about 20 mg of the sample and add enzyme dilution buffer to make a total of 20 ml. Dilute it with enzyme dilution buffer to adjust the concentration as required.

■ Procedure

1. Pipette accurately 3.0 ml of reaction mixture into a small test tube and preincubate at 37℃.
2. After 5 min, add exactly 40 μl of enzyme solution and mix to start the reaction at 37℃.
   ※ In the case of a test blank, add 40 μl of enzyme dilution buffer in place of enzyme solution.
3. After starting the reaction, measure the rate of increase per minute in absorbance at 340 nm. The rate must be measured within the linear portion of the absorbance curve.
   Absorbance sample : As/min
   blank : Ab/min
   △A/min = (As/min − Ab/min) ≦ 0.070 Abs/min

■ Calculation

$$\text{Activity (U/mg of powder)} = \frac{\triangle A/\text{min}}{6.22} \times \frac{3.04}{0.04} \times \frac{1}{X}$$

6.22 : millimolar extinction coefficient of NADH at 340 nm (cm^2 / μmole)
3.04 : final volume (ml)
0.04 : volume of enzyme solution (ml)
X : concentration of the sample in enzyme solution (mg/ml)

Storage

Storage at −20℃ in the presence of a desiccant is recommended.

3–HBDH Ⅱ活性測定法 (Japanese)

Ⅰ. 試薬液

1. 反応試薬混合液
   3–ヒドロキシ酪酸 126mg を 0.2M トリス –HCl 緩衝液 pH8.5 12.5ml で溶解した後、精製水 25ml と 10mM NAD 溶液 12.5ml を混合する。
2. 酵素溶解希釈用液
   0.1% (W/V) BSA を含む 20mM トリス –HCl 緩衝液 pH8.5
3. 試薬
   NAD (ニコチンアミドアデニンジヌクレオチド) :ナカライテスク製 #24334–84
   3– ヒドロキシ酪酸 [D– (–) –3– ヒドロキシ酪酸・ナトリウム塩] : シグマ製 #29836-0
   BSA: Millipore 製 Fraction V pH5.2 #81–053

Ⅱ. 酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液に溶解して全容 20ml とする。
その液を酵素溶解希釈用液で適宜希釈する。

Ⅲ. 測定操作法

1. 小試験管に反応試薬混合液 3.0ml を正確に分注して 37℃で予備加温する。
2. 5 分経過後、酵素試料液 40 μl を加えて混和し、37℃で反応を開始する。
   ※盲検は酵素試料液の代わりに酵素溶解希釈用液 40 μl を加える。
3. 反応開始後、340nm における吸光度を測定して直線的に反応している 1 分間当たりの吸光度変化を求める。
   求められた吸光度変化を試料液は As/min、盲検液は Ab/min とする。
   Δ A/min = (As/min − Ab/min) ≦ 0.070 Abs /min

Ⅳ. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A/\text{min}}{6.22} \times \frac{3.04}{0.04} \times \frac{1}{X}$$

6.22 : NADH の 340nm におけるミリモル分子吸光係数 (cm^2 / μmole)
3.04 : 反応総液量 (ml)
0.04 : 反応に供した酵素試料液量 (ml)
X : 酵素試料液中の検品濃度 (mg/ml)